<Final Report>

The first Korea-U.S. Seminar and Workshop on the Sustainable Marine Shrimp Culture:

Challenges and Opportunities for the Future of Marine Shrimp Farming

August 8-12, 2005
West Sea Fisheries Research Institute
Incheon, Republic of Korea

West Sea Fisheries Research Institute, NFRDI

National Oceanic and Atmospheric Administration

The first Korea-U.S. Seminar and Workshop on the Sustainable Marine Shrimp Culture

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Summary Report

The National Fisheries Research & Development Institute (NFRDI) of the Ministry of Maritime Affairs and Fisheries (MOMAF) of the Republic of Korea and the National Oceanic and Atmospheric Administration (NOAA) of the United States of America co-hosted the 1st Korea-U.S. Seminar and Workshop on the Sustainable Marine Shrimp Culture: Challenges and Opportunities for the Future of Marine Shrimp Farming, in Incheon, Republic of Korea, August 8-12, 2005. The seminar and workshop were organized and facilitated by the West Sea Fisheries Research Institute of NFRDI. The organizers invited four experts from U.S.A. and 14 experts including observers from Korea in the field of shrimp culture (i.e. pathologists, aquaculturists, nutritionists and geneticists. See the participants list in Appendix I). This seminar and workshop was fully supported by MOMAF-NOAA Joint Fund.

The seminar was held in the West Sea Fisheries Research Institute, Incheon on August 8, 2005 and the number of attendants was 88 people from institutes, universities, technical service centers, shrimp culture-related companies and shrimp farms. In the seminar four scientists from each country gave eight presentations on shrimp diseases, biosecurity for shrimp farming, shrimp feed and nutrition, sustainable shrimp production technologies and genetics (See Proceedings of Seminar in Appendix IV). During the seminar many questions and discussion were made between speakers and attendants. All presentations and discussion were made in English without interpreters. This seminar aimed to exchange information on the topics of ongoing workshop between scientists of two countries.

On August 9, 2005, Dr. In Kwon Jang and four US scientists visited shrimp feed manufacturer company, shrimp farms and hatchery. They visited CJ feed manufacturer company in Incheon. Dr. Park, CJ Feed Research Institute gave a tour of the feed production facility with an emphasis on shrimp feed nutrition and discussed on feed and nutrition with Dr. Allen Davis. On the way to Duksan, they visited shrimp and swimming crab farms and hatchery of *Fenneropenaeus chinensis* and *Litopenaeus vannamei* in Seosan, Chungcheongnam-do.

The workshop was held at the Crustacean Research Center under West Sea Fisheries Research Institute in Taean from August 10-11, 2005. Nine panelists including four experts from U.S.A. and five experts from Korea discussed on six topics on shrimp diseases, biosecurity, new technologies and practices, and feed and nutrition. One panelist from each side for each topic gave short introduction using power point presentation and leaded the discussion. About 20 shrimp farmers and researchers also attended at the workshop as observer. After discussion, both counter partners of each topic prepared summary report (See Summary Report of Workshop in Appendix V).

On August 12, 2005, Dr. In Kwon Jang and four U.S. scientists visited the National Fisheries Research & Development Institute (NFRDI), Busan and had a meeting with Mr. Young Kyu Park, director-general of NFRDI. In the afternoon three of U.S. scientists, Dr. Tzachi Samocha, Allen Davis and Craig Browdy, attended the symposium on 'the Present Status of Nutrion Research and the Future of Aquacuture Feed in Korea' organized by Korean Aquaculture Society and gave lectures on shrimp feed and nutrition (See Abstracts of Symposium in Appendix VI). Dr. Kenneth Hasson visited the department of Pathology, NFRDI and gave a lecture on Sampling, Diagnosis and Sources of the Principal Marine Penaeid Shrimp Viral Diseases.

In the 1st Korea-U.S. Seminar and Workshop on the sustainable marine shrimp culture, scientists of the two countries made good progress in the various fields of shrimp culture and agreed to collaboration on:

1. Exchange knowledge and experience in the are of shrimp diseases, biosecure for shrimp farming, SPF shrimp production technologies, sustainable shrimp production practices and shrimp feed and nutrition; and
2. Exchange of scientists between two countries to develop sustainable shrimp production techniques.

Appendix I.

List of Participants

<The United States>

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<Observer>

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Appendix II.

The first Korea-U.S. Seminar and Workshop on the Sustainable Marine Shrimp Culture

August 8-12, 2005
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Incheon, Republic of Korea

AGENDA

August 8, 2005

Seminar on "the Challenges and Opportunities for the Future of Marine Shrimp Farming" at West Sea Fisheries Research Institute, Incheon

09:30 **Registration**

PART I OPENING CEREMONY

10:00 **Opening and Welcoming Remarks**
Dr. Jong Yun Lee, General Director, West Sea Fisheries Research Institute, NFRDI

10:10 **Welcoming Remarks**
Dr. Im Gi Jeon, President of Korean Aquaculture Society

10:20 **Purpose, objectives and organization for the workshop**
Dr. In Kwon Jang, West Sea Fisheries Research Institute, NFRDI

10:30 **Coffee break**

PART II DISEASES AND BIOSECURITY OF SHRIMP FARMING

11:00 **Current infectious marine shrimp diseases of concern in the United States**
Dr. Kenneth Hasson, Texas Veterinary Medical Diagnostic Laboratory, Texas A & M University

11:30 **Current status of shrimp diseases and its control in Korea**
Dr. In Kwon Jang, West Sea Fisheries Research Institute, NFRDI

12:00 **Development of farm management protocols and regulatory controls for improved biosecurity and disease prevention in marine shrimp farming**
Dr. Craig Browdy, Marine Resources Research Institute, South Carolina Department of Natural Resources

12:30 **Biochemical haracteristics of local *F. chinensis* populations - RNA/DNA and Trypsin activity as condition indexes**
Dr. Su-Kyoung Kim, Crustacean Research Center, NFRDI

13:00 **Lunch hosted by General-Director of West Sea Fisheries Research Institute**

PART III NEW TECHNOLOGIES AND NUTRITION

14:00 **Super intensive shrimp production technologs for nursery and grow-out**
Dr. Tzachi Samocha, Texas Agriculture Experiment Station, Shrimp Mariculture Research Facility, Texas A & M University-Corpus Christi

14:30 **Genetic variability of Chinese white shrimp in Korea and genetic relationship with Chinese and Japanese shrimps**
Dr. Hyonsob Han, Biotechnology Research Bureau, NFRDI

15:00 **Historical review of feeding protocols for the Pacific hite hrimp *Litopenaeus vannamei* at Claude Peteet Mariculture Center, Gulf Shores, Alabama**
Dr. Allen Davis, Department of Fisheries and Allied Aquacultures, Auburn University

15:30 **Shrimp feed production in Korea**
Mr. Joo Min Kim, SCF Co., Ltd.

16:00 **Coffee Break**

PART IV CLOSING SESSION

16:20 **Discussion and Conclusion**

16:50 **Schedule for the next step**

17:00 **Closing Remarks**

18:00 **Dinner hosted by organizer**

Tuesday, August 9, 2005

Field trip to shrimp feed manufacture company, shrimp farms and hatchery

Wednesday, August 10, 2005

Workshop at Crustacean Research Center, WSFRI in Taean

10:00 **Diagnosis of shrimp diseases and its control technologies**
by Myoung Ae Park and Ken Hasson

11:00 **Biosecurity in shrimp production**
by Craig Browdy and Jong Sik Kim

12:00 Lunch

13:00 **Land based super intensive shrimp production technologies – water management, filtration, insulation, and feed and nutrition**
by and In Kwon Jang

14:00 **Development of SPF broodstock in *F. chinensis* and *L. vannamei***
by Craig Browdy and Su-Kyoung Kim

15:00 **Induced maturation and larval production of *L. vannamei***
by Tzachi Samocha and Dae Hyeon Kim

Thursday, August 11, 2005

Workshop contined

10:00 **Heterotrophic culture technologies in outdoor shrimp ponds**
by Tzachi Samocha and In Kwon Jang

11:00 **Shrimp feed and nutrition**
by Allen Davis and Sang-Min Lee

12:00 **Wrap-up and closing**

Before the discussion of each topic, discussion leaders will give general introduction using Power point or printed materials for 5-10 min.

August 12, 2005

Symposium on Aquculture Feed and Nutrition organized by Korean Aquaculture Society at National Fisheries Research and Development Institute (NFRDI), Busan

Three US attendants can give presentations in the symposium

13:00 **Development of marine fish meal and fish oil replacement diets for the Pacific white shrimp *Litopenaeus vannamei***
by Dr. Tzachi Samocha

13:25 **Evaluation of diets for the intensive culture of the Pacific white shrimp *Litopenaeus vannamei*: Experimental systems development and preliminary results from pond trials with fishmeal free feeds**
by Dr. Craig Browdy

13:50 **Use of Plant Protein Sources in Shrimp Feeds**
by Dr. Allen Davis

15:00 **Seminar on 'Sampling, diagnosis and sources of the principal marine penaeid shrimp viral diseases' at Department of Pathology, NFRDI**
by *Dr. Kenneth W. Hasson*

18:00 Dinner hosted by General-Director of National Fisheries Research & Development Institute

Appendix III.

OVERALL ITINERARY

Sunday, August 7, 2005

Afternoon US participants arrive Incheon, Korea

Dr. Tzachi Samocha
From San Francisco (DELTA AIR, DL 7866 operated by KAL): Aug 7, 7:00 p.m.

Dr. Kenneth Wolf Hasson
From Dallas FT Worth (DELTA AIR, DL 7868 operated by KAL): Aug 7, 4:40 p.m.

Drs. Donald Allen Davis and Craig L. Browdy
From San Francisco (United Air line, UA 0893): Aug 7, 4:20 p.m.

Hotel check-in:
Hyatt Regency Incheon Airport
2850 Woonseo-dong, Jung-gu, Incheon
Tel: (032) 745-1234

Monday, August 8, 2005: *09:30-17:00*

Seminar on "the Challenges and Opportunities for the Future of Marine Shrimp Farming" at West Sea Fisheries Research Institute, Incheon

Breakfast at hotel
Lunch hosted by Director-General of West Sea Fisheries Research Institute
Dinner hosted by organizer

Tuesday, August 9, 2005

Field trip to shrimp feed manufacture company, shrimp farms and hatchery

08:00 Hotel check-out
08:30 Depart Hotel
09:30 CJ Feed Manufacture Co.
12:00 Shrimp farms in Taean
14:00 Shrimp hatcheries in Seosan
18:00 Hotel check-in:
Duksan Spa Tourist Hotel
Sadong-ri 15, Duksan-Myeon, Chungnam
Tel: 041-338-5000

Wednesday, August 10, 2005

Workshop at Crustacean Research Center, WSFRI in Taean

08:00 Breakfast at hotel

08:30 Depart hotel
09:00 Arrive Crustacean Research Center in Taean
18:00 Return hotel

Thursday, August 11, 2005

08:00 Breakfast at hotel
08:30 Hotel check-out and depart hotel
09:00 Arrive Crustacean Research Center in Taean
12:00 Closing Workshop
13:00 Depart Crustacean Research Center (by car)
18:00 Arrive Busan and Hotel check-in:
Royal Kingdom Hotel
Jung-dong 1509
Haeundae, Busan
Tel (051) 744-1331, Fax (051) 741-5757

Friday, August 12, 2005

Symposium on "the Present Status of Nutrition Research and the Future of Aquaculture Feed in Korea" organized by Korean Aquaculture Society at NFRDI (National Fisheries Research and Development Institute), Busan

Three US participants can give presentations in the symposium

09:00 Depart hotel
10:00 Visit fish market
12:30 Attend to Symposium at NFRDI
13:00 Development of marine fish meal and fish oil replacement diets for the Pacific white shrimp *Litopenaeus vannamei*
by Dr. Tzachi Samocha
13:25 Evaluation of diets for the intensive culture of the Pacific white shrimp *Litopenaeus vannamei*: Experimental systems development and preliminary results from pond trials with fishmeal free feeds
by Dr. Craig Browdy
13:50 Use of plant protein sources in shrimp feeds
by Dr. Allen Davis
15:00 Seminar on shrimp diseases and diagnosis at Department of Pathology, NFRDI
by *Dr. Kenneth W. Hasson*

18:00 Dinner hosted by General-Director of National Fisheries Research & Development Institute

Saturday, August 13, 2005

Morning: three U.S. participants hotel check-out and departure to Incheon or Gimpo

Drs. Tzachi Samocha and Kenneth Wolf Hasson
From Busan to Incheon (Korea Air, KE 1402): Aug 13, 7:00 a.m.
From Incheon to Atlanta (Delta Air, DL 7851) operated by Korea Air: Aug 13, 10 a.m.

Dr. Donald Allen Davis
From Busan to Gimpo (Korea Air, KE 1112): Aug 13, 9:30 a.m.
From Incheon to San Francisco (United Air, UA 0892): Aug 13, 1:50 p.m.

Sunday, August 14, 2005

Dr. Craig L. Browdy
From Busan to Gimpo (Korea Air, KE 1112): Aug 14, 9:30 a.m.
From Incheon to San Francisco (United Air, UA 0892): Aug 14, 1:50 p.m.

Appendix IV.

Proceedings of the 1st Korea-U.S. Seminar

on

the Sustainable Marine Shrimp Culture:

Challenges and Opportunities for the Future of Marine Shrimp Farming

August 8, 2005
West Sea Fisheries Research Institute
Incheon, Republic of Korea

PROGRAM

The 1st Korea-U.S. Seminar on the Sustainable Marine Shrimp Culture

Monday, August 8, 2005
West Sea Fisheries Research Institute
Incheon, Republic of Korea

09:30 **Registration**

PART I **OPENING CEREMONY**

Announcer: Dr. Duk-Young Kang
West Sea Fisheries Research Institute, NFRDI

10:00 **Opening and Welcoming Remarks**
Dr. Jong Yun Lee, General Director, West Sea Fisheries Research Institute, NFRDI

10:10 **Welcoming Remarks**
Dr. Im Gi Jeon, President of Korean Aquaculture Society

10:20 **Purpose, objectives and organization for the workshop**
Dr. In Kwon Jang, West Sea Fisheries Research Institute, NFRDI

10:30 ***Coffee break***

PART II **DISEASES AND BIOSECURITY OF SHRIMP FARMING**

Chairman: Dr. Im Gi Jeon
President of Korean Aquaculture Society

11:00 **Current infectious marine shrimp diseases of concern in the United States**
Dr. Kenneth Hasson, Texas Veterinary Medical Diagnostic Laboratory, Texas A & M University

11:30 **Current status of shrimp diseases and its control in Korea**
Dr. In Kwon Jang, West Sea Fisheries Research Institute, NFRDI

12:00 **Development of farm management protocols and regulatory controls for improved biosecurity and disease prevention in marine shrimp farming**
Dr. Craig Browdy, Marine Resources Research Institute, South Carolina Department of Natural Resources

12:30 **Biochemical characteristics of local *F. chinensis* populations - RNA/DNA and Trypsin activity as condition indexes**
Dr. Su Kyoung Kim, Crustacean Research Center, NFRDI

13:00 ***Lunch***

PART III NEW TECHNOLOGIES AND NUTRITION

Chairman: Dr. Sang Min Lee
Kangnung National University

14:00 **Super intensive shrimp production technologies for nursery and grow-out**
Dr. Tzachi Samocha, Texas Agriculture Experiment Station, Shrimp Mariculture Research Facility, Texas A & M University-Corpus Christi

14:30 **Genetic variability of Chinese white shrimp in Korea and genetic relationship with Chinese and Japanese shrimps**
Dr. Hyonsob Han, Biotechnology Research Bureau, NFRDI

15:00 **Historical review of feeding protocols for the Pacific white shrimp *Litopenaeus vannamei* at Claude Peteet Mariculture Center, Gulf Shores, Alabama**
Dr. Allen Davis, Department of Fisheries and Allied Aquacultures, Auburn University

15:30 **Shrimp feed production in Korea**
Mr. Joo Min Kim, SCF Co., Ltd.

16:00 ***Coffee Break***

PART IV CLOSING SESSION

Chairman: Dr. In Kwon Jang
West Sea Fisheries Research Institute, NFRDI

16:20 **Discussion and Conclusion**

16:50 **Schedule for the next step**

17:00 **Closing Remarks**

Opening and Welcoming Remarks

Jong Yun Lee

Director-General of West Sea Fisheries Research Institute

Thank you, scientists from the United States and Korea.

Ladies and gentlemen, it's my greatest pleasure to present "my welcoming address" to your outstanding scientists. On behalf of the West Sea Fisheries Research Institute, I would like to express my warmest appreciation to invited speakers and all of participants attended in the seminar on the sustainable marine shrimp culture.

Shrimp is one of major farmed species in the west coast of Korea. More than 95% shrimp farms locates along the west coast. Farmed shrimp production in Korea was about 2,500mt in 2003 from 450 farms. However, outbreaks of viral disease is the most serious problem in shrimp farming industry in Korea like other shrimp producing countries in Asia and America. Because of virus-related diseases, about fifty percent of shrimp farms in Korea had total loss of farmed shrimp crops in 2003 and shrimp farmers suffered great economic loss.

Currently most of shrimp farms in Korea are culturing Chinese fleshy shrimp, *Fenneropenaeus chinensis*. To solve disease problems of this farmed shrimp, my institute introduced the Pacific white shrimp from Hawaii, U.S.A. because this species is known to be more resistant to diseases than Chinese fleshy shrimp. So far, culture with the white shrimp is very successful in many farms but it still causes disease problems in some farms.

To solve and overcome the problems facing with unexpected and unsustainable shrimp farming in Korea, we need to exchange information in the field of the shrimp diseases, sustainable shrimp production practices, and shrimp feed and nutrition.
In this seminar, I hope we can have the opportunity to share our knowledge and experience on the shrimp culture between two countries.

Again, I would like to appreciate all of you for joining this seminar and workshop. I'm sure we can strengthen our cooperative research activities for the sustainable marine shrimp culture between Korea and U.S.A.

Thank you very much.

Welcoming Remarks

Im Gi Jeon

President of Korean Aquaculture Society

Ladies and gentlemen!
It is my great honour and pleasure to congratulate all of you for holding the Korea-U.S. Joint Seminar on the Sustainable Marine Shrimp Culture. I would like to appreciate participation of scientists from the United States as invited speakers, many scientists of National Fisheries Research & Development Institute and universities, and all of other participants.

Aquaculture has provided us with more foods for human consumption in last decades and is poised to become an important source of marine protein that human needs in the future. This is because the capture fisheries productions have reached their maximum sustainable limits and is likely to decline as wild stocks are diminished.
Actually, aquaculture is one of the fastest growing food producing sectors of the world, and become already an important industry of food products in many countries including Korea and the United States. I think, its importance will keep going in the future.

It is true in shrimp. Production of farmed shrimp in the world is more than 1.5 million tons. Shrimp farming is one of the most competitive aquaculture industries in the world. Even though shrimp farming is not high competitive in Korean aquaculture, it is still important in the west coast provinces because, except for shrimp, there are few suitable farmed species in the west coast of Korea. Unfortunately shrimp farming industry is facing with serious problems, so called mass mortalities and unstable production because of poor management and technology.

Aquaculture is a comprehensive science. Here, we need the best aquaculture management techniques to overcome the present problems in shrimp farming. In this sense, I believe this seminar will be a golden opportunity to share knowledge and information on shrimp culture between two countries.
Finally, as a President of Korean Aquaculture Society, I'd like to express that I have total confidence in the success of this seminar and workshop.

Thank you.

Purpose, objectives and organization of the workshop:

In Kwon Jang

West Sea Fisheries Research Institute, NFRDI
Elwang-dong, Incheon 400-420, Republic of Korea

In accordance with the Arrangement for Scientific and Technical Cooperation in Integrated Coastal and Ocean Resources Management signed in 2000 between the National Oceanic and Atmospheric Administration(NOAA) of the United States of America and the Ministry of Maritime Affairs and Fisheries(MOMAF) of the Republic of Korea, the 1st U.S.-Korea Joint Coordination Meeting(JCM) for Aquaculture Cooperation between the National Fisheries Research & Development Institute(NFRDI) and NOAA was convened in Busan, Republic of Korea, from April 15-16, 2002.

In the 1st JCM, both sides shared mutual understanding that the two countries need a holistic and sustainable approach to manage marine living resources and coastal areas, and agreed that this cooperation would provide a formal mechanism to improve the level of coordination and collaboration among aquaculture scientists and mangers between the two countries.

In the 1st JCM, five contact scientists from both countries were chosen: Dr. In Kwon Jang, West Sea Fishery Research Institute and Dr. Myoung Ae Park, Pathology Division, NFRDI (Korea); Dr. Tzachi Samocha, Texas A&M University-Corpus Christi, Dr. Donald Lightner, University of Arizona and Mr. Josh Wilkenfeld, Arizona Mariculture Associates (U.S.). Three attendants, Drs. In Kwon Jang, Myoung Ae Park and Tzachi Samocha agreed to work plan for 2002-2003 for the field of shrimp culture (See Work Plan 2002-2003)

In the shrimp culture field, scientists of the two countries made good progress in 2003. As a follow up to the cooperative activities endorsed by the Joint Working Group of the 1st JCM, two reciprocal visits were arranged in July and September of 2003 for research tours on biosecure shrimp culture and improved closed recirculating systems, respectively. Professor Tzachi Samocha of the Texas Agricultural Experiment Station, Texas A&M University-Corpus Christi visited several Korean shrimp farms and provided useful information on bioseucre shrimp culture and gave a series of lectures to shrimp farmers and researchers during his visit in July, 2003. Drs. In Kwon Jang and Bong Lae Kim of the Crustacean Research Center, NFRDI visited shrimp farms and institutes in Florida, South Carolina and Texas in September, 2003. After these visits, Dr. jang used a biosecure intensive nursercy and induced maturation system for *Fenneropenaeus chinensis* at the Crustacean Research Center with promising results.

The 2nd U.S.-Korea Joint Coordination Panel Meeting for Aquaculture Cooperation was held in Honolulu, Hawaii, U.S.A from February 29 to March 5, 2004. Drs. In Kwon Jang and Myoung Ae Park of Korea and Dr. Tzachi Samocha of U.S.A. were attended in the meeting and agreed to propose the work plan for 2004-2005(See Work Plan 2004-2005). According to this work plan, Dr. In Kwon Jang of West Sea Fisheries Research Institute, NFRDI visited the Waddell Mariculture Center, South Carolina and Texas Agricultural Experiment Station Shrimp Mariculture Research Facility, Texas, U.S.A. on September 20th to November 8th, 2004,

With this background of shrimp culture field, this seminar and workshop, supported by MOMAF-NOAA Joint Fund, is held in the West Sea Fisheries Research Institute and the Crustacean Research Center from August 8-12, 2005. The purpose of the seminar and workshop is to exchange knowledge and experience in the area of the shrimp diseases, biosecure for shrimp farming, shrimp feed and nutrition and sustainable shrimp production practices, and discuss future cooperative research between two countries.

Work Plan 2002-2003

A. Nursery and grow-out

Researchers of the Texas Agricultural Experiment Station-Shrimp Mariculture Research Facility (TAES-SMRF), Corpus Christi, Texas have been involved for several years in intensive research program designed to develop cost-effective, sustainable and biosecured production practices for penaeid shrimp. This research was conducted at the TAES-SMRF and on shrimp farms in Texas, Arizona, Florida, Ecuador and Israel. Research conducted at the facility focused on native (e.g., *Farfantepenaeus aztecus*, *F. duorarum* and *Litopenaeus setiferus*) and exotic shrimp species (*L. vannamei*). These studies targeted wide range of research areas.

Both Dr. Samocha and Dr. In Kwon Jang of the West Sea Fisheries Institute (WSFRI) will work to establish a mutual research program in the following areas, and both feel that focusing their research program on these topics will be mutually beneficial for both countries. We suggest focusing on the following topics:

1. To continue the development of biosecure environmentally friendly shrimp nursery and grow-out technologies.
2. To develop closed recirculating systems for induced-maturation and postlarvae production penaeid shrimp.
3. To exchange scientists between the two countries to study biosecure shrimp farming technologies.

B. Collaborative work on SPF/SPR broodstock shrimp development

C. and shrimp disease control and diagnostic issues

In partial financial support from NOAA, Dr. In Kwon Jang from the West Sea Fishery Research Institute/NFRDI visited from November 6-12, 2001, the University of Arizona in Tucson and the Arizona Mariculture Associates, the Woods Brothers Shrimp Farm, and the Arizona Shrimp Farm in Dateland, Arizona, USA to explore ideas for potential research possibilities and learn about the status of U.S. shrimp virus disease control and the development of specific pathogen free (SPF) stocks of the Chinese white shrimp, *Fenneropenaeus chinensis*. Dr. Hui Gong of the Arizona Mariculture Associates visited the West Sea Fishery Research Institute, NFRDI, and Pukyung University to import and establish broodstock for SPF lines of *F. chinensis* for use as a winter crop in Arizona. Under auspices of MOMAF-NOAA Arrangement for Scientific and Technical Cooperation in Integrated Coastal and Ocean Resources Management, the Arizona Mariculture Associates imported successfully a total of 148 adult shrimp from Korea in July 2001 as the first attempt. The University of Arizona, in collaboration with the Arizona Mariculture Associates, has already begun some-level of cooperation with Dr. In Kwon Jang of the West Sea Fishery Research Institute on white spot syndrome virus (WSSV), and provided him with some purified WSSV and monoclonal antibodies to WSSV to supplement his research efforts on these topics in Korea. Both sides agreed to continue collaboration on:

1. Transfer of SPF broodstock of *L. vannamei* and implementation of production technology to Korea.
2. Development of SPF/SPR broodstock of *Fenneropenaeus chinensis*
3. Exchange of scientists between the two countries to develop diagnostic techniques of shrimp diseases.

Work Plan 2004-2005

During the year of 2003, progress was made in terms of implementing the use of biosecure and sustainable practices for the production of shrimp, in spite of the limited funds available. The activities included the use of closed recirculating induced-maturation and nursery systems with limited discharge of salt water. Both countries agreed to continue the on-going research on the use of a closed recirculating system, and in addition, to work on the development of SPF (specific pathogen free) *Fenneropenaeus chinensis* through:

1. A planned visit of a Korean scientist for two months in 2004 to the Texas Agricultural Experiment Station (TAES) in Corpus Christi and reciprocal visits of shrimp experts from both countries for approximately one month each trip in 2005 (supported from the MOMAF-NOAA Joint Fund);
2. One TAES graduate/Ph.D. student's visit to Korea to collaborate in research on *F. chinensis* for approximately 6 months up to one year during 2005 (supported from R&D grants of NFRDI);
3. Exchange of information for developing SPF *F. chinensis* strain in Korea and biosecure and sustainable high-density shrimp growout technology in both Korea and the U.S.;
4. Evaluation and analysis on the growth, survival and economic viability of producing *F. chinensis* in West Texas (funding to be sought); and
5. Submission of a proposal to USAID for the development of organic shrimp and other value added methods to improve economic viability of shrimp production under biosecure and sustainable conditions.

Korea contact: In Kwon Jang, NFRDI
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Current infectious marine shrimp diseases of concern in the United States

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There are currently 10 different states within the United States that farm the Pacific white shrimp, *Litopenaeus vannamei*. Texas is the largest producer of farmed and wild caught shrimp in the U.S., accounting for ~ 28% of the nation's annual production of 200 million pounds. In the U.S., the use of selectively bred, specific pathogen free (SPF) shrimp stocks and implementation of biosecurity measures have limited shrimp losses to infectious disease. However, even with these preventive measures in place, infrequent viral epizootics have occurred in the U.S. Shrimp are susceptible to a variety of infectious diseases that can be divided into four main categories, which include the viruses, bacteria, fungi and parasites. Of these four categories, viruses, and bacterial infections (both intracellular and extracellular), are the principal health concern in Texas.

Over 20 different viral diseases have been described in penaeid shrimp with seven of these infectious agents now designated as notifiable by the OIE (World Organization for Animal Health). The three most economically important viruses include White Spot Syndrome virus (WSSV), Taura Syndrome virus (TSV) and Yellowhead virus (YHV). White Spot Syndrome virus is the most lethal and widespread of the three, having caused billions of dollars in shrimp losses worldwide. An intranuclear, enveloped, dsDNA virus and member of the Nimaviridae virus family, WSSV is unique among the shrimp viral diseases due its wide host range that includes both freshwater and marine crustaceans. Originally detected in China during 1992, WSSV has now been reported throughout the Eastern and Western hemispheres. It was first detected in the Americas in 1995 in wild-caught *P. setiferus* stocks collected from the Gulf of Mexico. During 1999, the widespread incidence of WSSV epizootics throughout Central and South America resulted in devastating economic losses to the shrimp mariculture industry. The most recent outbreaks of WSSV occurred in Brazil and a single farm in Hawaii during 2004. Due to the economic importance of this disease, all U.S. aquatic diagnostic labs routinely screen for the presence of WSSV in both domestic and foreign submitted shrimp samples.

Taura Syndrome virus, an intracytoplasmic, ssRNA virus and member of the Disctroviridae, was first recognized in farmed *P. vannamei* in Ecuador during summer 1992. Within 5 years of its discovery, TSV spread to all 14 shrimp producing countries in the Americas, mainly through unrestricted international commerce of infected postlarvae and broodstock. Epizootics typically occur 15-40d post-stocking, resulting in 60-95% mortality among susceptible *P. vannamei* populations. TSV induces a unique disease cycle in *P. vannamei* consisting of three histologically and clinically distinct phases; a peracute to acute phase, a brief transition phase and a long term chronic phase among survivors. The introduction of *P. vannamei* stocks into Asia has resulted in TSV outbreaks in Taiwan, Thailand, Indonesia and China over the past 6 years. A recent TSV outbreak that occurred on five Texas farms in 2004 was associated with the stocking of TSV susceptible shrimp strains. Economic losses were minimized with the restocking of ponds with TSV-resistant strains. With the identification of four different TSV strains in the Americas, the high mutability of this virus poses a continued threat to both wild and farmed shrimp worldwide.

Yellowhead virus, an enveloped, intracytoplasmic, ssRNA virus that is classified as a member of the Ronaviridae, was first recognized in Thailand during 1991 and is found in shrimp farming regions throughout the Eastern hemisphere. Capable of infecting all of the important farmed penaeid species, YHV causes an acute infection that can result in 100% mortality among infected stocks. At least six different YHV serotypes have been isolated from Asia and Australia. While of concern, no definitive YHV infections have yet been detected in the Western hemisphere.

Infectious Myonecrosis virus (IMNV) is an intracytoplasmic, dsRNA virus, corresponding to the Totiviridae and the most recently described penaeid shrimp virus. This disease was first recognized in farmed *P. vannamei* stocks in Brazil during 2003 and was recently characterized by the University of Arizona. The histological characteristics of IMNV include severe muscle necrosis together with the formation of numerous ectopic and lymphoid organ spheroids. Mortality due to IMNV among infected farmed *P. vannamei* stocks can range from 60-85%. Epizootics occur among juvenile stage shrimp following periods of stress resulting from handling or abrupt environmental changes. However, the disease has also been reported in postlarvae and broodstock. Experimental exposure studies have demonstrated that *P. stylirostris* and *P. monodon* juveniles are also susceptible to this disease, which has not yet been classified by the OIE. Both RT-PCR and in situ hybridization assays have been developed for the diagnosis of IMNV, which has only been detected in Brazil to date.

Necrotizing hepatopancreatitis (NHP) is caused by an intracellular rickettsial-like organism that selectively targets hepatopancreocytes and is a recurring problem in Texas shrimp farms as well as in Central and South American countries. This disease was first described in Texas farmed *P. vannamei* in 1985. As a chronic disease, NHP causes necrosis of the hepatopancreatic tubules and can result in mortalities reaching 95% if left untreated. Little is known about the potential vectors of this disease. However, outbreaks are associated with high water temperature and salinity over 20ppt. Early detection and administration of oxytetracycline-medicated feed are presently the most effective means of controlling this disease.

The occurrence of opportunistic *Vibrio* infections has recently become a growing problem in shrimp mariculture that appears to be linked with the increasing practice of reduced water exchange as a common health biosecurity measure. The resulting high organic loads tend to lead to increased bacterial counts in the water, as well as an increased incidence of bacterial disease in the shrimp. Bacterial infections can manifest themselves in a variety of ways including localized infections within the hepatopancreas (hemocytic hepatopancreatitis) or midgut (hemocytic enteritis), generalized systemic infections involving multiple tissues or organs and infections of the exocuticle by chitinolytic *Vibrio* sp. resulting in grossly visible melanized lesions (Black Spot or Shell disease). Hemocytic hepatopancreatitis is a common chronic disease problem seen in Texas farmed shrimp populations that is associated with low pond mortality with the potential of leading to more serious systemic infections.

Current status of shrimp diseases and its control in Korea

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Shrimp farming in Korea initially began in the 1960s and the farming industry was developed in 1980s. Farmed shrimp production has been rapidly increased since the 1990s. Farmed shrimp production reached 3,256 mt from about 2,600 ha of 437 farms in 2001. However, due to more frequently occurring outbreaks of shrimp diseases, the farmed shrimp production decreased to 2,332 mt in 2004. The unit production of farmed shrimp was 1.25 mt/ha in 2001, but it was 1.11, 1.07 and 1.02 mt/ha in 2002, 2003 and 2004, respectively.

Two indigenous species, *Fenneropenaeus chinensis* (Chinese fleshy prawn) and *Marsupenaeus japonicus* (Japanese Kuruma shrimp) were cultured before the mid-1990s in Korea, but *M. japonicus* has not been cultured since the outbreak of WSSV (white spot syndrome virus) which occurred in 1993. In 2003, SPF broodstocks of the Pacific white shrimp, *Litopenaeus vannamei* were introduced from Hawaii to the Crustacean Research Center, NFRDI for the first time. About 1,400 SPF broodstocks of *L. vannamei* were imported to commercial hatcheries for mass postlarval production in 2004 and more than 30 million of the PL were produced and stocked to outdoor ponds. The results of culture with *L. vannamei* in Korea were very satisfactory. The growth rate was 30% to 50% higher than that of *F. chinensis* and the frequency of viral outbreaks was much lower than *F. chinensis*. However, mass mortalities from IHHNV and TSV outbreaks, of which have never been occurred in Korea, were reported from several shrimp farms with *L. vannamei* in 2004 and 2005. The route of introduction of these viruses was not confirmed but it is possible that some broodstocks and/or enriched *Artemia* or bloodworms imported from China carried these pathogens.

Parasitic, bacterial, and viral diseases cause serious economic damage to shrimp farms every year. The most frequent parasitic disease is a ciliate, *Zoothamnium* sp. which is found on shrimp gills from July to August.

Major bacterial diseases are caused by *Vibrio parahaemolyticus, V. harveyi, V. spp*., and *Aeromonas* spp. and occur from July to September. Three kinds of viral diseases, HPV (Hepatopancreatic Parvo-like Virus), BMN (Baculoviral Midgut Gland Necrosis Virus), and WSSV are found in shrimp farms of F. chinensis. HPV was reported from *F. chinensis* in 1991 and BMN was reported from *M. japonicus* in 1991. WSSV was first identified from a *M. japonicus* farm in and available evidence suggested the infection was derived from the *M. japonicus* broodstock for larval production imported from Taiwan in 1993. Outbreak of WSSV occurred from *F. chinensis* farms near the farm in 1994 and mass mortalities were followed by lots of farms along west coast in a few years. Among them, WSSV causes the biggest economic losses to shrimp industry in Korea. With the introduction of *L. vannamei*, however, new viral diseases, TSV and IHHNV are threatening the shrimp farming of *F. chinensis* as well as *L. vannamei* in Korea.

Larval production of *F. chinensis* is entirely dependent upon wild broodstocks in Korea. Domesticated broodstocks are not developed in commercial hatcheries. Farmers collect gravid females with bottom gill-nets during spring tides from late April. Since this species is a closed-thelycum species, fertilization is not a problem. From this point of view, the viral infection rate of wild broodstocks is closely related to the artificial shrimp seeds for stocking. In 2001 the infection rates of WSSV and HPV in wild females were 5.04% and 8.0%, respectively. However, these rates increased to 28.4% and 31.9% respectively in 2004.

To control viral diseases in shrimp farms, various technologies including rapid diagnosis and management of farms have been developed and will be discussed in the presentation.

Development of farm management protocols and regulatory controls for improved biosecurity and disease prevention in marine shrimp farming.

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Prevention of viral disease continues to represent one of the most formidable challenges to sustainable and profitable marine shrimp culture. Much effort has gone into development of management strategies to better assure the health of cultured shrimp. Success depends upon consistent and effective implementation of well conceived biosecurty assurance plan for shrimp farms along with reasonable and enforceable regulations aimed at controlling the spread of excludable pathogens.

To provide a framework for the controlled development of farm biosecurity strategies, hazard analysis critical control point (HACCP) principles were applied as a risk management tool for the control of exotic viruses at shrimp production facilities. This process allowed for systematic hazards analysis and determination of critical control points (with associated limits monitoring and verification) and areas for which best management practices were needed. Following analysis of flows in an integrated shrimp production system, a series of potential hazards were analyzed including: shrimp stocks, incoming water, feeds and other inputs, human and animal vectors, equipment etc. The development and proper use of specific pathogen free stocks forms the basis for biosecure production. Proper site selection, facilities designs and management controls can then be applied to control other potential vectors for disease introduction. Examples based on successful application of these principals in the US are provided.

Ultimately, improving health in the industry as a whole depends upon cooperation of growers and effective and enforceable regulations. With the emergence of new pathogens and potential for mutation of existing pathogens into new and more virulent strains, regional or national regulations to control importation and transfer of stocks can help reduce the potential for new epizootics. An example of a permitting structure developed in SC for the culture of non-indigenous shrimp *L. vannamei* is provided.

Through the consistent application of stock assessment, farm operations plans and contingencies for disease outbreaks, industry development can be promoted while managing risks associated with excludable pathogens.

Biological characteristics of local *Fenneropenaeus chinensis* populations -RNA/DNA ratio and Trypsin activity as condition indices

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The fleshy prawn, *F. chinensis*, occurs in the northwest Pacific region along Korea and Japan and to southern China, and is locally abundant in the coastal areas of Korea. Two different geographical populations of *F. chinensis* are found in the Yellow Sea; a small Korean population off the west coast of Korea and a larger Chinese population in the Yellow Sea and Pohai Sea.

F. chinensis is a main candidate for shrimp aquaculture in Korea, and its larval production begins with the selection of wild broodstocks because technologies of SPF production and domestication of broodstocks are not commercially developed. It means that successful production of these shrimps strongly depends on the condition of adult wild prawns, i.e. selecting the best healthy condition and not infected prawn stocks with viruses. Korean prawn populations are divided into three small local groups including Narado, Young-Kwang and Taean. The mated females of these local groups are observed each with the time-lag of around one month. The duration of this time-lag gives the extended chance of collecting and selecting adult females. Therefore, it is important to monitor the condition of each prawn groups and the state of gonad maturation.

In this presentation, the method of biochemical analysis to determine the condition of these marine organisms will be discussed. RNA/DNA (R/D) ratio in the gonad and in the body part can be used to identify the onset of maturity and nutritional conditions. Tryptic enzyme activity and protein concentration in digestive track will give useful information about the feeding and nutritional conditions between the virus infected and none-infected groups.

Super intensive shrimp production technologies for nursery and grow-out of the Pacific white shrimp *Litopenaeus vannamei*

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During the last two decades there has been a worldwide expansion of the shrimp farming industry. With the increase in world population, it is most likely that this trend will continue since contributions from fisheries are expected to stay unchanged or even decrease. This industry experiences heavy crop-losses due to disease outbreaks which sometimes are associated with receiving water degradation. Thus there is a need for the development of new production practices that are environmentally sustainable and biosecure.

Shrimp production can take place in single, two, or three-phase systems with the two-phase system being the one widely used by farmers and mostly referred to as the nursery phase. In a single-phase system, postlarvae are stocked directly into the grow-out ponds, while in a multi-phase system, shrimp are stocked into small tanks or ponds before moving them into the grow-out ponds. Shrimp production in a multi-phase system provides several advantages over direct stocking. Among others, one can find improved shrimp survival, more efficient facility utilization, better control over predators, and better feed and water quality management. Furthermore, many producers began to use shrimp nursery facilities as quarantines to minimize losses to viral disease outbreaks. The first part of the presentation will provide a description of the nursery system developed by the TAES and some examples from commercial operations that used the same system.

The second part of the presentation will focus on indoor super-intensive production systems using low salinity and ambient seawater. Isolation from the coast and potential use of effluent water for crop-irrigation makes inland production of shrimp in low-salinity water a potential cost-effective tool to reduce crop-losses to diseases with reduced negative environmental impact. Nevertheless, reported yields from inland facilities are still lagging behind production from coastal facilities. Current technology advancement suggests that yields greater than 10 kg/m^3 are feasible in small scale super-intensive production systems operating at ambient salinity. Nevertheless, the high construction and operating costs suggest that these systems may only satisfy specialty niche markets. The presentation will provide a description and production figures from different super-intensive grow-out systems used in research and commercial facilities in Texas, Arizona, Hawaii, Florida, South Carolina, Ecuador, China and Israel.

Genetic variability of Chinese white shrimp in Korea and genetic relationship with Chinese and Japanese shrimps

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Chinese white shrimp, *Fenneropenaeus chinensis* is a high-grade shrimp that belongs to the genus *Fenneropenaeus*, Family Penaeidae, mostly distributed around South Sea and West Sea of Korea and Bohai Bay of China. Recently, it has been confirmed that these shrimp inhabits in Ariake Sea of Japan. Also, Chinese white shrimp is economically-valuable, main aquaculture species of the west coast of Korea. In 1993, shrimp production was dramatically reduced as the White Spot Syndrome Virus (WSSV) that occurred in Taiwan and China spread to Korea. Nevertheless, with the gradual increase of aquaculture area and number involved in aquaculture, 3,269 tons were produced in 2001. Yet starting from 2002, production per unit (ha) decreased, and 2,728 tons and 2,368 tons were produced in 2003 and 2004 respectively, showing continuous reduction. Also, the amount of catch of Chinese white shrimp was 5,946 tons in 1975, but decreased dramatically from 1997, all the way down to 1,211 tons in 2000. In 2003, the amount of catch decreased even further, recording mere 148 tons.

In recent years, the amount of shrimps caught and aquaculture production have been decreasing every year due to illegal overfishing, worsening environment, and infectious viruses that attack aquaculture shrimps. Thus, biological and genetic research is necessary to efficiently manage and increase stock enhancement of shrimp.

This research analyzed the isozyme of wild shrimps inhabiting in the south and west sea of Korea in order to investigate the genetic characteristics and variability of Korean shrimps. In addition, the research evaluated genetic relation and differentiation amongst Korean shrimps, Chinese shrimps in the Bohai Bay, and Japanese shrimps in the Ariake Sea. Furthermore, this research analyzed the genetic characteristics of artificially-produced shrimps for release in order to compare it with that of wild populations and hatchery stock.

For the sample of the shrimp, 493 shrimps were collected from a total of 9 areas. Some parent shrimps used as spawners were collected in spring from 4 areas in Korea including Goheung Narodo, Heuksando, and Youngkwang Bubseongpo, Taean Cheseokpo which are main habitats of these shrimps. Others were caught in autumn from 3 areas in Seocheon, Muchangpo and Chunsu Bay. Other samples were collected from China and Japan.

The isozyme was extracted from the muscle from each sample by applying starch gel electrophoresis. A total of 13 loci were detected from 9 isozymes - G3PDH*, LDH*, MDH*, IDHP*, GPI*, MEP*, MPI*, PGDH*, PROT*- that have been confirmed of possessing variability. Genetic distance between each sample by local population was presumed based on Nei (1988), calculating alleles frequency in each locus.

Genetic characteristics of Korean shrimps are as follows: 22 alleles were presumed from 13 analyzed loci. An average of 1.4 alleles were detected, showing very low diversity, while multiple allelism rate (P) showed an average of 0.293, indicating very low variability. These figures are almost similar to that of Kuruma shrimp(1.72, 0.283). For Korean wild shrimps, the observed value(Ho) of heterozygosity rate for each group ranged between 0.048(Narodo) and 0.12(Youngkwang Bubseongpo). Japanese shrimps indicated 0.054, similar to that of Korea, but Chinese shrimps posted a higher rate at 0.158. The heterozygosity rate of released artificially - produced shrimps was 0.142. The ratio of observed value to expected value(Ho/He) regarding heterozygosity rate was 0.811-1.062 for Korean populations, showing genetic equilibrium relatively in place. The ratio of Chinese population was 0.913, similar to that of Korean group. Japanese groups, however, showed a very low ratio of 0.551, which one can make a prediction that enormous inbreeding is taking place while the size of the foundation population is small.

According to the genetic distance amongst Korean shrimp groups based on Nei's(1988), the figure amongst Narodo, Seocheon, and Heuksando, and Chunsu Bay population was the closest, followed by the figure between Youngkwang and Muchangpo populations. This is because the shrimps that were produced using the Youngkwang population as brood adult shrimp were released in Muchangpo, testifying the effect of release. The Chinese population was recognized to have a slightly higher genetic distance compared to that of Korea, but no research results have been found to verify genetic independence. Thus Korean and Chinese shrimps have no independent populations that are either isolated reproductively or geographically differentiated, but all populations were almost similar genetically. Japanese shrimps, however, have very thin genetic supply resource. It is presumed that the population is barely sustained by inbreeding, so without great efforts to recover the stock, the shrimps may be endangered. Furthermore, the artificially-produced shrimps for release used in this research showed even more distant genetic distance than that of Korea and Japan, which can be predicted that they cannot have a positive effect on the wild population. Also, in order to improve the release effect, genetic variability must essentially be taken into consideration for production and release of shrimps when producing artificially-produced shrimps for release into sea.

Historical review of feeding protocols for the Pacific White Shrimp *Litopenaeus vannamei* at Claude Peteet Mariculture Center, Gulf Shores, Alabama

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Subject: feeding strategy, feeding management, marine shrimp.

Feed management is one of the most important activities when considering water quality management and production economics. It has been estimated that feed accounts for 55 to 60% of the operation cost in intensive systems and around 40% in semi-intensive systems. Adequate management should aim to optimized feed inputs, feed conversion ratio (FCR) and minimize the potential impact on effluents. Over the last decade there has been a tendency toward greater intensity in shrimp production, resulting in higher stocking densities and greater feed inputs which commonly result in a higher FCR. Quite often production failures, have been blamed on PL quality, feed, water quality and /or disease, although in most cases the origin of the problem is poor feed management. Typically shrimp farms use feeding charts, which are adjusted according to shrimp size, climate conditions, water quality parameters, and/or possibly through observations of feed consumption from feed trays. These feed tables are based on biomass, survival, and growth or a combination of these and they tend to increase linearly the amounts of feed offered as the shrimp grows. Unfortunately, from a theoretical point of view most feed tables over supplement feed and do not take into consideration the nutritional quality of the diet. This presentation will focus on historical review of various feeding protocols which had been used at Claude Peteet Mariculture Center, Gulf Shores (Alabama). Based on previous production trials conducted in 0.1 ha ponds stocked with shrimp at about 35 shrimp/m^2 feed inputs as high as 140 kg/ha and as low as 60 kg/ha have been used with similar overall production. This presentation will discuss current thoughts on feed inputs and the effect on overall production.

Shrimp feed production in Korea

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In Korea, the total production of animal feed was about 15 million mt in 2004. Of this the feed production for aquaculture was around 100,000mt and the production of shrimp feed was 9,200mt.

Shrimp culture in Korea was begun from 1970's. Shrimp feed of extruded pellet form was produced by Doosan Co. and provided to it's daughter farms only. However most farmers provided frozen fish and crustaceans for shrimp until 1980's. The commercial feed of pellet type was produced for shrimp farms by SCF Co., Ltd in 1980 for the first time. Currently seven feed manufacturers are producing shrimp feed for domestic and oversea markets.

Major farmed shrimp is *Fenneropenaeus chinensis* in Korea. The Pacific white shrimp, *Litopenaeus vannamei* was introduced to Korea in 2003 and spreading very fast to many farms. Shrimp feed for *L. vannamei* was produced by SCF Co. Ltd. in this year and provided to many local farms.

The general results of shrimp farms are as follows.
The average feed conversion rate in shrimp farms is 1.4 to 2.0. Shrimp price on farm is about 10-15US$/kg and depends on season and location of farms. The feed price per kg is about 1.4US$ for fry, 1.2US$ for juvenile, 1.2US$ for subadult and 1.10US$ for adult.

The Major problem in shrimp farming in Korea is viral outbreak of WSSV and HPV, but the effective control technologies is not developed so far. To replace unexpected shrimp farming, new farmed species including swimming crabs and other fish should be considered.

Appendix V.

Summary Report of Workshop on the Sustainable Marine Shrimp Culture

August 10-11, 2005
Crustacean Research Center, Taean
Republic of Korea

Diagnosis and control of shrimp diseases

Kenneth Hasson and Myoung Ae Park

The major diseases of concern in the Western hemisphere include White Spot Syndrome virus (WSSV), Taura Syndrome virus (TSV), Yellowhead virus (YHV) and Infectious Myonecrosis virus (IMNV). Diagnostic methods routinely used in the U.S. for the detection of these and other important shrimp pathogens are routine histopathology, in situ hybridization, and the polymerase chain reaction (PCR). The merits and faults of each of the three techniques were briefly discussed. As each of these techniques have inherent limitations and are fallible it is recommended that important disease findings be confirmed by a second diagnostic method prior to issuing a definitive diagnosis. Potential sources of penaeid shrimp diseases and their modes of introduction were discussed together with possible solutions. The sources described included live PLs and broodstock, frozen commodity shrimp, frozen bait shrimp, shrimp packing plant wastes, roadside shrimp markets, shrimp processing wastes in landfills, marine birds, aquatic insects, ship ballast water, pond intake water, farm equipment and vehicles. Implementation of potential solutions aimed at preventing disease introduction is difficult. State or federally mandated regulations, that are both beneficial and cost effective, are needed together with an infrastructure to enforce these regulations. To be successful, all groups impacted by such regulations (i.e. shrimp farmers, shrimp processors, shrimp importers and retailers) need to understand the purpose of such regulations and that disease exclusion will be economically beneficial to their shrimp industry as a whole in the long term.

Recommendations that were made during the open discussion sections of the meeting and during conversations with researchers and/or farmers included the following

1. Use of UV sterilization and/or ozonation to disinfect hatchery water in addition to mechanical filtration.
2. Decapsulation and/or disinfection of recently hatched artemia prior to their delivery to larviculture tanks in order to reduce bacterial loads.
3. Daily use of Treflan to treat hatchery water to prevent larval mycosis.

4. Daily use of EDTA to treat hatchery water with the purpose of chelating heavy metals to eliminate effects of heavy metal toxicity on larvae.
5. Storage of PCR primers in a frost-free freezer to extend their shelf life.
6. Preparation of PCR master mix, nucleic acid extraction and handling of PCR products should be conducted in three separate areas that are physically isolated from one another to reduce the possibility of cross-contamination by aerosolized amplicons. Master mix preparation should be conducted under a hood equipped with UV for sterilization between uses. A dedicated set of pipettors should remain in this hood and not used for other preparations.

Biosecurity in shrimp production

Craig Browdy and Jong Sik Kim

A brief review was given of basic farm management strategies to improve the outlook for more biosecure production and control of disease. In the case of *F. chinensis*, the vertical transfer of disease in wild populations is likely. In this case viral infections may be latent in the farmed populations until triggered to amplify and begin horizontal transmission in the farms. Triggers include any type of stressor including temperature, salinity, alkalinity, pH, dissolved oxygen, transfer of juveniles, etc. in particular for white spot syndrome virus scientific data was presented which indicates that for *L. vannamei*, acute infection does not occur at temperatures exceeding 31 degrees C. However, when temperatures drop to 29, acute disease causes near total mortality. Thus by maintaining optimal conditions in the pond environment, it is possible to reduce occurrence of acute disease.

Achieving good on farm health depends upon the application of best management practices in all aspects of husbandry. Often disease outbreaks occur over and over at certain farms while others seem to produce good crops every year. This can be related to site specific problems and/or combinations of management deficiencies none of which would be significant on their own but which result in problems when combined. Thus education and extension can play a critical role in improving overall health management.

A series of standard operating procedure recommendations was discussed including farm location and design, pond preparation, stocking strategies, water exchange, feed management, health monitoring, and disease exclusion. Each of these was reviewed and specifics were shared in ensuing discussions. Recommendations included efforts to reduce disease levels through the selection of disease free broodstock, hatchery evaluation and testing of broodstock and PL for WSSV using commercial rapid test kits ("Shrimple kits"). Use of acclimation stations was suggested where weak PL can be eliminated before stocking of ponds and health of PL can be enhanced over the first several days by feeding of high quality PL starter feeds, immunostimulation etc. It was further recommended to continue research on application of nursery systems based on the successful trials at the CMRC. This could allow for stocking of larger more robust shrimp and for expanding the hatchery season for SPF shrimp by warehousing PL during the spring. Finally recommendations were discussed for pond stocking which included a reevaluation of the use of chlorine disinfection and further work on pond screening, use of reservoirs, and holding of water for several days before stocking PL.

Land Based super-intensive production systems development

In Kwon Jang and Craig Browd

Over the past ten years the US Marine Shrimp Farming Program has sponsored a broad array of research efforts aimed at development of sustainable shrimp production technologies for the United States. These research efforts have at their core, focused upon basic research into the fundamental importance of the microbial community in terms of pond water quality and shrimp growth and survival. Several key studies at the Oceanic Institute (OI) have demonstrated the importance of pond growth factors for the culture of *Litopenaeus vannnamei* while research at the Waddell Mariculture Center (WMC) has explored effects of various management regimes on the dynamics of these communities. In South Carolina studies reducing water exchange demonstrated advantages of zero exchange systems. Shrimp ponds are now routinely managed without water exchange and commercial super-intensive zero exchange systems are being developed. Advanced nursery systems developed in Texas are expanding stocking windows and extending the growing season. Basic studies on water quality have led to improving pond conditions and greatly reduced farm discharge.

All of these efforts have culminated in the development of next generation super-intensive enclosed biosecure systems for shrimp production. Recent production results from the super-intensive systems at the WMC were reviewed and discussed. The importance of the microbial community in these systems was emphasized. Microbial communities contribute to the recycling of waste materials within the system and to maintaining water quality while enhancing the growth of the target crop. Detailed discussions were conducted on the importance of oxygen in the system and the use of fast growing specific pathogen free shrimp stocks.

It is anticipated that over time, these advances in science and technology may increasingly provide opportunities for development of next generation systems in underutilized rural areas or in peri-urban aquaculture settings close to niche markets.

Development of SPF Broodstock in *F. chinensis* and *L. vannamei*

Craig Browdy and Su-Kyoung Kim

A review and discussion of the principles of development of an SPF broodstock population as implemented by the US Marine Shrimp Farming Program was discussed. The historical roots of the SPF *L. vannamei* population currently marketed from US broodstock suppliers came from the IHHNV infections diagnosed in the late 1980s. At that time it was decided to try to produce an SPF population to eliminate the runt deformity syndrome plaguing US producers. The model described in guidelines of the International Council for the Exploration of the Sea was adopted. Founder stocks were are fully studied and candidate populations were carried through a primary and a secondary quarantine for disease status assurance. Shrimp were reproduced in secondary quarantine and the F1 was tested for disease. Only disease free F1 animals were incorporated into the breeding program. A series of populations were evaluated in this way over the next 5-8 years until a diverse founder population was developed.

According to the USMSFP model, breeding takes place in an ultra secure nucleus breeding center. Broodstock are multiplied for sale at broodstock production centers. They are then distributed to hatcheries for SPF seed production for stocking farms. Levels of biosecurity are high at all stages but by the nature of the facilities decrease form one to the next. Thus no animals are ever moved back from one facility to the next (ie from farms to hatcheries or from hatcheries to broodstock centers).

A discussion was also conducted on the concept of specific pathogen resistance. Two types of resistant populations may be found. An SPF resistant population has never been exposed to the pathogen. Resistance has been achieved through family selection and the testing of siblings in disease bioassay laboratories. These animals may be imported to different regions as they do not carry pathogens. For some viruses resistant lines have been developed through the selection of individual survivors in the presence of disease. This population though refractory to the disease agent, in many cases, carry the agent in a chronic infection. Thus a resistant carrier would be less appropriate for transboundary shipment as it could spread disease agents into new geographic ranges. At the end of the discussion observations on the development of resistant lines for TSV was compared with the complete lack of documented success in generating resistance to WSSV to date.

Induced maturation and larval production of *Litopenaeus vannamei*

Tzachi M. Samocha and Dae Hyeon Kim

This paper summarizes work conducted at the Texas Agricultural Experiment Station, Shrimp Mariculture Research Facility, Corpus Christi, Texas. Although the research was done on two local species from the Gulf of Mexico, the information collected in these studies should be applicable for the Pacific white shrimp as well. The basic system design and management presented hear are based on the same system design developed for this species. Currently, there are no commercial hatcheries that produce postlarvae of native species in the southern region of the U.S. Furthermore, as all of the three native shrimp species in the Gulf of Mexico were found to be infected with a white spot like virus, any work with these species should take all precautions to minimize the spread of this deadly virus. Also, as wild *Fenneropenaeus chinesis* were found to be heavily infected with white spot virus, the information and the management concept describe in this presentation should also be applicable for this species. The work describes the results obtained with the brown shrimp *F. aztecus* and the pink shrimp *F. duorarum* using closed recirculating system in order to develop pathogen-viral-free stocks of the two species. This work was done in order to eliminate the need to collect wild spawners to produce the eggs for the production of postlarvae without running the risk of introducing viral pathogen into the system. The first step in the process was to evaluate the reproductive performance of the two species in captivity in closed recirculating system. The studies were conducted in three maturation tanks 4 m in diameter with total working volume of 34 m^3. This tank-system was constructed from inexpensive material in order to save on construction cost (total cost without taking into consideration the housing building was $12,000. It had a simple photo and temperature control and water treatment-polishing components. The system was operated with very limited water exchange (0.007% new water/day). Water from the maturation tanks was gravity fed into coarse filter bags (800, 100 µ). A pump was used to run the water over a trickling biofilter, diatom filter (5µ), carbon adsorption, and foam fractionator, with optional UV sterilizer. All wild populations collected were kept under quarantine until clearance was received from a diagnostic lab verifying the health status of the animals (e.g., free of known viral pathogens using PCR detection methods). For the brown shrimp the studies showed that good reproductive performance can be achieved with 2:1 and 1:1 male to female ratio using this setup. Furthermore, it was found that ablation was needed to obtain dependable supply of fertilized eggs. In terms of feed effect on the reproductive performance it was concluded that use of frozen enriched *Artemia* (8% body weight/d) and squid (12% body weight/d) provided overall better results than feeding bloodworms squid (8% body weight/d) and squid (12% body weight/d). Similar good results were obtained for the pink shrimp. Of interest is the fact that when operated without foam fractionator, pH adjustment was needed every 3 to 4 days using sodium bicarbonate. Nevertheless, after incorporation of foam fractionation, pH adjustment was reduced to every 4 to 5 weeks.

The use of closed recirculating systems for induced maturation of penaeid shrimp is only the first step in the development of biosecure shrimp production practices. Successful production of postlarvae in closed recirculating systems will enable operation of inland shrimp hatcheries, thus reducing the risk of viral disease outbreaks from contaminated coastal water. The use of these systems can also reduce nutrient releases to receiving streams. An experimental postlarval production system was constructed and used at the TAES-SMRF. The experimental test-system was made of ten larval rearing tanks (LRT) each with working volume of 200 L. Five LRT serve for raising PL's using routine water exchange. Each of the other five LRT was equipped with a self-sustained recirculating system similar to the one described earlier for the closed recirculating maturation system except for the diatom filter component. The studies showed that it was possible to re-use the culture water from at least six cycles without water exchange. Reused water was used to produce the algae needed to feed the larvae. Nevertheless, since this water had higher nutrient concentration than natural seawater, future studies will need to evaluated different fertilization regimes to enhance algal bloom. Biofiltration was needed to prevent the accumulation of nitrite build-up. The use of this system reduced labor by 40%. Animal health and stress tolerance was better in the recirculating tank system. Overall, survival in the recirculating tank-system was similar to control-system. Finally, the studies suggest that validation of the data using larger larval rearing tanks (10 to 20 m^3) is needed to make this technology commercially viable.

Use of similar system in Korea should enable the production of viral pathogen free postlarvae as the first step to develop biosecure shrimp farming industry in this country.

Heterotrophic culture technologies in outdoor shrimp ponds

Tzachi M. Samocha and In Kwon Jang

Operation and management of shrimp farms has changed substantially in the past 30 years. This rapid change has primarily been attributed to technological advances, high market demand and reduced supplies of wild stocks. After a catastrophic viral epizootic in 1990, many commercial shrimp production operations started to adopt management practices emphasizing biosecurity while maintaining profitability. Steps taken by the industry include the use of closed recirculating culture systems, production of high quality SPF shrimp stocks, and establishment of best management practices. In the past, acceptable WQ in grow-out ponds was maintained largely by release of pollutants via large-volume water exchange. This discharge issue has generated substantial interest in the shrimp farming industry to use zero or limited water exchange practices. Adoption of nursery & grow-out production practices with limited discharge can increase biosecurity while minimizing crop losses to disease outbreaks. Studies evaluating limited water exchange systems were initiated in the U.S. in 1985 by the Waddell Mariculture Center, South Carolina. For these systems to be effective, proper feed management, adequate aeration and circulation, natural productivity and nitrogen cycling processes are carefully managed as they ultimately determine quality of culture water. Furthermore, data suggest that water exchange could be greatly reduced without negative impact on shrimp performance. This management practice has been used successfully in farms in the Far East, Central America, Texas and other places. Belize Aquaculture Ltd. is another example where no negative impact was found from reduced water exchange management on survival and growth of the Pacific white shrimp in outdoor lined ponds.

Two experimental systems were used in our studies: the 1st system has 24 circular tanks under a shade (10.5-m^2; 6.8-m^3) each tank is provided with 10 airstones. The other system has four membrane-lined ponds (2,000-m^2 water surface area and about 2,000-m^3 water volume). Every pond is provided with paddlewheel and aspirator-type aerators. The objectives of the studies in both systems were to evaluate the effect of different dietary protein levels and ration-sizes on shrimp performance (e.g., growth, survival, FCR, yield) and selected WQ indicators under limited discharge management. All studies were conducted with the *L. vannamei* raised in the nursery system. Both test-systems were operated with no scheduled water exchange. Water was discharge only to avoid overflow of the culture tanks or at harvest. Municipal freshwater was used to maintain salinity and compensate for seepage.

The studies showed that production of the Pacific white shrimp can be done successfully in tanks and ponds operated with limited discharge. Furthermore, shrimp fed commercially available high-protein diets, which were fed on iso-nitrogenous basis to low-protein diets, performed as good as shrimp fed the low-protein diets. Of significant was the finding that feeding the shrimp the high-protein diets on iso-nitrogenous basis to the low-protein diets for the most part did not adversely affect water quality. Furthermore, in few cases it was demonstrated that feeding the low-protein diets resulted in greater deterioration of water quality compare to system where high-protein diets were used and fed on iso-nitrogenous basis to the low-protein diets. Thus feeding the high-protein can result in reduced load on receiving streams and at the same time can save the shrimp producers money as far less feed is needed to achieve the same shrimp performance as observed when shrimp were fed low-protein diets. For these systems to operate properly, the producer needs to observe his feed management very carefully to ensure effective use of the feed without overloading the culture medium. As these systems were operated with no water exchange, it is extremely important to provide adequate level of aeration and circulation in order to ensure that the bacteria flocs that develop in the water are kept suspended in the water column.

We strongly recommend evaluating the use of this technology in Korea as a tool to minimize losses to viral disease outbreaks. These testing can be conducted in facilities currently available in Korea, thus minimizing the cost associated with the evaluation. Furthermore, to facilitate the process, we recommend sending a scientist from Korea to work with Dr. Samocha for a year at the Texas Agricultural Experiment Station, Shrimp Mariculture Research Facility in Corpus Christi, Texas.

Shrimp feed and nutrition

Allen Davis

Dr. D. Allen Davis summarized previously presented data and then discussed important topics to the industry. The shrimp feed industry in Korea, clearly utilize state of the art production facilities and their use of extrusion processing conditions makes for very good quality feed in terms of physical factors such as size, density, durability, and stability. However, there are a number of areas that the industry could be improved upon.

Nutrient data: In general there is limited data regarding the nutritional requirements of chinensis. Consequently, nutrient requirement data needs to be established. In general for a variety of species there is good data on gross protein and energy requirements but we need precise data with regards to EAA requirements as well as EFA requirements. This is particularly critical as the use of marine protein sources and fish oils must be reduced if the industry is to continue to grow. Efforts should also emphasis the development of digestibility data sets for locally produced ingredients.

Ingredient use: There is considerable need to replace marine ingredients as sources of protein and lipids. Based on conversations with feed producers, current formulations have more marine products than are required to meet nutritional requirements of the shrimp. There are a variety of suitable plant and animal protein sources that could be used to replace marine ingredients. Hence, we must look into the use of alternative ingredients in terms of nutrient availability and acceptability / palatability.

Processing: Processing conditions are relatively well established but could use further refinement. There is limited data on the effects of various processing condition on nutrient availability as well as leaching of nutrients. It would be beneficial to the industry if the loss of water soluble nutrients is quantified. In general, a number of minerals are highly soluble for optimal delivery the industry should look at moving toward chelated mineral sources.

Feed management: As in most confined feed systems proper feed management is critical to profitability. In the aquatic environment, proper feed management also influences water quality and the culture environment. Based on the limited number of farms that we visited it would appear that the industry practices good feed management but it is my impression that this could be improved by at least 25% resulting in substantial savings to the industry.

In all cases it is critical that technologies are demonstrated to the farmer and that continuing education be pursued.

Appendix VI.

Abstracts of Symposium

on

the Present Status of Nutrition Research and the Future of Aquaculture Feed in Korea

August 12, 2005
National Fisheries Research & Development Institute
Busan, Republic of Korea

Organized by
Korean Aquaculture Society, National Fisheries Research & Development Institute and Kangnung National University

Development of marine fish meal and fish oil replacement diets for the Pacific white shrimp *Litopenaeus vannamei*

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World farmed shrimp production has expanded rapidly over the past two decades and the trend is expected to continue as the global population increases and demand for seafood continues to rise. In 2001, marine shrimp were the second most important world aquaculture species with a value of 8.4 billion US$. In the year 2000, it was estimated that the aquaculture industry used 35% of the fish meal and 57% of the global fish oil supply in the feed manufacturing industry. It is anticipated that as the aquaculture industry continues to expand, the demand for fish meal and oil will increase significantly. Since the supply of capture fisheries is likely to remain constant, this might lead to a shortage in supply along with price increases. If aquaculture is to continue to be sustainable, cost-effective, and responsible, fish meal and fish oil replacement sources need to be developed.

In previous studies a practical diet was developed without fish meal, containing marine fish oil. Since supplies of fish oil are also limited, our work focused on the replacement of the marine oil source. Tests were also conducted to develop an experimental organic diet to satisfy increased demand for organic products. In the first experiment, fish oil in two of the diets was substituted by oil originating from commercially produced algae containing approximately 50% oil. These diets and commercial feed were offered to juvenile *Litopenaeus vannamei* over a 15-week growth trial. At the conclusion of the trial, survival, final weight, and feed conversion ratios (FCR) were not significantly different among treatments. In a second experiment, the diet previously tested using plant and algae oils was tested against a diet using only plant oils. To examine the potential of an organic diet, a practical diet using primarily organic ingredients was also tested. The three diets were compared along with a commercial control diet in a 12-week study. At the conclusion of this trial, shrimp reared on the organic diet and the diet without algae oil supplements were significantly smaller than those offered the commercial control. In a third 15-week trial, the effect of fish-meal and fish-oil replacement strategies was evaluated using non-marine protein and oil sources and the subsequent influence on growth and survival of the shrimp. A practical diet formulated to contain 35% crude protein (no marine source) and 8% lipid (marine source) was compared to diets with no marine protein and no marine oil sources but containing highly unsaturated fatty acids (HUFA) originating from commercially produced algae meals containing approximately 50% oil. In addition, the study evaluated whether feeding a non marine HUFA-rich diet during the first part of the shrimp growth (0.95 g to 5 g size) followed by feeding a HUFA-deficient diet until market size can improve growth performance compared with shrimp fed a HUFA-deficient diet throughout the study duration. A commercial shrimp feed was included in the study to serve as a reference. At the conclusion of the trial, survival, yields and feed conversion ratio (FCR) were not significantly different among treatments. Final weights of shrimp reared on the diet with no fish-oil was as good as those of shrimp offered the commercial feed and the practical marine oil enriched diet. Final weights of the shrimp fed the HUFA-deficient diets were significantly lower than shrimp fed the commercial and the practical diet containing fish oil. Shrimp maintained on the diets without HUFA supplements were numerically lower but not statistically lower than those offered the diet containing algae meals. The poor performance of shrimp on diets without HUFA supplements confirms the need for HUFA's in practical diets and indicates that algae meals can be used to meet this requirement. The intermediate results of the diets containing the algae meals may indicate that further optimization of the lipid sources is required.

Based on the results of these studies, it would appear that both fish meal and marine oil sources can be removed from shrimp feeds if suitable alternative sources of protein and lipids are provided to meet essential amino acid and fatty acid requirements of the shrimp. Although this study confirms the biological feasibility of fish meal and fish oil replacement at the densities tested, commercial application will require further analysis of relative costs and marketing benefits of these technologies.

Evaluation of diets for the intensive culture of the Pacific white shrimp *Litopenaeus vannamei*: Experimental systems development and preliminary results from pond trials with fishmeal free feeds.

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Future economic viability of marine shrimp culture in the US will depend upon development of new super-intensive production systems for the marketing of high quality products. Recent intensive production trials for *Litopenaeus vannamei* at the Waddell Mariculture Center have demonstrated harvest biomass levels exceeding 6 kg/m^2 with excellent growth to harvest sizes exceeding 25g. The super-intensive production raceway is enclosed in a greenhouse allowing for year round production. Dissolved oxygen levels are maintained by injecting oxygen and organic materials are resuspended in a floc based system operated without water exchange. Microbial communities within the water column recycle waste materials while providing an additional nutritional boost to the target crop.

Practical diet development for well mixed intensive zero exchange shrimp production systems can be facilitated by the evaluation of the feed in the presence of water column organic matter. An experimental system was designed in which raceway water is recirculated through large numbers of small tanks stocked at densities mimicking those found in the super-intensive production systems. Shrimp performance, feed consumption and leaching rates, are carefully monitored providing precise data on feed performance in the system. Results from a trial carried out at the WMC during 2004 are reviewed demonstrating the use of the system for the evaluation of practical diets while exploring the potential nutritional contributions of the microbial community.

Pond trials at the WMC have been evaluating the potential for production of certified organic shrimp grown on innovative diets which replace fish meal and fish oil through the incorporation of protein and HUFAs from alternative sources. A recent experiment evaluated a 39% protein organic diet formulated from certified organic components (wheat flour, soybean meal, pea meal, flax seed oil, soy lecithin) augmented with algal components, squid meal and liquid fish solubles. The diet was compared with a conventional commercial feed consisting of 35% fish meal protein and 2.5% squid. Mean weight at harvest for the organic diet was 18.6 ± 3.0 g and for the conventional diet 17.9 ± 3.2 g. Survival between treatments was comparable (93% for the conventional diet; 88% for the organic diet). At the densities evaluated ($25/m^2$), the organic diet is palatable to shrimp, and growth was similar to the commercial feed control. Thus, it would appear that both fish meal and marine oil sources can be removed from shrimp feeds if suitable alternative sources of protein and lipids are provided to meet essential amino acid and fatty acid requirements of the shrimp.

Use of Plant Protein Sources in Shrimp Feeds

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As shrimp prices become more competitive and the profit margins keep declining, producers are looking to reduce their costs and to identify potential high value markets for their products. Shrimp produced under organic or environmentally friendly conditions have the potential to bring in higher prices through market differentiation. However, if one is to develop production schemes for such markets, one must also produce an organic feed or one that is based on sustainable ingredients. As organic standards have not been established for shrimp feeds in many countries, specific ingredients may or may not be approved. Until such standards are established, adopting production systems and feeds that are based on the general concepts of organic standards as well as good environmental standards could allow us to move into value added environmentally conscious markets. In either case it is clear that in most instances diets will have to be based on plant proteins with minimal levels of marine proteins and oils. Such restriction presents a challenge to both the feed manufacturer and the farmer. In this presentation we will discuss the concepts for replacement strategies to formulate practical diets with minimal levels of marine ingredients and summarize current published information on the use of plant proteins in shrimp feeds. Also we will provide examples of ongoing research designed to demonstrate the use of aquatic feeds with high levels of plant proteins, which utilization should reduce the cost of feeds in most countries and could easily be adopted to organic standards once they are established.

Abstract for the seminar at Pathology Department, NFRDI (August 12, 2005)

Sampling, diagnosis and sources of the principal marine penaeid shrimp viral diseases.

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Of the seven OIE-notifiable shrimp viral pathogens, three are of primary concern in the Americas because of the high mortality rate and economic losses associated with their occurrence. These include White Spot Syndrome virus (WSSV), Taura Syndrome virus (TSV) and Yellowhead virus (YHV). Both WSSV and TSV are now widespread in shrimp farming regions throughout the Western Hemisphere. YHV currently remains a disease problem exclusive to shrimp farms in the Eastern hemisphere. A relatively new viral disease, Infectious Myonecrosis virus (IMNV), was discovered in *P. vannamei* stocks in Brazil during 2003. Although limited to Brazil and not yet classified by the OIE, this disease causes significant mortalities and could pose a major health threat to shrimp farming enterprises if permitted to spread.

The principal diagnostic tools routinely used for the detection of shrimp diseases in the U.S. include histology, in situ hybridization and PCR. Histology has broad-based diagnostic capabilities by allowing evaluation of various different tissues via microscopic examination of hematoxylin and eosin-stained, 4-5 μm paraffin-embedded histologic sections of Davidson's AFA preserved shrimp tissue. Although this method is not as specific or sensitive as in situ hybridization and PCR, it does permit evaluation of the general health status of a shrimp specimen for a variety of pathogens (e.g. viral, bacterial, fungal, or parasites) and is invaluable in the identification of emerging new diseases. In situ hybridization (ISH) is a highly sensitive, molecular-based technique that combines visualization of histologic lesions with the uptake of a labeled, pathogen-specific, nucleic acid probe. Following denaturation and hybridization steps, binding of the probe to the pathogen's target genomic sequence (if present) is detected via application of a labeled antibody-enzyme conjugate (i.e. alkaline phosphatase) and substrate (i.e. NBT/BCIP), which results in a color change that is detected

visually by light microscopy. This technique is useful for confirmation of suspect histologic findings and/or presumptive diagnoses made by histopathology. The polymerase chain reaction (PCR) is another molecular-based technique used to exponentially amplify a small unique segment of a pathogen's genomic RNA or DNA. The resulting DNA copies are then visualized as distinct bands of a specified length by gel electrophoresis. Due to its high sensitivity and capacity for increased sample pooling, PCR is routinely used for surveillance testing or to confirm histological findings. A variety of ISH probes and PCR primers for the detection of the major shrimp viral pathogens are commercially available or procedures for their synthesis published. Standardized ISH and PCR methodologies for the principle shrimp diseases are described in the OIE Aquatic Disease manual and their use is recommended to minimize the possibility of generating false-positive or false-negative results. As all diagnostic techniques have limitations, inclusion of positive and negative controls for molecular-based assays is necessary and we routinely verify a disease-positive diagnosis using a second diagnostic method whenever possible.

Proper sampling and fixation is critical for accurate disease detection and diagnosis. Davidson's AFA fixative is used for the preservation of shrimp samples for histological and ISH analyses. Weekly pond-side examinations of shrimp should include examination of the exocuticle, gills, midgut, skeletal muscle and hepatopancreas for abnormalities, deformities, color changes, or lesions, particularly when selecting samples for disease analysis. For PCR analysis, either fresh, frozen or ethanol (95%) preserved tissues are utilized for nucleic acid extraction. Tissues can consist of excised pleopods, uncoagulated hemolymph, hepatopancreas or portions of the cephalothorax, depending on the pathogen of interest. In our laboratory PCR diagnostic fees are minimized without loss of detection sensitivity by pooling tissues from up to 60 individual shrimp for DNA analysis and a maximum of 30 samples for RNA analysis. For histopathology and ISH analyses, preservation of whole shrimp with Davidson's AFA fixative is recommended. However, fixation time should be kept to a minimum if ISH analysis for RNA viruses is anticipated in order to avoid problems of acid hydrolysis of the viral RNA genome. During a disease epizootic, submission of a select group of 5 to 10 affected shrimp (e.g. moribund or displaying clinical signs of disease) is recommended to diagnose the problem. For general health surveys of shrimp populations in excess of 100,000 animals, the analysis of 60 specimens permits detection of pathogens present at the 5% prevalence level or higher with 95% confidence. The practice of screening for diseases at the 5%

prevalence level is currently used in the U.S., but some countries now require analyses at the 2% prevalence level, requiring that 150 animals be tested from populations in excess of 100,000 shrimp.

The movement of shrimp viral pathogens between countries has mainly been attributed to the unrestricted international commerce of infected nauplii, postlarvae and broodstock. However, in the U.S., marine shrimp farms exclusively use domestically produced specific pathogen free (SPF)-derived *L. vannamei* stocks to minimize the potential of disease introduction by this route. Imported bait shrimp, ship ballast water harboring infected crustaceans, marine birds, aquatic insects, shrimp farm effluents, escaped farmed shrimp, non-shrimp animal importations, shrimp packing plant wastes and landfill dumping of these wastes have also been implicated or demonstrated as potential sources of virus introduction. It has been known for almost a decade that frozen imported commodity shrimp are often infected with viruses such as WSSV, TSV, IHHNV and YHV. During 2004, a USMSFC disease survey of grocery store shrimp in Hawaii found that 73% of the animals analyzed were WSSV-positive and 82% were IHHNV-positive by PCR. While imported, fresh-frozen, commodity shrimp have been the focus of a number of investigative studies, no similar reports or studies have been published regarding the potential health threat of virus - infected, fresh-frozen, imported bait shrimp to aquatic species in U.S. waters. A small disease survey was conducted at TVMDL on both fresh-frozen, native bait shrimp from the Gulf of Mexico and fresh-frozen, imported bait shrimp from China that are being sold in Texas for both fresh and saltwater sport fishing use. PCR, histopathology and ISH were utilized in virus detection and subsequent infectivity studies conducted on these samples. Infectious WSSV was found in two of 20 boxes of bait shrimp, originating from China. Considering these various modes of potential shrimp disease introduction, cooperative efforts need to be undertaken and established between exporters and importers of shrimp-producing countries to minimize the spread of penaeid shrimp diseases. Such measures have been undertaken by the Australian government to prevent WSSV introduction into their country and will be discussed.

Appendix VII.

REVIEW OF THE COOPERATIVE ACTIVITIES
IN SHRIMP CULTURE IN 2002-2004

A Visit to Korea by a Scientist of Texas A&M University-Corpus Christi

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Professor Tzachi Samocha of Texas A & M University-Corpus Christi visited a number of shrimp research institutes and local shrimp farms in Korea and an aquaculture site in Hainan Island, China from July 14-29, 2003. The purpose of his trip, supported by NOAA, was in three folds: 1) to visit existing shrimp production operations in Korea and China; 2) to help develop sustainable and biosecure shrimp farming practices in Korea; and 3) to evaluate the feasibility of producing the *Fenneropenaeus chinensis* in Texas.

Professor Samocha visited the West Sea Research Institute in Incheon to give a presentation on the development of sustainable shrimp practices with limited discharge. After this seminar, he visited Jeil Shrimp Farm raising the *Fenneropenaeus chinensis*. This farm had few earthen ponds and two HDPE-lined ponds that were built with fund from NFRDI. A few days before Professor Samocha's visit, mass shrimp mortality was observed in the earthen ponds. This shrimp mortality was attributed to the white spot syndrome virus outbreak. As the HDPE-lined ponds were stocked with WSSV-free postlarvae and because these ponds were maintained with very little water exchange, no shrimp mortality was observed in these ponds. In a meeting with the owner, Professor Samocha discussed different aspects associated with the operation of ponds with limited water discharge with an emphasis on the tolerance of the shrimp to high levels of ammonia and nitrite.

Professor Samocha traveled to the Taean Marine Hatchery Center. During this visit, Professor Samocha had reviewed the management and the system setting used by the Center staff. Recommendations were made in terms of improving growth and survival in the nursery phase with minimal water exchange. In addition, some modifications were made to improve spermatophore transfer and egg fertilization rates in a broodstock population of *Litopenaeus vannamei* that was kept at the facility. An immediate improvement in spermatophore transfer and fertilization rates was observed the following day. Two slide presentations was given to the staff: one dealt with the use of intensive nursery system with limited discharge and the other on the use of closed recirculating system for induced maturation of penaeid shrimp species.

Professor Samocha visited a shrimp hatchery that maintained a viral pathogen free broodstock population of *Litopenaeus vannamei* imported from Hawaii. This population was kept in a flow through system without treating the incoming water to avoid introduction of viral-pathogen. In a discussion with the owner following the visit, the significance of using closed recirculating system for induced maturation was emphasized. Recommendations were made to reduce the number of airstones in the maturation tanks to improve reproductive performance. In addition the owner agreed to convert several of his flow-maturation tanks to be operated in a closed recirculating mode.

Professor Samocha visited NFRDI, meeting with the General Director (Dr. Yeon Kang), the Director of the Aquaculture Division, the Director of Aquaculture, and the Head of the Training Department. In all these meetings he discussed the use of sustainable and biosecure shrimp production systems and the potential for expending the cooperation between TAES, Texas A&M University and NFDRI. After this meeting, Professor Samocha traveled with Dr. Inkwon Jang to Haikou, China to visit a fully integrated biosecured shrimp farm that used closed recirculating induced maturation system, larval rearing, intensive nursery system and HDPE-lined grow-out ponds.

Conclusion: In light of the major losses suffered by shrimp farmers in Korea due to viral disease outbreak it was obvious that in order for this industry to survive a biosecure and sustainable shrimp production methods have to be adopted. Conceptual designs for closed recirculating induced maturation system and intensive nursery raceway system were discussed with Dr. In Kwon Jang. One commercial hatchery was interested in converting part of its flow through maturation tanks into a closed recirculating system. Owners of a recirculating aquaculture system agreed to implement few modifications in their system to improve production. In addition they have expressed an interest in using the limited discharge system for nursery. It was decided that the final conceptual designs for closed recirculating system for the production of marketable size shrimp would be finalized after visiting few facilities in the U.S.

As for future cooperation, Professor Samocha and Dr. Jang had discussed potential ways to increase cooperation between NFDRI, TAES-SMRF and Texas A&M University-Corpus Christi. Among the different options discussed were students and researchers exchange programs, organizing seminars, and training programs. With respect to the introduction of the *Fenneropenaeus chinensis* to Texas, Professor Samocha and Dr. Jang will try to identify funding sources that will enable both countries to evaluate the reproductive performance and the growth potential of this species in West Texas.

A Visit to the U.S. by Two Shrimp Scientists from Korea

In Kwon Jang and Bong Lae Kim

Crustacean Research Center, West Sea Fisheries Research Institute, NFRDI, Korea

Professor Tzachi Samocha of Texas A&M University-Corpus Christi accompanied two Korean scientists, Drs. In Kwon Jang and Bong Lae Kim of the Taean Marine Hatchery Center, Korea, on travel to shrimp production facilities in Florida, South Carolina, and Texas, USA from September 19-30, 2003. The purpose of the trip, fully supported by MOMAF, was designed to expose Drs. Jang and Kim to biosecure limited discharge shrimp production technology in order for them to decide which technology will be suitable for implementation in South Korea.

Those scientists had meetings with Mr. David McMahon, President, OceanBoy Farms in Florida and visited one of the OceanBoy farms in Little Cypress. Mr. McMahon described the production process of shrimp in low salinity water with an emphasis on biosecurity protocol and sustainability issues. Dr. In Kwon gave a Power Point presentation describing the shrimp farming activities in Korea and the potential use of antibodies to overcome the white spot viral disease. In addition they discussed the potential importation of *Fenneropenaeus chinensis* to West Texas.

On the way to South Carolina, they visited the Harbor Branch Oceanographic Institute and received a detailed explanation from Dr. Megan of the experimental closed recirculating systems is use. Following this visit they had a meeting with Dr. Peter Van Wyk discussing the use of closed recirculating system for the production of marine shrimp, and visited the Harbor Branch shrimp, an indoor closed recirculating raceway system for the production of the Pacific white shrimp using low salinity water.

They visited the Waddell Mariculture Center, Bluffton, South Carolina. Mr. Al Stokes gave them a tour of the facility with an emphasis on shrimp production with limited discharge in outdoor ponds and greenhouse-enclosed raceways. After this meeting in South Carolina, Drs. Jang and Kim visited the TAES Shrimp Mariculture Research Facility in Corpus Christi. Professor Samocha gave a detailed explanation how the closed recirculating induced maturation system is working and describe the intensive nursery raceway system and the studies conducted in production of marketable shrimp with limited discharge under biosecure conditions. After visiting several other shrimp research facilities in Texas, Dr. Jag gave a presentation at Texas A&M University-Corpus Christi describing the status of the shrimp farming industry in South Korea and the potential use of antibodies to overcome the white spot viral outbreak.

Conclusion: Dr. Jang and Professor Samocha had a meeting at the TAES facility in Corpus to discus the next phase in the development of biosecure shrimp farming industry in Korea. This discussion included the use of closed recirculating induced maturation system, nursery and grow-out with limited.

A Visit to U.S. by a Scientist from Korea

In Kwon Jang

West Sea Fisheries Research Institute, NFRDI, Korea

Visiting period: September 20th to November 8th, 2004
Visiting places: Waddell Mariculture Center, Bluffton, South Carolina
Biotechnology Center, Univ. of Connecticut, Storrs, Connecticut
Texas Agricultural Experiment Station Shrimp Mariculture Research Facility, Corpus Christi, Texas.

Dr. In Kwon Jang visited the United States on September 20th to November 8th, 2004, as part of the cooperative activities agreed upon at the 2nd U.S.-Korea Joint Coordination Meeting for Aquaculture Cooperation held in Honolulu, Hawaii, USA, March 1st, 2004. The purpose of this trip, supported by MOMAF-NOAA Joint Fund, was to exchange knowledge and experience in the area of the biosecure and sustainable shrimp production practices, and discuss future cooperative research between two countries.

The visit to Waddell Mariculture Center, Bluffton, South Carolina took place from September 20th to September 30th, 2004. During this visit, Dr. Jang met Mr. Al Stokes, Production Manager, who gave a detailed description of the system and management strategies for super intensive shrimp culture in greenhouse-enclosed raceways under limited discharge. During this visit, Dr. Jang had the opportunity to have hands-on experience in operating the system including feed management and feeding, water management and monitoring shrimp performance. He also had the opportunity to participate in the harvest of four HDPE-lined ponds where testing with organic shrimp feed took place. In addition, Dr. Jang was able to visit the Marine Resources Research Institute, South Carolina Department of Natural Resources (SCDNR) in Charleston, South Carolina where Dr. Craig Browdy gave him a tour of the facilities. During the meeting with Dr. Browdy, they have discussed viral infection of wild populations as experienced in Korea and South Carolina and the potential development of antibodies against WSSV. Tentative plans were made to create a research group that will involve researchers from SCDNR, TAES, and NFRDI with an emphasis on development of biosecure shrimp production management in both countries.

On October 1st through the 6th, 2004, Dr. Jang visited Dr. Thomas Tom Chen at the Biotechnology Center, University of Connecticut in Storrs, Connecticut. In their meeting they discussed the potential development of transgenic shrimp in which the antimicrobial peptide (Cecropin B and/or analog CF-17) genes are transferred to boost shrimp resistance to infectious bacterial and viral diseases.

On October 7th through November 6th, 2004, Dr. Jang visited the TAES Shrimp Mariculture Research Facility, in Corpus Christi. Professor Tzachi Samocha provided working space in his office for Dr. Jang and arranged for him to have hands-on experience in shrimp production in HDPE-lined outdoor ponds under limited discharge. In addition, detailed description of the closed recirculating induced-maturation system and the intensive nursery raceway system with limited discharge under biosecure conditions were provided. During his visit to TAES, Dr. Jang attended graduate class, Aquaculture Techniques, given by Dr. Samocha on every Friday and joined a field trip to commercial shrimp farms, hatchery, and shrimp processing plant near Harlingen, Texas on October 14th through 15th, 2004. He also visited TAES Shrimp Mariculture Research Lab. in Port Aransas where Mr. William Bray and Dr. Frank Castille gave a detailed tour of the facilities with an emphasis on induced maturation in captivity and the experimental setup available for conducting diet related studies. On October 23rd through the 24th, Dr. Jang participated in harvest of shrimp from outdoor HDPE-lined ponds at the TAES SMRF. The total yield harvested from four 0.2-ha ponds was about 13,000 Lb. During the harvest, Dr. Jang had the opportunity to learn how to use fish-pump to harvest Pacific white shrimp, *Litopenaeus vannamei*.

Dr. Jang and Professor Samocha discussed the next phase of in their cooperative research with an emphasis on biosecurity and sustainability of shrimp production technology. They agreed as follows :

1. Dr. Jang will invite Professor Samocha as an Invited Expert to participate in a research project entitled: “Improvement of water quality and disease control in shrimp ponds using probiotic bacteria” funded by MOMAF, Korea. The visit is scheduled to take place from June 1st through the 10th, 2005;

2. Dr. Jang will try to work with Dr. Samocha on the development of intensive shrimp culture technology using heterotrophic and limited discharge methods in a current shrimp project funded by NFRDI from 2005; and

3. Drs. Jang and Samocha will try to submit to Korean Science and Engineering Foundation (KOSEF) a bi-national cooperative research proposal for the development of biosecure and sustainable intensive shrimp production technologies.