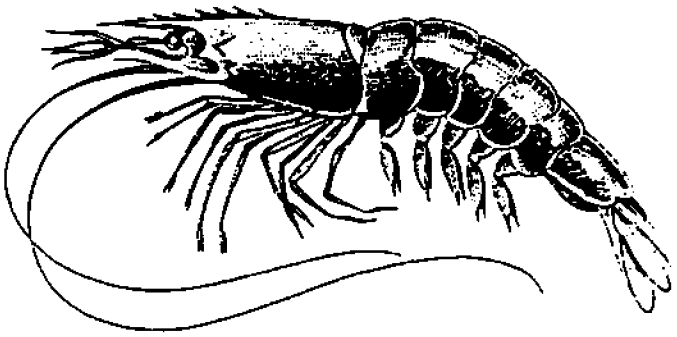
Diseases of Cultured Penaeid Shrimp in Asia and the United States



Edited by
Wendy Fulks
and
Kevan L. Main

The Oceanic Institute

Diseases of Cultured Penaeid Shrimp in Asia and the United States

Edited by

Wendy Fulks
and
Kevan L. Main

Proceedings of a Workshop
in Honolulu, Hawaii,
April 27-30, 1992

Published by

The Oceanic Institute
Makapuu Point
P.O. Box 25280
Honolulu, Hawaii 96825

Sponsored by

The National Oceanic and Atmospheric Administration
United States Department of Commerce

For additional copies, please contact:

The Oceanic Institute
Makapuu Point
P.O. Box 25280
Honolulu, Hawaii 96825

ISBN 0-9617016-5-X

Contents

Preface v

Part I: Introduction

Introduction 3

Part II: Contributed Papers - Country Situations

Major Diseases of Cultured Shrimp in Asia: An Overview
M. Shariff and R.P.Subasinghe 37

An Overview of the Disease Situation, Diagnostic Techniques, Treatments and Preventives Used on Shrimp Farms in China
Dou Chen 47

Occurrence, Diagnosis and Treatment of Shrimp Diseases in Thailand
Timothy Flegel, Daniel F. Fegan, Sumana Kongsom, Sompoach Vuthikornudomkit, Siripom Sriurairatana, Sitdhi Boonyaratpalin, Chaiyuth Chantanachookhin, Joan E. Vickers and Oliver D. Macdonald 57

Diseases of *Penaeus monodon* in Taiwan: A Review from 1977 to 1991
I-Chiu Liao, Mao-Sen Su and Cheng-Fang Chang 113

Prevalence and Geographic Distribution of MBV and Other Diseases in Cultured Giant Tiger Prawns (*Penaeus monodon*) in the Philippines
José M. Natividad and Donald V. Lightner 139

The Status of Culture and Diseases of Penaeid Shrimp In Korea
Myoung Ae Park 161

Baculovirus Infection of Penaeid Shrimp in Japan
Tokuo Sano and Kazuo Momoyama 169

Part II: Contributed Papers - Viral Diseases

Infection Route and Eradication of *Penaeus monodon* Baculovirus (MBV) in Larval Giant Tiger Prawns, *Penaeus monodon*
Chen, S.N., P.S. Chang and G.H. Kou 177

Viral Diseases of Cultured Penaeid Shrimp in Japan
Kazuo Momoyama 185

Part II: Contributed Papers - Bacterial Diseases

Studies on the Epizootiology and Pathogenicity of Bacterial Infections in Cultured Giant Tiger Prawns, *Penaeus monodon*, in Taiwan
S.N. Chen, S.L. Huang and G.H. Kou 195

Part II: Contributed Papers - Diagnostic Procedures

Current Diagnostic Methods for Agents and Diseases of Farmed Marine Shrimp
James A. Brock 209

New Developments in Penaeid Virology: Application of Biotechnology in Research and Disease Diagnosis for Shrimp Viruses of Concern in the Americas
D. V. Lightner, B. T. Poulos, L. Bruce, R. M. Redman, J. Mari, and J.R. Bonami 233

Part II: Contributed Papers - SPF Stocks

Selective Breeding of Specific Pathogen-Free (SPF) Shrimp for High Health and Increased Growth
James A. Wyban 257

Developing Specific Pathogen-Free (SPF) Animal Populations for Aquaculture: A Case Study for IHHN Virus of Penaeid Shrimp
Jeffrey M. Lotz 269

Growth and Survival of Virus-infected and SPF *Penaeus vannamei* on a Shrimp Farm in Hawaii
Nick Carpenter and James A. Brock 285

Shrimp Production in Texas Using Specific Pathogen-Free Stocks
Fritz Jaenike, Kieth Gregg and Louis Hamper 295

Part II: Contributed Papers - Research, Regulations, and Health Management

Shrimp Culture Technologies Inc.: Research to Improve Shrimp Genetics and Health
Rolland Laramore 305

Drugs and Chemotherapeutants for Shrimp Diseases: Their Present Status in the United States, with an Overview of Research and Approval Processes
Thomas A. Bell 311

Precautions for Importing and Culturing Non-native Shrimp
Carl J. Sindermann 325

Issues Related to Regulation of Penaeid Shrimp Diseases in Texas, U.S.A.
Sterling K. Johnson 333

Shrimp Health Management Procedures
Brad LeaMaster 345

Part III: Discussion Group Summaries

Discussion Group Summaries 359

Appendices

Appendix 1: Workshop Participants 379

Appendix 2: Workshop Agenda 381

Preface

The Asian Interchange Program was founded at The Oceanic Institute in 1989. The program's goal is to facilitate the exchange of applied aquaculture information and technology between the United States and Asia. This is accomplished, in large part, through the organization of international workshops and distribution of workshop proceedings to information networks throughout the United States and Asia.

This is the third workshop proceedings issued by the Asian Interchange Program. Previous volumes dealt with the culture of cold-tolerant shrimp in Asia, and the culture of rotifers and microalgae in Asia and the United States. For our third year, we chose to focus on the infectious and noninfectious diseases of cultured shrimp in Asia and the United States. Not only is this a timely topic in many Asian countries where shrimp disease problems have been mounting over the past five years, but it also ties in with recent efforts by the Gulf Coast Research Laboratory Consortium to develop and maintain specific pathogen-free stocks for shrimp farmers in the United States.

The Workshop

This year, participants traveled from Japan, Malaysia, the Philippines, the People's Republic of China, South Korea, Taiwan, Thailand and the United States mainland (Arizona, Florida, Mississippi and Texas) to attend the workshop in Honolulu, Hawaii from April 27 - 30, 1992 (see photo). Everyone presented a paper during the four morning sessions. Afternoons, by contrast, were spent in discussion groups, where participants had the opportunity to share information and ideas with their colleagues on a variety of disease-related issues.

WORKSHOP PARTICIPANTS. *Front row (from left):* Celia Pitogo, Alcian Omoso, Mohammed Shariff, Tokuo Sano, Jose Natividad, S.N. Chen, Thomas Bell, Myoung Ae Park, Kevan Main, Kazuo Momoyama, Josh Wilkenfeld, Faith Antiquiera, Wendy Fulks, Timothy Flegel; *Back row (from left)*: Donald Lightner, Jeffrey Lotz, Carl Sindermann, Ken Johnson, James Wyban, Cheng-Fang Chang, Rolland Laramore, Dou Chen, Nick Carpenter, James Brock, Fritz Jaenike, Steve Psinakis, and Brad LeaMaster.

The Proceedings

The volume is divided into three parts: the introduction, contributed papers and discussion group summaries. The introduction and discussion group summaries were written by Wendy Fulks and edited by Kevan Main.

The papers are grouped into six sections: Country Situations, Viral Diseases, Bacterial Diseases, Diagnostic Procedures, Specific Pathogen-Free Stocks, and Research, Regulations and Health Management. The 21 papers represent a wide range of perspectives — shrimp farmers in every country face unique problems. For example, growing conditions (e.g., temperature, water quality and soil conditions) vary between regions; also, different species may be grown and different pathogens may be encountered. Importantly, regulations governing culture practices, such as the use of drugs, and their enforcement are also different in every country. The range of viewpoints presented in this volume are those of researchers, farmers and/or extension agents from eight different countries.

Even with all of these differences, several common themes recurred in both the papers and the discussion group sessions. One of those issues was the spread of pathogens (especially viruses) via the uncontrolled movement of shrimp stocks. The transfer of stocks whose disease status is unknown may pose a threat to wild shrimp populations and could also harm shrimp culture ventures. Resolution of this problem will not be easy, and will most likely involve more widespread adoption of quarantine regulations and increased usage of specific pathogen-free (SPF) stocks. Everyone agreed that international cooperation is necessary to define and implement feasible, effective quarantine regulations. Furthermore, each country must determine which pathogens to target for exclusion, and there will probably be a different exclusion list for every species grown.

A quarantine system, however, is useless in the absence of standardized diagnostic techniques. Experts need to agree about which techniques are best for a given pathogen, and health inspectors and, eventually, hatcheries, will need to be certified. Furthermore, better (more reliable, more sensitive, easier, quicker and less expensive) diagnostic methods need to be developed and transferred to field diagnosticians.

Another common theme was the need for improved husbandry techniques. Since many serious diseases of cultured penaeids are caused by opportunistic organisms, disease losses can be prevented by providing optimal conditions for growth and by carefully monitoring the health of animals throughout the rearing cycle.

This is especially true in semi-intensive and intensive culture conditions. In the area of drug use, most participants agreed that a number of compounds, including antibiotics and so-called "probiotics," are being used irresponsibly in many shrimp farming areas. The prophylactic use of antibiotics in hatcheries can foster the spread of antibiotic-resistant bacterial strains and may also weaken larvae in the long run. Also, extreme care must be taken when using drugs during growout to ensure that no residues remain in shrimp that are sold for consumption.

Environmental awareness was another important issue that was raised several times during the workshop. Almost nothing is known about how shrimp farms affect their surroundings. In areas where there is a high concentration of farms, an answer is needed to the question, "How many shrimp farms can the ecosystem support over the long term?" In turn, we also need to know how fluctuations in the natural environment may affect the health of cultured shrimp. The dynamics involved are quite complex, and answers will not be immediately forthcoming.

Internationally, shrimp culture is recognized as a valuable industry; if it is to prosper, the problems of disease diagnosis, prevention and treatment must be dealt with immediately. Progress has been made toward resolving some of the vital issues mentioned above, and some of that progress is detailed in this book. For example, advances in disease diagnosis are described herein, as are recommended husbandry practices to prevent and treat diseases of cultured shrimp. SPF stocks of *Penaeus vannamei* are now being used throughout the United States, and awareness of the need for quarantine measures is increasing. In an effort to safeguard the future of shrimp farming, researchers in Asia and the United States are increasing our knowledge of known shrimp diseases, and identifying and characterizing new diseases and disease agents. It is hoped that this volume will further the efforts of both researchers and producers by making available the latest information and technology related to the diseases of cultured penaeid shrimp.

Acknowledgments

The Asian Interchange Program is funded by the National Oceanic and Atmospheric Administration, United States Department of Commerce (Grant # NA90AA-D-SG483). The editors thank the University of Hawaii Sea Grant College Program for its administrative support throughout the project.

A number of individuals contributed to this work. Most importantly, we would like to thank all the authors who participated in the workshop and prepared the papers included in this volume. Donald Lightner assisted in the identification of workshop participants and provided valuable guidance in the development of the discussion group agenda and throughout the workshop. In addition, Cheng-Sheng Lee, I-Chiu Liao, Ruiyu Liu and Byung-Ha Park assisted in the identification of Asian workshop participants. We acknowledge our capable interpreters, Stella Guillory, Hongja Harrison, Lynette Shi, Taeko Wellington and Masako Yamatani, for assisting in the implementation of the workshop.

Donald Lightner, James Brock and S.K. Johnson reviewed the introduction, and Donald Lightner and James Brock reviewed the discussion group summaries. We thank Stephanie Frank, Rose Marie Norton, and Debbie Fritz for proofreading portions of the text.

The final production of the proceedings was done by Patti Killelea-Almonte, with assistance from Alcian Omoso and Elizabeth Reynolds. The cover was designed by Elizabeth Reynolds.

Part I:

Introduction

Introduction

Worldwide, almost 700,000 MT of cultured shrimp were produced in 1991 — 28% of all the shrimp sold that year (Rosenberry, 1991). More than 80% of that record total represents shrimp grown in Asia, and production in Asia and in the western hemisphere is expected to increase again in 1992 (Rosenberry, 1991).

A practical way to increase production is to adopt more intensive culture methods, and this is exactly what is happening in many shrimp producing nations, especially those in which suitable land is expensive or scarce. Increasing the density at which shrimp are cultured, however, increases the amount of stress the animals experience, making them more likely to suffer from disease. Another concern resulting from the expansion of penaeid aquaculture is the transfer of pathogens, especially viruses, between culture locales and species. As the global shrimp culture grows, so will the importance of recognizing, treating and preventing disease in culture facilities.

For the purposes of this paper, disease is defined as "any departure from normal structure or function" and includes infectious diseases caused by microbial pathogens or parasites, and noninfectious diseases (genetic and environmentally induced abnormalities) (see Sindermann, 1990).

Shrimp Culture in Asia and the United States

In Asia, the largest producers of farm-raised shrimp (in order of importance) are the People's Republic of China, Indonesia, Thailand, India, the Philippines, Vietnam and Taiwan. In fact, China, Indonesia and Thailand are the three largest producers in the world (Rosenberry, 1991).

Eighty percent of the shrimp grown in China are *Penaeus chinensis* (also known as *P. orientalis*), but *P. merguiensis*, *P. penicillatus* and *P. monodon* are cultured on a large scale as well. Relatively small numbers of *P. japonicus* and *P. semisulcatus* are also grown in China (Liu, 1990). *Penaeus monodon*, a fast-growing, hearty species, dominates production in the remaining Asian countries, but *P. merguiensis*, *P. indicus* and *P. japonicus* figure prominently in some locales (Rosenberry, 1991). Most of the shrimp culture in Asia would probably be classified as extensive or semi-intensive, yielding between 500 and 5,000 kg/ha/crop.

The United States has a small, young shrimp culture industry. Almost all of the farm-raised shrimp are *P. vannamei*, which are grown either semi-intensively or intensively in South Carolina, Texas and Hawaii. The United States is the second largest consumer of shrimp,

however, importing much of its supply from farms in Asia.

Penaeid Shrimp Diseases

Several detailed reviews of the literature pertaining to diseases of penaeid shrimp have been published, including those by Couch (1978), Ruangpan (1978), Lightner (1983, 1985), Provenzano (1983), Baticados (1988), Sindermann and Lightner (1988), Johnson (1989), Lightner and Redman (1991) and Bell (1991). Nearly 30 diseases and disease syndromes of cultured penaeids, with both infectious and noninfectious etiologies, have been described (Sindermann and Lightner, 1988) but many are poorly understood. The most important pathogens of cultured shrimp are viruses, bacteria, fungi and protozoa.

Viruses

"Viral diseases and associated mortalities are emerging as one of the most important problems in penaeid shrimp culture" (Sindermann, 1990). Viruses in crustaceans were first reported in the mid-1960s, and to date, none have been adequately characterized (Sindermann, 1990). Thus far, 12 viruses have been identified from penaeid shrimp (Lightner et al., this volume), and six fatal viral diseases of penaeids have been reported. Infections in larvae and juveniles are most common. Some viruses are specific to one or only a few species of shrimp, while others appear to be capable of infecting all penaeids.

Viral disease outbreaks in populations in which a virus has remained latent can result from stress; common stressors are overcrowding, abnormal temperatures, and low dissolved oxygen levels. Prevention of viral diseases consists of quarantining all introduced stock to virus-free facilities, and disinfection of infected facilities. There are no known treatments for viral diseases; moreover, diagnosing some viral infections is currently an expensive, time-consuming task undertaken only by highly trained persons.

Rickettsia

Rickettsia are rod-shaped microorganisms known to cause diseases in a variety of taxa. Rickettsia or rickettsia-like organisms have been found in *P. vannamei* (Krol et al., 1991), *P. marginatus* and *P. merguiensis*. *Penaeus monodon* and *P. stylirostris* have been experimentally infected (Brock, pers. comm.). Tetracycline may be an effective treatment (Brock, pers. comm.). Rickettsia are not, however, usually considered serious pathogens of cultured penaeids.

Bacteria

Another important category of pathogens is bacteria. A group of Gram-negative, rod-shaped bacteria, most of which belong to the genus *Vibrio*, is associated with serious disease outbreaks in cultured populations of shrimp — mortalities of up to 100% have been reported (Lightner, 1983; Lightner et al., 1984). One disease

caused by these bacteria is called **vibriosis** (a type of bacterial septicemia), and infections can be chronic, subacute or acute. There are a number of pathogenic species and strains, and their virulence can vary markedly. All species of shrimp can be infected. Although bacteria have frequently been associated with mortalities, most bacterial infections are of secondary bacterial etiology and result from extreme stresses and opportunistic pathogens. A number of chemicals and antibiotics have been used to treat shrimp vibriosis, including EDTA, furanace, furazolidone (NF-180), erythromycin, terramycin and chloramphenicol.

Chitinolytic bacteria may invade existing breaks in the epicuticle, enlarging wounds through the secretion of extracellular enzymes (**bacterial necrosis** and **shell disease**), and filamentous bacteria (usually *Leucothrix mucor*) may attach to setae and gill lamellae. The latter can affect respiration, and their presence is considered indicative of stressful culture conditions.

Fungi

Fungal diseases are also common in shrimp. In many cases, infection results from opportunistic invasions of shrimp that have been injured or exposed to stressful conditions. Fungi can cause mass mortalities, especially in hatcheries, where the disease **larval mycosis** has proven to be quite deadly. Larval mycosis* is usually caused by either *Lagenidium callinectes, Sirolpidium* sp. or *Haliphthoros* sp., and mortalities can reach 100% within 48 h after the onset of infection. Treflan® and malachite green are commonly used to treat fungal infections in hatcheries.

Another important fungal pathogen is *Fusarium solani*, the most common etiological agent of **Fusarium disease** or **black gill disease**. All penaeids can contract Fusarium disease, but some, like *P. japonicus*, seem to be particularly sensitive and suffer a high rate of mortality when infected.

Protozoa

All shrimp are susceptible to fouling by epicommensal protozoa. These organisms are naturally present in the culture environment. Protozoa may attach to the gills, appendages and bodies of cultured penaeids. A number of genera may be encountered, including *Zoothamnium, Epistylis, Vorticella, Acineta* and *Lagenophrys*. Late larval through adult stages can all be affected, and high-density cultures are probably more susceptible to serious infestations. Extensive colonization of gills may interfere with respiration, resulting in mortalities.

Microsporida, internal parasitic protozoa, are responsible for the abnormality known as **cotton shrimp** or **milky shrimp**. Thus far, microsporidosis has not been a major problem in cultured shrimp, presumably because these pathogens require either an intermediate, or, more likely, a "conditioning" host to complete their life cycle. This disease is more apt to occur in extensive

shrimp culture situations, where intermediate hosts such as fish may be present in the culture environment.

Finally, gregarines are common inhabitants of penaeid intestinal tracts. Bell and Lightner (1991) reported that populations with a high prevalence of gregarine infestation sometimes exhibit reduced feeding and growth rates, increased surface fouling, and/or slight to moderate increases in mortality.

Nutritional, Toxic and Environmental Diseases

A few of the most common diseases in this category are **vitamin C deficiency**, also known as **black death; black gill disease**, a result of exposure to toxic levels of substances such as heavy metals, ozone, or ammonia (not to be confused with the black gill disease caused by infection with the fungus *Fusarium* sp.); and **gas bubble disease**, caused by supersaturation of culture water with atmospheric gases. All species of penaeids are presumably susceptible to these diseases, though some may be more sensitive than others to suboptimal environmental conditions or certain toxins.

Diseases of Unknown Etiology

In addition to the previously mentioned diseases and disease syndromes, there are a number of conditions reported from cultured penaeids for which no cause has been discovered. Examples include **black spermatophore disease**, affecting male *P. vannamei*, *P. stylirostris* and *P. setiferus*, and **gut-and-nerve syndrome** of *P. japonicus*.

The Status of Penaeid Diseases In Asia

Penaeus monodon

As the most important species of cultured shrimp in the world, *P. monodon* has been a frequent subject of disease investigations — pathologists have been studying *P. monodon* for years. Naturally distributed throughout the Indo-West Pacific region from 30°E to 155°E longitude and from 35°N to 35°S latitude, *P. monodon* is most abundant in the tropical waters of Indonesia, Malaysia and the Philippines. It has become an important culture species in countries within its range, especially Indonesia, Thailand, India, the Philippines, Vietnam and Taiwan. *Penaeus monodon* is normally considered to be exceptionally hearty, but increasing culture densities and environmental degradation have contributed to disease problems. Serious diseases of viral, bacterial, fungal, protozoan, rickettsial and unknown etiologies have been reported.

Infectious and Parasitic Diseases. From an economic standpoint, the most important viral pathogen of *P. monodon* may be *Penaeus monodon*-type baculovirus (MBV; Lightner and Redman, 1981). This baculovirus affects all life stages and has been implicated in mass mortalities, especially in shrimp that

Table 1. Viral disease agents of cultured *Penaeus monodon*.

Disease agents	Countries	Treatments	References
Penaeus monodon-type baculovirus (MBV)	Taiwan, Philippines, Malaysia, French Polynesia, Hawaii, Kenya, Mexico, Singapore, Indonesia, Israel, Thailand	None	Baticados, 1988; Ruangpan, 1978; Anderson et al., 1987; Colorni, 1990; Lightner and Redman, 1981; Boonyaratpalin, 1990
Infectious hypodermal and hematopoietic necrosis virus (IHHNV)	Ecuador, Guam, Tahiti, Philippines, Hawaii, Singapore, Israel, Panama, Costa Rica	None	Baticados, 1988; Lightner, 1985
Hepatopancreatic parvo-like virus (HPV)	Malaysia, Philippines, Kenya, Israel	None	Lightner and Redman, 1985; Baticados, 1988; Colorni, 1990
Reo-like viruses (REO)	Malaysia	None	Anderson et al., 1987

are cultured at high densities or exposed to some other stressor. According to Baticados (1988), MBV has been detected in *P. monodon* from Taiwan, the Philippines, Malaysia, French Polynesia, Hawaii, Kenya, Mexico, Singapore and Indonesia (Table 1, also see Colorni, 1990). Ruangpan (1978, cited in Anderson et al., 1987) reported an instance of 50% mortality in pond-reared *P. monodon* at a farm in Thailand (also see Fegan et al., 1991), and Anderson et al. (1987) stated that MBV is likely to be normally present in all cultured pond populations of *P. monodon* in Malaysia at low endemic levels.

Infectious hypodermal and hematopoietic necrosis virus (IHHNV) has also been found in cultured *P. monodon*. Lightner (1983) reported 80 - 90% cumulative mortalities within two weeks of onset of IHHNV disease in 0.05- to 1-g *P. monodon*. Postlarvae and juveniles are considered particularly susceptible, and *P. monodon* infected with IHHNV have been reported from Ecuador, Guam, Tahiti, the Philippines, Hawaii, Singapore, Israel, Panama and Costa Rica (Baticados, 1988; also see Colorni, 1990). Furthermore, IHHNV has been found in southeast Asian culture facilities that use only captive, wild *P. monodon* broodstock, suggesting that this region is within the natural geographic range of the virus and that *P. monodon* may be a natural host species (Lightner and Redman, 1991).

Hepatopancreatic parvo-like virus (HPV) is yet another viral pathogen of *P. monodon*. It has been reported from Malaysia (Lightner and Redman, 1985), Israel (Colorni, 1990), the Philippines and Kenya (Baticados, 1988). HPV-infected *P. monodon* exhibit poor growth rates, anorexia, reduced preening activity, increased surface fouling, and occasional opacity of tail musculature. A reo-like virus (REO) has also been found in *P. monodon* in Malaysia (Anderson et al., 1987).

There are no treatments for the diseases caused by MBV, IHHNV, HPV or REO. The only recourse is to prevent contamination of virus-free stock and facili-

Table 2. Bacterial and rickettsial diseases of cultured *Penaeus monodon*.

Diseases	Countries	Treatments[1]	References
BACTERIAL			
Vibriosis	Malaysia, Philippines	Erythromycin phosphate, streptomycin-bipenicillin, tetracycline chlorhydrate, sulfamethazin, furanace, chloramphenicol	Anderson et al., 1987; Ruangpan, 1982; PCARRD, 1985
Luminous bacterial disease	Philippines, Indonesia, Malaysia, Thailand	Rigid sanitary practices, such as the chlorination of culture water, removal of the wastes and sediments from the tank bottom, and more frequent water exchange	Baticados, 1988
Epicommensal filamentous bacteria	Philippines, but presumably widespread	Cutrine®-Plus given upon onset of disease at 0.1 mg Cu/L for 24 h or 0.25 -0.5 mg Cu/L for 4 - 8 h	Baticados, 1988
Bacterial exoskeletal lesions	Philippines, Taiwan	None reported	Lio-Po and Pitogo, 1990; Chen, 1990; Baticados, 1988
Necrosis of appendages	Philippines, Indonesia, Malaysia, Singapore, Thailand, Taiwan	None reported	Baticados, 1988
Bacterial induced hepatopancreatitis	Taiwan	Unknown	Liu, 1989
RICKETTSIAL			
Unknown sp.	Malaysia	None reported	Anderson et al., 1987

[1]Many countries regulate the chemicals and drugs used on shrimp farms. In the United States, FDA or EPA approval is required for drugs and chemicals used on shrimp grown for human consumption.

ties by destroying infected animals and quarantining all imported stock.

A number of bacterial pathogens have been reported for *P. monodon* (Table 2). Ruangpan (1982) and the Philippine Council for Agriculture and Resources Research Development (PCARRD) (1985, both cited in Baticados, 1988) reported that vibriosis affects protozoeal stages and causes heavy mortalities — up to 80% — in *P. monodon* hatcheries. Anderson et al. (1988) investigated several cases of vibriosis in juvenile *P. monodon* cultured in Malaysian brackishwater ponds. Anderson et al. (1988) isolated bacteria of the genus *Vibrio* from the hemolymph, and also found *Pseudomonas* sp. and other Gram-negative bacteria. They noted that vibriosis in juvenile shrimp has been treated successfully by adding antibiotics to the diet; for the semi-intensive ponds studied, however, chemotherapy was not an economical option. Mortalities were reduced after the farmers began to remove excess detritus

from dried ponds followed by applications of CaO at the rate of 0.5 kg/m^2.

Chitinoclastic bacteria were responsible for exoskeletal lesions in pond-cultured *P. monodon* (Lio Po and Pitogo, 1990; Baticados, 1988; Chen, 1990) and a bacterial disease of larvae and young postlarvae termed "necrosis of appendages" has been shown to cause mortalities in hatcheries in the Philippines, Indonesia, Malaysia, Singapore, Thailand and Taiwan (Baticados, 1988). Baticados (1988) discussed a disease caused by luminous bacteria that had caused significant problems in hatcheries in the Philippines, Indonesia, Malaysia and Thailand. Filamentous bacteria of the genus *Leucothrix* sometimes attach to the cuticles of cultured *P. monodon*. Finally, Liu (1989) studied the histopathology of bacterial induced hepatopancreatitis of cultured *P. monodon* in Taiwan.

With respect to the treatment of bacterial diseases in Asia, a number of drugs have been tested in the Philippines to combat larval necrosis, including erythromycin phosphate, streptomycin-bipenicillin, tetracycline chlorhydrate, sulfamethazin, furanace and chloramphenicol. Rigid sanitary practices, such as the chlorination of culture water, removal of wastes and sediments from the tank bottom, and increasing the rate of water exchange have effectively reduced mortalities from luminous bacteria. And finally, filamentous bacterial disease is controlled in the Philippines with Cutrine®-Plus, a copper compound, given upon onset of the disease at 0.1 mg Cu/L for 24 h or 0.25 - 0.5 mg Cu/L for 4 - 8 h (Baticados, 1988; Lightner, 1983).

Anderson et al. (1987) reported on a disease syndrome of *P. monodon* in Malaysia in which rickettsia were believed to be the primary pathogens. MBV and a reo-like virus were also detected in the diseased shrimp, and Gram-negative bacteria were implicated as secondary pathogens. Interestingly, this disease syndrome did not affect the *P. merguiensis* or *P. indicus* inhabiting the same ponds.

One of the most serious fungal diseases, larval mycosis, affects all cultured penaeids, *P. monodon* being no exception. *Lagenidium callinectes* is the most common agent, causing heavy mortalities in larvae and postlarvae in the Philippines, Thailand and Indonesia (Baticados, 1988). *Sirolpidium* sp. and *Haliphthoros* sp. have also been reported to cause larval mycosis (Table 3). If the incidence rate is low, Baticados (1988) reports, *Lagenidium* spp. infections can be managed by removing sediments and dead larvae, increasing water exchange, and reducing stocking densities. In addition, malachite green, Treflan® (trifluralin), Formalin and detergent have been used to combat the disease, and potassium permanganate is considered to be a useful disinfectant (Baticados, 1988). Water management techniques and Treflan® are used in the Philippines to combat *Sirolpidium* sp., and *Haliphthoros* sp. infections are treated with furanace, malachite green, Formalin and potassium permanga-

Table 3. Fungal diseases and disease agents of cultured *Penaeus monodon*.

Diseases/disease agents	Countries	Treatments	References
Larval mycosis (*Lagenidium callinectes*, *Sirolpidium* sp. and *Haliphthoros* sp.)	Philippines, Thailand, Indonesia	Malachite green, Treflan® (trifluralin), Formalin, detergent, potassium permanganate, furanace	Baticados, 1988 (also see Boonyaratpalin, 1990)
Fusarium spp.	Philippines, Taiwan	No known chemical treatments	Baticados, 1988
Hyphomyces sp., *Saprolegnia parasitica* and *Leptolegnia marina*	Philippines		Baticados, 1988

Table 4. Protozoan disease agents of cultured *Penaeus monodon*.

Disease agents	Countries	Treatments	References
Epistylis, *Vorticella*, *Zoothamnium*, *Ephelota gemmipara*, *Acineta*	Presumably widespread	Chloroquine diphosphate or 25 ppm Formalin bath, saponin (also see Johnson, 1976)	Baticados, 1988; Ruangpan, 1986; Liao et al., 1977; Gacutan et al., 1977
Microsporidians	Philippines	None reported	Baticados, 1988
Gregarines	Philippines, Thailand	None reported	Baticados, 1988

Formalin and potassium permanganate. Finally, detergent, calcium hypochlorite and Resiguard® are used to disinfect systems in which *Haliphthoros* sp. has been a problem (Baticados, 1988).

Adult *P. monodon* are sometimes infected by *Fusarium* sp., the agent responsible for black gill disease in penaeid shrimp. According to a report by Ruangpan (1982; cited in Baticados, 1988), about 50% of a sample population of cultured *P. monodon* suffered from the disease, which resulted in large losses. Liao et al. (1977), however, observed that *Fusarium* sp. was rare in cultured *P. monodon* in Taiwan. Other fungi known to infect *P. monodon* are *Hyphomyces* sp., *Saprolegnia parasitica* and *Leptolegnia marina* (Baticados, 1988).

The following epicommensal ciliates have been reported to infect *P. monodon*: *Epistylis* sp., *Vorticella* sp., *Zoothamnium* sp., *Ephelota gemmipara* and *Acineta* sp. (Baticados, 1988; Liao et al., 1977; Gacutan et al., 1977) (Table 4). *Epistylis* sp. is quite common in the Philippines and Taiwan, whereas *Zoothamnium* sp. is prevalent in Thailand, Indonesia, Malaysia and Taiwan, and on juveniles and adults in the Philippines. *Ephelota gemmipara* is less common, but, nevertheless, caused heavy mortalities at a SEAFDEC hatchery in 1976 (Gacutan et al., 1977). Ruangpan (1986) concluded that the most important pathogenic protozoa for *P. monodon* and *P. merguiensis* larvae were the peritrichs *Zoothamnium* sp. and *Epistylis* sp. In the Philippines, *Zoothamnium* sp. and *Epistylis* sp. at-

tacking juvenile *P. monodon* are controlled with chloroquine diphosphate or a Formalin bath (Table 4).

Gregarines, internal parasitic protozoa that use bivalves as either intermediate or "conditioning" hosts, together with luminous bacteria, were reported to infect *P. monodon* postlarvae, resulting in mass mortalities within two days. In Thailand, two species of gregarines were found in the guts of 94% of the *P. monodon* from a private farm (Baticados, 1988). Microsporidians also infect *P. monodon*. The disease was observed in female broodstock that manifested white ovaries and were subsequently found to be sterile (Baticados, 1988).

Nutritional, Toxic and Environmental Diseases. Like all cultured shrimp, *P. monodon* will suffer poor growth and, perhaps, mortality if exposed to excessive levels of toxins or if deprived of essential nutrients. The following diseases and disease syndromes were summarized by Baticados (1988) (Table 5).

Chronic soft-shell syndrome. Affecting both juveniles and adults, chronic soft-shell syndrome is a major cause of decreased production in ponds in the Philippines. It is caused by nutritional deficiencies, pesticides in the water and poor water and soil conditions. This syndrome also afflicts pond- and tank-reared shrimp in Indonesia, Malaysia and Thailand.

Red disease. This disease has caused heavy mortalities (Liao et al., 1977; Liao and Chao, 1983; Liao, 1984). It affects juveniles and adults in Taiwan, the Philippines, Thailand and Malaysia. Gradual mortalities in one case reached 98%. It may be caused by microbial toxins in either rancid diets or in organically rich pond detritus.

Fatty infiltration of the hepatopancreas. This condition has been observed in *P. monodon* in Texas, Mexico and Hawaii. Juveniles and adults, especially extensively cultured ones, had excessive deposits of lipid in their hepatopancreatic tubule epithelium. The condition may be due to improper dietary lipid levels, improper caloric-lipid balance, or dietary toxins.

Blue disease. First observed in broodstock in Tahiti where it caused mass mortalities in 1978-79, blue disease has also been diagnosed in *P. monodon* cultured in Indonesia, Malaysia, Taiwan and Thailand. Blue *P. monodon* have low levels of astaxanthin; hence, this disease is thought to be mainly due to a nutritional deficiency. A viral etiology, however, has not been discounted. Blue disease is controlled at Aquacop in the Philippines by using low densities, high-quality feeds and frequent water exchange in broodstock ponds and tanks.

Cramped tails. Reported in Taiwan, the Philippines and Indonesia, this condition is seen in juveniles and adults. Afflicted animals have a flexed, rigid abdomen. The cause is probably a sudden increase in temperature, such as that experienced by shrimp when they

Table 5. Nutritional, toxic and environmental diseases of cultured *Penaeus monodon*.

Diseases and disease syndromes	Countries	References
Chronic soft-shell syndrome	Indonesia, Malaysia, Thailand, Philippines	Baticados, 1988
Red disease	Taiwan, Philippines, Thailand, Malaysia	Liao et al., 1977; Liao and Chao, 1983; Liao, 1984
Fatty infiltration of the hepatopancreas	Texas, Hawaii, Mexico	Lightner, 1985 (cited in Baticados, 1988)
Blue disease	Tahiti, Indonesia, Malaysia, Taiwan, Thailand	Baticados, 1988
Cramped tails	Taiwan, Philippines, Indonesia	Baticados, 1988
Hemocytic enteritis	Philippines	Baticados, 1988
Heavy metal poisoning	Taiwan	Kou et al., 1984 (cited in Baticados, 1988)
Black gill disease	Thailand, Philippines, Indonesia, Malaysia, Taiwan	Lightner, 1985 (cited in Baticados, 1988)
Muscle necrosis/Tail rot	Indonesia, Malaysia, Taiwan, Thailand, Philippines	Lightner, 1985 (cited in Baticados, 1988)
Gas bubble disease	Not specified	Lightner, 1983 (cited in Baticados, 1988)

are handled on warm days (also see Liao et al., 1977).

Hemocytic enteritis. In the Philippines, blooms of blue-green algae belonging to the family Oscillatoriaceae caused hemocytic enteritis in primarily young juveniles as well as subadult prawns (Lightner, 1985).

Heavy metal poisoning. Exposure to excessive levels of mercury, copper, cadmium and zinc have caused disease in cultured *P. monodon*, and cadmium and copper poisoning were suspected to cause mortalities in hatcheries in Taiwan in 1980-81 (Kou et al., 1984).

Black gill disease. This morphological condition can be caused by a number of biotic and abiotic agents, including viral, bacterial, fungal and protozoan infections, vitamin C deficiency, and contamination with toxic pollutants like cadmium, copper, potassium permanganate, ozone, ammonia, and nitrate (Lightner, 1985). This condition has been observed in Thailand, the Philippines, Indonesia, Malaysia and Taiwan.

Muscle necrosis. Associated with stress, muscle necrosis is prevalent in high-density cultures. The chronic and infected form of the disease when it affects the distal portion of the abdomen is termed **tail rot** (Lightner, 1985). If large portions of the abdomen are affected, mortalities can result (Lightner, 1985). This is apparently a common problem in Indonesia, Malaysia, Taiwan, Thailand and the Philippines.

Gas bubble disease. This occurs as a result of supersaturation of sea water

Table 6. *Penaeus monodon* diseases and disease syndromes of unknown etiology.

Disease	Countries	Treatments	References
Red discoloration	Taiwan	Use only fresh feed	Liao et al., 1977
Larval black spot syndrome	Thailand	None	Flegel et al., this volume
Spongy muscle syndrome	Thailand	None	Flegel et al., this volume
One-month mortality syndrome	Thailand	Maintain healthy blooms of planktonic algae to discourage growth of benthic blue-green algae	Flegel et al., this volume
Yellowhead shrimp	Thailand	None	Flegel et al., this volume

with atmospheric gases (Lightner, 1983).

Finally, Nash et al. (1988) described the pathological changes occurring in *P. monodon* cultured in acidic, brackishwater ponds. The gills of the shrimp were brown, and upon closer examination, were found to be clogged with ferric hydroxide precipitate. Hypoxia and stress resulted, causing low yields.

Diseases of Unknown Etiology. Some of the previously mentioned diseases have uncertain etiologies. In addition to these, at least six diseases of unknown etiology have been reported to afflict *P. monodon* specifically (Table 6): **red discoloration** (Liao et al., 1977) **larval black spot syndrome, spongy tissue syndrome, one-month mortality syndrome,** and **yellowhead shrimp** (Flegel et al., this volume).

Red discoloration occurred in Taiwan where pond-reared shrimp gradually turned yellowish green, red and then pale. Heavy mortalities accompanied the symptoms, which also included loss of appetite and difficulty with respiration. *Penaeus penicillatus* and *P. semisulcatus* present in the same ponds did not become sick. After an effort was made to use only fresh fish as a feed ingredient, the disease did not recur; hence, red discoloration may be a nutritional disease, or it may have resulted from a toxin that was present in spoiled and rotting fish flesh.

Larval black spot syndrome was reported from Aquastar hatcheries in Thailand (Flegel et al., this volume). Zoea-1 or zoea-2 are occasionally found with a black lump of unidentified material at the junction of the stomach, hepatopancreas and midgut. The effect of this disease on larval survival is unknown.

The presence of very spongy muscle, nerve, and other tissues has been observed in *P. monodon* postlarvae, growout shrimp and broodstock in Thailand (Flegel et al., this volume). No other information is available on spongy tissue syndrome. Flegel and his coworkers also described one-month mortality syndrome, a growout disease characterized by sudden mortality four

to six weeks after stocking. This disease may be associated with transparencies greater than 40 cm and floating plaques of benthic blue-green algae.

Finally, yellowhead shrimp is the name of a disease whose victims present a swollen cephalothorax in the region of the hepatopancreas and an abnormally light yellow-colored hepatopancreas (Flegel et al., this volume). Flegel and coworkers reportedly have found a potential viral pathogen associated with this disease (Lightner et al., this volume).

The Taiwanese Shrimp Culture "Crash" of 1988. The most renowned shrimp culture disaster struck Taiwan's *P. monodon* industry in 1988. In 1987, Taiwanese farmers produced 95,000 MT of *P. monodon*. Due to a combination of factors, however, including pollution, imprudent culture practices, and disease, production in 1988 dropped by 70% and exports declined by 80% (Liao, 1989; Lin, 1989; Chen, 1990).

A special "disease task force" was commissioned to investigate the crisis and make recommendations. The group reported on a number of nonpathogenic and pathogenic factors believed to contribute to the culture crash. The following **nonpathogenic factors** were identified:

- The use of high temperatures to accelerate larval growth;
- Deterioration of growout ponds;
- Unreasonably high stocking densities;
- Poor artificial feed;
- Indiscriminate use of medicine and antibiotics;
- Inadvertent and sometimes unavoidable use of polluted water;
- Lack of technical training of farmers; and
- Absence of a reliable system of sanitation on the farms.

The following **pathogenic factors** were also suspected to contribute to the crisis:

- Bacterial infection of the hepatopancreas;
- Bacterial and protozoan epicommensal infestations;
- Concurrent bacterial infection of the hepatopancreas and bacterial and protozoan epicommensal infestations;
- MBV; or
- A combination of MBV and bacterial infections (Liao, 1989).

The publicity generated by the crash, as well as the investigations it spurred, have helped focus needed attention on penaeid diseases and the importance of culture practices that incorporate dis-

ease prevention. Meanwhile, Taiwan continues to recover from the 1988 disaster. Some farmers are stocking *P. monodon* at lower densities, while others have switched to different species.

Economic Impact of Disease. No one has estimated the extent to which disease affects the *P. monodon* culture industry in Asia. At best, isolated incidences of mortality have been attributed to certain diseases or disease syndromes. For example, Anderson et al. (1988) reported that vibriosis caused 70 - 95% reductions in the expected harvests for three farms in Johore, Malaysia, during 1986 and 1987. While the 1988 collapse of the Taiwanese shrimp industry could not be attributed entirely to disease, the cost of the crisis in its entirety was approximately US$ 376 million (given that exports worth US$ 470 million in 1987 decreased by 80% in 1988 [Liao, 1989]).

Penaeus chinensis

The Chinese have been culturing shrimp for centuries, and, in the 1980s, China became an important player in the global shrimp market. Approximately 80% of the shrimp grown in the People's Republic of China are *P. chinensis*. *Penaeus chinensis* (also known as *P. orientalis*) is a temperate species with a small natural distribution. The main fishing grounds are in the Bohai Bay in northern China's Yellow Sea, but the species extends to the mouth of the Pearl River in southern China and is also relatively abundant along the western coast of the Korean peninsula. In addition to the farms and hatcheries in China, there are several *P. chinensis* hatcheries and farms in South Korea, and the species is being investigated for production in Taiwan. The majority of production is extensive or semi-intensive, and high growth rates have been reported for this species (Main and Fulks, 1990).

Infectious and Parasitic Diseases. To date, the only viral disease agent reported to infect cultured *P. chinensis* is hepatopancreatic parvo-like virus (HPV, see Table 7) (Lightner and Redman, 1985). HPV-positive *P. chinensis* have been found from South Korea and China. Wang and Ma (1990) reported on a disease affecting shrimp in China whose gross pathology was similar to the baculoviral midgut gland necrosis virus (BMNV) that afflicts *P. japonicus*, but no viral pathogens were isolated from diseased individuals.

The usual bacterial pathogens have been reported to infect *P. chinensis* in China and South Korea, and in both countries, antibiotics are sometimes added to the feed to combat bacterial infections (Table 7) (Main and Fulks, 1990). *Vibrio* spp. and *Pseudomonas* sp. can cause serious mortalities in hatcheries, as well as during growout (Liu, 1990; Wang and Ma, 1990). Liu (1990) reported that infections are more serious in southern China where temperatures are higher, and that copper sulfate has been successfully used to control vibriosis in *P. chinensis* hatcheries. Treatment with 1 - 2 ppm chloramphenicol is also effective (Wang and

Table 7. Infectious and parasitic disease agents of cultured *Penaeus chinensis*.

Diseases	Countries	Treatments/Prevention	References
VIRAL			
Hepatopancreatic parvo-like virus (HPV)	China, South Korea	None	Lightner and Redman, 1985; Brock, pers. comm.
BACTERIAL			
Vibrio spp., *Pseudomonas* sp.	China, S. Korea	Treatments: antibiotics added to feed (during growout), copper sulfate, 1 - 2 ppm chloramphenicol	Liu, 1990; Wang and Ma, 1990; Main and Fulks, 1990
Leucothrix sp., *Thiothrix* sp.	China, S. Korea	Prevention: keep culture water clean	Wang and Ma, 1990
FUNGAL			
Lagenidium sp., *Sirolpidium* sp.	China, S. Korea	Prevention: disinfect broodstock, treat rearing water regularly with 6-10 ppb malachite green, clean tanks with 25 ppm Formalin. Treatment: nystatin	Wang and Ma, 1990; Main and Fulks, 1990
PROTOZOAN			
Paranophrys carcini	China	Prevention: disinfection with 25 ppm Formalin	Wang and Ma, 1990
Zoothamnium, Carchesium, Vorticella, Epistylis	China, S. Korea	Prevention: keep water clean, supply good diet, disinfect tanks with Formalin. Treatment: 90% water exchange	Wang and Ma, 1990; Main and Fulks, 1990
Pleistophora sp., *Thelohania* sp., *Nosema* sp.	China	No treatment; prevent by removing infected individuals, sterilize pond bottom	Meng and Yu, 1983; Wu et al., 1991 (both cited in D. Chen, this volume)
Unidentified flagellates	China	None reported	Wang and Ma, 1990

Ma, 1990). In addition, filamentous bacteria, *Leucothrix* sp. and *Thiothrix* sp., are sometimes found adhering to the surfaces of eggs and larvae, but keeping the culture water clean can prevent this (Wang and Ma, 1990).

As far as fungal infections are concerned, *P. chinensis* eggs and larvae may suffer from larval mycosis, caused by *Lagenidium* sp. or *Sirolpidium* sp. (Table 7). According to Wang and Ma (1990), in China these pathogens "do very serious damage and can destroy all the shrimp fry produced during the breeding season [in some hatcheries]." Preventive measures include carefully disinfecting female broodstock, and regular treatment of the rearing water with 6 - 10 ppb malachite green. Infected larvae are treated with nystatin, a "fairly effective" cure. Formalin (30

Table 8. Nutritional, toxic and environmental diseases reported from cultured *Penaeus chinensis*.

Diseases	Countries	References
Bubble disease	China	Wang and Ma, 1989
Deformity disease	China	Wang and Ma, 1990
Black gill disease	China	Meng and Yu, 1982; Liu, 1990
Body cramp	China	D. Chen (this volume)
Muscle necrosis	China	D. Chen (this volume)
Floating head syndrome	China	D. Chen (this volume)

ppm) is used to disinfect rearing tanks in South Korea to prevent both fungal and protozoan infections (Main and Fulks, 1990).

Wang and Ma (1990) discussed **parasitic ciliate disease**, caused by a new strain of *Paranophrys carcini*, an endoparasitic ciliate (Table 7). In China, the disease is found in larvae and overwintering adults. Parasitic ciliate disease often causes 100% mortality in infected tanks. While no effective treatments have been found, the disease can be prevented if tanks are disinfected with 25 ppm Formalin prior to use.

D. Chen (this volume) listed three microsporidians that occasionally infect cultured *P. chinensis* in China, causing cotton shrimp disease. They are *Pleistophora* sp., *Thelohania* sp. and *Nosema* sp. (Table 7).

Epicommensal ciliates also cause problems in China, and to a lesser degree in South Korea (Main and Fulks, 1990). In China, 38 species of ciliates have been identified from cultured *P. chinensis*. *Zoothamnium* sp., *Carchesium* sp., *Vorticella* sp. and *Epistylis* sp. are the most common (Wang and Ma, 1990). Serious infestations are prevented by keeping the culture water clean and supplying a good diet. In addition to disinfection with Formalin, the Koreans treat protozoan infestations by changing 90% of the water in affected tanks. Finally, Wang and Ma (1990) made a brief reference to another disease caused by a protozoan pathogen, "flagellate adhesive disease."

Nutritional, Toxic and Environmental Diseases. The diseases in this category reported for *P. chinensis* are **bubble disease, deformity disease** (Wang and Ma, 1990), **body cramp, muscle necrosis** and **floating head syndrome** (D. Chen, this volume) (Table 8). The latter disease occurs on hot summer days in high-density growout ponds that have insufficient phytoplankton blooms and poor water quality. Severe mortalities can result; the cause is probably an insufficient supply of dissolved oxygen.

Table 9. Diseases of unknown etiology reported from cultured *Penaeus chinensis*.

Diseases	Countries	References
Red leg disease	China	Liu et al., 1989
White-black spot disease	China	D. Chen (this volume)
White spot disease	China	D. Chen (this volume)
Soft shell syndrome	China	D. Chen (this volume)
Black gill disease	China	Meng and Yu, 1982

Diseases of Unknown Etiology. Although they did not mention them by name, Wang and Ma (1990) said that there are some diseases of *P. chinensis* for which no cause is known. Also, Liu et al. (1989) reported on **red leg disease** of *P. chinensis* and *P. penicillatus*. It was reportedly rampant in the Fujian Province of southern China, and may be caused by a bacterium. Other diseases in this category are **white-black spot disease, white spot disease**, and **soft shell syndrome** (see D. Chen, this volume). Finally, *P. chinensis* cultured in China sometimes suffer from a poorly understood condition that has been called **black gill disease** which may or may not be the same as either Fusarium disease or the toxic disease bearing the same name (Meng and Yu, 1982) (Table 9).

Economic Impact of Disease. The economic impact of disease on cultured *P. chinensis* is unknown.

Penaeus japonicus

Penaeus japonicus is a colorful shrimp grown primarily in Japan, Taiwan, and South Korea for consumption in Japan. In Japan, 95% of the shrimp (live weight) produced in 1991 were *P. japonicus*, and in Taiwan, that species comprised 30% of production (Rosenberry, 1991). Although the species is relatively expensive to culture due to a high protein requirement and its need for a clean, sandy substrate, *P. japonicus* nonetheless fetch a high price, tolerate low temperatures, and can survive long journeys out of water.

Infectious and Parasitic Diseases. Larval and postlarval *P. japonicus* are susceptible to a severe epizootic viral disease, baculoviral midgut gland necrosis virus (BMNV) disease (Table 10) (Sano et al., 1981, 1983, 1984, 1985; Momoyama and Sano, 1989; Lightner, 1988). Mortalities of up to 90% have been reported in some hatcheries (Sano et al., 1984). Known since approximately 1971, BMNV infections occur orally or by exposure to water-borne pathogens, resulting in drastically high mortality within the first week of an outbreak.

Prevention consists of rinsing and transferring eggs to a disinfected tank immediately after spawning. According to Momoyama and Sano (1989), "rinsing eggs with sea water has now been carried out on an industrial scale in Japan since 1985 and, to date, BMN

Table 10. Infectious diseases and disease agents of cultured *Penaeus japonicus*.

Diseases	Countries	Treatments	References
VIRAL			
Baculoviral midgut gland necrosis virus (BMNV)	Japan	None	Sano et al., 1981, 1983, 1984, 1985; Momoyama and Sano, 1989; Lightner, 1988; Tsing and Bonami, 1987; Lightner et al., 1985
Reo-like virus (REO)	Japan, Hawaii	None	
BACTERIAL			
Vibrio sp.	Japan, S. Korea	Oxytetracycline added to feed	Sano and Fukuda, 1987; Shigueno, 1975; Davy, 1991; Main and Fulks, 1990; Takahashi et al., 1985
Bacterial gill disease [= gill rot disease?]	Japan	Bathing in furazolidone at a conc. of 2 - 3 ppm for two to four consecutive nights	Shigueno, 1975
"Bacterial infections of the hepatopancreas"	Taiwan	None reported	Main and Fulks, 1990
Leucothrix sp.	Japan, S. Korea	None reported	Davy , 1991; Main and Fulks, 1990
FUNGAL			
Fusarium solani, *Fusarium moniliforme*	Japan	None reported	Shigueno, 1975; Main and Fulks, 1990
PROTOZOAN			
Epicommensal ciliates	S. Korea, Japan, Taiwan	In Taiwan: Formalin, saponin and methylene blue	Main and Fulks, 1990

has never occurred in the hatcheries where it has been practised" (also see Sano and Momoyama, this volume; Momoyama, this volume).

A reo-like virus (REO) was first discovered by Tsing and Bonami (1987) in juvenile *P. japonicus* in France. Reo-like viruses have also been reported in Hawaii, among *P. japonicus* imported from Japan (Lightner et al., 1985; Lightner and Redman, 1991). Tsing et al. (1985) suggested that infection by REO may be associated with **gut-and-nerve syndrome** (GNS), an idiopathic condition, which was found in chronically ill populations of *P. japonicus* cultured in Hawaii (Lightner and Redman, 1991).

Two serious bacterial diseases have been reported in *P. japonicus* grown in Japan, **vibriosis** and **bacterial gill disease** (Table 10). The former has caused remarkably high mortality, especially in the summer and autumn seasons. The pathogen is apparently an unidentified

species of the *Vibrio* genus, and affected shrimp manifest brown pigmentation in the lymphoid organ and the gill as well as cloudiness in the muscular tissue of the 3rd to 6th abdominal segment (Takahashi et al., 1985; Sano and Fukuda, 1987). During growout, Japanese farmers treat vibriosis in *P. japonicus* by including oxytetracycline in the feed at the rate of 50 mg/kg shrimp weight per day. By law, however, treatments must end 25 days before harvesting if the shrimp are to be used for human consumption (Sano and Fukuda, 1987; also see Main and Fulks, 1990; Shigueno, 1975; Davy, 1991).

Shigueno (1975) reported that an unidentified bacterium is responsible for **bacterial gill disease**. This condition is prevalent in high density culture tanks and is most commonly seen in the spring. Losses from bacterial gill disease are not as high as from vibriosis, and treatment consists of bathing the shrimp in 2 - 3 ppm furazolidone for 2 to 4 consecutive nights. Bacterial gill disease may be the same as **gill rot disease**, which was reported by Sano and Fukuda (1987) to inflict heavy losses on the Japanese *P. japonicus* industry (see Table 10).

Bacterial infections have also been noted in *P. japonicus* cultured in South Korea and Taiwan, and *Leucothrix* sp. attach to cultured *P. japonicus* in Japan and South Korea (Davy, 1991; Main and Fulks, 1990).

According to Lightner (1983), *P. japonicus* is particularly susceptible to *Fusarium solani*, the etiological agent of **Fusarium disease** or **black gill disease** (Table 10). It has recently been discovered that *F. moniliforme* can also cause Fusarium disease (Rhoobunjongde et al., 1991). No effective chemotherapies are in use for *P. japonicus* infected with *Fusarium* sp. While mortalities may approach 100%, Shigueno (cited in Main and Fulks, 1990) reported in 1990 that Fusarium disease was rare in *P. japonicus* cultured in Japan. Rearing at lower densities may prevent the disease (Lightner, 1983; also see Momoyama, 1987 and Bian and Egusa, 1981).

Finally, epicommensal ciliates have been observed in *P. japonicus* raised in Taiwan, South Korea and Japan (Main and Fulks, 1990) (Table 10).

Nutritional, Toxic and Environmental Diseases. ***Penaeus japonicus*** is susceptible to those diseases with nutritional, toxic and environmental etiologies, such as gas bubble disease and muscle necrosis, that affect other species of shrimp.

Diseases of Unknown Etiology. Black spot disease and gut-and-nerve syndrome (GNS) are two diseases of *P. japonicus* for which etiological agents have not been discovered (Table 11). The latter is known from France and Hawaii where shrimp exhibited anorexia, lethargy, poor feed conversion, epibiotic fouling and muscle opacity (Brock, pers. comm.). Black spot disease is poorly described, and affected *P. japonicus* cultured in Taiwan (Main and Fulks, 1990).

Table 11. Diseases of unknown etiology reported from cultured *Penaeus japonicus*.

Diseases	Countries	Treatments/Preventions	References
Black spot disease	Taiwan	Unknown	Main and Fulks,1990
Gut-and-nerve syndrome (GNS)	Hawaii, France	None	Brock, pers. comm.

Table 12. Disease-related losses of *Penaeus japonicus* in Japan in 1984 (Fisheries Agency; cited in Sano and Fukuda, 1987).

Diseases	Losses (MT)	Losses (million Yen)
Vibriosis	30.8	231.4
Fusarium disease	5.9	30.7
BMN	5.7	11.2
Gill rot disease	0.5	7.7
Unknown	27.3	185.1
Total	70.2	466.1

Economic Impact of Disease. In 1984, 70.2 MT of *P. japonicus* worth ¥ 466.1 million were lost in Japan due to disease (Sano and Fukuda, 1987). The greatest losses were attributed to vibriosis (30.8 MT) and unknown diseases (27.3 MT) (see Table 12). Fusarium disease, BMN, and gill rot disease were the other major diseases impacting Japan's cultured *P. japonicus* industry in 1984.

Penaeus merguiensis

This species ranges from the Arabian Gulf and Pakistan through the Malay Archipelago and South China Sea to Australia (Dore and Frimodt, 1987). *Penaeus merguiensis* is an important culture species in Indonesia, Vietnam, Thailand and the Philippines (Rosenberry, 1991).

Infectious and Parasitic Diseases. There are few references to the viral diseases of *P. merguiensis* in the literature, although the species is known to be susceptible to MBV (observed in Singapore and Malaysia) and HPV (observed in Singapore and Australia) (Lightner, 1985; Roubal et al., 1989) (Table 13). Lightner and Redman (1985) stated that in juvenile *P. merguiensis*, HPV has caused cumulative mortalities of up to 50%. An unidentified baculovirus was also isolated from a wild specimen in Australia (Doubrovsky et al., 1988).

No references to the bacterial or fungal diseases of *P. merguiensis* were found in the literature. Presumably, this species is susceptible to the same bacteria and pathogenic fungi infections that affect other shrimps. *P. merguiensis* is, however, one of four penaeid species to be

Table 13. Disease agents of cultured *Penaeus merguiensis*.

Diseases	Countries	Treatments	References
VIRAL			
Penaeus monodon-type baculovirus (MBV)	Malaysia, Singapore	None	Lightner, 1985
Hepatopancreatic parvo-like virus (HPV)	Singapore	None	Lightner, 1985; Lightner and Redman, 1985
BACTERIAL			
None reported			
RICKETTSIAL			
Unidentified species			Lightner et al., 1985
FUNGAL			
None reported			
PROTOZOAN			
Thelohania sp.	Thailand	Unknown	Ruangpan, 1986
Zoothamnium sp., *Epistylis* sp.	Thailand	Unknown	Ruangpan, 1986

diagnosed with rickettsial infections (Lightner et al., 1985).

Ruangpan (1986) studied the pathogenic protozoa affecting cultured *P. monodon* and *P. merguiensis* in Thailand. She discovered that in *P. merguiensis* "cotton" or "milky disease" produced by the microsporidian, *Thelohania* sp., caused losses up to 55%. Furthermore, two epicommensal protozoans were identified as important pathogens, *Epistylis* sp. and *Zoothamnium* sp. (Table 13).

Nutritional, Toxic and Environmental Diseases. *P. merguiensis* is susceptible to those diseases with nutritional, toxic and environmental etiologies, such as gas bubble disease and muscle necrosis, that affect other species of shrimp.

Diseases of Unknown Etiology. Little information was found regarding *P. merguiensis* diseases that have unknown or uncertain etiologies. Liu (1990), however, did report that *P. merguiensis*, along with *P. chinensis* and *P. penicillatus*, cultured in China suffer from red leg disease. This disease may be caused by a bacterium.

Economic Impact of Disease. The economic impact of disease on cultured *P. merguiensis* is unknown.

Penaeus penicillatus

Penaeus penicillatus is a minor culture species. Grown only in the southern provinces of China and in Taiwan, its culture techniques are similar to those of *P. monodon* and *P. chinensis*. *P. penicillatus* reportedly has a low protein re-

Table 14. Disease agents of cultured *Penaeus penicillatus*.

Disease	Countries	Treatments	References
VIRAL			
Penaeus monodon-type baculovirus (MBV)	Taiwan	None	Main and Fulks, 1990
Hepatopancreatic parvo-like virus (HPV)	Brazil (in stock imported from Taiwan)	None	Lightner et al., 1990
BACTERIAL			
"Bacterial infections of the hepatopancreas"	Taiwan	None reported	Main and Fulks, 1990
Ectocommensal bacteria	Taiwan	None reported	Main and Fulks, 1990
FUNGAL			
None reported			
PROTOZOAN			
Ectocommensal ciliates	Taiwan	Add the following chemicals and flush; up to 25 ppm formalin, 5 ppm of 10% soln. saponin, and 8 ppm methylene blue.	Main and Fulks, 1990

quirement and an unusually favorable head to tail ratio (Liao and Chien, 1990).

Infectious and Parasitic Diseases

Very little has been published about the diseases of cultured *P. penicillatus*. In Main and Fulks (1990) it was reported that cultured *P. penicillatus* in Taiwan have been diagnosed with the following: MBV, bacterial infections of the hepatopancreas and epicommensal ciliates and bacteria (Table 14).

Nutritional, Toxic and Environmental Diseases

P. penicillatus is susceptible to those diseases with nutritional, toxic and environmental etiologies that affect other species of shrimp.

Diseases of Unknown Etiology

The only diseases of uncertain or unknown etiology observed in *P. penicillatus* are black spot disease and red leg disease. The former afflicts pond cultured *P. penicillatus* and *P. japonicus* in Taiwan (Main and Fulks, 1990). A bacterium is suspected to cause red leg disease, a serious problem in *P. chinensis* and *P. penicillatus* grown in China's Fujian Province (Liu et al., 1989).

Economic Impact of Disease

The economic impact of disease on cultured *P. penicillatus* is unknown.

Table 15. Disease agents of cultured *Penaeus vannamei* in the United States.

Diseases	Treatments	References
VIRAL		
Baculovirus penaei (BP)	None	(Lightner, 1988; Overstreet et al., 1988)
Infectious hypodermal and hematopoietic necrosis virus (IHHNV)	None	Lightner et al., 1983a, 1983b; Lightner, 1988
Reo-like virus (REO)	None	Krol et al., 1990
BACTERIAL		
Filamentous bacteria	Increase rate of water exchange, adjust feeding schedules	Wyban and Sweeny, 1991
Unidentified acid-fast bacterium	None reported	Krol et al.,1989
FUNGAL		
Sirolpidium sp.	0.1 ppm Treflan®	Wyban and Sweeny, 1991
PROTOZOAN		
Epicommensal species	Prevent by performing larval rearing in discrete cycles and with good water filtration; Formalin	Wyban and Sweeny, 1991; Code of Federal Regulations, 1991

The Status of Penaeid Diseases in the United States

Penaeus vannamei

Penaeus vannamei is the dominant penaeid cultured in the western hemisphere. Seventeen percent of the shrimp produced worldwide in 1991 was *P. vannamei* (Rosenberry, 1991). The natural distribution of this species extends from the Pacific coast of southern Mexico to Peru; Ecuador is the largest producer of farm-raised *P. vannamei*. Because it has outperformed native shrimps, *P. vannamei* is grown in the United States, where, in 1991 there were 22 farms and 3 hatcheries operating in Texas, South Carolina and Hawaii (Rosenberry, 1991).

Infectious and Parasitic Diseases. At least four viruses infect cultured *P. vannamei*: *Baculovirus penaei* (BP), infectious hypodermal and hematopoietic necrosis virus (IHHNV), REO and HPV (Table 15). BP causes high, acute mortalities (Lightner, 1988; Overstreet et al., 1988), but the effects of IHHNV on *P. vannamei* are more subtle, often resulting in lower growth rates and an uneven size distribution. IHHNV may be the etiological agent of **runt-deformity syndrome** (RDS) in *P. vannamei* (Kalagayan et al., 1991). A reo-like virus has only been reported once in *P. vannamei* cultured in the United States, and then it was found concurrently with shrimp experimentally infected with BP

(Krol et al., 1990). HPV, by contrast, has only been found in cultured *P. vannamei* from Brazil, Ecuador and Mexico (Lightner et al., 1990, Lightner, pers. comm). Viral diseases are considered to be a serious threat to the shrimp culture industry in the United States; hence, intense efforts are underway to ensure that *P. vannamei* cultured in the United States are free of all detectable viruses (see Wyban, this volume).

Penaeus vannamei are susceptible to all the common bacterial diseases. Vibriosis and filamentous bacteria, for example, are commonly encountered on farms and in hatcheries. In addition, infection of cultured *P. vannamei* with an acid-fast bacterium was described by Krol et al (1989). Outbreaks of filamentous bacteria are treated at The Oceanic Institute hatchery by increasing the rate of water exchange and "fine tuning" feeding schedules (Wyban and Sweeny, 1991) (Table 15).

Larval mycosis has caused severe mortalities in *P. vannamei* hatcheries. At The Oceanic Institute, the agent responsible has been identified as *Sirolpidium* sp., and mortalities can be as high as 100%. The disease is now controlled by the addition of 0.1 ppm Treflan® once per day. The incidence of larval mycosis can also be reduced by periodically disinfecting reservoirs and water lines (Wyban and Sweeny, 1991) (Table 15).

Outbreaks of epicommensal protozoans are prevented at The Oceanic Institute's *P. vannamei* hatchery by 1) completely drying out the hatchery between rearing cycles, and 2) water filtration (Table 15). Formalin was recently approved by the U.S. Food and Drug Administration for controlling epicommensal protozoans in cultured shrimp (Code of Federal Regulations, 1991).

Nutritional, Toxic and Environmental Diseases

Lightner and Redman (1982) studied the histopathology of aflatoxicosis in juvenile *P. vannamei* and *P. stylirostris*, and found the penaeids to be remarkably resistant to aflatoxin. Aflatoxin is created by fungi and is occasionally present in the ingredients used to make fish and shrimp feeds. *Penaeus vannamei* are, however, susceptible to gas bubble disease, body cramp and other shrimp diseases having nutritional, toxic or environmental etiologies.

Diseases of Unknown Etiology. There are two noteworthy disease syndromes that fall into this category: Z-1 syndrome and Texas necrotizing hepatopancreatitis (alias "Texas pond mortality syndrome"). The former was first encountered in 1987 at The Oceanic Institute's hatchery where high mortality rates at the zoea-1 stage were coupled with severe deformities (Wyban and Sweeny, 1991). While the definitive agent is unknown, the disease was effectively prevented by the addition of the chelating agent EDTA to the rearing water, prompting speculation that heavy metals may have been responsible.

Texas necrotizing hepatopancreatitis has thus far been observed only in *P. vannamei* cultured in Texas. The disease is characterized by increases in mortality and morbidity, poor growth, soft shells, empty intestinal tracts, thin tails, lethargy, surface fouling and elevated food conversion ratios in pond-cultured shrimp (Bell et al., submitted). While the disease is poorly understood and probably has an environmental component, *Vibrio* or *Vibrio*-like bacteria have been identified from diseased shrimp during epizootics, and researchers have found several other potentially pathogenic microbes in diseased individuals. Texas necrotizing hepatopancreatitis has been treated with oxytetracycline with promising results (Bell et al., submitted).

Economic Impact of Disease

The economic impact of disease on *P. vannamei* culture is unknown.

Current and Future Avenues of Research

Shrimp pathologists around the world are currently engaged in research to improve the prevention, diagnosis, and treatment of diseases in cultured penaeids. This section will discuss some of the most current advances in penaeid disease research.

Disease Prevention

There are a number of ways to prevent the occurrence and spread of disease among cultured penaeid shrimp, including adopting better culture practices, instituting quarantine procedures, stocking only specific pathogen-free (SPF) shrimp, and vaccination. Preventive culture practices are necessary to exclude or reduce the number of pathogens present in the culture environment and to minimize the stress experienced by the animals. Examples include disinfecting tanks and drying ponds between cycles, optimizing feeding regimes, providing high-quality feeds, etc. An example of research in this area is the work of LeBlanc and Overstreet (1991a and b). The authors tested the means by which culture facilities could be disinfected to prevent *Baculovirus penaei* (BP) infections. They found that 48 h desiccation inactivated BP in hepatopancreatic tissue (LeBlanc and Overstreet, 1991a) and concluded that desiccation may be, in many cases, the most practical means of preventing BP infections in aquaculture facilities. BP was also inactivated by treatment with chlorine at concentrations of 200 mg/L for 1 h or 1,600 mg/L for 20 s (LeBlanc and Overstreet, 1991b).

Another disease prevention tool is quarantining imported stocks and using only stock that has been certified as SPF. A number of viruses have already been introduced into previously "clean" areas via infected imports; these introductions are regarded as serious setbacks to the global shrimp culture industry.

Finally, it is theoretically possible to prevent the onset of some diseases — vibriosis for example — by immunization. Even though marine invertebrates

have nonspecific immune systems, a number of bacterial vaccines have been developed and tested on shrimp (e.g.. Giorgetti, 1990; Itami and Takahashi, 1991; Laramore, this volume), with mixed results. Vaccination is an expensive prospect, however, and one problem that must be solved is the efficient delivery of the vaccine to a cultured population (Dunn et al., 1990).

Diagnostic Techniques

In recent years, a number of researchers have brought attention to the need for better diagnostic techniques for shrimp viruses (Lewis, 1986; Lightner et al., 1983b, 1990; Bell et al., 1990a; Thurman et al., 1990; Baticados et al., 1991). Commonly, health experts employ light microscopy to detect characteristic signs of viral infection (e.g., occlusion bodies) in stained preparations of tissues. Electron microscopy is important in some applications as well. In addition to the problems of cost, time, and accessibility, these techniques, practically employed, are not sensitive enough to detect latent infections, necessitating the use of enhancement and bioassays. Enhancement is accomplished by stressing a suspect population of shrimp to trigger any latent viral infections into patency. To conduct a bioassay, one feeds the suspect shrimp to a specific pathogen-free (SPF) laboratory population of an extremely sensitive species, stresses the population, and subsequently tests for the presence or absence of the virus. Bioassays are extremely labor- and time-intensive; furthermore, SPF shrimp are increasingly difficult to find, and they must be maintained.

Ideally, new diagnostic methods should be rapid, simple, inexpensive, more sensitive than existing techniques, and easily standardized (Lightner et al., 1990). Lightner et al. (1990) state that "methods using tissue culture, serologic methods, and gene probe diagnostic techniques that have become common place in human and veterinary medicine are being developed for penaeid shrimp" (also see Lightner et al., this volume). An example of one of these "high-tech" approaches to penaeid viral detection is the use of the enzyme-linked immunosorbent assay (ELISA) method to detect BP in *Penaeus duorarum* (Lewis, 1986; Lightner et al., this volume). Furthermore, the first documented primary cell cultures for shrimp were described by Chen et al., 1986.

Improvements have also been made in the standard techniques. For example, Bell et al. (1990a) recently developed a nonlethal biopsy procedure for IHHNV that involves the excision of the first pereiopod, followed by standard histological examination of the appendage nerve cord. Animals tested by this method need not be sacrificed and remain available for use as broodstock. Thurman et al. (1990) tested the efficacy of fluorescent microscopy with wet-mount tissue squashes stained with phloxine to detect baculoviral occlusion bodies. They achieved good results and concluded that the time required to diagnose baculoviral infections could

be reduced using the new technique (also see Sano et al., 1985 and Momoyama, 1988).

In the meantime, new diseases continue to be discovered. Owens et al. (1991) recently found evidence for a new shrimp virus, lymphoidal parvo-like virus (LOPV) in farmed *P. monodon*, *P. merguiensis* and *P. esculentus*. The virus appears to be closely related to IHHNV.

Drugs/Chemotherapy

> "Chemotherapy should be considered as an emergency or last-resort measure. Although chemicals may reduce the incidence of pathogens or control the abundance of facultative organisms, they also may have negative effects on desirable pond biota and on the flora of biological filters. Some chemicals may be hazardous to the user or leave undesirable or harmful residues in the cultured animals" (Meyer, 1991).

When preventative measures fail, it may be necessary to treat diseases with antibiotics or chemicals. A number of treatments are currently being used in many Asian countries, where the use of compounds like saponin, Formalin, malachite green, Treflan® , chloramphenicol, oxytetracycline and furanace is commonplace. Such therapy is most practically applied in hatcheries, where dense groups of animals are present in small volumes of water. It is simply not cost-effective to combat diseases encountered in extensive and semi-intensive ponds with antibiotics or other expensive drugs.

In the United States, Cutrine-Plus® is approved for use as an algicide, and Formalin may be used to combat epicommensal protozoans. Since these are the only formally approved substances for the prevention or treatment of diseases on U.S. shrimp farms, efforts are underway to develop new drugs and to obtain regulatory approval for those which have been shown to be safe. The following statement by Meyer (1991) represents the views of many U.S. aquaculturists (also see Sindermann, 1986):

> "An urgent need exists for regulatory approval of therapeutic drugs for use in combating diseases in aquaculture . . . increased federal support and greater cooperation and involvement by regulatory agencies are vital if producers are to be able to control the economic losses presently caused by diseases. Without such support, the aquaculture industry in the United States will fail to achieve its full potential and be unable to compete in the world market" (Meyer, 1991).

Conclusions

According to Meyer (1991), "Disease problems constitute the largest single cause of economic losses in aquaculture." Whether this holds true for shrimp culture remains to be seen; since, as this paper has shown, few estimates of the economic impact of shrimp diseases are available. Clearly, diseases can cause serious problems in the culture of all shrimp species, wherever they are are grown. In the future, it will be important to have accurate estimates of the economic damage

wrought by the various diseases in order to effectively manage them. Such estimates will, in essence, help define the problem so that it can then be solved. Accurate estimates will depend upon accurate diagnoses. Identification of the causes of diseases for which no etiological agents have been found will enhance efforts to define and manage "the disease problem" as well.

In addition to documenting their disease situation, it may in the best interest of shrimp producing nations to institute quarantine regulations wherever they have not already been adopted. Such regulations will slow the spread of viruses and other pathogens from their natural geographic ranges and host species.

There is also an indisputable need for more research into all aspects of penaeid diseases. Penaeid culture generates billions of dollars every year and diseases can seriously impact production levels. Key research areas involve preventing infectious and noninfectious diseases, developing better (quicker, less expensive, more sensitive) diagnostic techniques and finding safe, effective treatments for infectious diseases.

Finally, in some areas, shrimp farmers need to be better educated about matters related to disease. For example, the panel investigating the 1988 culture crisis in Taiwan concluded that rapid growth in the *P. monodon* industry in Taiwan encouraged inexperienced persons to try to make quick, easy money growing shrimp. Too many farmers had little or no training in culture practices that prevent disease. In addition to prevention, shrimp farmers should also be educated about how to recognize and quickly respond to those diseases for which sophisticated equipment and/or training is not needed. A farmer would probably be unable to diagnose a viral disease, for example, but he or she could be trained to recognize and respond to vitamin C deficiency, gas bubble disease, infestations of filamentous bacteria, etc.

Literature Cited

Anderson, I.G., M. Shariff, G. Nash and M. Nash. 1987. Mortalities of juvenile shrimp, *Penaeus monodon*, associated with *Penaeus monodon* baculovirus, cytoplasmic reo-like virus, and rickettsial and bacterial infections, from Malaysian brackishwater ponds. Asian Fisheries Science. 1:47-64.

Anderson, I.G., M. Shamsudin, M. Shariff and G. Nash. 1988. Bacterial septicemia in juvenile tiger shrimp, *Penaeus monodon*, cultured in Malaysian brackishwater ponds. Asian Fisheries Science. 2: 93-108.

Baticados, M.C.L. 1988. Chapter 6: Diseases. *In*: Biology and Culture of *Penaeus monodon*. SEAFDEC Aquaculture Dept. Iloilo, Philippines.

Baticados, M.C.L., C.L. Pitogo, M.G. Paner, L. de la Peña and E.A. Tendencia. 1991. Occurrence and pathology of *Penaeus monodon* baculovirus infection in hatcheries and ponds in the Philippines. Bamidgeh. 43(1): 35-41.

Bell, T.A. 1991. Overview of diseases and drug needs for major aquaculture species: Shrimp. Vet. Hum. Toxicol. 33 (Supplement 1): 19-23.

Bell, T.A., D.V. Lightner and J. A. Brock. 1990a. A biopsy procedure for the nondestructive determination of infectious hypodermal and hematopoietic necrosis virus (IHHNV) infection in *Penaeus vannamei*. J. Aquatic Animal Health. 2: 151-153.

Bell, T.A. and D.V. Lightner. 1991. Chemotherapy in aquaculture today—Current practices

in shrimp culture: Available treatments and their efficacy. *In*: Problems in Chemotherapy in Aquaculture: From Theory to Reality. Office International Des Épizooties, Conference Proceedings. 12-15 March, 1991, Paris, France. pp. 35-42.

Bian, B.Z. and S. Egusa. 1981. Histopathology of black gill disease caused by *Fusarium solani* (Martius) infection in the Kuruma prawn, *Penaeus japonicus* Bate. J. Fish. Dis. 4: 195-201.

Boonyaratpalin, S. 1990. Shrimp larval diseases. *In*: M.B. New, H. de Saram and T. Singh (Eds.). Technical and Economic Aspects of Shrimp Farming. Proceedings of the Aquatech '90 Conference, Kuala Lumpur, Malaysia, 11-14 June, 1990. pp. 158-163.

Chen, L. 1990. Aquaculture in Taiwan. Fishing News Books.

Chen, S.N., S. Chi, G.H. Kou and I.C. Liao. 1986. Cell culture from tissues of grass prawn, *Penaeus monodon*. Fish Pathol. 21: 161-166.

Code of Federal Regulations. 1991. Vol. 56(87): 20818.

Colorni, A. 1990. Penaeid pathology in Israel: problems and research. Advances in Tropical Aquaculture, Tahiti, Feb. 20-March 4, 1989, Aquacop. IFREMER, Actes de Colloque. 9:113-126.

Couch, J. 1978. Diseases, parasites, and toxic responses of commercial shrimps of the Gulf of Mexico and South Atlantic Coasts of North America. Fish. Bull. 76: 1-44.

Davy, B. 1991. Mariculture Research and Development in Japan. IDRC-MR298e.

Dore, I. and C. Frimodt. 1987. An Illustrated Guide to Shrimp of the World. Osprey Books, New York.

Doubrovsky, A., J.L. Paynter, S.K. Sambhi, J.G. Atherton and R.J.G. Lester. 1988. Observations on the ultrastructure of baculovirus in Australian *Penaeus monodon* and *Penaeus merguiensis*. Australian J. Marine and Freshwater Res. 39: 743-749.

Dunn, E., A. Polk, D. Scarrett, G. Oliver, S. Lall and M. Goosen. 1990. Vaccines in aquaculture: The search for an efficient delivery system. Aquacultural Engineering. 9: 23-32.

Fegan, D.F., T.W. Flegel, S. Sriurairatana and M. Waiyakruttha. 1991. The occurrence, development and histopathology of monodon baculovirus in *Penaeus monodon* in southern Thailand. Aquaculture. 96: 205-217.

Gacutan, R.Q., A. Llobrera, C. Santiago, P.J. Gutierrez and G.L. Po. 1977. A suctorean parasite of *Penaeus monodon* larvae. Quart. Res. Rep. Aquaculture Dept. SEAFDEC (no. 1) 6-11.

Giorgetti, G. 1990. Disease problems in farmed penaeids in Italy. *In*: Advances in Tropical Aquaculture. Tahiti, Feb. 20 - March 4, 1989. Aquacop, IFREMER. Actes de Colloque 9. pp. 75-87.

Itami, T. and Y. Takahashi. 1991. Survival of larval giant tiger prawns *Penaeus monodon* after addition of killed vibrio cells to a microencapsulated diet. J. Aquatic Animal Health. 3: 151-152.

Johnson, S.K. 1976. Chemical control of peritrichous ciliates on young penaeid shrimp. Texas A&M University, Extension Fish Disease Diagnostic Laboratory, No. FDDL-S7, 4 p.

Johnson, S.K. 1989. Handbook of Shrimp Diseases. Texas A&M Sea Grant College Program, Texas A&M University, College Station, TX. 25 p.

Kalagayan, H., D. Godin, R. Kanna, G. Hagino, J. Sweeney, J. Wyban and J. A. Brock. 1991. IHHN virus as an etiological factor in runt deformity syndrome of juvenile *Penaeus vannamei* cultured in Hawaii. J. World Aquacult. Soc. 22(4): 235-243.

Kou, G.H, Y.S. Lin, H.C. Chen and C.F. Lo. 1984. Diseases and mortalities of cultured marine fish and shellfish in Taiwan. *In*: I.C. Liao and R. Hirano (Eds.). Proceedings of ROC-Japan Symposium on Mariculture; 1981 December 14-15; Taipei, Taiwan. Pingtung, Taiwan. Tungkang Marine Laboratory; pp. 173-192.

Krol, R.M., W.E. Hawkins, W. Vogelbein and R.M. Overstreet. 1989. Histopathology and ultrastructure of the hemocytic response to an acid-fact bacterial infection in cultured *Penaeus vannamei*. J. Aquatic Animal Health. 1: 37-42.

Krol, R.M., W.E. Hawkins and R.M. Overstreet. 1990. Reo-like virus in white shrimp *Penaeus vannamei* (Crustacea: Decapoda): co-occurrence with *Baculovirus penaei* in experimental infections. Dis. Aquat. Org. 8: 45-49.

Krol, R.M., W.E. Hawkins and R.M. Overstreet. 1991. Rickettsial and mollicute infections in hepatopancreatic cells of cultured Pacific white shrimp (*Penaeus vannamei*). J. Invertebr. Pathol. 57: 362-370.

LeBlanc, B. and R.M. Overstreet. 1991a. Effect of desiccation, pH, heat and ultraviolet irradiation on viability of *Baculovirus penaei*. J. Invertebr. Pathol. 57: 277-286.

LeBlanc, B. and R.M. Overstreet. 1991b. Efficacy of calcium hypochlorite as a disinfectant against the shrimp virus *Baculovirus penaei*. J. Aquatic Animal Health. 3:141-145.

Lewis, D. 1986. An enzyme-linked immunosorbent assay (ELISA) for detecting penaeid baculovirus. J. Fish. Dis. 9: 519-522.

Liao, I.C. 1984. Status and problems of grass prawn culture in Taiwan. *In*: I.C. Liao and R. Hirano (Eds.). Proceedings of ROC-Japan Symposium on Mariculture; 1981 December 14-15; Taipei, Taiwan. Pingtung, Taiwan. Tungkang Marine Laboratory; pp. 81-98.

Liao, I.C. 1989. *Penaeus monodon* culture in Taiwan: Through two decades of growth. Int. J. Aq. Fish. Technol. 1: 16-24.

Liao, I.C., F. Yang and S. Lou. 1977. Preliminary report on some diseases of cultured prawns and their control methods. JCRR Fish. Ser. 29: 28-33 (Reports on Fish Disease Research, I). Translated from Chinese to English.

Liao, I.C. and N. Chao. 1983. Hatchery and grow-out: penaeid prawns. *In*: J. McVey (Ed.) CRC Handbook of Mariculture. Volume 1. Crustacean Aquaculture. CRC Press, Inc., Boca Raton, FL. pp. 161-167.

Liao, I.C. and Y.H. Chien. 1990. Evaluation and comparison of culture practices for *Penaeus japonicus*, *P. penicillatus* and *P. chinensis* in Taiwan. *In*: K.L. Main and W. Fulks (Eds). The Culture of Cold-Tolerant Shrimp: Proceedings of an Asian-U.S. Workshop on Shrimp Culture. The Oceanic Institute. Honolulu, HI. pp. 49-63.

Lightner, D.V. 1983. Diseases of cultured penaeid shrimp. *In*: J. McVey (Ed.) CRC Handbook of Mariculture. Volume 1. Crustacean Aquaculture. CRC Press, Inc., Boca Raton, FL. pp. 289-320.

Lightner, D.V. 1985. A review of the diseases of cultured penaeid shrimps and prawns with emphasis on recent discoveries and developments. *In*: Y. Taki, J.H. Primavera and J.A. Llobrera (Eds.). Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps, Iloilo City, Philippines. pp. 79-103.

Lightner, D.V. 1988. Diseases in cultured penaeid shrimps and prawns. *In*: C.J. Sindermann and D.V. Lightner (Eds.) Disease Diagnosis and Control in North American Marine Aquaculture. Elsevier, Amsterdam. pp. 11-37.

Lightner, D.V. and R.M. Redman. 1981. A baculovirus caused disease of the penaeid shrimp, *Penaeus monodon*. J. Invertebr. Pathol. 38: 299-302.

Lightner, D.V. and R.M. Redman. 1982. Histopathology of aflatoxicosis in the marine shrimp *Penaeus stylirostris* and *P. vannamei*. J. Invertebr. Pathol. 40: 279-291.

Lightner, D.V., R.M. Redman and T.A. Bell. 1983a. Infectious hypodermal and hematopoietic necrosis (IHHN) a newly recognized virus disease of penaeid shrimp. J. Invertebr. Pathol. 42: 62-70.

Lightner, D.V., R.M. Redman, T.A. Bell and J.A. Brock. 1983b. Detection of IHHN virus in *Penaeus stylirostris* and *P. vannamei* imported into Hawaii. J. World Maric. Soc. 14: 212-225.

Lightner, D.V., R.M. Redman, D.A. Danald, R.R. Williams and L. Perez. 1984. Major diseases encountered in controlled environment culture of penaeid shrimp at Puerto Peñasco, Sonora, Mexico. *In*: C.J. Sindermann (ed.). Proc. Ninth and Tenth US/Japan Meetings on Aquaculture. NOAA Tech. Rep. NMFS, 16: 25-33.

Lightner, D.V. and R.M. Redman. 1985. A parvo-like virus disease of penaeid shrimp. J. Invertebr. Pathol. 45:47-53.

Lightner, D.V., R.M. Redman, R.R. Williams, L.L. Mohney, J.P.M. Clerx, T.A. Bell and J. A. Brock. 1985. Recent advances in penaeid virus disease investigations. J. World Maric. Soc. 16: 267-274.

Lightner, D.V., T.A. Bell and R.M. Redman. 1990. A review of the known hosts, geographical range and current diagnostic procedures for the virus diseases of cultured penaeid shrimp. Advances in Tropical Aquaculture, Tahiti, Feb. 20-March 4, 1989, Aquacop. IFREMER, Actes de Colloque. 9: 113-126.

Lightner, D.V. and R.M. Redman. 1991. Hosts, geographic range and diagnostic procedures for the penaeid virus diseases of concern to shrimp culturists in the Americas. *In*: P. DeLoach, W.J. Dougherty and M.A. Davidson (Eds.) Frontiers in Shrimp Research. Elsevier, Amsterdam. pp. 173-196.

Lio-Po, G.D. and C.L. Pitogo. 1990. Bacterial exoskeletal lesions of the tiger prawn *Penaeus monodon*. *In*: R. Hirano and I. Hanyu (Eds). The Second Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines. 991 p.

Lin, C.K. 1989. Prawn culture in Taiwan. What went wrong? World Aquaculture. 20(2): 19-20.

Liu, K.C. 1989. Histopathological study of the bacterial induced hepatopancreatitis of cultured shrimp (*Penaeus monodon* Fabricius). Fish Disease Research. 9: 34-41.

Liu, R. 1990. Present status and future prospects for shrimp mariculture in China. *In*: K. Main and W. Fulks (Eds). The Culture of Cold-Tolerant Shrimp: Proceedings of an Asian–U.S. Workshop on Shrimp Culture. The Oceanic Institute. Honolulu, HI. pp. 16-28.

Liu, T., G. Su and W. Huang. 1989. Pathogenic and biological research on the cause of "red leg disease" in *Penaeus penicillatus*. Fujian Fisheries. 2: 1-3.

Main, K.L. and W. Fulks (Eds.). 1990. The Culture of Cold-Tolerant Shrimp: Proceedings of an Asian–U.S. Workshop on Shrimp Culture. The Oceanic Institute, Honolulu, Hawaii. 215 p.

Meng, Q. and K. Yu. 1982. On the black gill disease of *Penaeus orientalis*. J. Shandong Coll. Oceanogr. 12(4): 95-100. (In Chinese with English abstract).

Meyer, F. 1991. Aquaculture disease and health management. J. Anim. Sci. 69: 4201-4208.

Momoyama, K. 1987. Distributions of the hyphae in kuruma shrimp, *Penaeus japonicus*, infected with *Fusarium solani*. Fish Pathol. 22(1): 15-23.

Momoyama, K. 1988. Infection source of baculoviral mid-gut gland necrosis in mass production of kuruma shrimp larvae, *Penaeus japonicus*. Fish Pathol. 23: 105-110. (In Japanese with English abstract).

Momoyama, K. and T. Sano. 1989. Developmental stages of kuruma shrimp, *Penaeus japonicus* Bate, susceptible to baculoviral mid-gut gland necrosis (BMN) virus. J. Fish. Dis. 12: 585-589.

Nash, G., I.G. Anderson and M. Shariff. 1988. Pathological changes in the tiger prawn, *Penaeus monodon* Fabricius, associated with culture in brackishwater ponds developed from potentially acid sulphate soils. J. Fish. Dis. 11: 113-123.

Overstreet, R.M., K. Stuck, R.M. Krol and W. Hawkins. 1988. Experimental infections with *Baculovirus penaei* in the white shrimp *Penaeus vannamei* (Crustacea: Decapoda) as a bioassay. J. World Aquacult. Soc. 19: 175-187.

Owens, L., S. De Beer and J. Smith. 1991. Lymphoidal parvovirus-like particles in Australian penaeid prawns. Dis. Aquat. Org. 11: 129-134.

PCARRD. 1985. State of the Art and Abstract Bibliography of Shrimp and Prawn Researches. Los Banos, Laguna: Philippine Council for Agriculture and Resources Research Development and National Science and Technology Authority. 102 p.

Provenzano, A. 1983. Pathobiology. The Biology of Crustacea. Vol. 6. Academic Press, New York. 290 p.

Rhoobunjongde, W., K. Hatai, S. Wada and S. Kubota. 1991. *Fusarium moniliforme* (Sheldon) isolated from gills of kuruma prawn *Penaeus japonicus* (Bate) with black gill disease. Nippon Suisan Gakkaishi. 57(4): 629-635.

Rosenberry, B. 1991. World Shrimp Farming 1991. San Diego, CA. 45 pp.

Roubal, F.R., J.L. Paynter and R.J.G. Lester. 1989. Electron microscopic observation of hepatopancreatic parvo-like virus (HPV) in the penaeid prawn, *Penaeus merguiensis* de Man, from Australia. J. Fish. Dis. 12: 199-201.

Ruangpan, L. 1978. Diseases and parasites of *Penaeus monodon* Fabricius. Presented at the First Workshop on Tropical Fish Diseases, 28 November - 1 December 1978, Bogor, Indonesia.

Ruangpan, L. 1982. Diseases and parasites of *Penaeus monodon* Fabricius. Thai. Fish. Gaz. 35: 385-387.

Ruangpan, L. 1986. Pathogenic protozoa in shrimp hatcheries. *In*: 24th Kasetsart University Conference, Bangkok, Thailand, 27-29 January 1986. pp. 235-245. (In Thai with English abstract).

Sano, T, T. Nishimura, K. Oguma, K. Momoyama, and N. Takeno. 1981. Baculovirus infection of cultured Kuruma shrimp *Penaeus japonicus* in Japan. Fish Pathol. 15: 185-191.

Sano, T., T. Nishimura, H. Fukuda and K. Momoyama. 1983. Baculoviral mid-gut gland necrosis of cultured kuruma shrimp larvae *Penaeus japonicus* in Japan. *In*: G.H. Kou and S. Egusa (Eds.). Proceeding of Republic of China-Japan Cooperative Science Seminar on Fish Diseases. National Science Council, Taipei, Taiwan, R.O.C.

Sano, T., T. Nishimura, H. Fukuda, T. Hayashida and K. Momoyama. 1984. Baculoviral mid-gut gland necrosis (BMN) of kuruma shrimp (*Penaeus japonicus*) larvae in Japanese intensive culture systems. Helgoländer Meeresunters. 37: 255-264.

Sano, T., T. Nishimura, H. Fukuda and T. Hayashida. 1985. Baculoviral infectivity tri-

als on kuruma shrimp larvae, *Penaeus japonicus*, of different ages. *In*: A.E. Ellis (Ed.). Fish and Shellfish Pathology. Academic Press, London. pp. 397-403.

Sano, T. and H. Fukuda. 1987. Principal microbial diseases of mariculture in Japan. Aquaculture. 67: 59-69.

Shigueno, K. 1975. Shrimp Culture in Japan. Association for International Technical Promotion. Tokyo, Japan. 153 p.

Sindermann, C.J. 1986. The role of pathology in aquaculture. *In*: M. Bilio, H. Rosenthal, C.J. Sindermann (Eds.). Realism in Aquaculture: Achievements, Constraints, Perspectives. European Aquaculture Society, Bredene. pp. 395-419.

Sindermann, C.J. 1990. Principal Diseases of Marine Fish and Shellfish. Vol. 2. 2nd edition. Academic Press, San Diego. 516 p.

Sindermann, C.J. and Lightner, D.V. (Eds.). 1988. Disease Diagnosis and Control in North American Marine Aquaculture. Elsevier, Amsterdam. 431 p.

Takahashi, Y., Y. Shimoyama and K. Momoyama. 1985. Pathogenicity and characteristics of *Vibrio* sp. isolated from cultured Kuruma prawn *Penaeus japonicus* Bate. Bull. Jap. Soc. Sci. Fish. 51(5): 721-730.

Thurman, R., T.A. Bell, D.V. Lightner and S. Hazanow. 1990. Unique physicochemical properties of the occluded penaeid shrimp baculoviruses and their use in diagnosis of infections. J. Aquatic Animal Health. 2: 128-131.

Tsing, A., D.V. Lightner, J.R. Bonami and R.M. Redman. 1985. Is "gut and nerve syndrome" (GNS) of viral origin in the tiger shrimp *Penaeus japonicus* Bate? *In*: Programme and Abstracts of the 2nd International Conference of the European Association of Fish Pathologists. Montpellier, France. 91 p.

Tsing, A. and J.R. Bonami. 1987. A new viral disease of the shrimp, *Penaeus japonicus* Bate. J. Fish. Dis. 10: 139-141.

Wang, K. and S. Ma. 1990. Advances in larval rearing techniques for *Penaeus chinensis* in China. *In*: K. Main and W. Fulks (Eds). The Culture of Cold-Tolerant Shrimp: Proceedings of an Asian–U.S. Workshop on Shrimp Culture. The Oceanic Institute. Honolulu, HI. pp. 16-28.

Wu, D.H., H.W. Zeng, X. Hong and H. Huang, 1991. Investigation on diseases of cultured penaeid shrimp in southern part of Fujian province. Xiamen Aquatic Science and Technology. 2: 1-10. (In Chinese).

Wyban, J. and J. Sweeny. 1991. Intensive Shrimp Production Technology: The Oceanic Institute Shrimp Manual. The Oceanic Institute, Honolulu, Hawaii. 158 p.

Part II:

Contributed Papers -

Country Situations

Major Diseases of Cultured Shrimp in Asia: An Overview

M. Shariff and R.P. Subasinghe
Faculty of Fisheries and Marine Science
Universiti Pertanian Malaysia
43400 Serdang, Selangor, Malaysia

Abstract

Shrimp aquaculture is a major industry in a number of countries in Asia, where 89% of the global output was produced in 1989. However, diseases and health management have been identified as major constraints to the sustainable development of the industry. Viruses, bacteria, fungi and protozoa are all common pathogens causing morbidity and mortality in cultured Asian shrimp. Besides the infectious diseases, which involve pathogens, a number of noninfectious diseases that stem from poor nutrition, poor water quality and environmental pollution are also important. This paper reviews the current knowledge of the major diseases of cultured shrimp in Asia with respect to their importance to the Asian shrimp culture industry.

Introduction

Although the capital expenditure may be US$ 10,000/ha or more to enter the industry, the short culture period of less than 120 days, which allows production of 2.5 crops/year, and the farm-gate profit margin of US$ 2.00 - 5.00/kg, have made the shrimp aquaculture industry extremely attractive to entrepreneurs. This is still true, even though shrimp prices have recently begun to decline (Chong, 1990). According to Rosenberry (1990), the global output from shrimp aquaculture grew rapidly, from 300,000 MT in the early 1980s to 565,000 MT in 1989; 89% of this total comes from Asia.

In spite of the disaster in Taiwan, when its share of Asian farmed shrimp production dropped from 21% in 1987 to 4% in 1989, shrimp production in Asia increased from 200,000 MT in 1985 to over 500,000 MT in 1989 (Csavas, 1990). However, the episode in Taiwan will be remembered by all shrimp-producing countries because disease and health management were among the most significant causes of this painful experience (Liao, 1990). This paper reviews existing knowledge on the major diseases of cultured shrimp in Asia.

Common Pathogens

The important, but common, pathogens that cause significant morbidity

and mortality among cultured Asian shrimp include viruses, bacteria, fungi and protozoa. Although it has been said that viruses and bacteria are, by far, the most important causative agents, emerging trends and recent literature show that mycosis and parasitosis also play significant roles in mortality and morbidity of both larval and growout shrimp (Boonyaratpalin, 1990; Flegel et al., 1992).

Viruses

Baculoviruses

Six diseases of baculoviral etiology have been described in Asia. Rod-shaped, enveloped, DNA viruses approximately 70 nm by 300 nm in size are the members of the *Penaeus monodon* baculovirus group. This group consists of *Penaeus monodon* baculovirus (MBV), *Penaeus monodon* baculovirus-type (MBV-type), baculovirus midgut gland necrosis virus (BMNV) and baculovirus midgut gland necrosis-type virus (BMNV-type). MBV is infectious to a wide range of cultured Asian penaeid species, including *Penaeus esculentus, P. kerathurus, P. merguiensis, P. monodon, P. penicillatus* and *P. semisulcatus* (Lightner and Redman, 1991). Plebejus baculovirus (PBV), a monodon-type baculovirus, has been reported from *P. plebejus* in Australia by Paynter et al. (1985). Although BMNV is reported to cause serious epizootics among hatchery-reared *P. japonicus* in southern Japan (Sano and Fukuda, 1987), this virus has not been reported in *P. japonicus* cultured outside Japan (Lightner and Redman, 1991). Kuruma shrimp, *P. japonicus*, have been successfully infected with BMNV by Momoyama and Sano (1988) in Japan. However, BMN-type agents have been reported from Australia, Indonesia, Japan and the Philippines.

MBV-type baculoviruses appear to be extensively distributed and have become established in cultured shrimp populations in almost every country in Asia. Recent surveys by Dana and Sukenda (1990), M.D. Hassan (unpublished data) and Natividad and Lightner (1992) suggest that MBV-type baculoviruses are widely spread among shrimp culture enterprises in Indonesia, Malaysia and the Philippines, respectively.

Shrimp baculoviruses cause high mortalities in cultured penaeids. BMNV generally infects larvae and early postlarvae, while MBV-type baculoviruses cause mortalities in late postlarvae and juvenile shrimp (Brock and Lightner, 1990). Baculoviruses infect hepatopancreatic and midgut epithelial cells, which are of endothelial origin, and replicate within the nuclei. MBV and MBV-type viruses form single or multiple spherical inclusion bodies, causing hypertrophied nuclei, but BMNV and BMNV-type viruses are nonoccluded. Infected cells undergo necrosis and slough into the gut lumen. Free and occluded viruses are shed in the feces (Chen et al., 1989) and during spawning (Sano et al., 1985), causing transmission through ingestion (Over-

street et al., 1988). The unregulated transfer of subclinically infected wild-caught spawners and larvae appears to be the primary mode of entry of shrimp baculoviruses to hatcheries and culture facilities (Brock, 1991). A recent transmission experiment conducted by Paynter et al. (1992) showed that exposure of one day-old postlarvae to postlarval homogenates with MBV-type virus resulted in development of inclusion bodies after two days. The number of inclusion bodies reached a peak by eight days, a second peak in 16 days and disappeared in 23 days.

The importance of MBV to Asian shrimp culture has been well documented. It has caused heavy mortalities of all stages of *P. monodon* in the Philippines (Baticados, 1988; Natividad and Lightner, 1992) and is considered partially responsible for the collapse of the shrimp culture industry in Taiwan in late 1980s (Lin, 1989). The prevalence of MBV among juvenile shrimp in Malaysia was about 30% in the late 1980s (Anderson, 1988), but it is now almost 100% (M.D. Hassan, pers. comm.). The disease is more pronounced under intensive and semi-intensive culture practices, where heavily stocked shrimp live under environmental, nutritional, behavioral and other stressors. The occurrence of MBV has little or no consequence to shrimp grown under extensive conditions (Nash et al., 1988b). The diagnosis, control and management of baculoviral infections in Asian shrimp culture are well documented by Brock (1991).

Parvo-like Viruses

These isometric DNA viruses, including hepatopancreatic parvo-like virus (HPV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV), are the known parvo-like viruses of cultured Asian marine shrimp. HPV is 22 - 24 nm in diameter and occurs within intranuclear inclusion bodies in hepatopancreatic and epigastric caecal epithelium (Brock, 1991). The geographical distribution of HPV is similar to that of MBV, and HPV is found in *P. esculentus, P. indicus, P. chinensis, P. merguiensis, P. monodon, P. penicillatus, P. semisulcatus* and *P. vannamei* (Lightner and Redman, 1991). The main route of entry of HPV into shrimp farms is believed to be via postlarvae. Although HPV has been documented from nine species of penaeids, its significance in causing epizootics and economic losses is not fully understood.

IHHNV is 20 - 22 nm in diameter and considered highly contagious and infectious to many penaeid species (Brock, 1991). IHHNV has been recognized from *P. japonicus, P. chinensis, P. monodon* and *P. semisulcatus* cultured in Malaysia, Philippines, Singapore, Sri Lanka and Taiwan (Lightner and Redman, 1991). Anderson (1988) observed a few foci of IHHNV-like nuclear inclusions in two *P. monodon* juveniles from a farm in Sabah in Malaysia. However, the etiological and economic significance of IHHNV in Asia remains unclear. The diagnosis, control and management of parvo-like viruses are described by Brock (1991).

Reo-like Viruses

Reo-like viruses (REO) of penaeid shrimp are nonenveloped, icosahedral cytoplasmic agents with an average size of 70 nm. Within Asia, REO have only been recorded from *P. monodon* in Malaysia in conjunction with MBV, a rickettsia and Gram-negative bacteria (Anderson et al., 1987). However, their status as pathogens of penaeid shrimp remains to be established. Brock (1991) mentioned that the establishment of rapid, sensitive diagnostic methods for REO and determination of the distribution of REO in cultured shrimp stocks is important in understanding more about the control and management of these pathogens.

Bacteria

Perhaps as a consequence of the large number of *Vibrio* species in the normal shrimp microflora, opportunistic *Vibrio* spp. are the most common bacterial pathogens of cultured shrimp. *Vibrio* spp. appear to establish lethal infections following primary infections with other pathogens, environmental stress, nutritional imbalance and/or predisposing lesions (Lightner, 1988). According to Lightner et al. (1992), some more recently occurring disease syndromes of penaeid shrimp are caused by *Vibrio* spp. that behave more like true pathogens than opportunistic invaders. Involvement of other Gram-negative bacteria and *Cytophaga*-type filamentous bacteria in serious disease outbreaks of cultured shrimp in Asia has rarely been documented.

Luminescent Vibriosis

Significant larval mortalities associated with luminescent vibriosis, caused by *Vibrio harveyi* and *V. splendidus*, were reported from *P. monodon* and *P. merguiensis* hatcheries in Indonesia (Sunaryanto and Mariam, 1986), the Philippines (Baticados et al., 1991) and in Thailand (Tansutapanit and Ruangpan, 1987). Lavilla-Pitogo et al. (1992) found that the main source of luminescent bacteria in *P. monodon* is the midgut contents of the mother, which are shed into the water almost simultaneously with the eggs during spawning. Lavilla-Pitogo et al. (1990) reported that near-shore sea water could be a major source of *V. harveyi* and *V. splendidus* for shrimp hatcheries in the Philippines. Baticados et al. (1991) tested 24 antibacterials against *V. harveyi* and *V. splendidus* in *P. monodon* larvae and showed that only chloramphenicol, sodium nifurstyrenate and the nitrofurans cause reasonable growth inhibition of the bacteria. Based on their results, they concluded that the most reliable and effective way of controlling luminous vibriosis in penaeid shrimp hatcheries is through rigorous water renewal and by excluding luminous vibrios from the culture water.

Miscellaneous Vibriosis and Other Bacteria

Takahashi et al. (1985a) and Egusa et al. (1988) reported serious *Vibrio* sp. epizootics in postlarval and juvenile *P. japonicus* in Japan. This condition, which

existed since 1981, is characterized by cloudiness of the hepatopancreas in postlarvae, and cloudiness of muscle and brown spots in the gills and lymphoid organ in juveniles (Takahashi et al., 1984; 1985a). The serious epizootic in *P. japonicus* has been controlled with oxytetracycline-medicated feed at 50 - 100 mg/kg body weight/day for four to six days (Takahashi et al., 1985b). *In vitro* vaccination of juvenile *P. japonicus* with Formalin-killed *Vibrio* sp. has been shown to provide some protection against subsequent challenge by live *Vibrio* sp. (Itami et al., 1989).

In Malaysia, Anderson et al. (1988) experienced heavy mortalities in almost market-size (25 - 33 g) *P. monodon* associated with multifocal necrosis, hemocytic inflammation and nodule formation in the lymphoid organ, heart, gills, hepatopancreas, antennal gland, cuticular epidermis and subcutis, and in other connective tissues. Some hemocyte nodules contained Gram-negative bacteria within the granulomas, or within intracytoplasmic vacuoles. From the hemolymph of such shrimp, *V. alginolyticus, V. parahaemolyticus* and *Pseudomonas* sp. were isolated. According to Lightner et al. (1992), this condition could be the same or related to "red disease" in *Penaeus monodon*. Anderson et al. (1988) recommended decreasing shrimp density by partial harvesting and increasing water exchange toward the end of the production cycle to prevent mortalities. They further suggested that proper draining and drying of ponds and application of CaO at 0.5 kg/m^2 to the pond bottom could be effective in controlling the condition.

Anderson (1988), in addition to *Vibrio* spp., isolated *Pseudomonas* sp., *Moraxella* sp. and *Alcaligenese* sp. from the hemolymph of affected shrimp in Malaysia and considered them secondary invaders.

Filamentous Bacteria

Leucothrix mucor and similar filamentous, bacterial, ectocommensal fouling organisms have been reported to cause mortalities in all stages of shrimp under poor water conditions. Baticados (1988) mentioned that *L. mucor* in larval shrimp in the Philippines has been successfully controlled by vigilant water management, while postlarvae and adults have been effectively treated with Cutrine®-Plus, a copper compound, at 0.1 mg Cu/L for 24 h or 0.25 - 0.5 mg Cu/L for 4 - 8 h. Anderson (1988) found that *L. mucor* is a common secondary invader in *Penaeus monodon* postlarvae affected by MBV in Malaysian shrimp hatcheries, causing 100% mortality overnight. He suggested that early diagnosis is essential to minimize losses.

Rickettsias and Chlamydias

The true taxonomic position of these small, coccoid to rod-shaped, intracellular Gram-negative microorganisms is not fully understood. The presence of these organisms in diseased Asian penaeid shrimps has only been reported from Singapore (Chong and Loh, 1984) and Malaysia (Anderson et al., 1987).

Although switching from *P. monodon* to *P. merguiensis* culture has been suggested to control rickettsial infections (Anderson et al., 1987), detailed knowledge on the distribution, pathogenicity and the diagnosis of these organisms in shrimp has yet to be ascertained.

Fungi

Larval mycosis, caused by *Lagenidium* sp., *Sirolpidium* sp. and *Haliphthoros* sp., and subadult and adult mycosis caused by *Fusarium* sp., are the two major fungal diseases of cultured shrimp reported in Asia.

Larval Mycosis

Boonyaratpalin (1990) stated that larval mycosis commonly occurs in zoea and mysis stages, causing mortalities up to 100% within two days. The affected larvae show extensive, nonseptate, highly branched fungal mycelia throughout the body and appendages. In heavy infections, the larval tissues turn pale and yellowish-green. The fungi that cause this condition are *Lagenidium* sp. and *Sirolpidium* sp. Anderson (1988) noted that larval mycosis causes 100% mortality of larvae within 24 h in Malaysia. He also noted that larval mycosis is generally followed by bacterial necrosis, and that there is a close association between antibiotic use and larval mycosis. Antibiotics remove bacterial epibiont competitors, producing an increase in fungal pathogenicity.

Larval mycosis involving *Lagenidium callinectes*, *L.* sp., *Haliphthoros philippinensis* and *Sirolpidium* sp. has been reported from larval shrimp in the Philippines by Baticados (1988). Baticados (1988) recommended using Treflan®, a 5-ppm bath for 1 h for spawners before spawning, and/or treatment of eggs (before stocking in hatchery tanks) with 20-ppm tide detergent for 2 h. Boonyaratpalin (1990) recommends daily prophylactic treatment of penaeid larvae with Treflan® at 0.01 - 0.05 ppm. A number of other fungicides have also been tested against *Lagenidium* sp. and *H. philippinensis* (Lio-Po and Sanvictores, 1986). Although the losses due to larval mycosis seem to be substantial, data on economic losses in Asian shrimp culture are not available (Brock, 1991).

Fusarium Disease

The causative agent of this disease, *Fusarium solani*, has been reported from *P. japonicus* in Japan (Ishikawa, 1968; Egusa and Ueda, 1972) and *P. monodon* in the Philippines (Baticados, 1988). *Fusarium solani* affects all stages of shrimp, causing locomotory difficulties as a result of its mycelial growth. The fungus also causes blackening and destruction of gill tissue (hence, the name "black gill disease"), resulting in heavy mortalities (Bian and Egusa, 1981; Baticados, 1988). Chemotherapy and chemoprophylaxis of Fusarium disease is not well documented and needs further research.

Protozoa

Protozoan parasites, both facultative and obligate, have been frequently reported to cause significant economic

losses in Asian shrimp culture. Two major groups, peritrichus ciliates and other epibionts that colonize cuticular surfaces, and systemic Microsporidia have been recognized.

Peritrichus Ciliates and Other Epibionts

All species and life stages of penaeids are susceptible to epibiotic fouling by one or more protozoa. They include *Zoothamnium* sp., *Epistylis* sp., *Carchesium* sp., *Vorticella* sp. and, less commonly, *Acineta* sp. and *Euphelota* sp. (Anderson, 1988; Boonyaratpalin, 1990; Nash, 1990; Brock, 1991). They are found on unhatched *Artemia* cysts, and in tank and pond-bottom sediments. The presence of protozoan epibionts on cuticular surfaces of various stages of penaeid shrimp, in small numbers, is a common phenomenon in shrimp hatcheries and growout facilities. However, an abundance of these organisms indicates heavy bacterial loading, organic pollution, nutritional and/or environmental stress in shrimp, and inadequate hygiene. Under such conditions, heavy infections could cause difficulty in molting and hypoxia in larval shrimp leading to death. Formalin, 15 - 20 ppm for 30 min to 1 h, prevents infection without adverse effects (Boonyaratpalin, 1990).

Systemic Microsporidia

Microsporidian infections in Asian penaeid shrimps have been reported from Malaysia (Anderson, 1988; Anderson et al., 1989), the Philippines (Baticados and Enriques, 1982 [cited by Anderson et al., 1989]) and from Thailand (Donyadol et al. [cited by Flegel et al., 1992]). In the Philippines and Thailand, microsporidians with eight capsules were found in adult *P. merguiensis* and were identified as *Agamasoma* (= *Thelohania*) sp. Anderson et al. (1989) reported a microsporidian belonging to the genus *Ameson* infecting the hepatopancreas of pond-reared adult *P. monodon*. More recently, Flegel et al. (1992) identified a microsporidian (*Agamasoma penaei*) from pond-reared *P. monodon* in Thailand that caused mortalities up to 24%. The artificial infection of *P. monodon* by feeding and injection of tissue homogenates with spores were carried out with limited success (Flegel et al., 1992). Future research on the mode of infection and transmission of microsporidians is recommended.

Noninfectious Diseases

A few noninfectious diseases and conditions of environmental and nutritional origin have been documented in Asian shrimp culture. The following section briefly outlines the most important of those.

Chronic Soft-shell Syndrome

This has been repeatedly found in *P. monodon* in the Philippines (Baticados et al., 1986; Baticados et. al., 1987). The syndrome, characterized by a persistently soft exoskeleton for several weeks, results from a nutritional deficiency, exposure to chemical pesticides,

poor pond soil and water conditions, and inadequate management practices (Baticados et al., 1987). Histopathological and histochemical studies revealed that the multi-layered exoskeleton of soft-shelled shrimp is significantly thinner than that of hard-shelled shrimp. Furthermore, the calcium content of the hepatopancreas of soft-shelled shrimp is lower than that of normal shrimp (Baticados et al., 1987). Baticados (1988) and Bautista and Baticados (1989) mentioned that the syndrome can be controlled by adequate nutrition and by maintaining good water and soil quality.

Blue Disease

This has been reported from Malaysia (M. Shariff, pers. comm.), the Philippines (Baticados, 1988) and Thailand (Nash, 1990) and is characterized by bluish discoloration of the exoskeleton. Affected shrimp often become soft, thin and lethargic and eventually die. The etiology of the condition is believed to be nutritional (inadequate astaxanthin) and reduced stocking density, improved diet quality and frequent water exchange have been recommended for controlling it.

Pathological Conditions Related to Acid-sulfate Soils

Brown discoloration of the gills, soft shells and decreased survival were among the problems affecting *P. monodon* adults cultured in ponds with potentially acid-sulfate soils in Malaysia. Histological and ultrastructural study revealed that lamella ferric hydroxide accumulation and associated gill changes, which led to hypoxic damage in other tissues, were probably responsible for many of the clinical abnormalities observed (Nash et al., 1988a).

Other Diseases of Relative Importance

Red disease of *P. monodon* caused by aflatoxin has been detected in the Philippines by Baticados (1988) and de la Cruz (1989), and gas bubble disease and cramped tail syndrome in *P. monodon* in the Philippines, which are of uncertain etiologies, have also been reported. Detailed information on these diseases is limited, and their economic impact on Asian shrimp culture is not understood.

Literature Cited

Anderson, I.G. 1988. Disease problems faced by marine prawn farms and hatcheries. *In*: T. Singh and K.J. Ang (Eds.). Marine Prawn Farming in Malaysia — Present Problems, Future Prospects. Malaysian Fisheries Society Occasional Publication No. 1. pp. 67-83.

Anderson, I.G., M.N. Shamsudin, M. Shariff and G. Nash. 1988. Bacterial septicemia in juvenile tiger shrimp, *Penaeus monodon*, cultured in Malaysian brackishwater ponds. Asian Fisheries Science. 2: 93-108.

Anderson, I.G., M. Shariff, G. Nash and M. Nash. 1987. Mortalities of juvenile shrimp, *Penaeus monodon*, associated with *Penaeus monodon* baculovirus, cytoplasmic reo-like virus, and rickettsial and bacterial infections, from Malaysian brackishwater ponds. Asian Fisheries Science. 1: 47-64.

Anderson, I.G., M. Shariff and G. Nash. 1989. A hepatopancreatic microsporidian in pond-reared tiger shrimp, *Penaeus monodon*, from Malaysia. J. Invertebr. Pathol. 53: 278-280.

Baticados, M.C.L. 1988. Diseases of prawns in the Philippines. SEAFDEC Asian Aquaculture. 10: 1-8.

Baticados, M.C.L., R.M. Coloso and R.C. Duremdez. 1986. Studies on the chronic

soft-shell syndrome in the tiger prawn, *Penaeus monodon* Fabricius, from brackishwater ponds. Aquaculture. 56: 271-285.

Baticados, M.C.L., R.M. Coloso and R.C. Duremdez. 1987. Histopathology of the chronic soft-shell syndrome in the tiger prawn, *Penaeus monodon*. Dis. Aquat. Org. 3: 13-28.

Baticados, M.C.L., C.R. Lavilla-Pitogo, E.R. Cruz-Lacierda, L.D. de la Peña and N.A. Suñaz. 1991. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. Dis. Aquat. Org. 9: 133-139.

Bautista, M.N. and M.C.L. Baticados. 1989. Dietary control of soft-shelling in tiger prawn. SEAFDEC Asian Aquaculture. 11: 1-2.

Bian, B.Z. and S. Egusa. 1981. Histopathology of black gill disease caused by *Fusarium solani* (Martius) infection in the Kuruma prawn, *Penaeus japonicus* Bate. J. Fish Dis. 4: 195-201.

Boonyaratpalin, S. 1990. Shrimp aquaculture development in Asia. *In*: M.B. New, H. de Saram and Tharlochan Singh (Eds.). Technical and Economic Aspects of Shrimp Farming. Proceedings of the AQUATECH Conference '90 Kuala Lumpur, Malaysia, 11-14 June, 1990. pp. 158-163

Brock, J.A. 1991. An overview of diseases of cultured crustaceans in the Asia Pacific region. *In*: ADB/NACA, 1991. Fish Health Management in Asia-Pacific. Report on a Regional Study and Workshop on Fish Disease and Fish Health Management. pp. 347-395. ADB Aquaculture Department Report Series No. 1. Network of Aquaculture Centres in Asia-Pacific. Bangkok, Thailand.

Brock, J.A. and D.V. Lightner. 1990. Microbial pathogens and diseases of marine crustaceans. *In*: O. Kinne (Ed.). Diseases of Marine Animals, Vol. 3. John Wiley & Sons, N.Y. pp. 245-349.

Chen, S.N., P.S. Chang and G.H. Kou. 1989. Observations on pathogenicity and epizootiology of *Penaeus monodon* baculovirus (MBV) in cultured shrimp in Taiwan. Fish Pathol. 24: 189-195.

Chong, K.C. 1990. Asian shrimp aquaculture at cross roads. INFOFISH International. 5: 40-47.

Chong, Y.C. and H. Loh. 1984. Hepatopancreas chlamydial and parvoviral infections of farmed marine prawns in Singapore. Singapore Veterinary. 9: 51-56.

Csavas, I. 1990. Shrimp aquaculture development in Asia. *In*: M.B. New, H. de Saram and Tharlochan Singh (Eds.). Technical and Economic Aspects of Shrimp Farming: Proceedings of the AQUATECH Conference '90 Kuala Lumpur, Malaysia, 11-14 June, 1990. pp. 207-222

Dana, D. and Sukenda. 1990. The occurrence of viral agents in diseased shrimp *Penaeus monodon* reared in ponds. Symposium on Diseases in Asian Aquaculture, 26-29 November 1990. Scientific Session Abstracts. Fish Health Section, Asian Fisheries Society, Manila, Philippines. p. 99.

de la Cruz, M.C. 1989. Aflatoxin in commercial prawn feeds. SEAFDEC Asian Aquaculture. 9: 4.

Egusa, S.Y., Takahashi, T. Itami and K. Momoyama. 1988. Histopathology of vibriosis in the Kuruma prawn, *Penaeus japonicus* Bate. Fish Pathol. 23: 59-65.

Egusa, S. and T. Ueda. 1972. A *Fusarium* sp. associated with black gill disease of the Kuruma prawn, *Penaeus japonicus* Bate. Bull. Jap. Soc. Sci. Fish. 38: 1253-1260.

Flegel, T.W., S. Boonyaratpalin, D.F. Fegan, M. Guerin and S. Sriurairatana. 1992. High mortality of black tiger prawns from cotton shrimp disease in Thailand. *In*: M. Shariff, R.P. Subasinghe and J.R. Arthur (Eds.). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila, Philippines. pp. 181-197.

Ishikawa, Y. 1968. Preliminary report on black gill disease of the Kuruma prawn, *Penaeus japonicus* Bate. Fish Pathol. 3: 34-38. (In Japanese).

Itami, T., Y. Takahashi and Y. Nakamura. 1989. Efficacy of vaccination against vibriosis in cultured Kuruma prawns *Penaeus japonicus*. J. Aquatic Animal Health. 1: 238-242.

Lavilla-Pitogo, C.R., L.J. Albright, M.G. Pañer and N.A. Suñaz. 1992. Studies on the sources of luminescent *Vibrio harveyi* in *Penaeus monodon* hatcheries. *In*: M. Shariff, R.P. Subasinghe and J.R. Arthur (Eds.). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila, Philippines. pp. 157-164.

Lavilla-Pitogo, C.R., M.C.L. Baticados, E.R. Cruz-Lacierda and L.D. de la Peña. 1990. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. Aquaculture. 91: 1-13.

Liao, I.C. 1990. The world's marine prawn culture industries: today and tomorrow. *In*: R. Hirono and I. Hanyu (Eds.). Second Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines. pp. 11-28

Lightner, D.V. 1988. Diseases of cultured penaeid shrimp and prawns. *In*: C.J. Sindermann

and D.V. Lightner (Eds.). Disease Diagnosis and Control in North American Marine Aquaculture. Elsevier, Amsterdam. pp. 8-127.

Lightner, D.V. and R.M. Redman. 1991. Hosts, geographic range and diagnostic procedures for the penaeid virus diseases of concern to shrimp culturists in the Americas. *In*: P. Deloach, W.J. Dougherty and M.A. Davidson (Eds.). Frontiers in Shrimp Research. Elsevier Science Publishers. pp. 173-196.

Lightner, D.V., T.A. Bell, R.M. Redman. L.L. Mohney, J.M. Natividad, A. Rukyani and A. Poernomo. 1992. A review of some major diseases of economic significance in penaeid prawns/shrimps in the Americas and Indopacific. *In*: M. Shariff, R.P. Subasinghe and J.R. Arthur (Eds.). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila, Philippines. pp. 57-80.

Lio-Po, G.D. and E.G. Sanvictores. 1986. Tolerance of *Penaeus monodon* eggs and larvae to fungicides against *Lagenidium* sp. and *Haliphthoros philippinensis*. Aquaculture. 51: 161-168.

Lin, C.K. 1989. Prawn culture in Taiwan. What went wrong? J. World Aquaculture Soc. 20(2): 19-20.

Momoyama, K. and T. Sano. 1988. A method of experimental infection of kuruma shrimp larvae, *Penaeus japonicus* Bate, with baculoviral midgut gland necrosis (BMN) virus. J. Fish Dis. 11: 105-111.

Nash, G.L. 1990. *Penaeus monodon* grow out diseases. *In*: M.B. New, H. de Saram and Tharlochan Singh (Eds.). Technical and Economic Aspects of Shrimp Farming: Proceedings of the AQUATECH Conference (1990: Kuala Lumpur). pp. 172-190.

Nash, G., I.G. Anderson and M. Shariff. 1988a. Pathological changes in the tiger prawn, *Penaeus monodon* Fabricius, associated with culture in brackishwater ponds developed from potentially acid-sulphate soils. J. Fish Dis. 11: 113-123.

Nash, G., A. Poernomo and M.B. Nash. 1988b. Baculovirus infection in brackishwater pond cultured *Penaeus monodon* Fabricius in Indonesia. Aquaculture. 73: 1-6.

Natividad, J.M. and D.V. Lightner. 1992. Susceptibility of the different larval and postlarval stages of black tiger prawn, *Penaeus monodon* Fabricius, to monodon baculovirus (MBV). *In*: M. Shariff, R.P. Subasinghe and J.R. Arthur (Eds.). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila, Philippines. pp. 111-125.

Overstreet, R.M., K.C. Stuck, R.A. Krol and W.E Hawkins. 1988. Experimental infections with *Baculovirus penaei* in the white shrimp *Penaeus vannamei* (Crustacea: Decapoda) as a bioassay. J. World Aquaculture Soc. 19: 175-187.

Paynter, J.L., D.V. Lightner and R.J.G. Lester. 1985. Prawn virus from juvenile *Penaeus esculentus*. *In*: P.C. Rothisberg, B.J. Hill and D.J. Staples (Eds.). Second Australian National Prawn Seminar NPS2. Cleveland, Queensland. pp. 61-64.

Paynter, J.L., J.E. Vickers and R.J.G. Lester. 1992. Experimental transmission of *Penaeus monodon*-type baculovirus (MBV). *In*: M. Shariff, R.P. Subasinghe and J.R. Arthur (Eds.). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila, Philippines. pp. 97-110.

Rosenberry, J.H. 1990. World shrimp farming 1989. Aquaculture Digest, California, USA.

Sano, T. and H. Fukuda. 1987. Principal microbial diseases of mariculture in Japan. Aquaculture. 67: 59-69.

Sano, T., T. Nishimura, H. Fukuda, T. Hayashida and K. Momoyama. 1985. Baculoviral infectivity trials on Kuruma shrimp larvae, *Penaeus japonicus*, of different ages. *In*: A.E. Ellis (Ed.). Fish and Shellfish Pathology. Academic Press, N.Y. pp. 397-403.

Sunaryanto, A. and A. Mariam. 1986. Occurrence of a pathogenic bacteria causing luminescence in penaeid larvae in Indonesian hatcheries. Bull. Brackishwater Aqua. Devl. Centre. 8: 64-70.

Takahashi, Y., H. Nagoya and K. Momoyama. 1984. Pathogenicity and characteristics of *Vibrio* sp. isolated from diseased postlarvae of Kuruma prawn, *Penaeus japonicus*. Bate. J. Shimonoseki Univ. Fish. 32: 23-31.

Takahashi, Y., Y. Shimoyama and K. Momoyama. 1985a. Pathogenicity and characteristics of *Vibrio* sp. isolated from diseased postlarvae of Kuruma prawn, *Penaeus japonicus* Bate. Bull. Jap. Soc. Sci. Fish. 51: 721-730.

Takahashi, Y., T. Itami, A. Nakagawa, H. Nishimura and T. Abe. 1985b. Therapeutic effects of oxytetracycline trial tablets against vibriosis in cultured Kuruma prawns *Penaeus japonicus* Bate. Bull. Jap. Soc. Sci. Fish. 51: 1639-1643.

Tansutapanit, D.S.O.A. and L. Ruangpan. 1987. *Vibrio harveyi* a causative agent of white shrimp nauplii, *Penaeus merguiensis*. *In*: Abstract. Tech. Paper No. 6/30. Third Natl. Seminar on Marine Science, 6-8 August 1986, NSRC, Bangkok, Thailand.

An Overview of the Disease Situation, Diagnostic Techniques, Treatments and Preventives Used on Shrimp Farms in China

Dou Chen
Institute of Oceanology, Academia Sinica
7 Nan-hai Road, Qingdao
People's Republic of China

Abstract

The infectious and noninfectious diseases and disease syndromes affecting cultured shrimp in China are summarized. More than 20 diseases, some of which are newly discovered, are described herein. Comprehensive strategies for preventing penaeid diseases and the use of ecological control instead of chemical control to prevent Vibrio diseases are also discussed.

Introduction

The incidence of penaeid shrimp diseases is closely related to the local environment and culture patterns. In China, the growout ponds of most shrimp farms are earthen, 1.5 - 2.0 m in depth. The ponds range in size from 2 - 10 ha; a few are larger than 20 ha. The majority of farms pump their sea water from coastal areas, but some employ natural tidal flow. Ponds are rarely equipped with aerators. This is the basic type of culture system used in China, in which the initial stocking density of juveniles during growout is usually more than 150,000/ha, and the average production per unit area is quite low — only 1,275/ha in 1990.

As the penaeid shrimp culture industry in China expands, the incidence of disease increases year by year. Some diseases, such as those caused by certain *Vibrio* species, have become serious problems that hamper the development of the industry. To reduce the impact of disease, initial investigations began in 1979, but comprehensive research projects on shrimp diseases were not begun until after 1985 in the research institutes and universities of the coastal provinces. Research focused on identifying the various shrimp diseases and investigating their etiology, diagnosis, treatment, prevention and pathogenic ecology. Research dealt principally with *Penaeus chinensis*, but *P. penicillatus* and *P. merguiensis* were also included.

In 10 years, more than 20 shrimp diseases were identified, most of which had already been described in the literature (Sindermann and Lightner, 1988). However, new diseases continue to emerge. Standard treatment and prevention techniques for shrimp diseases cannot be applied to some newly discovered diseases, such as white-black spot disease, because their etiology is unknown (Xu and Liu, 1988).

Disease is a serious problem, and the situation in China is rather grim. This paper will provide an overview of the shrimp disease situation, and discuss treatments and preventives used on shrimp farms in China.

Diseases and Disease Syndromes

The most common and important diseases and disease syndromes of cultured penaeid shrimp in China will be listed according to their etiology: infectious diseases, noninfectious diseases and diseases whose etiology is unknown. Some important diseases that remain to be investigated are not included.

Infectious Diseases

Hepatopancreatic Parvo-like Virus, HPV

Lightner and Redman (1985) first reported that hepatopancreatic parvo-like virus had been found in *P. chinensis*. At the same time, Wang et al. (1985) confirmed this observation. Gross signs of HPV infection include atrophy of the hepatopancreas, poor growth rate, anorexia and reduced preening activity. Mortality is higher if shrimp have both HPV and either Vibrio disease, black gill syndrome or epicommensal fouling.

Intranuclear inclusion bodies in hypertrophied nuclei of hepatopancreatic tubule epithelial cells and the presence of virus particles in the intranuclear inclusion bodies of affected hepatopancreatocytes have been observed in cultured penaeid shrimp and in wild shrimp from the Huanghai Sea (Wang et al., 1985). Viral diseases have not been taken seriously in China because their impact is subtle and it is difficult to distinguish their symptoms from other diseases. Thus far, few works have dealt with the viral diseases of penaeid shrimp in China.

Vibrio Disease of Larval Shrimp

Septicemic vibriosis is the most serious and ubiquitous disease of larval shrimp in China. Most pathogenic strains appear to be *Vibrio parahaemolyticus, V. alginolyticus*, other *V.* spp. and other related bacteria. Affected shrimp appear turbid, exhibit reduced motility, reduced phototaxis, empty guts, increased surface fouling and a tendency to sink. Microscopic examination shows an abundance of bacteria. In serious cases, mortality can reach 100% within one or two days (Meng and Yu, 1982a).

Application of 1 - 1.5 ppm chloramphenicol, 2 - 3 ppm terramycin or 1 ppm furacin to the tank water is effective both as a treatment and to prevent this disease. Live animal feeds such as

Artemia nauplii are believed to be the principal means of transmission of pathogenic *Vibrio* spp.; therefore, all animal feeds should be thoroughly sterilized before feeding. Certain microalgal feeds, however, have an inhibitory effect on pathogenic *Vibrio*; hence, the application of selected microalgae to the shrimp rearing system not only serves as supplemental feed, but also helps to control the *Vibrio* population. Therefore, it is possible to prevent Vibrio disease in larvae with ecological measures (Chen et al., 1989; Chen et al., In press).

Red-leg Disease

The important pathogenic species are *V. parahaemolyticus, V. alginolyticus, V. anguillarum, V. campbellii*, other *V.* spp., *Proteus vulgaris* and related bacteria. Gross signs of affected shrimp include anorexia, lethargy and expansion of red chromatophores on the periopods and pleopods, giving these appendages a reddish coloration. Affected shrimp often swim in shallow water, and dead shrimp are frequently found along the pond side.

Pathogenic strains are frequently isolated from the hepatopancreas, which becomes discolored and soft. Hemocyte numbers may be drastically reduced, and hemolymph appears turbid and clots slowly or not at all. Gills become yellow or pink, and affected shrimp usually have empty, reddish guts.

Red-leg disease is the most harmful and ubiquitous shrimp disease in China, especially during warm seasons. Mortality can be as high as 95% during severe outbreaks. The disease occurs frequently in growout ponds in which the top layer of bottom mud has not been removed prior to stocking, or when the density of shrimp is too high.

Application of 2% mashed garlic and 0.2% terramycin (or 0.1% chloramphenicol) to the feed for 7 to 14 days, in conjunction with the addition of 1 - 2 ppm calcium hypochlorite in the pond water, is an effective means of treatment (Meng et al., 1989; Hong and Chen, 1991; Wu et al., 1991c; Xu et al., In press).

Eyeball Necrosis Disease

Zheng (1986) described eyeball necrosis disease of penaeid shrimp and determined that it was caused by *Vibrio cholerae* (non-01). Affected shrimp float motionless, lying prone and rolling over at the surface of the water from time to time. The eyes of affected shrimp swell, and the cornea turns from black to brown, then festers and falls away, causing mortality within a few days. *Fusarium* sp. is occasionally isolated from the eyes of affected shrimp.

Adding 0.2% terramycin or 0.1% chloramphenicol to the feed and treating the pond water with malachite green and formalin can prevent this disease (Zheng, 1986).

Brown Spot Disease

Brown spot disease is caused by chitin-degrading bacteria and a variety of related bacteria that enter through shell wounds. This is a ubiquitous disease, especially in overwintering shrimp. The

affected part of the body can become secondarily infected with *Vibrio* sp. or *Fusarium* sp. Over time, the ovaries may atrophy and turn red, and *Fusarium* sp. can cause tumors which invade the muscular tissue. In China, brown spot disease is treated with 25 ppm formalin for 24 h (Meng and Yu, 1980; Wu et al., 1991c).

Filamentous Bacterial Disease

The species of filamentous bacteria identified in China include *Leucothrix mucor* and *Thiothrix* sp. (Wu et al., 1991c). This disease is frequently found in larvae and juvenile shrimp. If the water quality is poor, both filamentous bacteria and fouling ciliates adhere to the gills, and mortality is caused by difficulty in respiration and molting. Application of 10 ppm teaseed cake to promote molting, and a high rate of water exchange are effective means of prevention and treatment (Meng and Yu, 1983; Wu et al., 1991c).

Larval Mycosis

The etiologic agents of larval mycosis reported in China are *Lagenidium* sp. and *Sirolpidium* sp. *Lagenidium* sp. has a terminal vesicle that is missing in *Sirolpidium* sp. Larval mycosis is ubiquitous in larval rearing systems throughout China. In serious cases, cumulative mortality reaches 100% in one to two days if treatment is not provided in time. The following have been reported as effective in the prevention of larval mycosis: 10 - 40 ppb methylene blue, 2 ppm gallnut (a traditional type of Chinese medicine) or 0.008 - 0.01 ppm malachite green (Wu et al., 1988; Meng and Yu, 1982a).

Fusarium Disease

This disease occurs principally in overwintering shrimp. The etiologic agents reported in China are *Fusarium solani*, *F. oxysporum*, *F. tricinctum* and *F. graminearum*. Conidiospores enter through wounds and produce hyphae that extend into muscle tissue, creating tumor-like swellings. Mortality is the inevitable result — there is no cure for Fusarium disease.

Gills are most susceptible to infection by *Fusarium* spp.; hyphae and conidia fill the gills of infected shrimp. Application of mycostatin can reduce the mortality rate in the early stages of infection, but it is also necessary to remove diseased shrimp early (Yu et al., 1989; Hong et al., 1988; Meng and Yu, 1983).

Cotton Shrimp Disease

Cotton shrimp disease is caused by microsporidians, including *Pleistophora* sp., *Thelohania* sp. and *Nosema* sp. (Meng and Yu, 1983; Wu et al., 1991c). *Nosema* sp. was also found in wild *Penaeus chinensis* taken from the coastal waters of Qingdao (Hao and Mou, 1984). Shrimp tissue infected with microsporidians turns white and becomes soft. In general, the mortality rate is low. There are no treatments, only prevention. For example, one should remove all diseased shrimp and sterilize the pond bottom to prevent cotton shrimp disease.

Parasitic Ciliate Disease

Paranophrys carcini Leglise is a marine facultative parasite that inhabits decaying organic matter and occasionally infects shrimp through wounds. The optimum temperature for propagation of this ciliate is 10°C.

In the early stages of the infection, the ciliate can only be found in the wounds of the shrimp. In later stages, it can infect hemolymph and damage other organs, even the gills. Shrimp exhibit reduced hemocyte numbers, and hemolymph becomes turbid and doesn't clot.

Parasitic ciliate disease only occurs in overwintering shrimp in northern China, where mortality due to the disease can exceed 90%. To prevent this disease, damage to the shrimp should be avoided, diseased or dead shrimp should be removed quickly, and live animal feeds should be treated with fresh water. Formalin and malachite green are also effective preventives (Meng et al., 1988).

Parasitic *Nematopsis* and *Cephalolobus* Diseases

Parasitic *Nematopsis sinaloensis* and *Cephalolobus penaeus* have been found in digestive tracts of penaeid shrimp in China (Meng and Yu, 1980; Wu et al., 1991b).

Epicommensal Ciliate Disease

Heavy fouling by epicommensal protozoa on the surfaces of gills and appendages may cause mortalities. Thirty-eight species of epicommensal ciliates belonging to nine genera (*Zoothamnium, Epistylis, Vorticella, Rhabdostyla, Myschiston, Pseudocarchesium, Intrastylum, Vaginicola* and *Cothurnia*) have been observed on penaeid shrimp in China (Song, 1986). *Zoothamnium, Vorticella* and *Epistylis* are most common, and the most serious fouling organisms affecting penaeid shrimp. The following treatments can remove ciliates from gill filaments and body surfaces of penaeid shrimp (Meng and Yu, 1983; Zheng et al., 1987; Wu et al., 1991c):

- Exchange a great deal of water;
- Teaseed cake at 10 ppm in pond water;
- Potassium permanganate at 5 ppm; and
- Formalin at 25 ppm for 24 h.

Miscellaneous Fouling Organisms

This large group of surface fouling organisms includes a variety of bacteria, algae and other protozoa. *Licmophora ehrenbery, L. paradoxa, Synedra tabulata, Nitzschia* sp., *Amphora* sp., *Belanus* sp., *Enteromorphra* sp., *Ectocarpus* sp. and *Cladophora* sp. have all been found attached to the gills or body surfaces of penaeid shrimp (Wu and Zeng, 1988; Wu et al., 1991a). *Acineta polymorpha, A. tuberosa* and *Ephelota* sp. also cause fouling diseases (Meng and Yu, 1980; Wu et al, 1991a).

Some treatments used to combat filamentous bacterial disease and epicommensal ciliate disease can also control fouling organisms listed in this section.

Noninfectious Diseases

Black Gill Syndrome

Most species of penaeid shrimp in China are susceptible to black gill syndrome, which may be caused by toxins or biotic agents in the water that harm the gills. In serious cases, most of the gill lamellae are affected and necrosis of the gills may be apparent. It is noteworthy that black gill syndrome is always accompanied by infection by *Vibrio* spp., *Fusarium* spp., *Zoothamnium* spp., *Lagenophrys* spp. or HPV, and eventually results in high levels of mortality. Therefore, to prevent the disease, comprehensive and appropriate measures must be considered in advance (Meng and Yu, 1982b).

Gas-bubble Disease

Gas-bubble disease may occasionally occur in hatcheries as a result of supersaturation of sea water with atmospheric gases. Prevention is accomplished by controlling the level of dissolved gases at all times (Meng and Yu, 1982a; Wu et al., 1991c).

Body Cramp Syndrome

Fully or partially cramped shrimp can be found in growout ponds during summer months when both air and water temperatures are high, especially when the air is warmer than the water. Avoidance of handling during the hottest hours and timely exchange of water in the summer months are suggested means of prevention (Meng and Yu, 1983; Wu et al., 1991c).

Muscle Necrosis

Muscle necrosis usually occurs in growout ponds during summer months and is caused by overcrowding, low oxygen, sudden changes in temperature or salinity and other unstable conditions. Keeping pond water clean and using moderate stocking densities may prevent muscle necrosis and secondary bacterial and fungal infections (Meng and Yu, 1983; Wu et al., 1991c).

Floating Head Syndrome

Floating head syndrome of penaeid shrimp always occurs in high-density growout ponds with poor phytoplankton blooms, poor water quality and polluted bottom conditions, during calm, sweltering summer days. Shrimp usually float at the surface of the water in the early morning due to lack of oxygen. Floating head syndrome results in severe mortalities at some shrimp farms if it is not treated in time. The following are effective means of prevention:

- Raising the shrimp at moderate densities;
- Judicious application of feed;
- Exchanging water; and
- Using chemical and physical measures to increase the level of dissolved oxygen.

Diseases of Unknown Etiology

White-black Spot Disease

The cause of this disease is unknown, but it usually occurs in warmer seasons.

At first, white spots appear on both sides of the abdomen. As the disease progresses, the white spots rapidly turn black, at which time a number of necrotic hemocytes can be observed. In serious cases, the mortality rate is higher than 90%. The seriousness of this disease is second only to red-leg disease (Xu and Liu, 1988; Wu et al., 1991c).

White Spot Disease

White spot disease is a chronic disease that often occurs in early stages of overwintering shrimp. Irregular white spots usually appear on the carapace, and occasionally on the dorsal surface of the abdomen. A thin, hard spot develops between the shell and the muscle, making separation difficult, but the surface of the shell remains undamaged. In the early stage of infection, shrimp behave normally. In the latter stages, shrimp become lethargic and some die. Although fungal mycelia have been observed in affected parts, the cause of the disease is still uncertain. White spot disease has been observed on all shrimp farms in China, but the infection rate is only 5% or less.

Soft Shell Syndrome

The main symptoms include softening of the shell, thin and weak muscles, poor growth rate and reduced activity. The disease may be caused by using poor quality water (water with a low pH or high levels of H_2S) or it could be a nutritional deficiency. High mortality results from difficulty in molting. Soft shell syndrome can be prevented by improving feeds and water quality.

Comprehensive Prevention

Larval Rearing

The hatchery can be considered a microecosystem in which different types of microorganisms are conditioned to one another. The production of healthy penaeid shrimp larvae requires a stable and suitable environment.

Vibrio disease is the most serious problem for shrimp larviculture in China. It is common knowledge that using chemicals to prevent shrimp diseases has many disadvantages. *Vibrio* spp. are opportunistic pathogens; therefore, it is possible to apply ecological or biological control instead of chemical control to prevent Vibrio disease. The most important means of biological control are:

- Select and treat broodstock before spawning and discard diseased shrimp quickly;
- Keep hatchery water clean and supply filtered sea water continuously;
- Provide sterilized live animal feeds and check for the transmission of pathogens. Vacuum hatchery tank bottoms frequently to prevent sludge accumulation;
- Use chemoprophylactics, especially antibiotics, responsibly; and
- Select microalgal feeds with anti-Vibrio microflora and add during all larval stages.

Growout

Shrimp growout ponds also can be considered ecosystems in which cultured shrimp and associated organisms, including live animal and microalgal feeds, related microorganisms and a variety of pathogens, live together and are conditioned to one another. Disease situations at certain shrimp farms are closely associated with culture patterns. Noninfectious diseases of penaeid shrimp can be prevented by controlling some environmental factors or improving nutrition. To prevent infectious diseases, ecological control measures must be instituted instead of chemical ones. The most important ecological measures are:

- Dry the bottom of growout ponds during the winter after harvest, then remove the surface layer;
- Sterilize the ponds with calcium hypochlorite or teaseed cake before stocking;
- Always keep pond water clean, pay close attention to water temperature, water color, salinity, dissolved oxygen, etc. and modify these parameters when necessary;
- Change pond water in a timely fashion;
- Ponds should be deeper than 1.5 - 2.0 m;
- Select healthy larvae for stocking;
- Raise shrimp at moderate densities in growout ponds;
- Give appropriate quantities of feeds to avoid deterioration of the bottom environment;
- Sterilize or treat animal feeds before use to avoid transmission of pathogenic *Vibrio* spp.;
- Give medicated feeds only during hot seasons or disease outbreaks;
- Control the transfer of pathogens at all times; and
- Study the ecology of pathogens and apply ecological control instead of chemical control.

Literature Cited

Chen, D., X.Y. Liu, Y.D. Yu, Z.H. Yang, T. Xiao and Q.X. Wang. 1989. Variation of vibrio population in larval penaeid shrimp rearing system and its controlling measure. Fifth International Symposium on Microbial Ecology. (abstracts) 159.

Chen, D., X.Y. Liu, Y.D. Yu, Z.H. Yang, T. Xiao and Q.X. Wang. In press. The vibrio disease of penaeid shrimp in the larval rearing systems and its comprehensive prevention. Oceanol. Limnol. Sinica.

Hao, B. and R.P. Mou. 1984. Preliminary study of the parasite microsporidian from the Chinese Prawn *Penaeus orientalis*. Mar. Sci. 2: 47-48. (In Chinese with English abstract).

Hong, X. and X.F. Chen. 1991. Histopathologic observation on red-leg disease of *Penaeus penicillatus*. Xiamen Aquatic Science and Technology. 2: 36-39. (In Chinese).

Hong, X., D.H. Wu and H.W. Zeng. 1988. Studies on Fusarium disease of penaeid shrimp and etiology. Bull. Fish Dis./Yubing jianxun. 3-4: 91-92. (In Chinese).

Lightner D.V. and R.M. Redman. 1985. A parvo-like virus disease of penaeid shrimp. J. Invertebr. Pathol. 45: 47-53.

Meng, Q.X., C.H. Song and K.K. Yu. 1989. Studies on red-leg disease. I Gross Signs, cause, epizootiology and pathogenic identification. Bull. Fish Dis./Yubing jianxun. 2-3: 36-38. (In Chinese).

Meng, Q.X. and K.K. Yu. 1980. Investigations on diseases and parasites of the saltwater shrimp, *Penaeus orientalis* Kishinouye. Fisheries Research 1: 31-45. (In Chinese).

Meng, Q.X. and K.K. Yu. 1982a. Diseases of penaeid shrimp in larval rearing period. Mar. Fish. 4: 149-152. (In Chinese).

Meng, Q.X. and K.K. Yu. 1982b. Notes on the "black gill disease" of penaeid prawn. J. Shandong College of Oceanology. Vol. 12. 4: 95-100. (In Chinese with English abstract).

Meng, Q.X. and K.K. Yu. 1983. Prevention and control of diseases in growout period of penaeid shrimp. Mar. Fish. 3: 110-116. (In Chinese).

Meng, Q.X., L. Zhou, K.K. Yu, Z.H. Luo and H.Z. Liu. 1988. Studies on the parasitic ciliate disease of overwintered penaeid shrimp. Bull. Fish Dis./Yubing jianxun. 3-4: 17-72. (In Chinese).

Sindermann C.J. and D.V. Lightner. 1988. Disease Diagnosis and Control in North American Marine Aquaculture. Second revised edition. Elsevier. 333 p.

Song, W.B. 1986. Description of seven new species of peritrichs on *Penaeus orientalis*. Acta Zootaxon, Sinica. 11: 225-235. (In Chinese with English abstract).

Wang, W.X. et al. 1985. Studies on hepatopancreatic parvo-like virus infection of *Penaeus chinensis* (unpublished report).

Wu, D.H., X. Hong and H.W. Huang. 1991a. Preliminary studies on a new penaeid shrimp disease—Synedra disease. Xiamen Aquatic Science and Technology. 2: 32-35.

Wu, D.H., X. Hong and H.W. Zeng. 1988. Studies on larval mycosis of penaeid shrimp and prevention. Bull. Fish Dis./Yubing jianxun. 3-4: 78-81. (In Chinese).

Wu, D.H., H. Huang and H.W. Zeng. 1991b. Studies on parasitic *Nematopsis* of cultured penaeid shrimp. Xiamen Aquatic Science and Technology. 2: 46-49. (In Chinese).

Wu, D.H. and H.W. Zeng. 1988. Licmophora disease of penaeid shrimp. Bull. Fish Dis./Yubing jianxun. 3-4: 40-42. (In Chinese).

Wu, D.H., H.W. Zeng, X. Hong and H. Huang. 1991c. Investigation on diseases of cultured penaeid shrimp in southern part of Fujian province. Xiamen Aquatic Science and Technology. 2: 1-10. (In Chinese).

Xu, B., W.S. Ji, M.Y. Ding, X. Li and H.S. Xu. In press. Comparison of antibacterial agents for control of pathogens in culture prawn. J. Ocean University of Qingdao.

Xu, G.J. and M.X. Liu. 1988. Preliminary observation on white-black spot disease of penaeid shrimp. Mariculture. 4: 19-22. (In Chinese).

Yu, K.K., W.B. Zhan and Q.X. Meng. 1989. Studies on etiology of Fusarium disease of penaeid shrimp. Bull. Fish Dis./Yubing jianxun. 2-3: 19-22. (In Chinese).

Zheng, G.X. 1986. Identification and pathogenicity of *Vibrio cholerae* (non-01) isolated from diseased penaeid shrimp. J. Fish China/Shuichan Xuebao. 10(2): 195-203. (In Chinese with English abstract).

Zheng, G.X., Y.L. Shen and H. Li. 1987. Preliminary studies on treatment of Zoothamnium disease of penaeid shrimp with potassium permanganate. Mar. Fish. 3: 102-105. (In Chinese with English abstract).

Occurrence, Diagnosis and Treatment of Shrimp Diseases in Thailand

Timothy W. Flegel[1], Daniel F. Fegan[1], Sumana Kongsom[1], Sompoach Vuthikomudomkit[1], Siripom Sriurairatana[2], Sitdhi Boonyaratpalin[3], Chaiyuth Chantanachookhin[3], Joan E. Vickers[4] and Oliver D. Macdonald[5]

DEDICATION

We dedicate this article to the memory of our dear colleague, Dr. Chaiyuth Chantanachookhin. His untimely accidental death in early May 1992 was a great loss to us all.

Abstract

Diseases of commercially cultured *Penaeus monodon* in Thailand are reviewed with emphasis on recent research results. The major causes of economic loss in growout ponds can be ultimately attributed to environmental stress resulting from poor management practices or external factors. The most common virus infection reported is *Penaeus monodon* baculovirus (MBV), but this is well tolerated by *P. monodon* under unstressful rearing conditions. Two new viruses of unknown impact are described in normal broodstock specimens of *P. monodon* captured from the Andaman sea. One is a nonenveloped, polyhedral particle (diameter 40 nm) that occurred in the cytoplasm of lymphoid organ cells at high incidence (90% of randomly selected stock examined between 1989 and 1991). The other, also in the lymphoid organ, was a rod-shaped, enveloped virus resembling the baculoviridae.

Bacteria of the genus *Vibrio* (*V. parahaemolyticus* and *V. harveyi* accounted for 83% of isolates) caused most growout shrimp mortality in opportunistic infections, and the incidence of antibiotic resistance in the isolated strains was high. Fungal infections in growout shrimp are also opportunistic and have not caused extensive economic loss. However, *Lagenidium* sp. is infectious in the hatchery and can sometimes cause heavy losses if it is not controlled with trifluralin (10 ppb). With respect to environmental factors, preliminary results from insecticide tests showed that the synthetic pyrethroid, cypermethrin, was extremely toxic to *P. monodon*. It was acutely toxic, causing significant mortality in growout shrimp at 1 ng/L, and in larvae (zoea) at 10 pg/L. In light of this, the effect of insecticides on crustaceans is briefly reviewed. Other environmental problems discussed include toxic algae, crude oil, release of rearing pond wastewater, and treatment of post-harvest pond bottoms. Finally, a series of mystery disease syndromes of unknown etiology are described. Two of these show indications of viral etiology.

[1]BPN Aquaculture Research Centre, 577 Thanadee Complex, Koryor-Songkhla Road, Amphur Muang Songkhla 90000, Thailand
[2]Faculty of Medicine, Siriraj Hospital, Mahidol University, Rama VI Road, Bangkok 10400, Thailand
[3]National Institute of Coastal Aquaculture, Songkhla 90000, Thailand
[4]Dept. Veterinary Pathology, University of Queensland, Brisbane, Australia
[5]Hadda Farm Ltd., Iaigh-Iaighoch II1 1SK, Scotland

Introduction

General

This paper is an overview of the impact of diseases on the cultivation of *Penaeus monodon* in Thailand. However, it would be pointless to simply repeat much of the information that has already been published in recent excellent reviews by Sindermann and Lightner (1988) and by Brock (1991), and wherever appropriate, we will refer to these works. There is also a recent publication in Thai (Limsuwan, 1991) that has a good section on diseases and their control. The emphasis here will be on recent investigations in Thailand, some of which have not yet been published.

We will also present some abnormal phenomena that we have observed in *P. monodon* but have, as yet, been unable to explain.

As an overall limitation, we caution the reader that the context of this article applies to the authors' experiences with shrimp farming on the "semi-intensive" scale in earthen ponds in southern Thailand. According to our definition, semi-intensive ponds are piscicide-treated ponds stocked with hatchery-raised larvae at 15 - 80 larvae/m^2 and supplied with dry commercial feeds as a major part of their diet. In the BPN (Aquastar) system, the average pond is approximately 1.5-m deep and 1 ha in area, with an average water exchange (over the whole cultivation cycle) of approximately 30%.

Management Practices and Environmental Stress

At recent meetings in Kuala Lumpur (New et al., 1990) and Korea (Workshop on Fish Health Management in the Asia-Pacific Region, 8 - 15 October 1990, Pusan, Korea), there was consensus among disease experts that many of the disease problems encountered in aquaculture can be avoided by good management practices. We strongly concur with this opinion. The two major causes of problems encountered by shrimp farmers in Thailand are poor management and environmental stress. Management problems are usually solved through assistance from government extension workers (e.g., from the Department of Fisheries) or from the technical sales staff of the various feed companies. Environmental problems, however, often cannot be solved, especially when they result from natural phenomena (e.g., red tides). Where they could conceivably be solved, major changes in social behavior and legislation would be required, and it is difficult to imagine any improvement for some time to come. Even so, a move has been made in that direction in Thailand. In February 1992, legislation was passed requiring all shrimp farms to be registered and all farms over 50 *rai* (approximately 8 ha) to provide a minimum of 10% of the farm area (the area of the discharge canal can be included) for the treatment of discharge water. The BOD limit of the outlet water has been set at 10 mg/L by this legislation.

In principle, we agree with such legislation since it is desirable for the stable future of the whole industry that the carrying capacity of the environment in each farming area be determined and that the water resources be managed in such a way that conflicts between various users (including nonaquaculturists) are avoided. To formulate such legislation, data from sound environmental studies are required, and these studies should be carried out as quickly as possible. The hasty drafting of laws in the absence of such data can result in the setting of arbitrary limits, such as the 10-ppm BOD limit for discharge water in Thailand. If such limits are not necessary or realistic, they can be damaging to the growth and competitiveness of the industry.

Serious environmental problems have developed in some shrimp farming areas in Thailand because of the unrestrained construction and operation of ponds. In the most extreme situations, single canals extending several km from the sea are used for both pond supply and discharge. In such situations, water quality deteriorates progressively from the seaside inwards, and, consequently, farmers at the end of the line can face serious disease problems as a direct result of poor water quality. Sometimes, farms are located near industrial areas, so residual wastes from factory discharges may also be significant underlying causes of disease (Menasveta, 1987; Summonok, 1990). The problems faced are not always on the side of the shrimp farmers. There have been extreme examples of shrimp farmers discharging saline waters into freshwater canals. In a few cases, this has led to physical clashes with rice farmers and police intervention.

The most significant poor management activity in Thailand is stocking ponds beyond their carrying capacity. In the standard Aquastar pond, farmers are stocking 15 - 80 larvae/m^2. BPN Aquaculture recommends stocking a maximum of 30 larvae/m^2 for such pond systems, but suggests that stocking 25 larvae/m^2 would be optimal. This optimal number was arrived at through discussions with experts of long practical experience in shrimp farming. Through experience, they found that such stocking levels allowed for stable, long-term productivity from these ponds. They found that higher stocking densities increased the risk of failure in the long term, although short-term gains could be higher under ideal conditions.

Unfortunately, many Thai farmers have adopted the goal of higher short-term gains, and average stocking densities have risen to the current level of approximately 50 - 60/m^2, contrary to the advice of almost all disease experts. This practice has been adopted almost universally, regardless of the constraints (environmental and infrastructural) that vary from pond to pond. Thus, it is entirely predictable that many farmers reach a point in the cultivation cycle where they eventually face disease problems that either re-

quire extreme treatment measures or, more often, are untreatable.

Infectious Versus Opportunistic Diseases

In the few years we have been working with *P. monodon*, we have been impressed by the hardiness of the animal and its high level of resistance to disease under good cultivation conditions. Under these conditions, there is a rather low level of mortality from diseases caused by infectious agents (i.e., agents that can surmount the defenses of a healthy animal). Such agents include viruses and parasites, and they usually cause relatively low financial loss; i.e., survival for good ponds (harvested shrimp/stocked larvae x 100%) is usually 70 - 80%, including losses from causes other than infectious agents (e.g., physical injury, predation, etc.). Thus, we believe that studying the infectious agents and formulating control or elimination strategies could raise the average survival by 10%. Better growth rates and feeding efficiency could also accrue, and, altogether, these factors could significantly improve production efficiency.

In contrast to the viruses and parasites, we consider all of the bacterial and fungal diseases that we have encountered in growout ponds in Thailand to be opportunistic infections. We exclude rickettsia, which has not yet been found in Thailand, from this generalization. Our conclusion is based on the experience that these infections can always be directly or indirectly attributed to an extended period of stress, which led to general weakening and a lowering of normal defenses. This applies equally to secondary infections via viral lesions, which can be exacerbated by stress to such an extent that bacterial infection can follow (see next section). Mortality from secondary infections usually causes the greatest losses in shrimp rearing. In some cases, the situation can be so severe that an entire crop is lost or must be terminated prematurely. Often in these situations, massive mortality begins before assistance is sought, and it is too late to devise a treatment that will improve survival.

With respect to the hatchery, we currently reserve judgment as far as the infectivity of bacteria is concerned, but we can confirm that oomycete diseases are definitely infectious for healthy, unstressed larvae.

Viruses

Lightner et al. (1990) and Brock (1991) have reviewed information on shrimp viruses, and only information relevant to Thailand will be included here. Only *Penaeus monodon* baculovirus (MBV) has been widely reported in Thailand. Other viruses are either uncommon or do not cause high losses. Alternatively, they may be overlooked when they occur. Below is a summary of recent information on viruses we have found.

MBV

We recently published a summary of our work with this virus infection in *P. monodon* in southern Thailand (Fegan et al., 1991). Although the disease is well tolerated in *P. monodon* as long as rearing conditions are optimal, its elimination from the hatchery will improve production. A recent publication by Liao et al. (1990) shows that the incidence of MBV in the hatchery can be substantially reduced by using clean sea water to wash the eggs or nauplii before they are transferred to rearing tanks. We are accumulating data to determine whether washing significantly improves the overall, long-term survival in a hatchery, but initial results concur with those of Liao et al. (1990).

Our procedure is as follows: after harvest from the hatching tank, several million nauplii are transferred by net to a 500-L fiberglass tank filled with clean, filtered sea water. The tank is fitted with an overflow filter consisting of a perforated pvc cylinder (approx. 25 cm dia. x 90 cm long), closed at one end, with an outlet line (approx. 7.5 cm dia.) at the other, and covered with 150-mesh nylon screening. The larvae are washed for 3 h at a rate of 500 L/h (total of three tank changes), before transfer to the rearing tanks.

In an effort to further control and understand this disease, we have been trying to develop a DNA dot hybridization probe for the rapid, on-site detection of MBV. Vickers et al. (1990) reported preliminary steps towards preparation of such a probe but, according the most recent issue of the Asian Shrimp Culture Council (ASCC) newsletter (issue #8, fourth quarter 1991), Dr. S.N. Chen has already developed such a kit. As a byproduct of the DNA probe effort, we now have excellent electron micrographs of MBV virions (Figs. 1-3), and these may help in the comparative characterization of MBV from various geographical locations.

Upon examining the negatively stained viral occlusion (VO) polyhedra of MBV (Figs. 1-3), we were struck by the large size of the polyhedron subunits (about 20 nm) and how they could appear to be "empty" and "full" (Fig. 1a, b; Fig. 2a, b; Fig. 3b, d, f), rather like negatively stained parvovirus (densovirus) virions (Tijssen and Arella, 1991). The VO polyhedra appeared to arise by the association of these globular polyhedron units (Fig. 2d-f; Fig. 3b, c, e) rather than the coalescence of fibers, as occurs in the insect baculoviruses (Adams and McClintock, 1991).

It is possible that the polyhedra from these MBV preparations are paracrystalline arrays of parvovirus particles with embedded baculovirus virions, and that what we see is the result of a dual infection, perhaps even involving interdependent replication of a parvovirus and a baculovirus. Further investigation of this possibility may be warranted. For example, the polyhedral subunits are close in size and shape to parvovirus virions (Adams, 1991). There are also reports of mixed or multiple infections with insect polyhedrosis

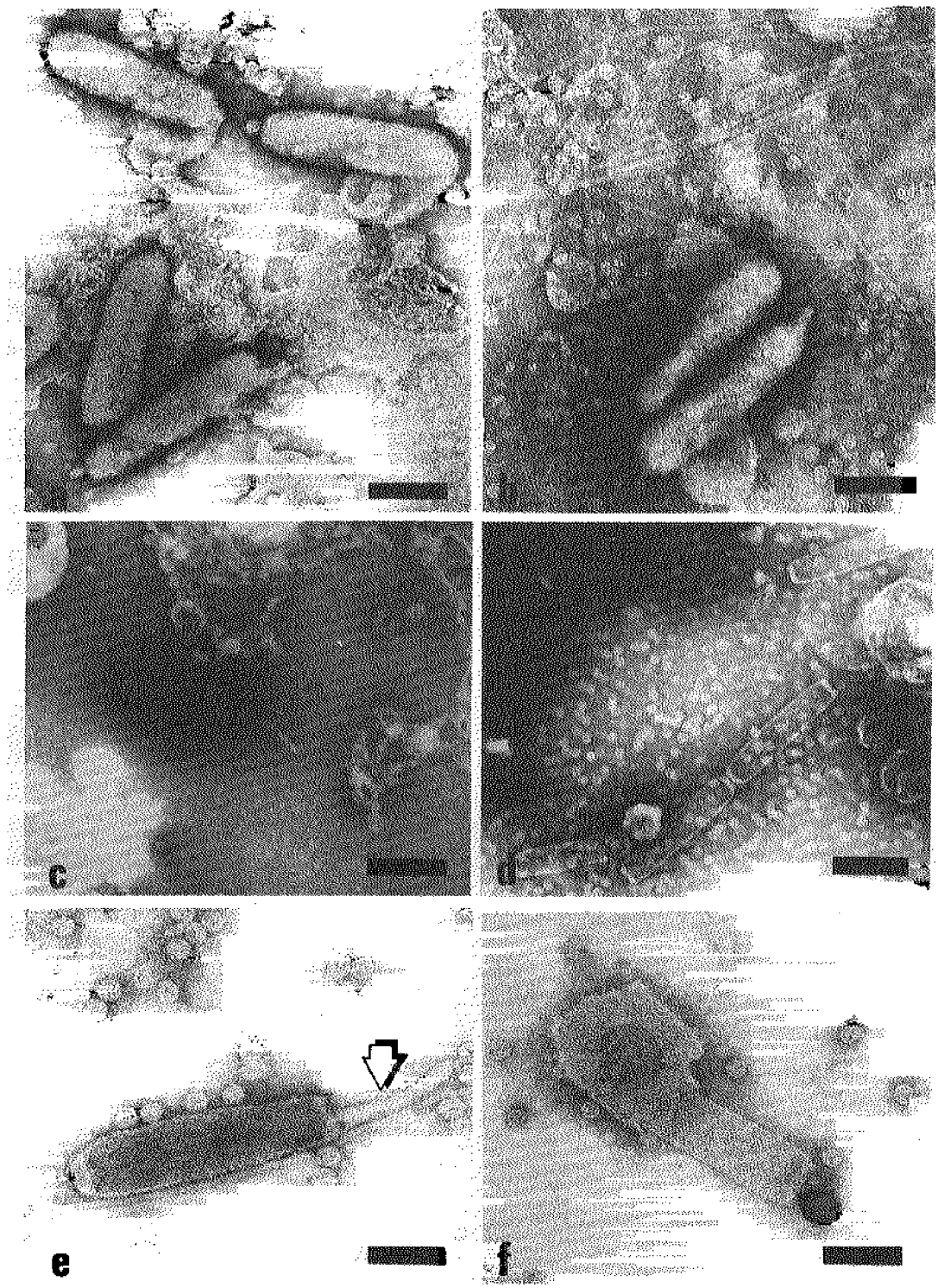


Figure 1. Negatively stained (molybdophosphate) preparations of MBV virions viewed with the electron microscope. (a) Fully enveloped virions (bar = 100 nm). (b) Fully enveloped virions accompanied by unknown filaments (bar = 100 nm). (c) Unenveloped virion with extruded filament (bar = 150 nm). (d) Empty nucleocapsids showing caps at both ends (bar = 150 nm). (e) Unenveloped nucleocapsid showing extruded filament adjacent to narrower filament of unknown origin (bar = 60 nm). (f) Nucleocapsid showing spiral arrangement of protein subunits (bar = 80 nm).

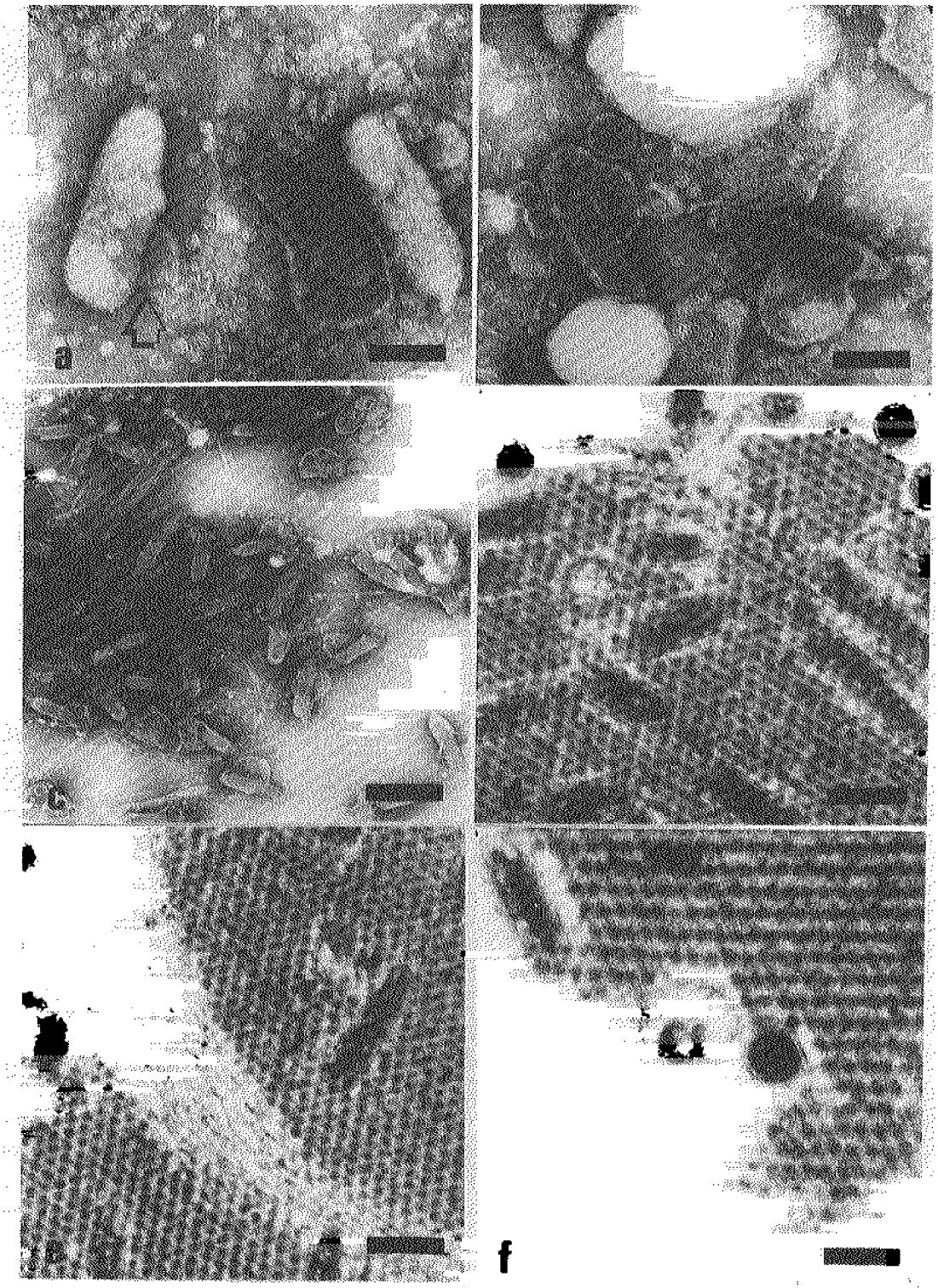


Figure 2. Negatively stained (molybdophosphate) preparations of MBV virions viewed with the electron microscope. (a) Complete and unenveloped virions. Arrow indicates partially torn envelope (bar = 100 nm). (b) Virions with torn envelopes (bar = 120 nm). (c) Low magnification of many virions with ruptured envelopes. Note also the bundles of unknown filaments (bar = 300 nm). (d-f) Transmission electron micrographs of MBV occlusion bodies. Note the presence of inserted filaments at the "fracture line" in (e) (bars = 120, 120 and 80 nm, respectively).

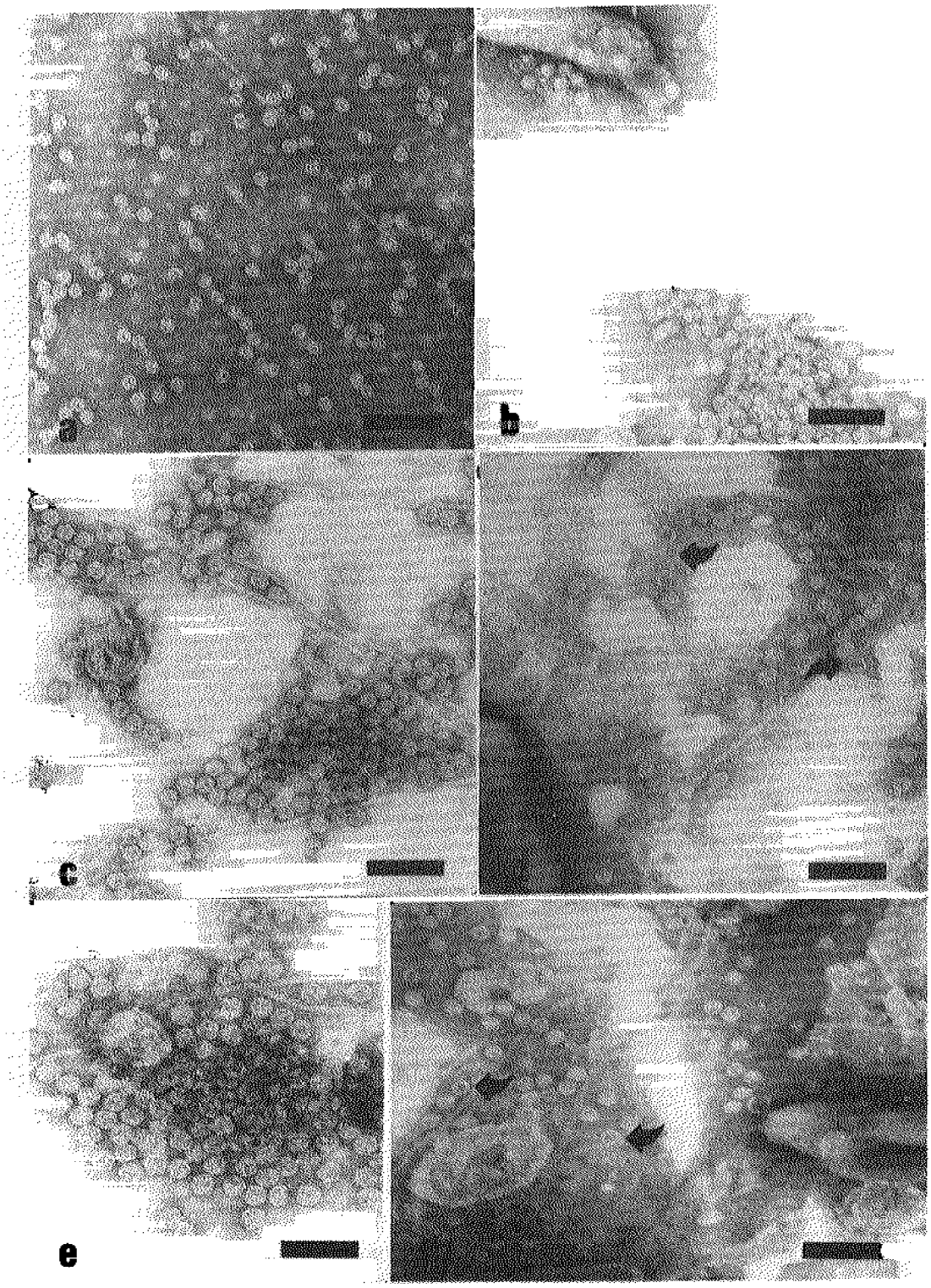


Figure 3. Negatively stained (molybdophosphate) preparations of MBV virions showing details of the polyhedron component of the occlusion bodies. Arrows indicate particles that appear "empty" (bars: a = 100 nm; b = 100 nm; c = 75 nm; d = 100 nm; e = 60 nm).

viruses (Adams and McClintock, 1991), and there are examples of dependent parvovirus (dependovirus) infections in vertebrates (Davis et al., 1990). A simple test would be to digest the virus preparations with nucleases and determine whether this treatment increases the number of "empty polyhedron subunits." Although the probability of such a dual infection is low, the ramifications would be far-reaching and, thus, it should not be discounted without proper assessment.

Lymphoid Organ Virus

Since August 1989, when we began to do histological examinations of captured broodstock to determine the frequency of MBV infection, we have noticed abnormal lymphoid organs (Fig. 4a-c) with clumps of basophilic cells scattered among the normal eosinophilic tubule tissue. These clumps of cells showed very densely staining basophilic inclusions that we first interpreted as pycnotic nuclei (Fig. 4b, c). However, a closer inspection of 1-µm, toluidine blue-stained sections, and transmission electron micrographs (Fig. 4d-f; Figs. 5, 8, 9, 10) showed that these inclusions were cytoplasmic and consisted of amorphous material (probably in secondary phagosomes) next to greatly enlarged nuclei. We propose that the phagosomes arose from the digestion of virions and virogenic stromata that had earlier developed near nuclei of "normal" tubule cells (Figs. 6, 7). Although the abnormal basophilic cells showed inflated nuclei, they did not appear to be necrotic in transmission electron micrographs and were uninucleate rather than multinucleate (i.e., each cell was characterized by a distinctive cell membrane separating it from other cells in the clump).

Virions and virogenic stromata were seen in electron micrographs (Figs. 6, 7) of lymphoid organ tubule cells that appeared to be normal by the light microscope in H&E - stained sections, and were adjacent to the abnormal basophilic regions. The virions were nonenveloped, approximately 40 nm in diameter and located around virogenic stromata adjacent to noninflated nuclei. Often, multilamellar structures consisting of concentric layers of membranes were located next to the virogenic stromata.

This lymphoid organ abnormality was found in 60/67, or 90%, of randomly selected broodstock specimens examined between August 1989 and December 1991 (9 different sets ranging from 4 to 11 animals, each including approximately equal numbers of males and females). The animals were regular production broodstock taken at the end of their usefulness for the hatchery, so they were outwardly healthy animals and the occurrence of the abnormal tissues could not be linked to any identifiable disease condition.

The description of the H&E staining reaction for our abnormal lymphoid organ material is very similar to that for abnormal lymphoid organs described by Owens et al. (1991) in Australian penaeids. However, the detailed mor-

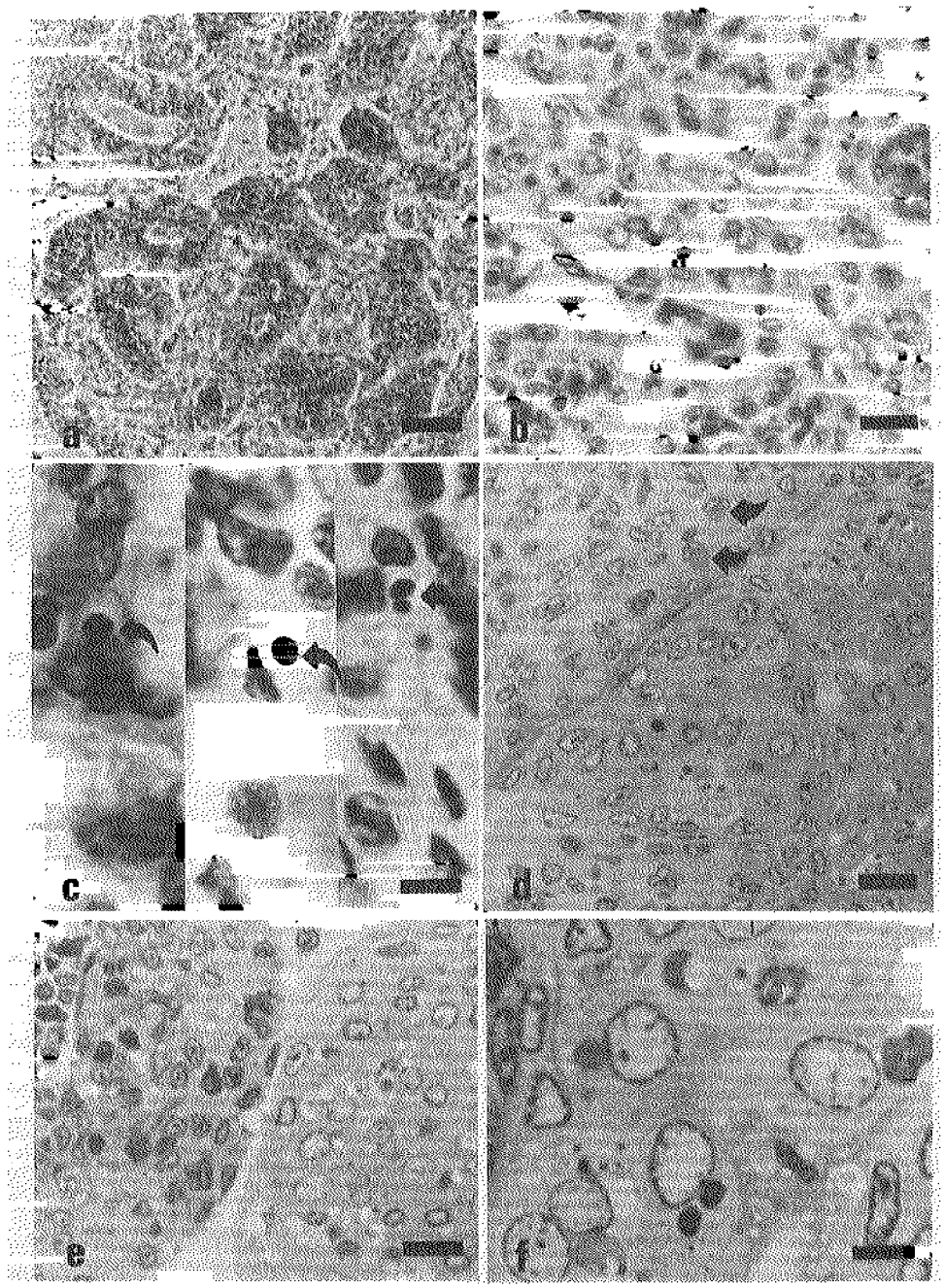


Figure 4. Light microscope photographs of abnormal lymphoid organs from healthy broodstock of Penaeus monodon. *(a) Low magnification view of an H&E-stained tissue section showing normal tubules separated by abnormal, solid clumps of cells with enlarged nuclei. These abnormal areas have a more basophilic staining reaction than the normal tubule tissue (bar = 50 μm). (b) Higher magnification of the same tissue showing deeply basophilic inclusions in the abnormal cells (bar = 12.5 μm). (c) High magnification of the basophilic inclusion bodies showing clearly that they are cytoplasmic (bar = 5 μm). (d) Toluidine blue-stained, thin section of cells with hypertrophic nuclei next to tubule cells with nuclei of normal size. Arrows indicate differentially stained areas next to "normal" nuclei (bar = 12.5 μm). (e) As in (d), except that a tubule lumen is visible to the left (bar = 12.5 μm). (f) As in (d) but a higher magnification clearly showing the hypertrophied nuclei and inclusion bodies that are cytoplasmic and have a variable appearance (bar = 5 μm).*

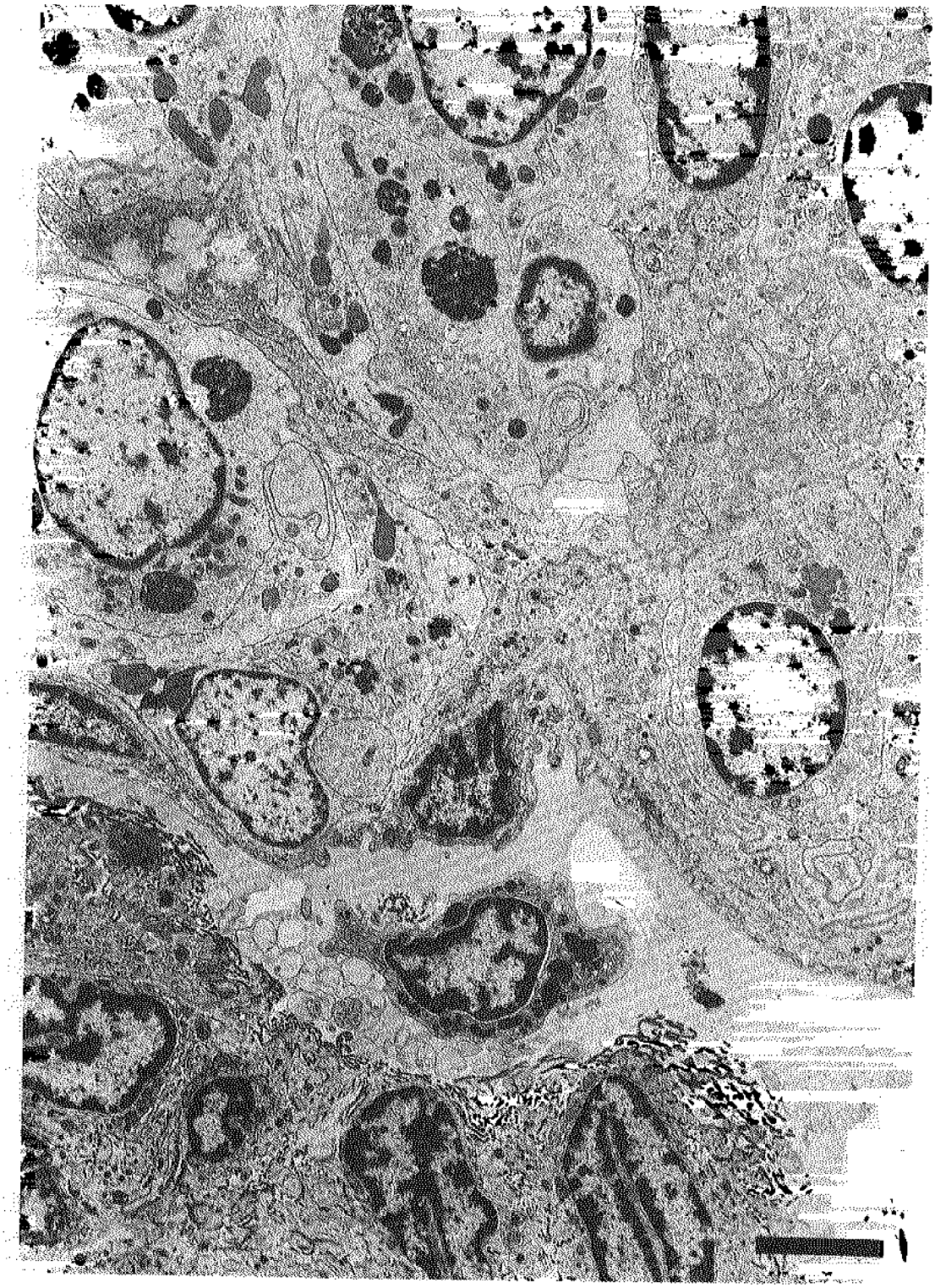


Figure 5. Transmission electron micrograph of an abnormal lymphoid organ from healthy broodstock of Penaeus monodon. *The cells with small indented nuclei in the lower part of the photograph are from a region of tissue that appears normal with the light microscope. In the upper part of the picture are round, hypertrophied nuclei typical of the areas that appear basophilic in H&E preparations (bar = 4* μm).

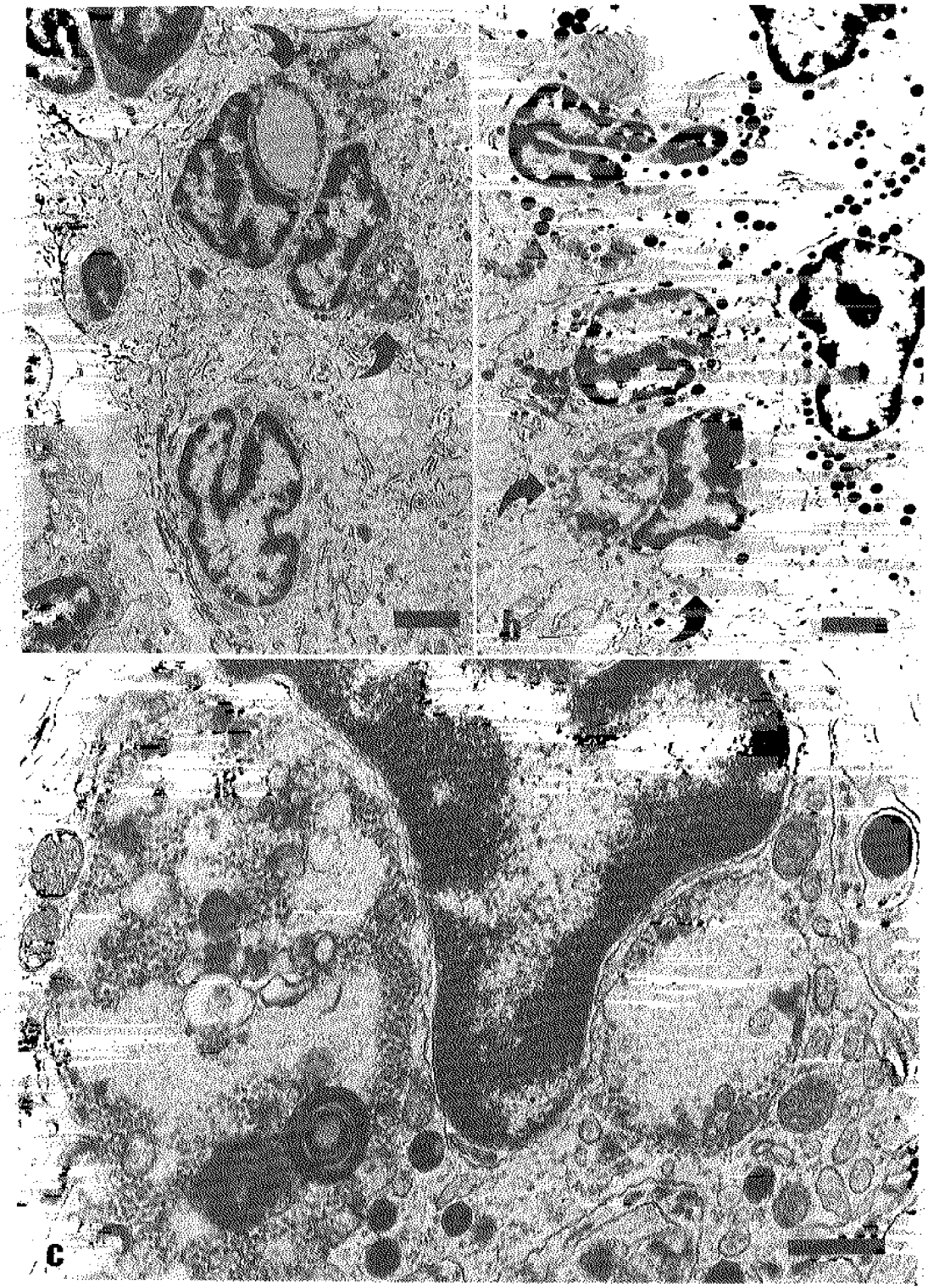


Figure 6. Transmission electron micrographs of an abnormal lymphoid organ from healthy broodstock of Penaeus monodon. *The cells shown were from tubules that appeared normal in H&E preparations, but here show cytoplasmic virogenic areas adjacent to the nuclei. (a-b) Cells showing two virogenic areas each (bars in both = 2 μm). (c) High magnification of the lowest nucleus in (b), clearly showing two virogenic areas next to the nucleus. Note the multilamellar membrane structures (bar = 0.6 μm).*

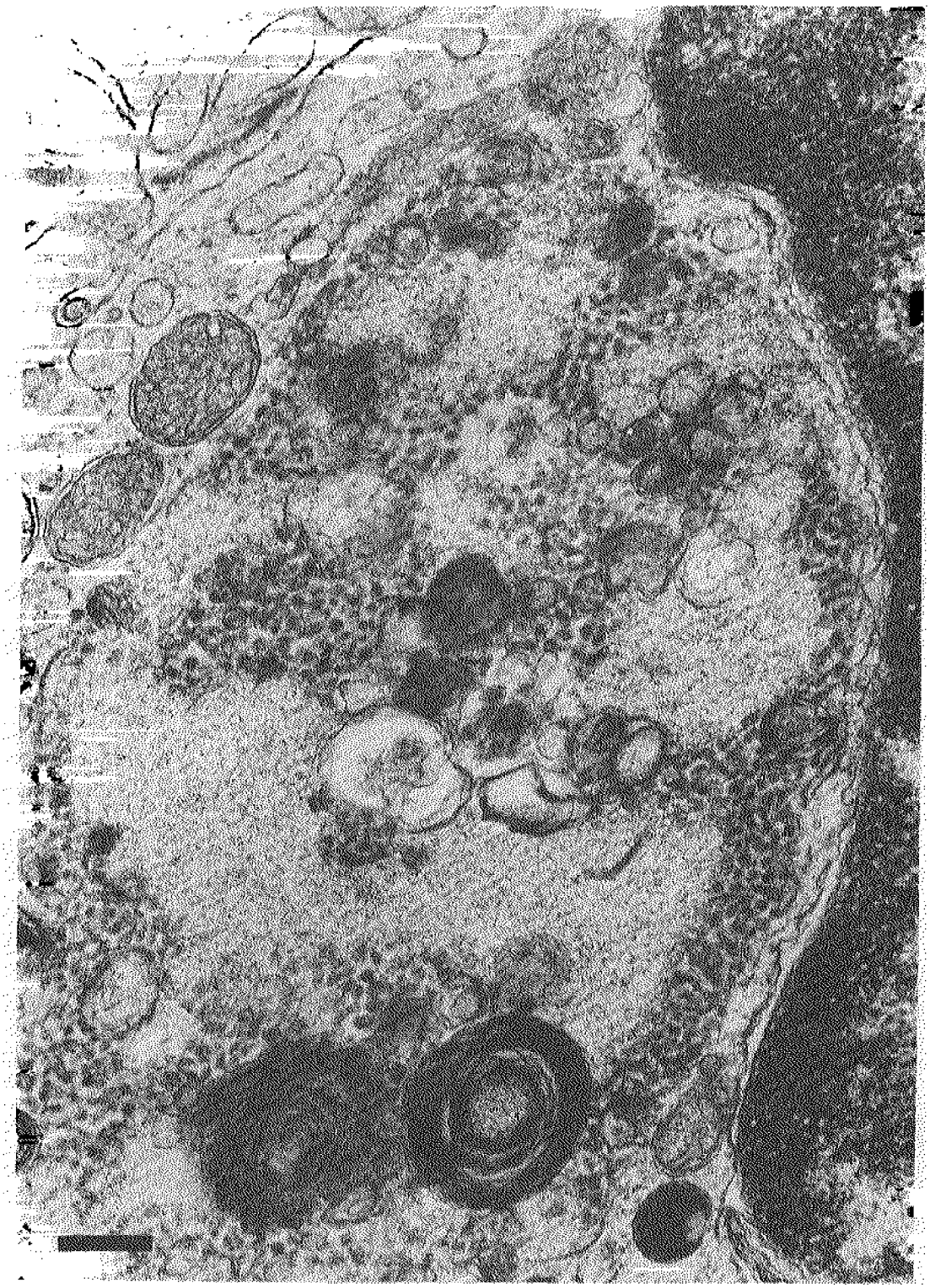


Figure 7. High magnification of a virogenic area from the cytoplasm of the cell in Figure 6c clearly showing unenveloped virions approximately 40 nm in diameter (bar = 300 nm).

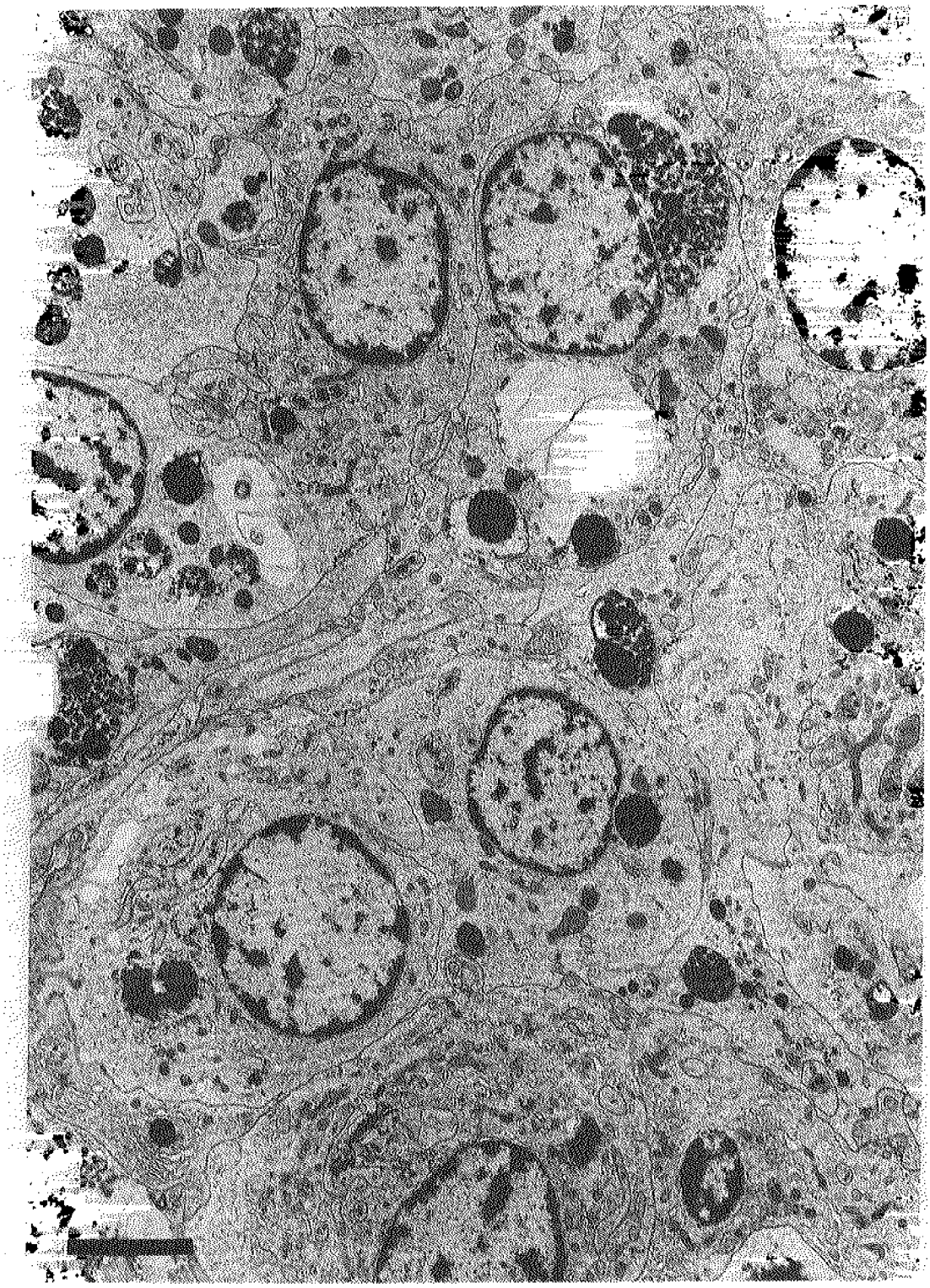


Figure 8. Low magnification of abnormal lymphoid organ tissue from healthy broodstock of Penaeus monodon. *Note the hypertrophied nuclei and the variety of different types of cytoplasmic inclusions that resemble secondary phagosomes (bar = 4 µm).*

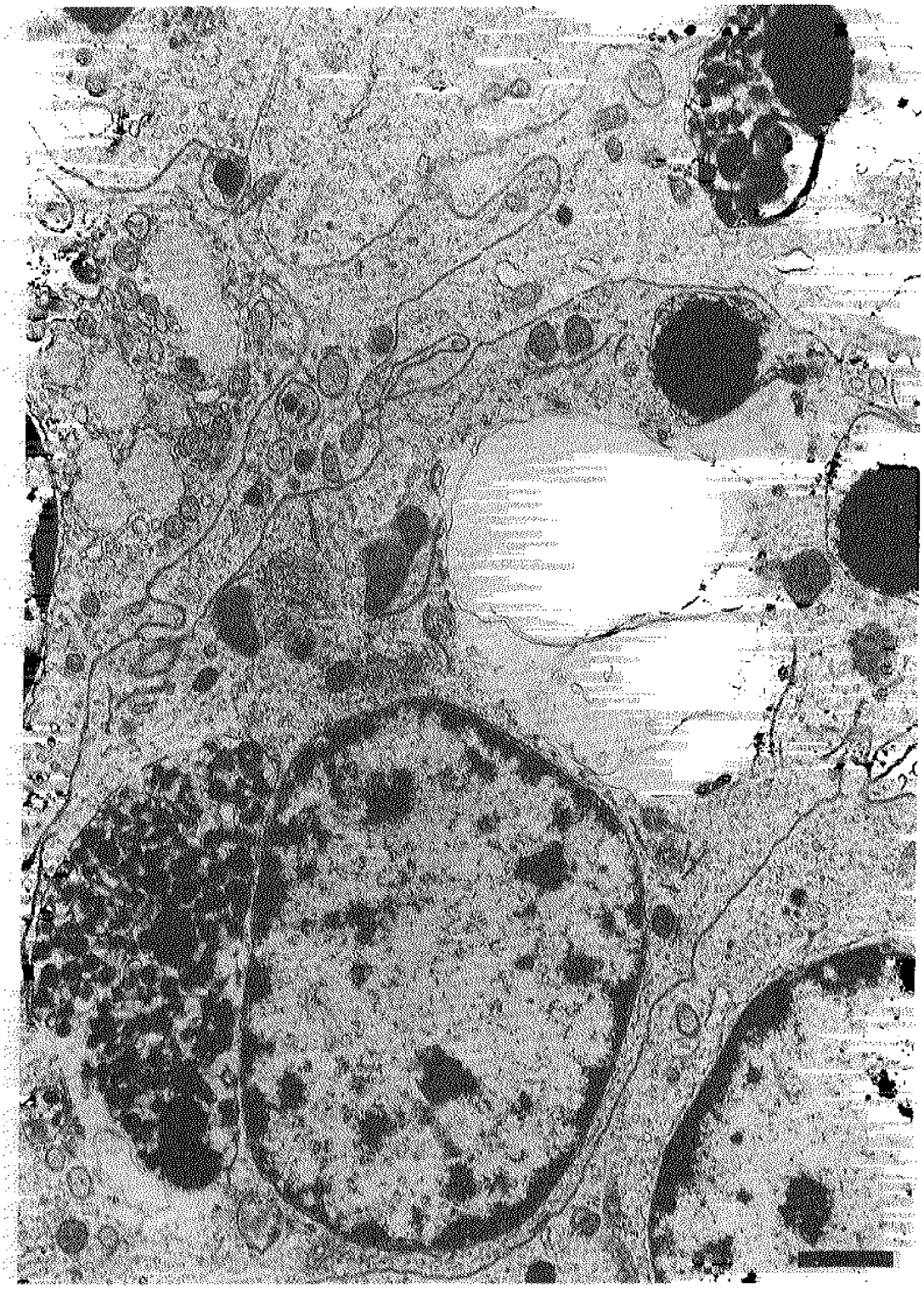


Figure 9. High magnification of a portion of Figure 8 showing two hypertrophied nuclei and a variety of inclusions in a single cell (bar = 1.2 μm).

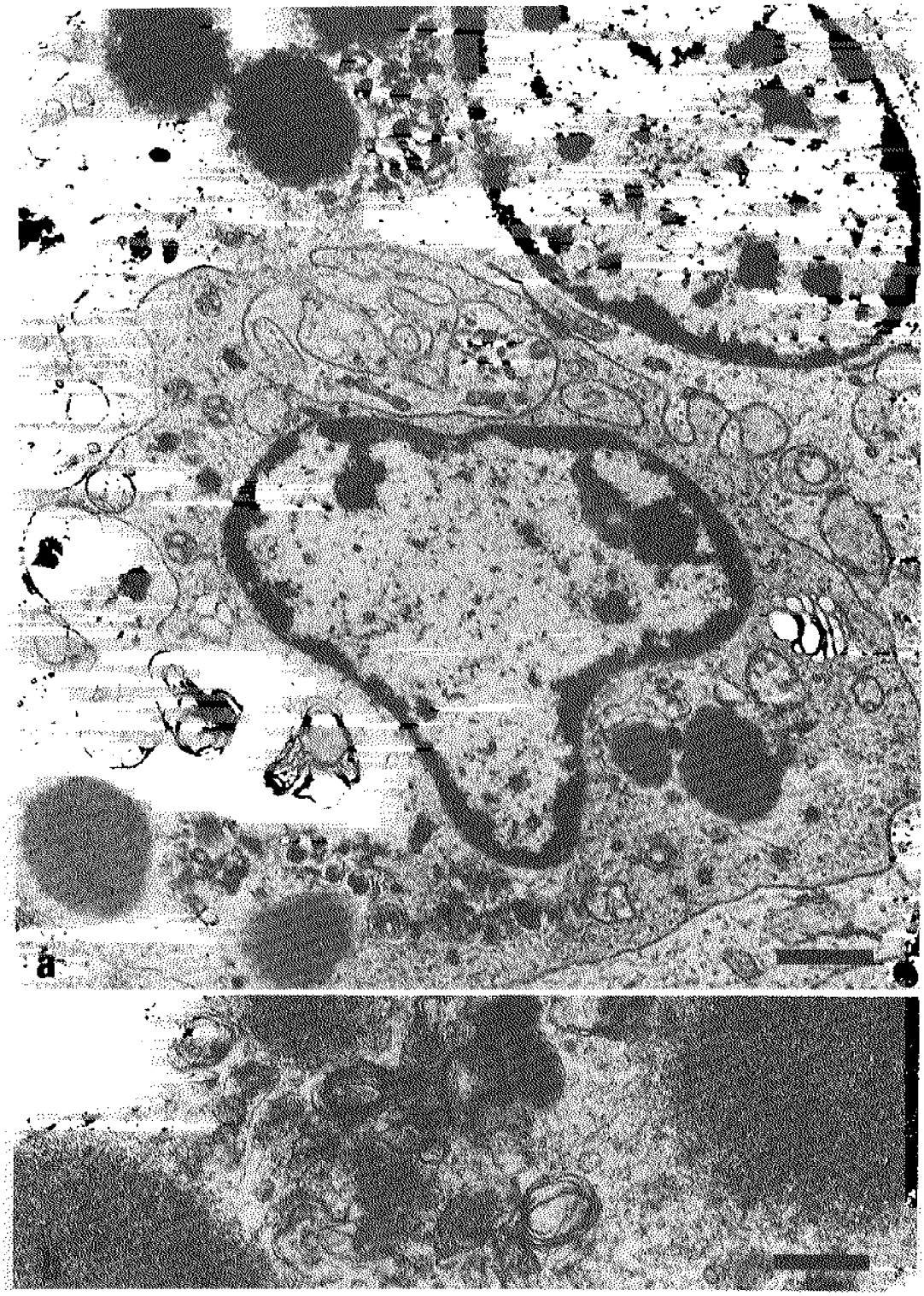


Figure 10. High magnification of hypertrophied nuclei showing (a) what may be incompletely synthesized viral material adjacent (bar = 1.2 μm). (b) High magnification of the "viral" material in (a) (bar = 0.3 μm).

phology of our material differs markedly from that reported from Australia. For example, the inclusions in our material are cytoplasmic rather than nuclear and the virions are much larger (40 nm in our specimens as opposed to about 20 nm in the Australian specimens). Thus, it appears that different viruses may give rise to superficially similar staining reactions in lymphoid organ tissue sections, and that a careful examination may be necessary to distinguish between them.

As to the nature of the virus in our Thai specimens, we may assume that it is an RNA virus because of its cytoplasmic location, but it is difficult to relate it to other unclassified viruses reported for crustaceans (Bonami and Lightner, 1991). If size is any indicator, it may be a picornavirus or a reovirus.

Although we could not relate the abnormal organs to a disease condition in the broodstock, we are concerned that the virus may be carried by the broodstock in a chronic infection, be passed on to the larvae, and cause problems later in the production cycle (e.g., see section below on yellow-head shrimp). More work is required to characterize the virus and to determine whether it has any impact on production.

IHHNV

Although infectious hematopoietic and hypodermal necrosis virus (IHHNV) has been reported from *P. monodon* (Sindermann and Lightner, 1988; Brock, 1991), we have found no sign of it (Cowdry A bodies) in specimens of diseased pond shrimp in the past four years. However, in one recently completed set of vitamin C feeding trials (proprietary company study), healthy shrimp were fixed and examined histologically at the end of the trial, and some of the animals (15/49) were found to have typical Cowdry A bodies in the antennal gland only. The incidence appeared to be higher in animals that had been given vitamin C polyphosphates, but the number of animals examined histologically for each group was too small to make statistical comparisons. We will repeat this trial with test animals receiving vitamin C polyphosphate at 500 ppm in feed (five times the maximum dose in the previous trial) and control animals receiving no supplementary vitamin C. We will include sufficient numbers of animals to allow statistical analysis.

HPV

In a preliminary vitamin C trial similar to that described in the previous section, five of eleven animals examined histologically showed distinctive basophilic (H&E staining) and Feulgen-positive inclusion bodies in the nuclei of cells in the hepatopancreatic epithelium (especially E cells). These resembled typical inclusions of hepatopancreatic parvo-like virus (HPV) (Sindermann and Lightner, 1988; Brock, 1991). As with the trial described above, these shrimp were normal for growth, external appearance, and behavior, and would not have been subjected to disease examination. Inclusions were

found in both test and control animals, and the numbers examined were too small to make any conclusion about the relationship between vitamin C dose and appearance of the inclusion bodies. This is the only occasion where we have seen HPV in histological preparations from southern Thailand.

Bacteria

Vibriosis in Growout Shrimp

As stated previously, most of the shrimp mortalities we see are caused by bacterial septicemia that has resulted either directly or indirectly from relatively long-term stress. It is possible that antibiotic treatment will help with these infections, but only for the interval during which the cause for the underlying stress is removed. If the stress is not alleviated, the treatment will fail. This is important when one considers the recommended withdrawal periods for antibiotics. For example, withdrawal periods recommended in Thailand for oxytetracycline and oxolinic acid in shrimp are 14 days and 20 to 30 days, respectively (Limsuwan, 1991), so one must be able to carry on with the cultivation for at least this period after drug administration. Obviously, the stress must be removed if this is to be accomplished.

Even if all of these conditions can be met, it may be difficult to proceed. We have found, for example, that individuals with septicemia collected from a single pond can be infected with different species of bacteria (unpublished). If one elected to carry out antibiotic treatment in such a case, it would necessitate doing sensitivity tests with each of the isolates in order to find an effective antibiotic(s) for all.

The use of antibiotics by shrimp farmers is not strictly regulated by the Thai government. Most antibiotics are widely and freely available and often promoted by unscrupulous salesmen who do not care whether they are effective. Fortunately, most of the *P. monodon* cultivated in Thailand is purchased by frozen-storage plants and sold by export agents who have their own screening procedures for antibiotic residues. Thus they protect themselves against potential rejection of shipments upon inspection by importing countries such as Japan and the United States. After a few Thai farmers had their crops rejected for export by frozen-storage plants, they had to sell them on the local market at a small fraction of the premium price for export-quality shrimp. The word traveled fast, and the result has been a sharp reduction in the indiscriminate use of these drugs.

The species of bacteria most often isolated from moribund shrimp are *Vibrio* species. Ruangpan and Kitao (1991) indicated that approximately 83% of isolates from 204 diseased shrimp comprised *V. parahaemolyticus* (47%) and *V. vulnificus* (36%). The following table (Table 1) was prepared with data drawn from Kongsom (1991). It gives details of antibiotic sensitivity testing with bacteria isolated from diseased

Table 1. Antibiotic sensitivity of bacteria isolated from diseased *Penaeus monodon* in Thailand.

Bacterial Strain	Amp. 10	Strep. 10	Oxy. 30	Ery. 15	Poly. B. 300 u	Chlor. 30	Suff./Tri 1.25	Nitro. 200	Nali. 30	Multip.
V. algin.	1/0/0	1/0/0	0/0/1	1/0/0	0/1/0	0/0/1	0/0/1	0/0/1	0/0/1	1/1
V. angui.	0/0/10	9/1/0	7/0/3	8/2/0	2/0/8	0/0/10	0/0/10	0/0/10	0/2/8	9/10
V. harv.	3/1/1	3/2/0	4/1/0	5/0/0	4/0/1	0/0/5	0/0/5	0/0/5	0/0/5	5/5
V. mari.	1/1/0	2/0/0	1/0/1	2/0/0	0/0/2	0/0/2	0/0/2	0/0/2	0/0/2	2/2
V. para.	4/0/0	3/1/0	2/3/0	1/4/0	5/0/0	0/0/5	0/0/5	1/2/2	0/0/5	5/5
V. vul.	2/0/2	2/2/0	1/2/1	2/2/0	1/1/2	0/0/4	0/0/4	0/0/4	0/0/4	3/4
V. sp.	9/0/10	15/4/0	12/3/4	13/5/1	4/1/14	0/0/19	0/0/19	1/5/13	0/0/19	17/19
Subtotal	20/2/23	35/10/0	27/9/10	32/13/1	16/3/27	0/0/46	0/0/46	2/7/37	0/2/44	42/46
Aer. sp.	1/0/1	2/0/0	2/0/0	1/1/0	0/0/2	2/0/0	2/0/0	0/1/1	0/0/2	2/2
P. shig.	0/0/3	3/0/0	2/1/0	2/1/0	1/2/0	0/0/3	0/0/3	0/1/2	0/0/3	3/3

Numbers indicate the number of strains Resistant/Intermediate/Sensitive, based on standard tables (Becton Dickinson, Antimicrobial susceptibility testing: a system for standardization, 1985) for diameter of inhibition zones in Mueller-Hinton agar (NaCl 2.5%) around paper discs impregnated with the quantities of antibiotics indicated (µg/disk except for polymixin B given as units). Also given is the number of strains with multiple resistance (i.e., resistance to more than one antibiotic) (#/total) and the subtotal for all the *Vibrio* isolates.

Note: V. = *Vibrio*; algin. = *alginolyticus*; angui. = *anguillarum*; harv. = *harveyi*; mari = *marinus*; para. = *parahaemolyticus*; vul. = *vulnificus*; Aer. = *Aeromonas*; P. shig. = *Pleisiomonas shigelloides*; amp. = ampicillin; strep. = streptomycin; oxy. = oxytetracycline; ery. = erythromycin; poly. B = polymyxin B; chlor. = chloramphenicol; suf./tri. = sulfamethoxazole 23.75/trimethoprim 1.25; nitro. = nitrofurantoin; nali. = nalidixic acid.

shrimp in Thailand, showing that the incidence of multiple antibiotic resistance was rather high. A similar pattern of sensitivity/resistance was found for the luminous hatchery pathogens *V. harveyi* and *V. splendidus* in the Philippines (Baticados et al., 1990). Dixon et al. (1990) have also found a high incidence of antibiotic resistance in *Aeromonas* species in tropical fish from Singapore.

In addition to mortality caused by bacteria, there are bacterial infections that cause black discoloration of the shrimp carapace and erosion of the appendages and the telson. These imperfections can reduce the sale value of the shrimp, and they are described in the references given in the introduction to this paper. These superficial imperfections are usually sloughed off with the molted exoskeleton and, again, their presence in significant quantity is an indication of underlying stress that is interfering with the regular molt cycle.

One syndrome that has been reported for shrimp in Thailand by Limsuwan (1988, 1991) is called "*sien dum*" in Thai, which translates to "black splint" in English. The gross appearance of the lesions is tough, black filaments up to 2 mm or more in width that arise just under the carapace in the tail muscle segments and penetrate inwards, usually through the connective tissue between body segments, producing finer

branch filaments in a rootlike fashion as they progress inwards. These filaments are very ugly, persist after cooking and make the shrimp unmarketable.

According to Limsuwan (1988), this disfiguration is caused by *V. vulnificus*, and according to Ruangpan and Sae-Oui (1988), infection occurred only during periods when the salinity in the rearing ponds dropped below 10 ppt. Although lesions could be found in shrimp from ponds at higher salinities, the authors reported that pond histories showed an earlier period of exposure to 10 ppt or less. They proposed that infections occurred during the low salinity period but were overlooked. Progression after the salinity was restored to higher levels eventually resulted in the gross symptoms. According to Limsuwan (1991), this disease can be treated with oxytetracycline at 2 - 3 g/kg feed for 7 to 10 days.

Another symptom we have observed is black lymphoid organs (Oki organs) in some pond-reared shrimp. Histological examination revealed that the animals have extensive bacterial septicemia caused by *Vibrio* species (unpublished).

The types and doses of antibiotics commonly used in shrimp ponds in Thailand have been reviewed by Tonguthai and Chanratchakool (1992). Recommended withdrawal periods are sometimes shorter than those used in more temperate countries such as Japan (Aoki, 1992), and it would be better if the periods were given in degree days.

Possible alternative treatments to antibiotics include the prophylactic use of vaccines (Itami et al., 1989; Song and Sung, 1990; Adams, 1991; Sung et al., 1991), immunostimulants (Robertsen et al., 1990; Raa et al., 1992; Nikl et al., In press) and probiotics (Asian Shrimp Culture Council Newsletter, issue #8, fourth quarter, 1991). Any of these approaches would be superior to the use of antibiotics, but some have not yet been tested with shrimp and those that have are still in the experimental phase.

Vibriosis In the Hatchery

Although we believe that vibriosis in growout shrimp arises as a secondary or tertiary phenomenon through stress, we are still uncertain whether *Vibrio* spp. can be infectious in the hatchery. There are many reports that they can, but the great sensitivity of the larvae to nutritional and environmental changes and toxic substances, makes it difficult to eliminate them as the underlying cause of infection. The species causing difficulties are the same as those given for growout ponds above, but special attention is often paid to the luminous species *V. harveyi* and *V. splendidus* (Baticados et al., 1990).

After reading the publication by Adams (1991) on the exposure of *P. monodon* to a bath of killed *Vibrio* cells, we wondered whether shrimp (including larvae) were continuously invaded by whole bacterial cells at a fixed rate. If so, high total numbers of bacterial cells in the bathing water would easily swamp the larval hemocytes, and even

if only a small fraction of the total count consisted of pathogenic *Vibrio* cells, the results would be disastrous. To test this possibility, we made daily bacterial counts (marine TSA plate counts and marine TCBS counts) in eight hatchery tanks over 15 days and recorded percent changes in larval survival during the following 24-h period after the count. The results are shown in Figures 11 and 12. We found no correlation between survival and total count, *Vibrio* count or a combined index of the two counts (i.e., total count x *Vibrio* count). From this preliminary test, we feel that the hypothesis is flawed or that other factors in the rearing are more critical to larval survival.

Treatment of *Vibrio* infections in Thai hatcheries usually consists of adding antibiotics directly to the rearing water. The antibiotics used are similar to those employed in growout ponds, although the quantities are usually lower. Even chloramphenicol (officially banned for use by the Thai government) is readily available. Because of uncertainty with respect to potency, we test all new lots of antibiotics for safety levels with larvae and for antibacterial activity. In Thai facilities such as ours, use is controlled by qualified personnel and is permitted only when necessary, and only after isolation of a disease organism followed by sensitivity testing and determination of the minimum lethal dose. We also prohibit the use of chloramphenicol. In other facilities, antibiotics are used indiscriminately (often for routine "prophylaxis") by unqualified operators, who use them at low doses to "slow down the bacteria but not harm the weak larvae." This is a formula for rapid development of resistant strains.

Other Bacteria

Although *Vibrio* spp. are the most common cause of problems in the hatchery, we have also had difficulties with *Aeromonas* sp. during periods of concomitantly high salinity (around 35 ppt) and high temperature (around 34°C). The problems disappeared when conditions returned to optimum (30 ppt and 30°C).

At some intervals during hatchery operation, we have found zoea with abundant quantities of sessile bacteria attached to their fine feeding appendages. We have not further isolated or characterized these bacteria. They are fouling organisms that can be seen easily with the phase contrast microscope (Fig. 13), and they are a good early indicator of a problem in the hatchery tank. If the underlying cause is not removed, massive mortalities can follow within one or two days. Underlying causes can be nutritional deficiencies, toxicity, lack of water exchange, etc.

Fouling by filamentous bacteria in the hatchery and in growout ponds occurs in Thailand as reported elsewhere (Sindermann and Lightner, 1988; Brock, 1991). The occurrence of these organisms on the shrimp is associated with poor water quality (especially high organic nutrient content) concomitant

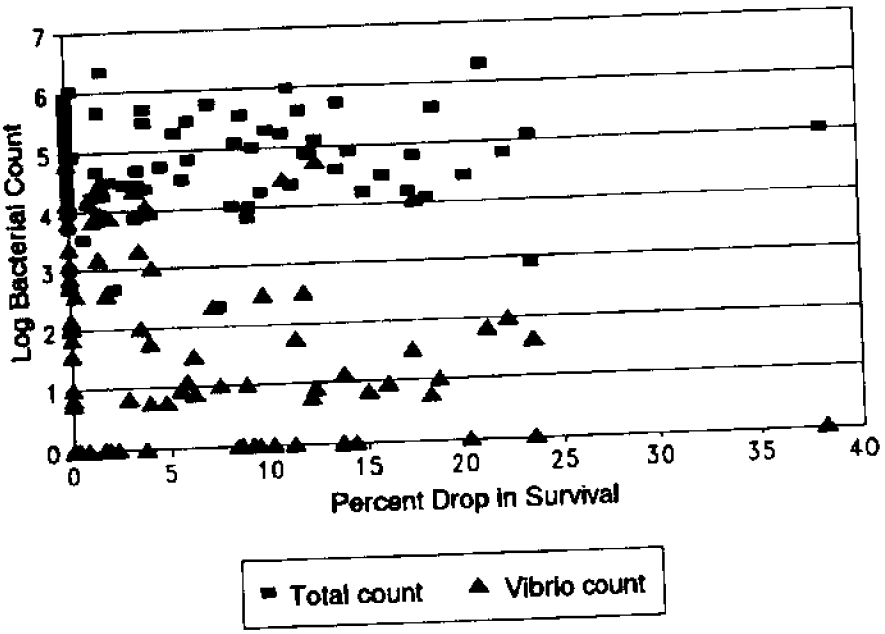


Figure 11. Relationship between log10 of the daily total bacterial count (TSA marine agar) and the "Vibrio" count (TCBS agar), to the drop in survival of shrimp larvae during the succeeding 24-h period. The counts (triplicate spread plates) were carried out daily over a period of 15 days for 8 production tanks. TCBS counts of 0 were converted to 1 and are shown on the graph as 0.

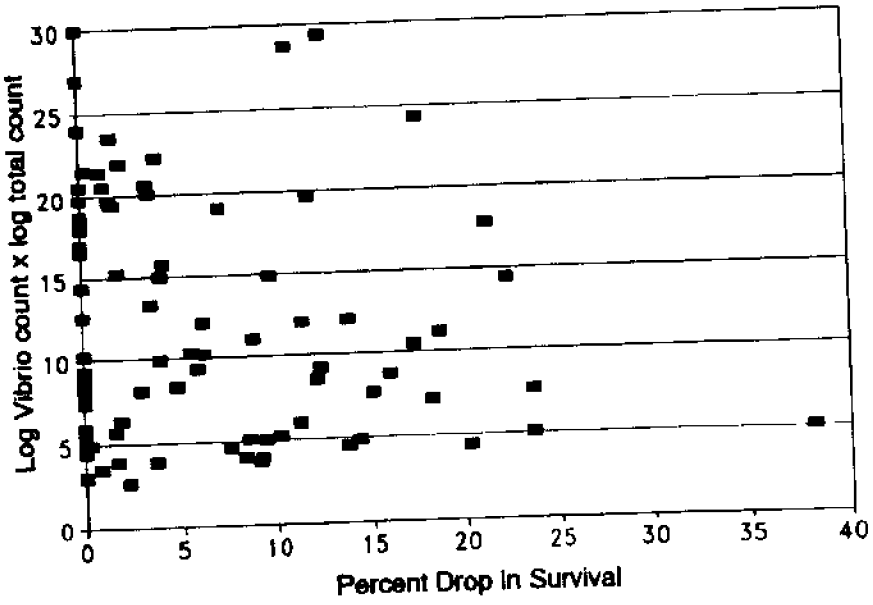


Figure 12. Relationship between a combined index of TSA count and TCBS count to the drop in larval survival during the following 24-h period. The data is the same as in Figure 11, except that the counts were combined to obtain an index calculated as (log of the TSA count) x (log of the TCBS count + 1). TCBS counts of 0 were converted to 1, giving log = 0.

with reduced molting frequency. The best treatment is to improve the water quality (water exchange) and this often induces molting.

Fungi

Oomycetes

We sometimes have difficulties with *Lagenidium* sp. in the hatchery, but not with other oomycetes reported to infect shrimp (Sindermann and Lightner, 1988; Brock, 1991). The source of the outbreaks has not been determined, but when they occur they can be handled easily by the administration of trifluralin (Treflan) at 10 ppb directly to the rearing water every four hours for as long as the problem persists. However, the report by Gil-Turnes et al. (1989) suggests that it may be possible to use a probiotic bacterium to solve the problem of oomycete infections. They found that embryos of *Palaemon macrodactylus* were protected from *Lagenidium* sp. infections by a species of *Altermonas* commensal on the embryo surface.

We have not yet found an example of loss caused by these fungi in growout shrimp.

Other Fungi

Fusarium sp. infections have been reported to afflict pond-reared shrimp in Thailand (Sindermann and Lightner, 1988; Brock, 1991; Limsuwan, 1991), but losses have been low and the infections are clearly precipitated by unfavorable rearing conditions. The problems resolve when these conditions are improved.

Parasites

External Parasites

The external parasites most commonly encountered in Thailand are stalked protozoans such as *Zoothamnium* sp., *Epistylis* sp., *Vorticella* sp. and *Acineta* sp. (Sindermann and Lightner, 1988; Brock, 1991). As with the filamentous bacteria, they are fouling organisms related to poor water quality and re-

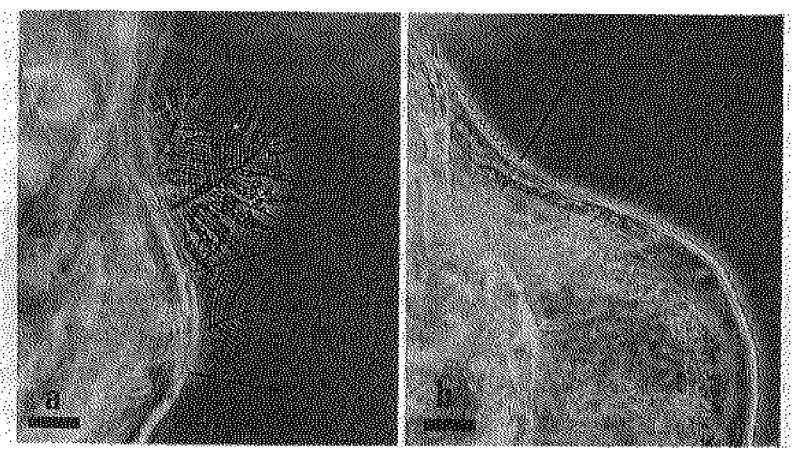


Figure 13. Fresh mount of zoea larvae showing unidentified fouling bacteria on the feeding appendages of Penaeus monodon. *(a) Fouled appendages (bar = 25 µm). (b) Normal appendages (bar = 25 µm).).*

duced molting frequency and treatments are similar.

We have found only malachite green at 0.05 ppm to be useful against these organisms for early larval stages. Effective doses for postlarvae and older stages are harmful to earlier larval stages; the earlier the stage, the more harmful the treatment. Compounds we have tested include Formalin, chloramine T, benzalkonium chloride and povidone iodine.

Internal Parasites

The only internal parasite that has caused significant losses in cultivated penaeids in southeastern Thailand is the microsporidian, *Agmasoma penaei* (Flegel et al., 1992). The parasite causes infections in both *Penaeus merguiensis* and *P. monodon*, but it is especially prevalent in *P. merguiensis*, which seems to have a high incidence of infection at all times, in reared and captured animals. By contrast, high levels of infection in *P. monodon* seem to be related to high rainfall. For both species, high levels of infection seem to occur only in farms on the southwest Gulf of Thailand in the area of Songkhla. They have not been reported from the southeastern Gulf in shrimp farms around Chantaburi, even though rainfall there is seasonally very high. The most likely reason for the difference is absence of the unknown intermediate host on the southeastern coast.

We would like to have a chemotherapy for this infection; hence, we have been trying to test a number of coccidiostatic drugs that are commonly used with poultry. The problem with this program is that we have not found a method of artificial infection and so we are limited by the availability of animals of suitable infection state from ponds or from the wild. As a result, the work is progressing slowly.

In the meantime, one Ph.D. student at Mahidol University in Bangkok has isolated and purified sporal DNA of *Agmasoma penaei* from both *P. merguiensis* and *P. monodon*, and he has identified sequences that we believe will be useful in preparing a DNA probe to help trace the life cycle. He is also comparing the variable region of ribosomal DNA from the two isolates to help determine whether the infections are caused by a single species of parasite.

Environmental Factors

Pesticides

In May 1990, we were informed that rice farmers in Thailand sometimes use compounds to eradicate freshwater crabs that attack rice seedlings. Since shrimp farms are often located in rice-growing areas, we became concerned that these compounds might possibly harm farmed shrimp.

From a local vendor in Songkhla province, we obtained two retail products sold to kill crabs in rice fields. One of these carried the following statement on the label, "absolutely guaranteed to

Table 2. Effect of a single addition of cypermethrin (µg/L) on survival of *P. monodon* postlarvae in a 24-hour test in 1-L beakers. Control animals showed no mortality.

Concentration		% Mortality	Time
µg/L	ppm/ppb/pptr		
10,000	10 ppm	100	<10 min
5.000	5 ppm	100	<10 min
1,000	1 ppm	100	<10 min
500 - 1	500 - 1 ppb	100	<1 h
0.5 - 0.01	500 - 10 pptr	100	<4 h
0.005	5 pptr	100	<24 h
0.001 or less	1 pptr or less	none	24 h

kill crabs in rice fields." Both of these products contained well known insecticides as active ingredients. One contained methyl-parathion; the other, cypermethrin. The methyl-parathion was recommended for use as a 1-ppt (active ingredient) mixture with cooked rice bait, while the cypermethrin preparation was recommended for use as a spray solution containing 200 ppm of active ingredient. We immediately carried out aquarium trials with postlarvae of *P. monodon* and found that these compounds were extremely toxic.

We wondered whether such compounds might be causally related to the recent occurrence in Thailand of unexplained mortality that is commonly referred to as "one month death syndrome." This syndrome refers to the sudden catastrophic death of juvenile shrimp of approximately 1 - 3 g about one month after stocking in growout ponds (see section below). To date, no specific cause for this syndrome has been found, but many Thai scientists suggest that it results from one or more unknown environmental stressors that leads to death from a variety of secondary infections.

Cypermethrin

The first test with cypermethrin was conducted using 20 postlarvae in 1-L beakers with various concentrations of active ingredient, from 10 ppm to 0.0001 ppb. Survival over 24 h was recorded to obtain some idea of the range of toxicity. The results are shown in Table 2. Clinical symptoms for the affected animals were restlessness, swirling with uncontrolled movement, swimming to the surface followed by sinking to the bottom of the tank, muscle cramps and death. These behavioral changes suggested involvement of the nervous system in the cause of death.

The results showed that cypermethrin was extremely toxic for larvae of *P. monodon*. Because of this, a second test was conducted to determine the effect of sublethal concentrations of this insecticide (i.e., active ingredient concentra-

Table 3. Survival of *P. monodon* juveniles (1 - 3 g fresh weight) upon exposure to sublethal concentrations of cypermethrin (ng/L) for 10 days in 20-L aquaria. Trials were carried out in triplicate but the control consisted of only one tank.

Concentration		% Mortality
ng/L	pptr	
1.0	1.0	50 ± 10
0.5	0.5	15 ± 11
0.1	0.1	10 ± 3
0	0	15

tions of 1 pptr or less) on juvenile shrimp.

In this 10-day trial, 1- to 3-g juvenile shrimp (the size reached approximately 1 month after stocking in growout ponds) were kept in 20-L aquaria containing 20 animals each. Concentrations of cypermethrin used were 1.0, 0.5 and 0.1 pptr of active ingredient, these sublethal concentrations being based on the results of the first trial. Water in the test aquaria was changed every two days for new water containing the same concentration of insecticide that was used at the start of the test. The aquaria were observed for mortalities, and surviving shrimp at the end of the experiment were preserved in Davidson's fixative. These specimens were later prepared for standard histological examination (Bell and Lightner, 1988). The results for mortality are shown in Table 3.

Because the control tank was not replicated, we could not test the statistical significance of our results. However, there is a strong indication of significant mortality within 10 days at 1 pptr for this insecticide.

Within the last three months, we have also tested cypermethrin with zoeal stages and found that it gave significant toxicity (30% mortality in 12 h) at 10 pg/L. Electron micrographs of moribund animals from these tests are shown in Fig. 14. They show extensive cellular damage and appear to have distinctively enlarged nuclei with tubular inclusions. These are preliminary results and the tests are being repeated. If the results are repeatable, these tubular nuclei may serve as a marker for detecting mortality caused by cypermethrin poisoning.

Methyl-parathion

The first test with methyl-parathion was conducted using 20 postlarvae in 1-L beakers with various concentrations of active ingredient from 5 ppm to 1 ppb. Survival over 24 h was recorded to obtain some idea of the range of toxicity. The results are shown in Table 4. Clinical symptoms for the affected animals were the same as for cyper-

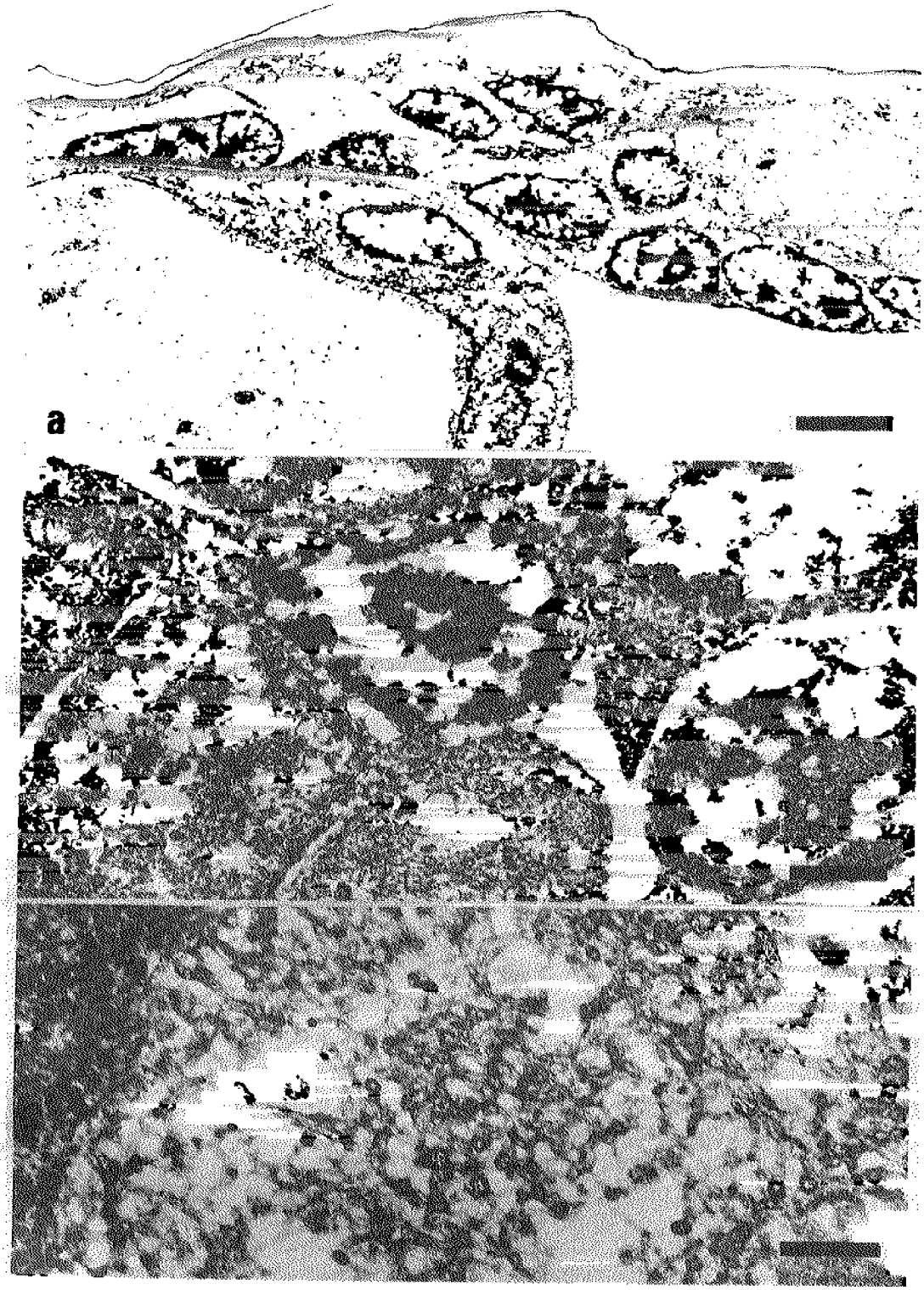


Figure 14. Transmission electron micrographs of moribund zoea 1 of Penaeus monodon *exposed to 10 pg/L of cypermethrin for 24 h in a recent preliminary test. (a) Low magnification showing generalized cellular damage. Note the collapse of the microvilli in the gut (bar = 4 μm). (b) Higher magnification of cells in a more advanced state of degeneration. Note that the enlarged nuclei almost completely fill the cells (bar = 1.2 μm). (c) High magnification of a nucleus from (b), showing details of the tubular inclusions (bar = 0.3 μm).*

Table 4. Effect of a single addition of methyl-parathion on survival of *Penaeus monodon* postlarvae in a 24-h test in 1-L beakers. Control animals showed no mortality.

Concentration		% Mortality	Time
µg/L			
5,000	5 ppm	100	<1 h
500	500 ppb	100	<1 h
150-90	150 - 90 ppb	100	<2 h
50	50 ppb	100	<4 h
30	30 ppb	100	<9 h
15	15 ppb	100	<24 h
10 or less	10 ppb or less	none	24 h

methrin above, and also suggested involvement of the nervous system in the cause of death.

Methyl-parathion was 3,000 times less toxic than cypermethrin in this acute toxicity test. Based on the results of this trial, a 10-day exposure test was conducted using the same protocol and the same control tank as for the cypermethrin test described above. The results are shown in Table 5.

Because the control tank was not replicated, we could not test the statistical significance of the differences in the results for the various treatments in this experiment (as with cypermethrin above). However, there is a strong indication of significant mortality within 10 days at 5 ppb for this insecticide. This is three times less than the concentration that caused 100% mortality.

Histological examination of the surviving juvenile shrimp from both insecticide treatments showed multiple anomalies, including enlarged, vacuolated ventral nerve ganglia, and general necrosis of the hepatopancreas and the skeletal muscles. These characteristics were shared by some of the animals

Table 5. Survival of *P. monodon* juveniles (1 - 3 g fresh weight) exposed to sublethal concentrations of methyl-parathion for ten days in 20-L aquaria. Trials were conducted in duplicate and in parallel to the triplicate cypermethrin tests above; the control consisted of the same single tank used for the cypermethrin tests.

Concentration		% Mortality
µg/L	ppb	
5.0	5	78 ± 11
1.0	1	23 ± 11
0	0	15

from the control tank. We found no specific indicators for insecticide or type of insecticide poisoning at the light microscope level. No preparations of the juveniles were made for the electron microscope. Obviously, more detailed studies with more extensive histological work are required.

Brief Review of Insecticide Studies

Table 6 gives a summary of pesticide toxicity data for crustaceans taken from the literature.

An early study by Eisler (1969) examined the acute toxicities of seven organochloride insecticides, including DDT, and five organophosphorus insecticides, including methyl-parathion, against three different decapod crustaceans. DDT was the most toxic of the organochloride insecticides (24-h LC_{50} 3 - 12 ppb and 96-h LC_{50} 0.6 - 6 ppb, depending upon species), while methyl-parathion was the most toxic of the organophosphorous compounds (24-h LC_{50} 11 - 23 ppb and 96-h LC_{50} 2 - 7 ppb, depending upon species). He also showed that temperature and salinity could alter the effect of these insecticides. In general, an increase in temperature resulted in increased sensitivity. With respect to changes in salinity, sensitivity to three organochlorides decreased with increasing salinity over the range of 12 - 36 ppt while it increased for two organophosphorous compounds over the same range.

A more recent study on the freshwater shrimp, *Paratya compressa improvisa* (Hatakeyama and Sugaya, 1989), gave 48-h LC_{50} values for three organophosphorous insecticides in the range of 1 - 8 ppb as compared to generally much higher values (up to 60 ppb in some cases) for the crustaceans usually employed in toxicity tests (e.g., cladocerans such as *Daphnia magna* and *Moina macrocopa*). Together with the earlier study by Eisler (1969), it is clear that sensitivities for individual species can vary by up to 10 times or more. Thus, specific tests are required wherever information is desired for a particular species.

In a rather extensive study, Kuhn et al. (1989) reported on the toxicity and "no observed effect concentration" (NOEC) for 73 potential water pollutants towards *Daphnia magna*. This included not only insecticides but also a long list of other organic compounds and heavy metals. Among the chemicals tested, the insecticide, ethyl-parathion, was the most potent, giving an NOEC (21-day reproduction test) of 2 ng/L. This was followed by bis (tri-n-butyltin) oxide at 160 ng/L, cadmium at 600 ng/L and perchloro-cyclopentadiene at 9 µg/L. No synthetic pyrethroid compounds were included in the chemicals tested, but other reports (see below) indicate that these compounds are up to three logs more potent against crustaceans than parathion-containing insecticides. This would translate into possible NOEC values in the range of pg/L.

A report by Armstrong et al. (1976) on the toxicity of the insecticide methoxy-

Table 6. Some insecticide toxicities reported for crustaceans.

Insecticide Type	Test Organism	Order	LC_{50} or ED_{50}* in µg/L (h)	Reference
ORGANOCHLORIDE				
Aldrin	*Crangon septemspinosa*	Decapoda	30 (24)	Eisler, 1969
	Palaemonetes vulgaris	Decapoda	>2,000 (24)	Eisler, 1969
	Pagurus longicarpus	Decapoda	300 (24)	Eisler, 1969
P,P'-DDT	*Crangon septemspinosa*	Decapoda	3 (24)	Eisler, 1969
	Palaemonetes vulgaris	Decapoda	12 (24)	Eisler, 1969
	Pagurus longicarpus	Decapoda	7 (24)	Eisler, 1969
Dieldrin	*Crangon septemspinosa*	Decapoda	68 (24)	Eisler, 1969
	Palaemonetes vulgaris	Decapoda	>107 (24)	Eisler, 1969
	Pagurus longicarpus	Decapoda	70 (24)	Eisler, 1969
Endrin	*Crangon septemspinosa*	Decapoda	2.8 (24)	Eisler, 1969
	Palaemonetes vulgaris	Decapoda	10.3 (24)	Eisler, 1969
	Pagurus longicarpus	Decapoda	27 (24)	Eisler, 1969
Heptachlor	*Crangon septemspinosa*	Decapoda	110 (24)	Eisler, 1969
	Palaemonetes vulgaris	Decapoda	>6,500 (24)	Eisler, 1969
	Pagurus longicarpus	Decapoda	470 (24)	Eisler, 1969
Lindane	*Crangon septemspinosa*	Decapoda	14 (24)	Eisler, 1969
	Palaemonetes vulgaris	Decapoda	62 (24)	Eisler, 1969
	Pagurus longicarpus	Decapoda	38 (24)	Eisler, 1969
Methoxychlor	*Crangon septemspinosa*	Decapoda	9 (24)	Eisler, 1969
	Palaemonetes vulgaris	Decapoda	16 (24)	Eisler, 1969
	Pagurus longicarpus	Decapoda	9 (24)	Eisler, 1969
	Cancer magister	Decapoda	0.42 - 130 (96)	Armstrong et al., 1976
ORGANOPHOSPHORUS				
Benthiocarb	*Mysidopsis bahia*	Mysidacea	330 (96)	Schimmel et al., 1983
Carbophenothion (Trithion)	*Mysidopsis bahia*	Mysidacea	46 (96)	Cripe et al., 1989
Chlorpyrifos (Dursban, Lorsban)	*Mysidopsis bahia*	Mysidacea	0.035 (96)	Schimmel et al., 1983
Dichlorvos (DDVP, Vapona)	*Crangon septemspinosa*	Decapoda	18 (24)	Eisler, 1969
	Palaemonetes vulgaris	Decapoda	390 (24)	Eisler, 1969
	Pagurus longicarpus	Decapoda	150 (24)	Eisler, 1969
Diaoxathon (Delnav)	*Crangon septemspinosa*	Decapoda	307 (24)	Eisler, 1969
	Palaemonetes vulgaris	Decapoda	500 (24)	Eisler, 1969
	Pagurus longicarpus	Decapoda	300 (24)	Eisler, 1969

Table 6. Continued.

Insecticide Type	Test Organism	Order	LC_{50} or ED_{50} in µg/L (h)	Reference
Diazinon (Spectracide, Donx Out)	*Daphnia magna*	Cladocera	2 (48)	Hatakeyama and Sugaya, 1989
	Moina macrocopa	Cladocera	9 (48)	Hatakeyama and Sugaya, 1989
	Paratya compressa improvisa	Decapoda	6 (48)	Hatakeyama and Sugaya, 1989
Ethyl-parathion	*Daphnia magna*	Cladocera	2* (24)	Kuhn, et al., 1989
Fenitrothion (Accothion, Folithion, Sumithion)	*Daphnia magna*	Cladocera	>50 (48)	Hatakeyama and Sugaya, 1989
	Moina improvisa vulgaris	Cladocera	37.8 (48)	Hatakeyama and Sugaya, 1989
	Pagurus longicarpus	Decapoda	40 (24)	Eisler, 1969
	Paratya compressa improvisa	Decapoda	1.2 (48)	Hatakeyama and Sugaya, 1989
Fenthion (Baytex, entex, Tiguvon)	*Daphnia magna*	Cladocera	>50 (48)	Hatakeyama and Sugaya, 1989
	Moina macrocopa	Cladocera	35.3 (48)	Hatakeyama and Sugaya, 1989
	Paratya compressa improvisa	Decapoda	1 (48)	Hatakeyama and Sugaya, 1989
Malathion (Cythion)	*Crangon septemspinosa*	Decapoda	246 (24)	Eisler, 1969
	Palaemonetes vulgaris	Decapoda	131 (24)	Eisler, 1969
	Pagurus longicarpus	Decapoda	118 (24)	Eisler, 1969
	Mysidopsis bahia	Mysidacea	5.3 (96)	Cripe et al., 1989
Methyl-parathion	*Crangon septemspinosa*	Decapoda	11 (24)	Eisler, 1969
	Palaemonetes vulgaris	Decapoda	15 (24)	Eisler, 1969
	Pagurus longicarpus	Decapoda	23 (24)	Eisler, 1969
	Mysidopsis bahia	Mysidacea	0.78 (96)	Schimmel et al., 1983
	Penaeus duorarum	Decapoda	1.2 (96)	Schimmel et al., 1983
	Oziotelphusa senex senex	Decapoda	1,000 (48)	Reddy et al., 1986a
Mevinphos (Phosdrin)	*Crangon septemspinosa*	Decapoda	13 (24)	Eisler, 1969
	Palaemonetes vulgaris	Decapoda	131 (24)	Eisler, 1969
	Pagurus longicarpus	Decapoda	40 (24)	Eisler, 1969
PYRETHROID				
AC 222,705 or Flucythrinate	*Mysidopsis bahia*	Mysidacea	0.008 (96)	Schimmel et al., 1983
	Penaeus duorarum	Decapoda	0.22 (96)	Schimmel et al., 1983

Table 6. Continued.

Insecticide Type	Test Organism	Order	LC_{50} or ED_{50} in µg/L (h)	Reference
Cypermethrin	*Mysidopsis bahia*	Mysidacea	0.019 (96)	Cripe et al., 1989
			0.005 (96)	Hill, 1985
			0.056 (96)	Clark et al., 1989
	Homarus americana	Decapoda	0.01 (96)	Schimmel et al., 1983
	Daphnia magna	Cladocera	1.0-5.0 (24)	Day, 1989
	Crangon septemspinosa	Decapoda	0.01 (96)	McLeese et al., 1980
	Palaemonetes pugio	Decapoda	0.016 (96)	Clark et al., 1989
	Penaeus duorarum	Decapoda	0.036 (96)	Clark et al., 1989
	Uca pugilator	Decapoda	0.2 ((6)	Clark et al., 1989
Fenvalerate	*Homarus americana*	Decapoda	0.14 (96)	McLeese et al., 1980
	Daphnia magna	Cladocera	0.3 (24)	Day, 1989
			0.8 - 2.5 (48)	Day, 1989
	Daphnia galeata mendotae	Cladocera	0.16 - 0.29 (48)	Day, 1989
	Ceriodaphnia lacustris	Cladocera	0.21 (48)	Day, 1989
	Diaptomus oregonensis	Cladocera	0.12 (24)	Day, 1989
	Mysidopsis bahia	Mysidacea	0.008 (96)	Schimmel et al., 1983
	Penaeus duorarum	Decapoda	0.84 (96)	Schimmel et al., 1983
	Nitocra spinipes	?	0.38 (96)	Clark et al., 1989
	Palaemonetes pugio	Decapoda	<0.003 (96)	Clark et al., 1989
Permethrin	*Crangon septemspinosa*	Decapoda	0.13 (96)	McLeese et al., 1980
	Mysidopsis bahia	Mysidacea	0.02 (96)	Schimmel et al., 1983
	Penaeus duorarum	Decapoda	0.22 (96)	Schimmel et al., 1983
	Penaeus aztecus	Decapoda	0.34 (96)	Clark et al., 1989
	Menippe mercenaria	Decapoda	0.02 (96)	Clark et al., 1989
	Nitocra spinipes	?	0.15 (96)	Clark et al., 1989
	Uca pugilator	Decapoda	2.2 (96)	Clark et al., 1989
CARBAMATE				
Carbaryl (Sevin)	*Daphnia magna*	Cladocera	12 (48)	Hatakeyama and Sugaya, 1989
	Moina macropa	Cladocera	200 (48)	Hatakeyama and Sugaya, 1989
	Paratya compressa improvisa	Decapoda	20 (48)	Hatakeyama and Sugaya, 1989
BMPC (2-sec-butylphenyl N-methyl-carbamate)	*Daphnia magna*	Cladocera	90 (48)	Hatakeyama and Sugaya, 1989
	Moina macrocopa	Cladocera	220 (48)	Hatakeyama and Sugaya, 1989
	Paratya compressa improvisa	Decapoda	8 (48)	Hatakeyama and Sugaya, 1989

chlor to the Dungeness crab, *Cancer magister*, showed that toxicity was inversely related to age after hatching, and that it increased with length of exposure. The 24-h LC_{50} values were >0.92, 12.0 and >920 µg/L for zoea, juveniles and adults, respectively. The 96-h LC_{50} values were 0.42, 5.1 and 130 µg/L, respectively, for the same stages. Since most reports of insecticide toxicity to crustaceans deal with adult stages, aquaculturists working at the hatchery stages or rearing should be aware that larval stages may be many times more sensitive than adult stages.

Vogt (1987) has shown that histological diagnosis of crustacean midgut glands (hepatopancreas) can be used to assess the effect of low levels of insecticides. At a concentration of 1 ppm of an insecticide preparation containing dimethoate as the active ingredient, there was no mortality in four days but damage to the midgut gland was evident at both the light microscope and electron microscope levels. Vogt points out that pesticide behavior and biological impact in the environment are complicated by transfer rates, chemical and biochemical transformations and health of an animal. As yet, there is no clear histological indicator to specifically indicate insecticide toxicity in pond-reared animals, as opposed to toxicity from other compounds (e.g., algal toxins, rancid feed, etc.) (Sindermann and Lightner, 1988; Brock, 1991).

Animal health is related to nutrition, and poor nutritional status in crustaceans has been shown to increase the sensitivity of crustaceans to insecticides. For example, Cripe et al. (1989) showed that low food availability increased the sensitivity of *Mysidopsis bahia* to various insecticides. This has implications not only for those facing real situations in hatcheries and growout ponds but also for those conducting toxicity test protocols.

Ware (1986) defines organophosphate insecticides as chemically unstable compounds containing phosphorous and derived from phosphoric acid. They are generally the most toxic of all pesticides to vertebrate animals, and they are related to "nerve gases" by chemical structure and mode of action (i.e., they inhibit the action of acetyl choline esterase or ACH-esterase). Although they are more acutely toxic to vertebrates than organochlorides, they have the advantage of being less persistent; this is part of the reason for their popularity in agriculture. One of this family of compounds, dichlorvos, or DDVP, is used widely for treatment of sea lice in salmon. From Table 6, it can be seen that dichlorvos has an LC_{50} of 18 - 390 µg/L (18 - 390 ppb) and that these concentrations are approximately 5,000 to 200,000 times higher than the LC_{50} concentration of the most potent of the synthetic pyrethroid compounds. However, the generally lower toxicity of the organophosphates does not mean that they can be disregarded. These compounds are used in considerable quantity in agriculture. Methylparathion alone was imported into Thailand to the amount of 1,452 MT (active ingredient) in 1989. Thus, these

compounds also have the potential to cause damage in shrimp culture if they contaminate the aquatic environment.

Metabolic and physiological studies of parathion and methyl-parathion action in crustaceans have shown that it is not limited to inhibition of ACH-esterase activity. Reddy et al. (1986a, b; 1988) have reported wide-ranging effects including evidence of tissue damage and alteration in enzyme activities. Their most interesting observation was that methyl-parathion at sublethal exposure (200 µg/L for 48 h) could lead to the accumulation of lactic acid in muscle tissues through inhibition of aerobic metabolism. This may relate to the fact that we have found sporadic occurrences of idiopathic muscle necrosis (IMN) in samples of shrimp from some farmers' ponds (unpublished); this syndrome has been linked to lactic acid accumulation in crustacean muscle as a result of unknown environmental stress factors (Nash et al., 1987). It is possible that organophosphate insecticides, although not entirely responsible for the syndrome, may, at very low, sublethal concentrations, aggravate it in an additive or synergistic way.

In detailed studies on the mechanism of parathion action on muscle of the crab *Carcinus* sp., Bradbury (1973a, b) found that "parathion does not activate muscle by a transient depolarization, but it does significantly depress reticular calcium uptake. In addition, parathion greatly modifies actomyosin superprecipitation, and fine-structural studies show great disruption of reticular organization, suggestive of massive calcium loss from the reticulum into the myoplasm" (Bradbury, 1973b). In tests on actomyosin superprecipitation in the presence of various mixtures of ATP, Ca^{++} and parathion, he found that any preparation containing parathion showed a massive increase in superprecipitation. Thus, there were two quite different effects from the insecticide. One was destruction of the sarcoplasmic reticulum and mitochondria causing release of vesicular calcium and the other was a direct action on actomyosin. The concentration of parathion used to obtain maximal contracture tension in these studies was 100 µm, about 25 times less than the recommended dosage for general agricultural use. It is possible that methyl-parathion or other organophosphorous insecticides have similar methods of action.

In mammals, parathion is metabolized to nontoxic compounds via the intermediate paraoxon, which is an even more potent ACH-esterase inhibitor than the parent compound (Elmamlouk and Giessner, 1976). However, it has been shown (Elmalouk and Giessner, 1976) that hepatopancreatic tissue of the lobster cannot convert parathion to paraoxon or p-nitrophenol, suggesting that lobster, and perhaps other crustaceans, may not be capable of transforming parathion and methyl-parathion to nontoxic compounds. This could help to explain why they are so much more toxic to crustaceans than mammals.

Most of the steps in the environmental degradative pathway for methyl-para-

thion are biotic transformations. In a laboratory model flow-through system, Bourquin et al. (1979) showed that methyl-parathion was rapidly and irreversibly bound to sediments, and that it was not chemically or biologically degraded in the absence of sediments, even under various sterile and nonsterile conditions. Degradation was fastest with nonsterile sediments, and the principal transformation product was amino methyl-parathion. The calculated half-life for methyl-parathion in the system used was 40 - 50 h.

Using another model ecosystem with parathion, Yu and Sanborn (1975) gave a half-life of 15 to 16 days in water and showed a gradual transformation from the insecticide to unextractable polar degradation products (45% of the total added radioactivity over the 38-day experiment). They suggested that the insecticide would not present a threat from environmental accumulation. They quoted sources indicating that the residual amount of 0.3 ppb (0.3 g/L) in water at the end of their 38-day experiment was 10 times less than that recommended as an acceptable residue for water. However, upon reference to Table 6, it is not certain that such a level would be harmless to crustaceans, if the period of exposure were long.

In conclusion, it is clear that the aquatic contamination from the organophosphate pesticides used in agriculture probably presents a much lower threat to reared crustaceans than do synthetic pyrethroid insecticides.

Cypermethrin is a fourth generation derivative of the natural insecticide, pyrethrum, a mixture of four related compounds extracted from *Chrysanthemum* (family Compositae, the sunflower family) (Ware, 1986). These natural compounds are good household insecticides because of quick knock-down and relatively rapid decomposition (e.g., instability to sunlight). The first synthetic pyrethroid insecticide, allethrin, was manufactured in 1949 but it, like several others to follow, was not successfully used as an agricultural chemical. The first successful agricultural compounds in the group (introduced in 1972 and 1973, respectively) were the fourth generation chemicals fenvalerate and permethrin. These were applied in the field at approximately 100 - 200 g (active ingredient)/ha and were active against a wide variety of insects. They have been followed by much more potent compounds such as cypermethrin which are applied at only 10 - 20 g/ha (Ware, 1986).

Since cypermethrin is one of the crabicides used by Thai farmers, and since it showed the highest toxicity in our preliminary tests with *Penaeus monodon*, we have concentrated our search of the literature on this compound. The effects of synthetic pyrethroids on freshwater zooplankton have been reviewed by Day (1989). He reported the range of acute toxicities for cladocerans and copepods as 0.12 - 5.0 µg/L, while reduced reproduction and filtration rates could be observed at concentrations as low as 0.01 µg/L.

Clark et al. (1989) have reviewed the effect of pyrethroids on marine invertebrates and fish; data from their study are in Table 6. They are amongst the most toxic insecticides for crustaceans. With whatever species or test conditions, LC_{50} values reported for 24- to 96-h tests are in the range of ng/L. For example, the most toxic compound listed is cypermethrin with an LC_{50} value (96-h test) of 5 ng/L for *Mysidopsis*. Our test with *P. monodon* (above), however, indicated that the tiger prawn is even more sensitive to cypermethrin, since 5 ng gave 100% mortality for larval shrimp in 24 h, and since 10-day exposure to 1 ng/L (1 pptr) gave approximately 50% mortality with juveniles. These lethal concentrations contrast with all of the other insecticides in Table 6 for which LC_{50} values are in the range of µg or mg/L (i.e., concentrations several logs higher are required to obtain the same level of mortality).

To obtain 50% mortality with fish (including fry), concentrations of pyrethroids in the range of hundreds of µg or even in mg/L are sometimes required, but there are exceptions (Schimmel et al., 1983; Clark et al., 1989). Oysters appear to be very insensitive, with LC_{50} values in the range of 1 - 2 mg/L. They also showed very high bioconcentration factors (BCF) up to 4,700 for fenvalerate (Clark et al., 1989). Finally, blue-green and green algae were shown in laboratory studies to be unaffected by concentrations of fenvalerate and cypermethrin up to 5 mg/L (i.e., 5,000 µg), a concentration that is in the range of one million times higher than that which can harm crustaceans (Megharaj et al., 1987). Growth inhibiting effects were not seen until concentrations exceeded 10 mg/L. Thus, it is possible that natural shrimp feeds or feed components derived from fish, algae or plants may contain sufficient residues to cause difficulties.

To understand the reason for the extraordinary potency of pyrethroid insecticides towards crustaceans, it is necessary to examine their mode of action. Chalmers and Osborne (1986) reported that like DDT, symptoms of pyrethroid poisoning in insects include excitation, ataxia and convulsions that correlate with repetitive discharges in many areas of the nervous system. They also noted that some DDT-resistant insect strains show cross-resistance to pyrethroids, and they, therefore, suggest that the chemicals may share a common mode of action. However, Gammon and Sander (1985) reported that Type I pyrethroids cause repetitive firing of neurons while Type II pyrethroids rarely do and that the two classes of compounds may therefore have different physiological targets. Their data suggests that Type I pyrethroids act on sites where calcium controls sodium inactivation, while Type II compounds may involve interaction with the GABA (gama amino butyric acid) receptor complex. Different pyrethroids may have different specificities. They also state that "there is growing evidence that Type II syndrome involves effects on one or more neurotransmitter-modulated channels as well

as affecting voltage-sensitive (axonal) ion channels."

Rashatwar and Matsumura (1985) showed direct inhibitory effects of pyrethroids on calmodulin. Since calmodulin plays a vital role in a wide variety of physiological reactions dependent upon calcium, it may mean that the mode of action of pyrethroids will turn out to be quite complex. For example, Mulla et al. (1982) reported a rather unusual phenomenon during cypermethrin application to control mosquitoes in the field. Even 14 days after application of the insecticide, the larval population prevailed at a high level, but no pupae occurred. This hints at a rather specific effect on a target associated with morphogenesis, a process that is regulated by neurosecretory hormones. If calmodulin plays a central role in this neurohormonal process, pyrethroids would be very disruptive.

Although pyrethroids are known to be extremely toxic to crustaceans in laboratory tests, several factors must be taken into consideration in assessing their impact in the environment. One important factor is persistence (governed by rates of degradation and metabolism) and another is sorption (Clark et al., 1989). In the study by Schimmel et al. (1983) the sediment half-lives for the pyrethroids AC 222,705 (flucythrinate), fenvalerate and permethrin were 16, 31 and 2.5 days, respectively. Those for the organophosphorous insecticides methyl-parathion, benthiocarb and chlorpyrifos were 1.2, 6.4 and 24 days, respectively. Agnihorti et al. (1986) also report persistence of permethrin, cypermethrin, fenvalerate and deltamethrin in sediments beyond 30 days; although, 75 - 95% was lost from the water column within 24 h of application. Perhaps most alarming from the point of view of feed formulators is the report by Joia et al. (1985) of cypermethrin and fenvalerate residues in wheat and its milled fractions. Half-lives in stored grain ranged from 69 to 385 weeks depending upon the moisture content and storage temperature. Reduction in residues through bread baking were low (79 - 84% cypermethrin and 87 - 88% fenvalerate remained after cooking). This means that any residues in shrimp feed ingredients would probably pass processing conditions and remain relatively undiminished in finished pellets, where they would pose a significant threat to reared shrimp.

In their laboratory study on sediment-sorbed fenvalerate and cypermethrin, Clark et al. (1989) concluded that "direct contact with, or ingestion of, contaminated sediment did not appear to enhance the toxicity of fenvalerate or cypermethrin to mysids, grass shrimp or pink shrimp" and that "sediment sorbed chemicals were not acutely toxic . . . until concentrations in sediment were increased to the point where partitioning into overlying water resulted in acutely lethal concentrations." Thus, they suggested that "chemical partitioning between contaminated sediments and overlying water may be modeled to predict acute lethal effects on marine crustaceans for habitats with

well-defined physicochemical and hydrodynamic characteristics." This information indicates that the threat of pyrethroid residues to aquaculture may be reduced by sorption.

A preparation of pyrethrum called Py-Sal 25 has recently been introduced in Great Britain for the treatment of sea lice on salmon, by Vetrepharm of Hampshire. The product is marketed under license from Norsk Pyrethrum of Norway (Fish Farming Intl. vol. 17 no. 6: 2). Although the preparation is purported to cause little environmental damage, it would be useful to determine if this product affects shrimp.

Toxic Algae

Limsuwan (1991) has reported that dinoflagellates, blue-green algae and some diatoms can cause problems in shrimp growout ponds. He states that some effects are indirect (e.g., the effects of *Oscillatoria* sp., *Trichodesmium* sp. and *Noctiluca* sp.) in that difficulties arise from low oxygen and high ammonia after an algal crash. In other cases, such as the dinoflagellates, toxins act directly on the shrimp.

In southern Thailand, we have had difficulties during some periods (but not others) when dinoflagellates are present in ponds in significant numbers (unpublished), and it is difficult to make conclusions without detailed testing with specific isolates. However, *Noctiluca scintilans* (Suvapepun, 1989) and *Protogonyaulax* spp. (Fukuyo et al., 1989) have been reported in the Gulf of Thailand, and these organisms are considered to be toxic to some marine animals. We have exposed postlarvae to *N. scintilans* in high-density beaker trials at our hatchery laboratory and found no indication of acute toxicity. However, reports concerning the possible dependence on commensal bacteria for toxin production in dinoflagellates (Kodama et al., 1989; Tamplin, 1990) may explain the variation in observed effect of dinoflagellates.

Within the last few months, Dr. Piamsak Menasvata (Dept. Marine Science, Chulalongkorn University, Bangkok) informed me that his group has isolated from shrimp ponds a strain of *Alexandrium* (= *Gonyaluax*) that rapidly causes 100% mortality to postlarvae of *Penaeus monodon* at levels of under 100 cells/L.

With respect to other algal species, Limsuwan (1991) reports that *Rhizosolenia* red tides can cause problems in growout ponds through physical irritation of the gills, but we have also found indications of toxicity. During one "red tide" dominated by this diatom, farmers reported a drop in feeding activity of pond-reared shrimp, but no significant mortality. In preliminary beaker trials at our hatchery site (unpublished) with supernatant liquid from heavy suspensions of the alga, we found acute toxicity to mysis larvae (100% mortality in 2 h) at dilutions of 1/1000. This warrants more rigid tests.

By contrast to *Rhizosolenia*, we have found the cyanobacterium, *Trichodesmium erythraeum*, is not acutely toxic in

similar beaker trials; but we concur with Limsuwan (1991) that it can produce low oxygen tensions. On one occasion during a "red tide" dominated by *T. erythraem*, we were called to a shrimp farm where the inlet canal was covered with a thick, tan-colored scum of this alga mixed with copious quantities of a large (up to 500-µm dia.) foraging protozoan of the family Chilodonellidae. The farmers closed the inlet gates when the scum appeared, and the water in the canal became stationary. As a consequence, a situation of low dissolved oxygen occurred beginning in the late afternoon, resulting in the death of fish and crustaceans in the canal (unpublished). This could probably even occur during mid-day, if a bloom were in the declining phase with large quantities of dead algal biomass in the water. In addition to alarm caused by the scum, the alga produced a noticeable red color through the release of copious quantities of phycocyanin and phycoerythrin when it died, which further alarmed farmers.

Crude Oil

With increasing frequency, we have found small (0.5 - 1.0 cm dia.) to large (3.0 - 6.0 cm dia.) lumps of what appear to be crude oil washed up on the beach in front of our hatchery. Inspection of another site 30 km south on the same coast revealed similar lumps, so the phenomenon was spread over a wide area and the quantity of oil that caused this deposition must have been large. We have tested samples of this material (three tests in triplicate) for toxicity to the larvae by stirring the oil (5 g/L) in normal sea water at 60°C for 1 h followed by separation and millipore filtration (0.45 µm) of the clear water "extract." This water is acutely toxic to postlarvae (100% mortality at no dilution and 30% mortality at 1/1000 dilution in 24 h) when compared to no mortality in controls (unpublished). We could not remove the toxicity by treatment with up to 5 g/L of activated charcoal (unpublished). We are now monitoring the beach for oil to determine whether its presence correlates with production difficulties in the hatchery.

We do not know the source of the oil. It could originate from passing ships or from undersea well heads approximately 200-km offshore in the Gulf of Thailand.

Waste Water and Pond Bottoms

In Thailand, it is customary at the end of a cultivation to *"cheet lane,"* that is, to flush out the loose material on the pond bottom with water jets and/or pumps. Farmers believe the residue in the pond bottom will jeopardize the next crop because of the high amount of organic residue remaining from shrimp feces and uneaten food. The problem with this practice is that it harms the water quality in the receiving canal, which may be the source of inlet water for another farmer. Because of the environmental damage, the govern-

ment has prohibited this activity, but enforcement is difficult.

To determine whether bottom removal is necessary, we sought the assistance of Dr. Claude E. Boyd of Auburn University to measure the residual organic matter at the end of harvest in ponds stocked with 25 larvae/m^2. It was in the range of 0.5- to 1.0-g organic carbon/kg dry weight of residue. Dr. Boyd concluded that most of the loose material in the center of the pond was clay that had been lifted by the paddle wheels, or sand and clay that had settled out from the inlet water (high suspended solids content in the monsoon season and average water exchange of 30%/day).

Based on these results, Dr. Boyd recommended tilling the dampened pond bottoms after harvest to promote maximum microbial degradation of the remaining organic matter. He also recommended tilling only in the center of the pond to avoid disturbing the scoured areas under the paddle wheels. This would reduce the quantity of clay lifted by the paddle wheels during the next cultivation cycle. These recommendations have been tested and found to be workable, so flushing the pond at the end of the harvest is not necessary. However, we have not tested ponds that are stocked at higher densities and subjected to higher feed inputs.

Mysteries

Larval Black Spot Syndrome

At unpredictable intervals in our hatchery, we find that zoea 1 or zoea 2 larvae carry a dense black lump of material at the junction of the stomach, hepatopancreas and midgut (Fig.15a, b). Examination of this material in squash mounts with the light microscope reveals it to be amorphous in nature. Sometimes the material loosens and is passed in the feces. We have not studied this phenomenon enough to conclude whether it reduces survival. During preparation of specimens for examination with the electron microscope, these black inclusions were dissolved by the solvents.

Lymphoid Organ Rod-shaped, Cytoplasmic, Enveloped Virus

During inspections of normal broodstock lymphoid organs for viral material, we found one strange cell in abnormal lymphoid tissue (Fig. 16). The cytoplasm of this cell contained what looked like enveloped baculovirus virions.

Spongy Muscle Syndrome

Histological examination of normal broodstock, pond-reared shrimp and postlarvae (PL20 and older) sometimes reveals the presence of spongy muscle, nerve and other tissues (Fig. 17). We do not know the cause or significance of this abnormal phenomenon.

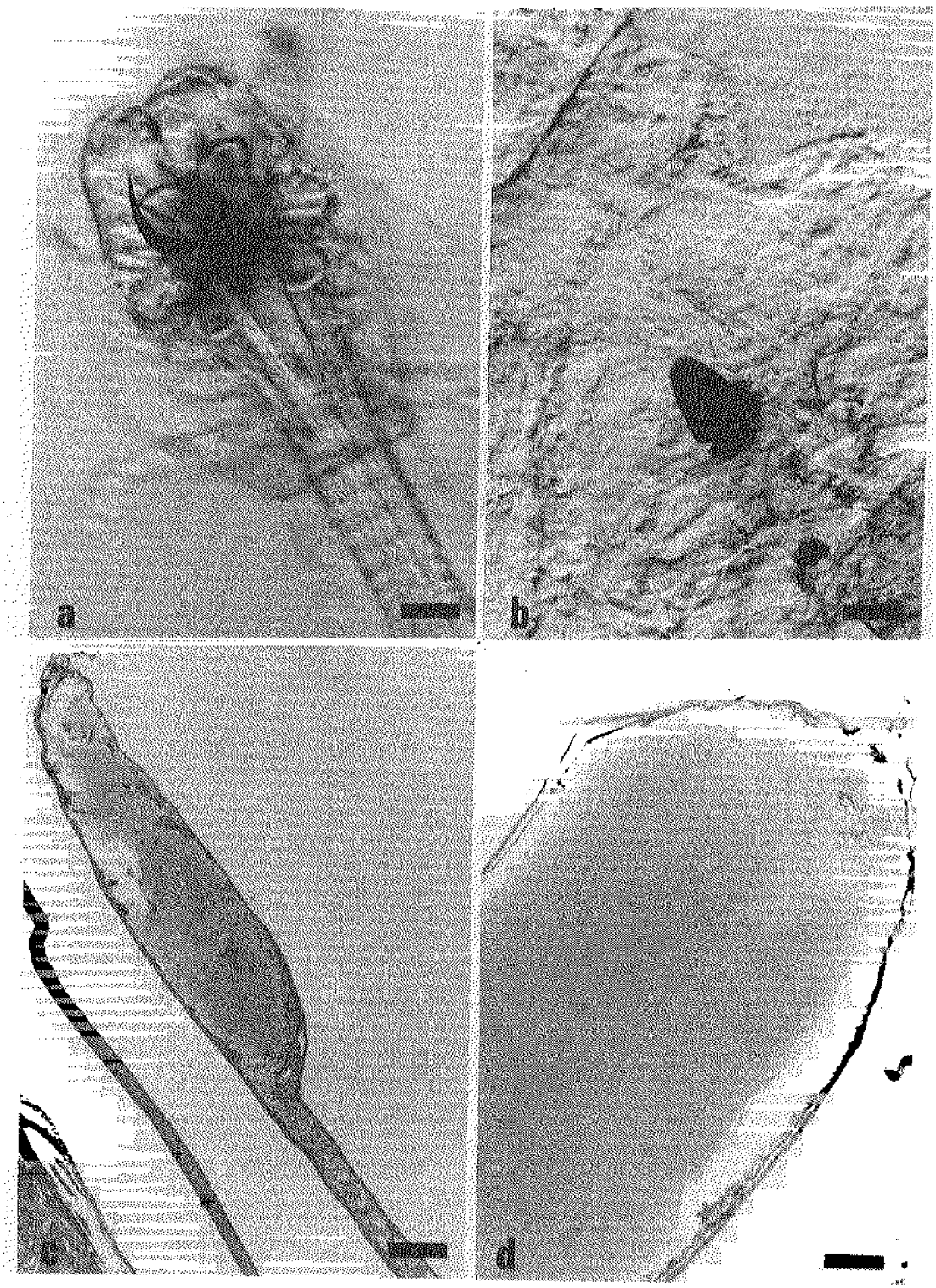


Figure 15. Two mystery syndromes found in Penaeus monodon *in Thailand. (a-b) Fresh mount and squash mount, respectively, of black amorphous material found lodged at the junction of the stomach, hepatopancreas and midgut of zoea 1 and zoea 2 larvae (bars = 125 µm and 50 µm, respectively). (c-d) H&E preparation of small blisters located on the fine appendages of* P. monodon *juveniles. The animals were removed from a "One month mortality syndrome" pond (see text). The blisters were filled with hemolymph (bars = 125 µm and 50 µm, respectively).*

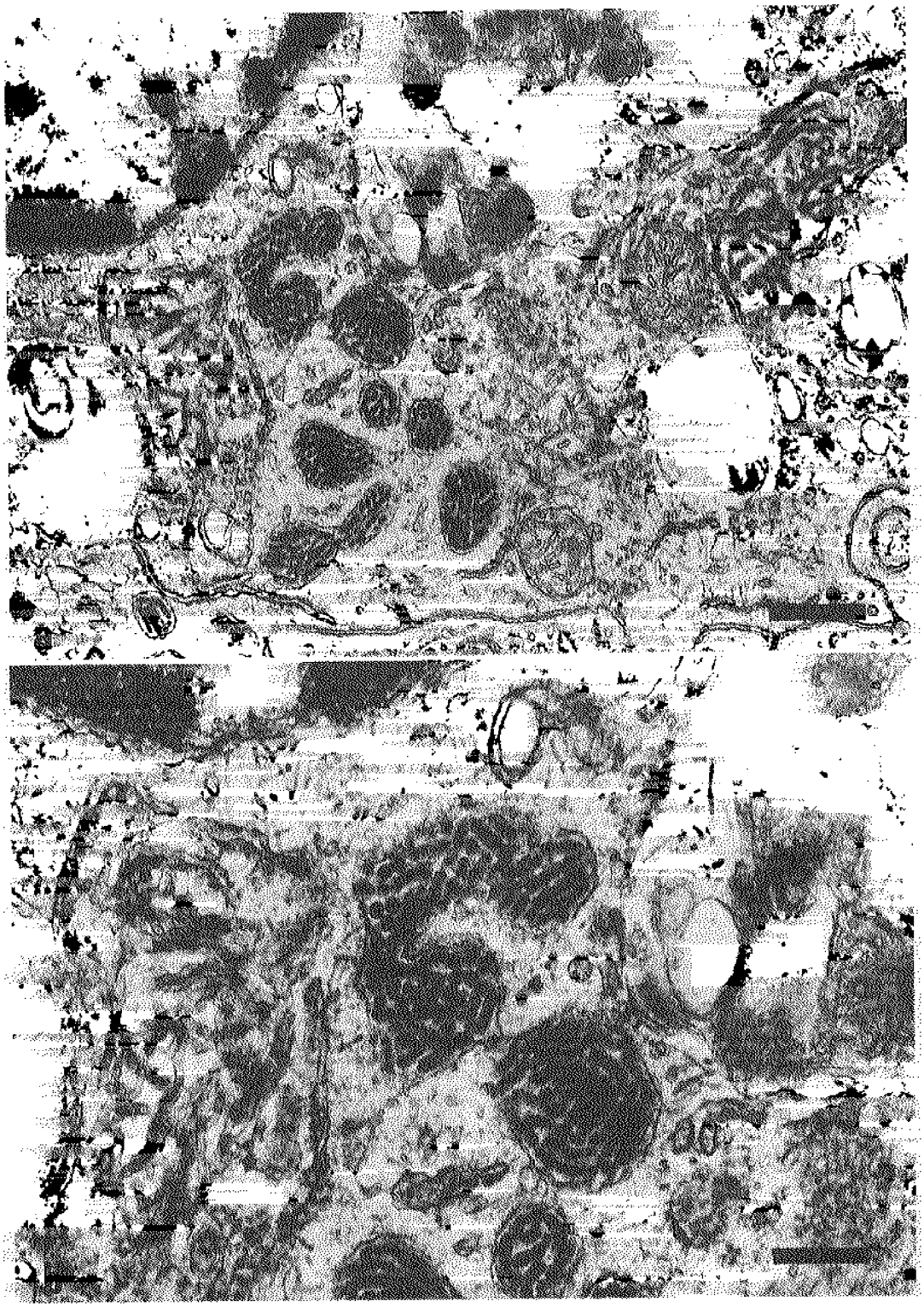


Figure 16. Unknown virus found in the cytoplasm next to the hypertrophied nucleus of an abnormal lymphoid organ cell. This was found by chance during examination of the abnormal tissues described in the section on lymphoid organ virus (bars: a = 2.4 μm; b = 0.3 μm).

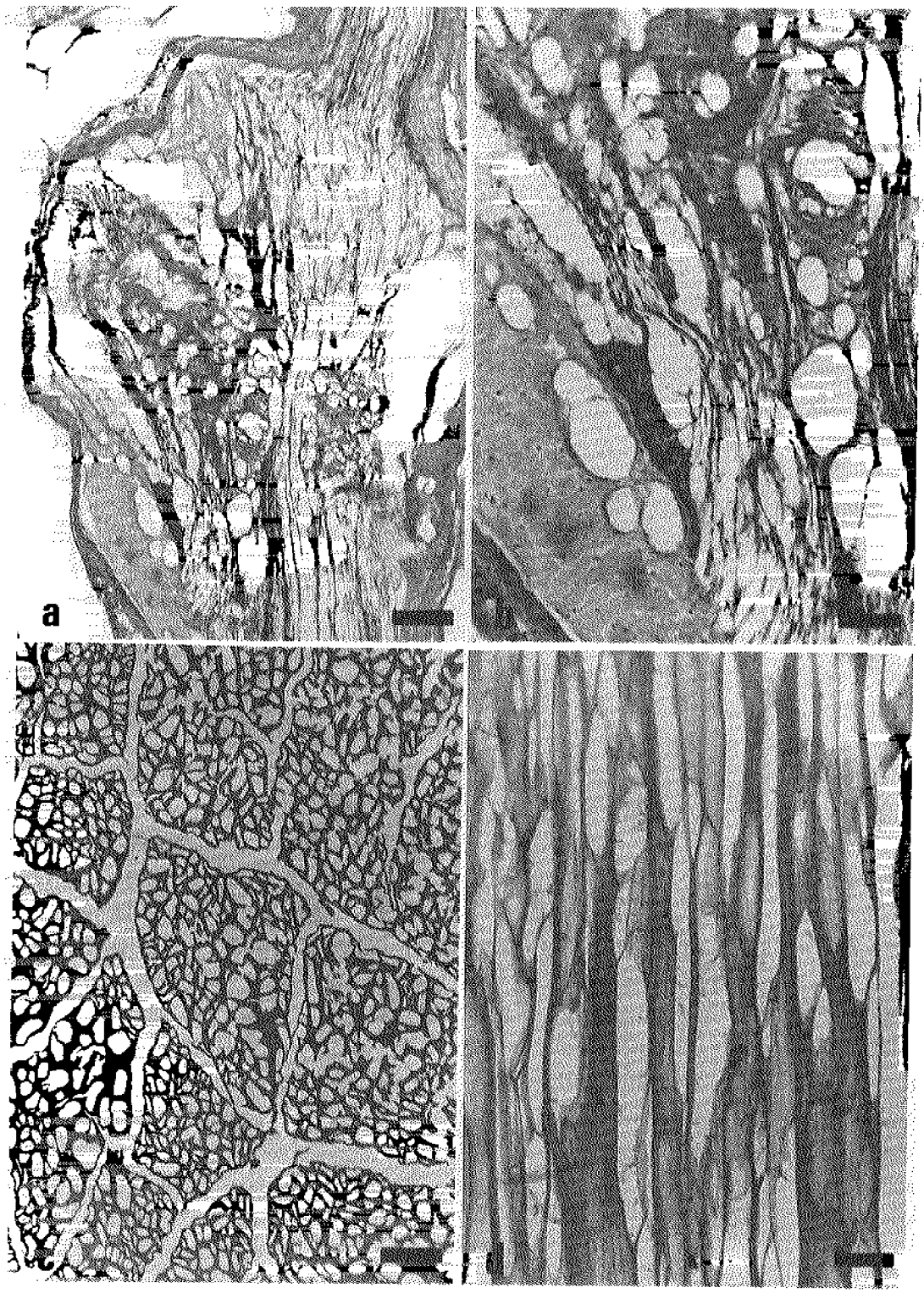


Figure 17. Photomicrographs of spongy muscle and nerve tissue from a normal broodstock specimen (H&E preparation). The animal was fixed for a general examination after it had been used for egg production in the hatchery. It showed no outward signs of disease. (a-b) Low and high magnifications of spongy nerve tissue (bars = 125 µm and 25 µm, respectively). (c-d) Transverse and longitudinal sections of spongy muscle tissue (bars = 25 µm and 12.5 µm, respectively).

Brown Muscle Syndrome (Idiopathic Muscle Necrosis or IMN)

During some harvests of *P. monodon*, up to approximately 5% of the shrimp are found to have gross, patchy brown discolorations of the muscle tissue in the tail region. The color remains after cooking, rendering the shrimp unmarketable. Since there are no external signs of the abnormality, it is discovered only after deheading at the frozen storage plant.

Histological examination of the animals with the light microscope (H&E staining) reveals massive hemocytic aggregation and melanization in areas of muscle necrosis (Figs. 18 and 19). A general breakdown of the sarcoplasm and muscle fibers can be seen. The picture closely resembles idiopathic muscle necrosis (IMN) reported for *Macrobrachium rosenbergii* by Nash et al. (1987), except for the absence of melanization in *M. rosenbergii*.

According to Nash et al. (1987), this disease arises from stress, leading to excess lactic acid accumulation, followed by tissue damage and hemocytic aggregation. They found that reduction in stocking densities eliminated the problem. (See earlier comments on the organophosphate insecticide methylparathion).

Electron micrographs (Figs. 20 - 22) have provided evidence of viral elements in the cytoplasm of hemocytes between the muscle fibers; these elements are somewhat similar to those described above in the lymphoid organ. There are cytoplasmic virogenic stromata and what appear to be unenveloped virions approximately 45 nm in diameter. In contrast to the lymphoid organ, however, there are also paracrystalline arrays of what may be proteinaceous material (Fig. 20) in some of the cells. It remains to be established whether the virus is responsible for the brown muscle syndrome and whether there is anything more than a superficial relationship between the virus in the muscle and in the lymphoid organ.

One Month Mortality Syndrome

One month to six weeks after stocking larvae in growout ponds, moderate to massive mortality can occur. This is characterized by a sudden reduction of feed intake and the appearance in the feeding nets of very lethargic shrimp that otherwise appear normal. Anecdotal information suggests that the phenomenon is related to transparencies in excess of 40 cm and the presence of floating plaques of benthic blue-green algae (*"ki dat"*) that rise from the pond bottom during the day, die on the surface and fall back to the bottom of the pond if they cannot be removed.

For this syndrome, Dr. Chalore Limsuwan (1989) coined the phrase "one month mortality syndrome" (*"roak tai deun"*) and the term is now widely used by farmers in an indiscriminate way, so it is difficult to know the real incidence of the phenomenon. Our Technical Services Division for the month of Janu-

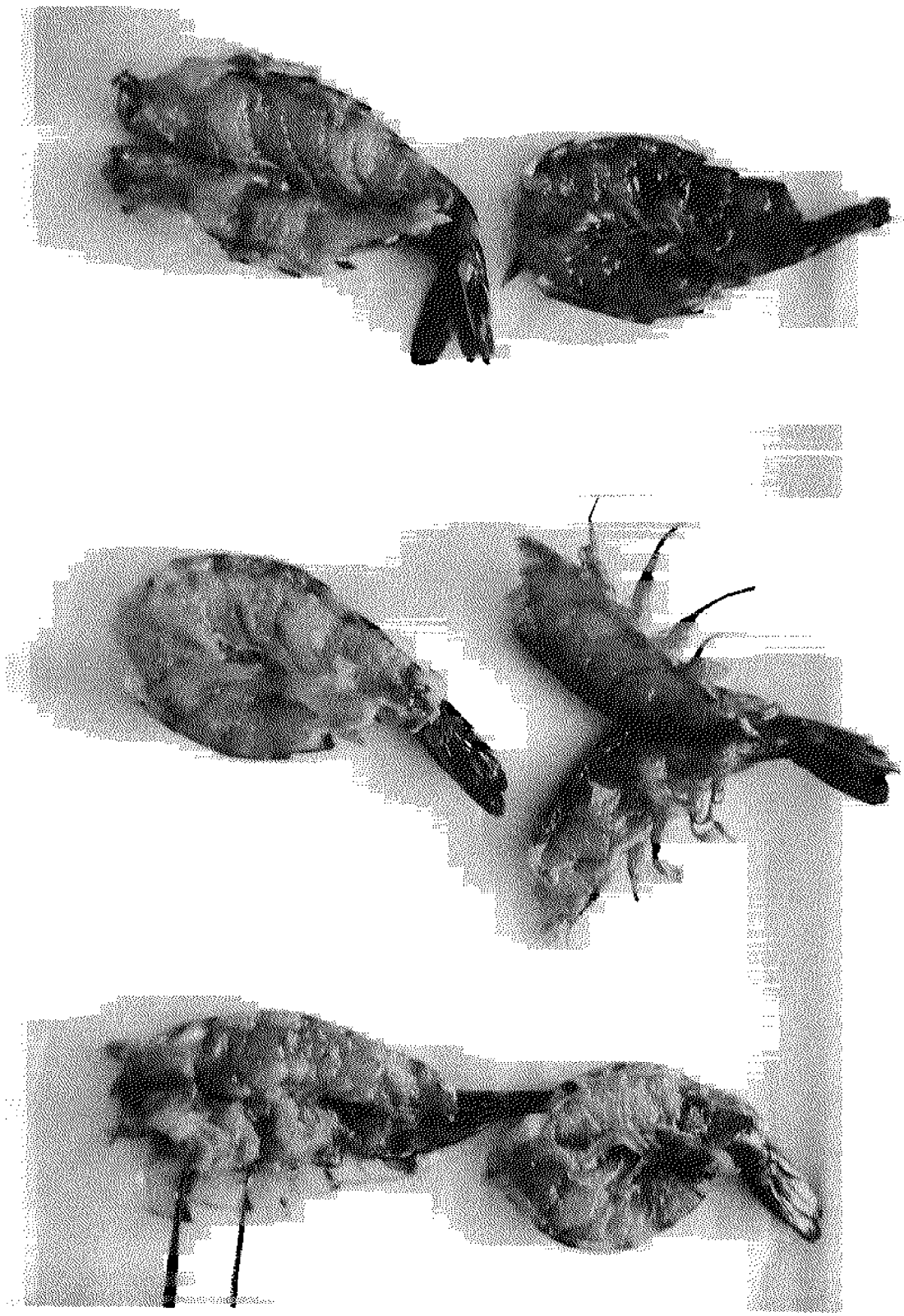


Figure 18. Photographs of gross symptoms of melanized muscle tissue from adolescent Penaeus monodon.

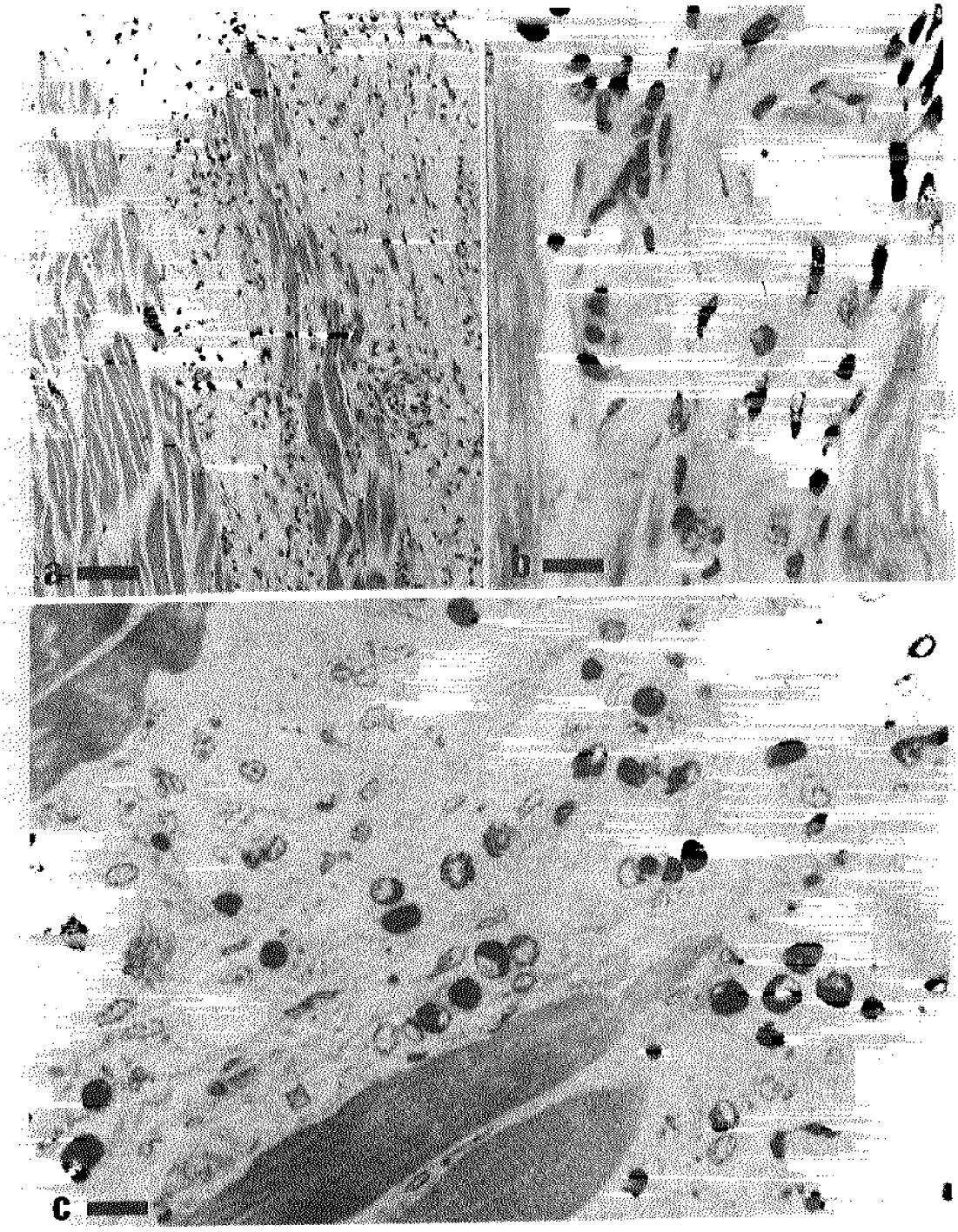


Figure 19. Photographs with the light microscope from an adolescent shrimp specimen with melanized muscle tissue. (a) Low magnification of disintegrating muscle tissue with H&E staining (bar = 40 µm). (b) High-dry view of area where hemocytes are aggregating between disintegrating muscle fibers (bar = 10 µm). (c) Toluidine blue-stained thin section showing large numbers of granulocytes (bar = 10 µm).

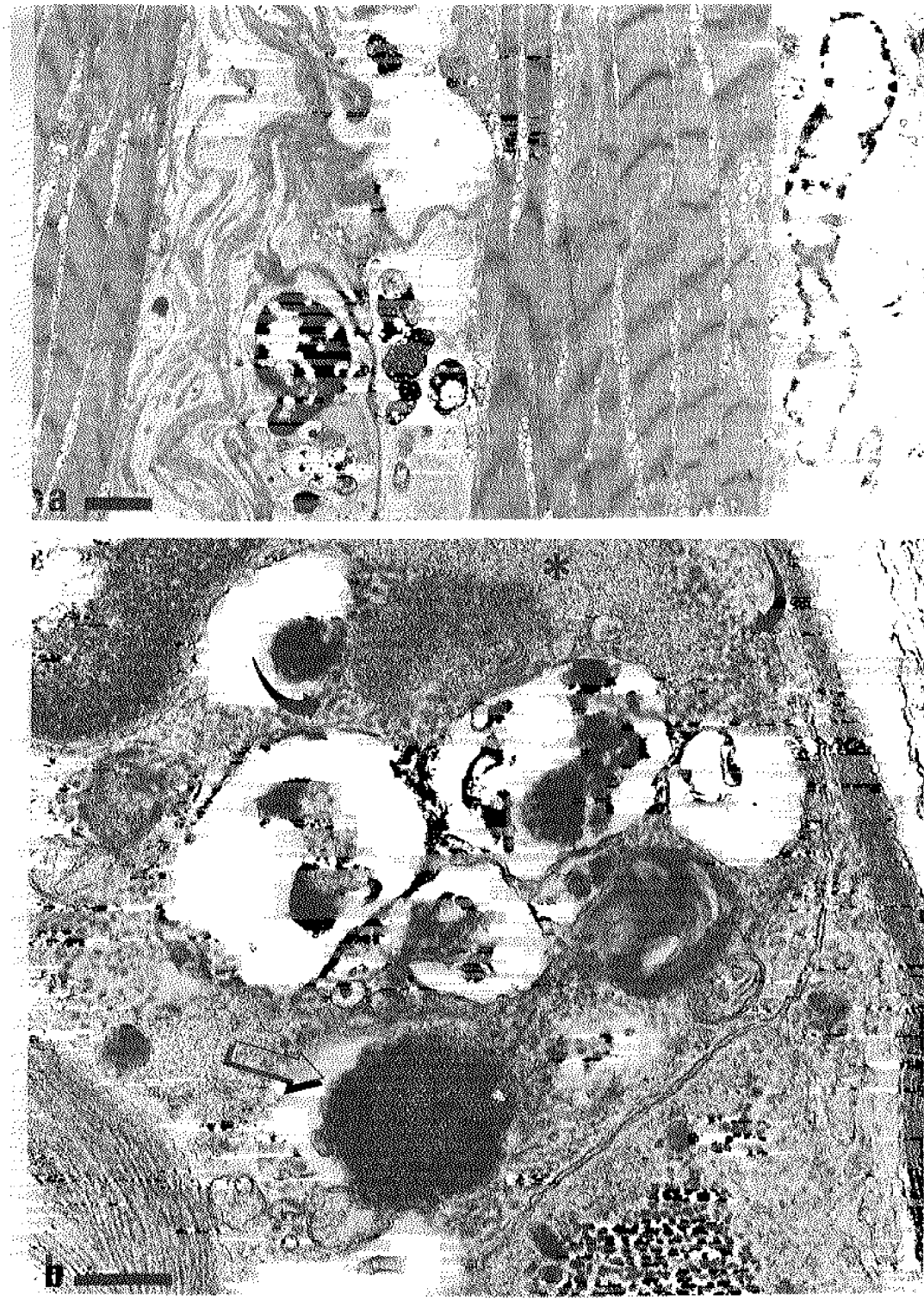


Figure 20. Electron micrographs of tissue from an adolescent shrimp with brown muscle syndrome. (a) Low magnification showing disintegrating sarcoplasm and muscle filaments (bar = 3 µm). Note the lymphocyte in the lower left corner of the micrograph. (b) High magnification of the cytoplasm of the lymphocyte noted in the preceding micrograph, showing a virogenic stroma (asterisk) virions (curved arrows) and paracrystalline arrays (open arrow) (bar = 0.4 µm).

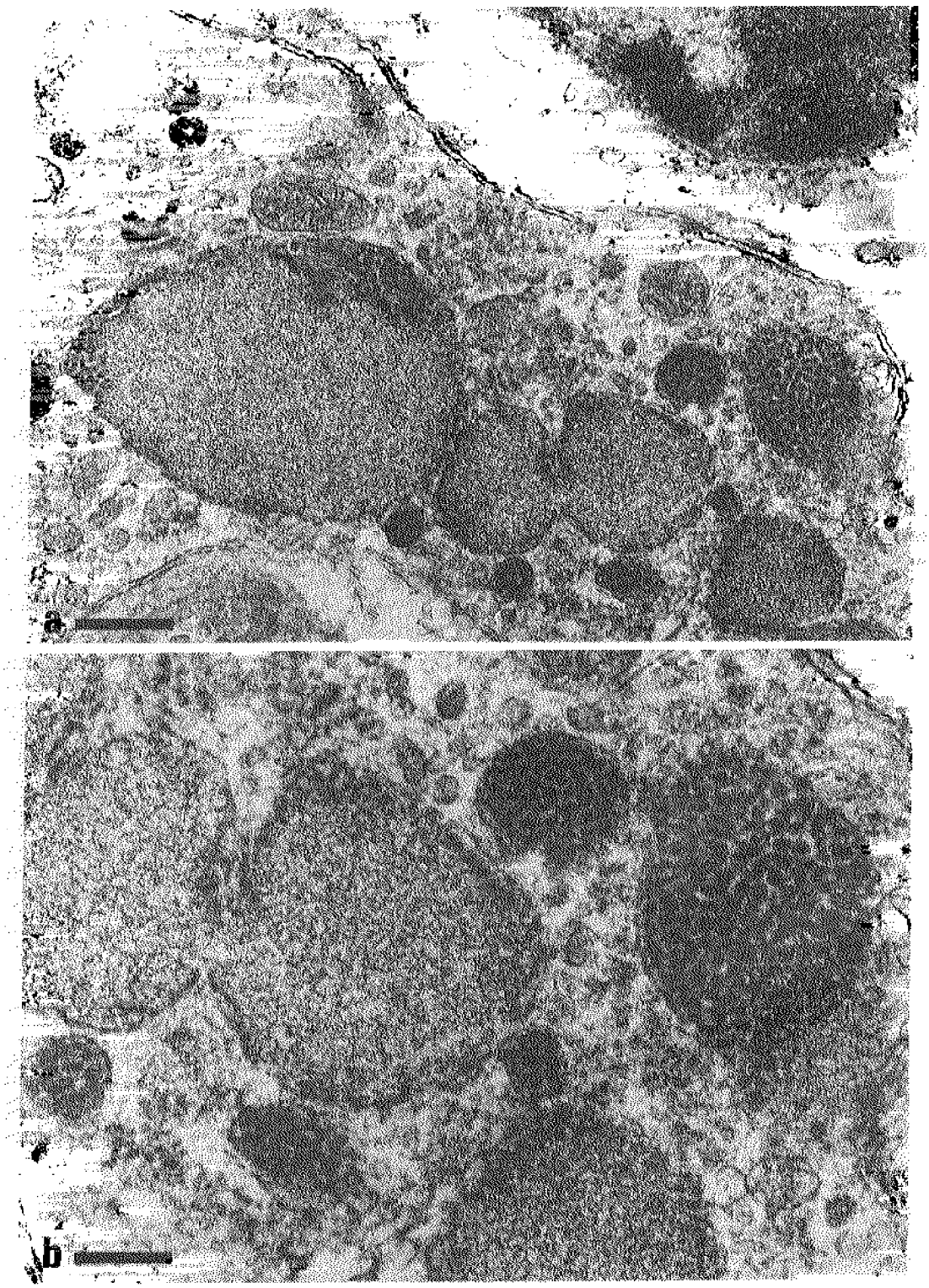


Figure 21. High magnifications of a virogenic stroma and virions (approximately 45 µm in diameter) in the cytoplasm of a lymphocyte from tissue samples of melanized muscle. Compare the appearance of the viral material with that from the lymphoid organ shown in Figures 6 and 7. (Bar in a = 0.4 µm, and in b = 0.2 µm).

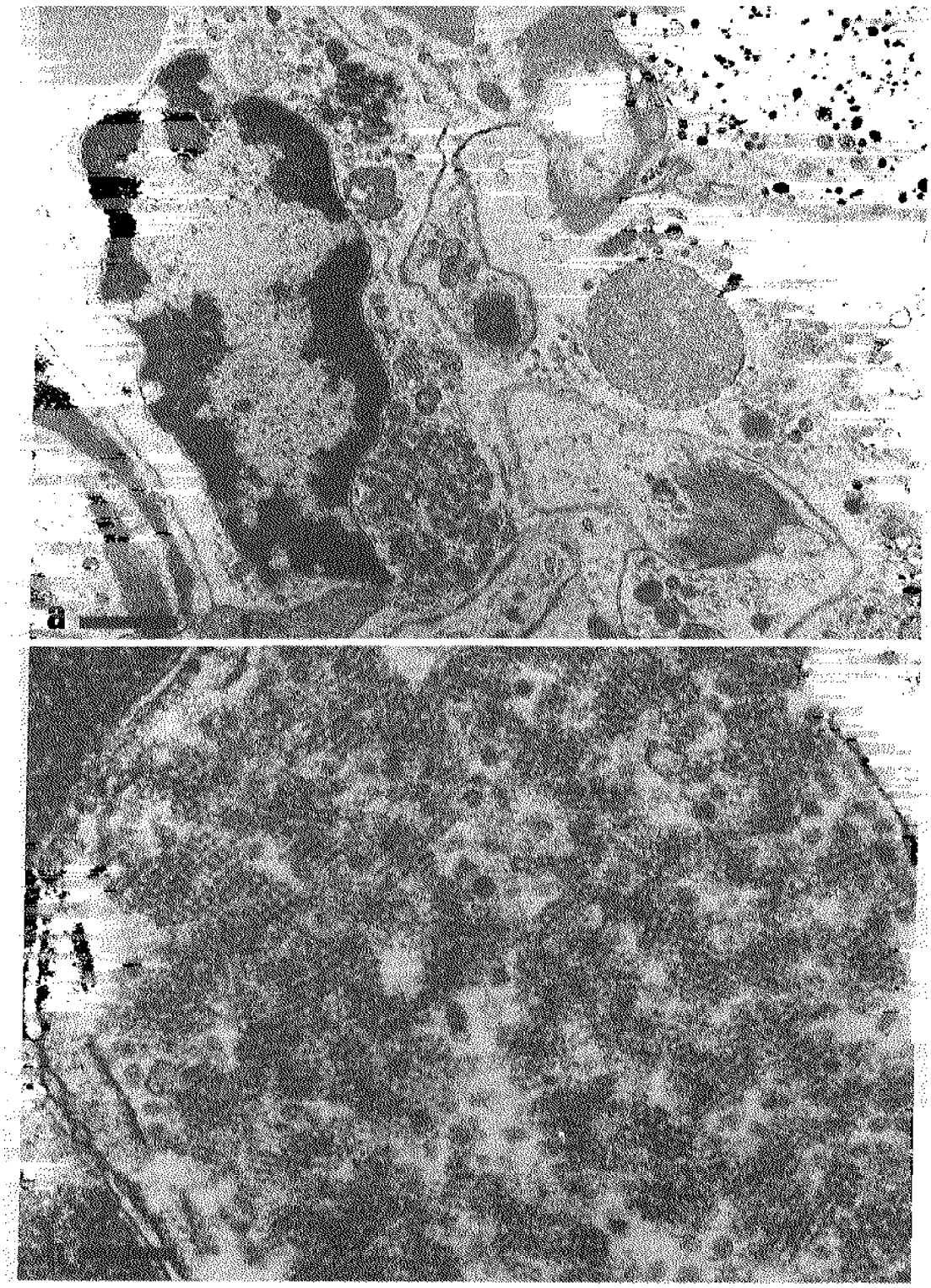


Figure 22. Electron micrographs showing a lymphocyte from melanized muscle tissue of an adolescent shrimp. (a) A lymphocyte with what appears to be a paracrystalline inclusion in the early stages of formation (bar = 1.2 µm). (b) Higher magnification of the paracrystalline array showing what appear to be virions. It is not clear if they are enveloped (bar = 0.2 µm).

ary 1992, reported six out of 83 problem ponds (7%) with this syndrome.

Histological examination of moribund shrimp from one month mortality syndrome (OMMS) ponds around Thailand revealed a variety of infections, but MBV and bacterial septicemia are most often reported. The consensus of participants at a recent meeting convened by the Faculty of Veterinary Science at Chulalongkorn University in Bangkok (June 1989) was that the diseases were a secondary result of unknown environmental stressors.

In one detailed study of five OMMS ponds (unpublished), we histologically examined 25 randomly selected animals in one day from feeding nets and found that 92% (23/25) had small blisters on small appendages (Fig. 13c, d) and what appeared to be a general edema. Other abnormalities were necrotic antennal glands (18/25, or 72%), bacterial septicemia (5/25, or 20%) and MBV (19/25, or 76%). In a separate sample of five animals taken during the same interval from Chantaburi on the opposite coast of the Gulf of Thailand, three animals showed similar blisters, one had a necrotic antennal gland and three had MBV. From our earlier work (Fegan et al., 1991), we did not believe that MBV could be the cause of mortality. However, the blisters and antennal gland necrosis were new, and we believe that these features should be further investigated to determine the causes.

Limsuwan (1991) proposed that OMMS can be avoided by maintaining a stable algal bloom of green/blue-green algae (ideal) or diatoms (less ideal) so that the transparency of the water remains at 20 - 40 cm. If this cannot be done, he recommends the use of oxytetracycline at 2 - 3 g/kg feed for five to seven days in mild cases, and 4 - 5 g/kg feed for five to seven days in severe cases, as the only efficacious treatment.

As an alternative treatment, we have recently begun testing the dye Aquashade for temporary relief when algal blooms crash or when they are slow to build up. Our working hypothesis is that benthic blue-green algae are either directly or indirectly toxic to the shrimp and that Aquashade will prevent or limit their growth and reduce or eliminate the incidence of floating plaques. To date, we have insufficient data to determine if this treatment is successful.

Yellow-head Shrimp

Since early 1990, there have been reports in Thailand of a phenomenon called "yellow-head" shrimp (Limsuwan, 1991). In affected ponds, the shrimp begin by growing very fast and eating more than normal. They then abruptly stop eating, and within one or two days, a few animals are found moribund or dead near the edge of the pond. By the following day, the number of dead shrimp increases to 100 or more, and within the succeeding day all of the shrimp die. The affected animals present a swollen cephalothorax in the region of the hepatopancreas,

and this has a yellowish appearance when viewed through the carapace. Upon removal of the carapace, the hepatopancreas is abnormally light-yellow in color.

Histological examination (H&E staining) of affected shrimp with the light microscope reveals massive changes in the lymphoid organ (LO) with inclusions similar to those described above (Figs. 23 and 24). However, all regions of the LO are affected, even tubules still with open lumens. In addition, other tissues are affected, including interstitial tissues of the hepatopancreas, the antennal gland and hematopoietic tissue.

This may be an infectious outbreak of the virus revealed in the chronically infected broodstock described in the previous section on the lymphoid organ virus. If this syndrome is indeed caused by a virus, it will be the first dangerous viral disease reported for *P. monodon* in Thailand, and urgent action is required to understand the epidemiology and limit its spread.

Conclusions

There are more answers than questions when it comes to disease problems of *P. monodon*. Obviously, many microbial diseases are still inadequately characterized and many others remain to be discovered. Much basic work remains to be done on mechanisms of pathogenicity, sources of infection, life cycles, etc. At the same time, we lack a set of simple, nondestructive, quantitative measures of shrimp health that could be used easily on a farm to detect problems early. Perhaps studies on crustacean and shrimp immunity (e.g., types of hemocytes, enzyme activities, hemolymph factors) will give us some of the needed tools. Work is underway on rapid diagnostic techniques for various diseases to help close the windows of infection.

Although they will never replace good management practice as the best prevention for disease, we should urgently pursue potential benefits from the use of immunostimulants, probiotics and "vaccines" as desired preventives to replace antibiotics.

Environmental diseases caused by pollutants such as insecticides should be a top priority. Rapid and simple detection methods are available for some of these compounds (e.g., EnzyTech detection tickets) but the levels of sensitivity are too low to be of use for shrimp.

Since most of the diseases discussed herein are either induced or exacerbated by pond management activities, more attention needs to be focused on the total dynamics of pond biota interactions and how they influence the health of the shrimp. Computer models may derive some first principles. Included in the total management picture is the urgent need to determine the capacity of the environment to cycle the waste from shrimp ponds. Once this capacity is exceeded, the desired goal of long-term production will be unachieveable. We have the tools at hand,

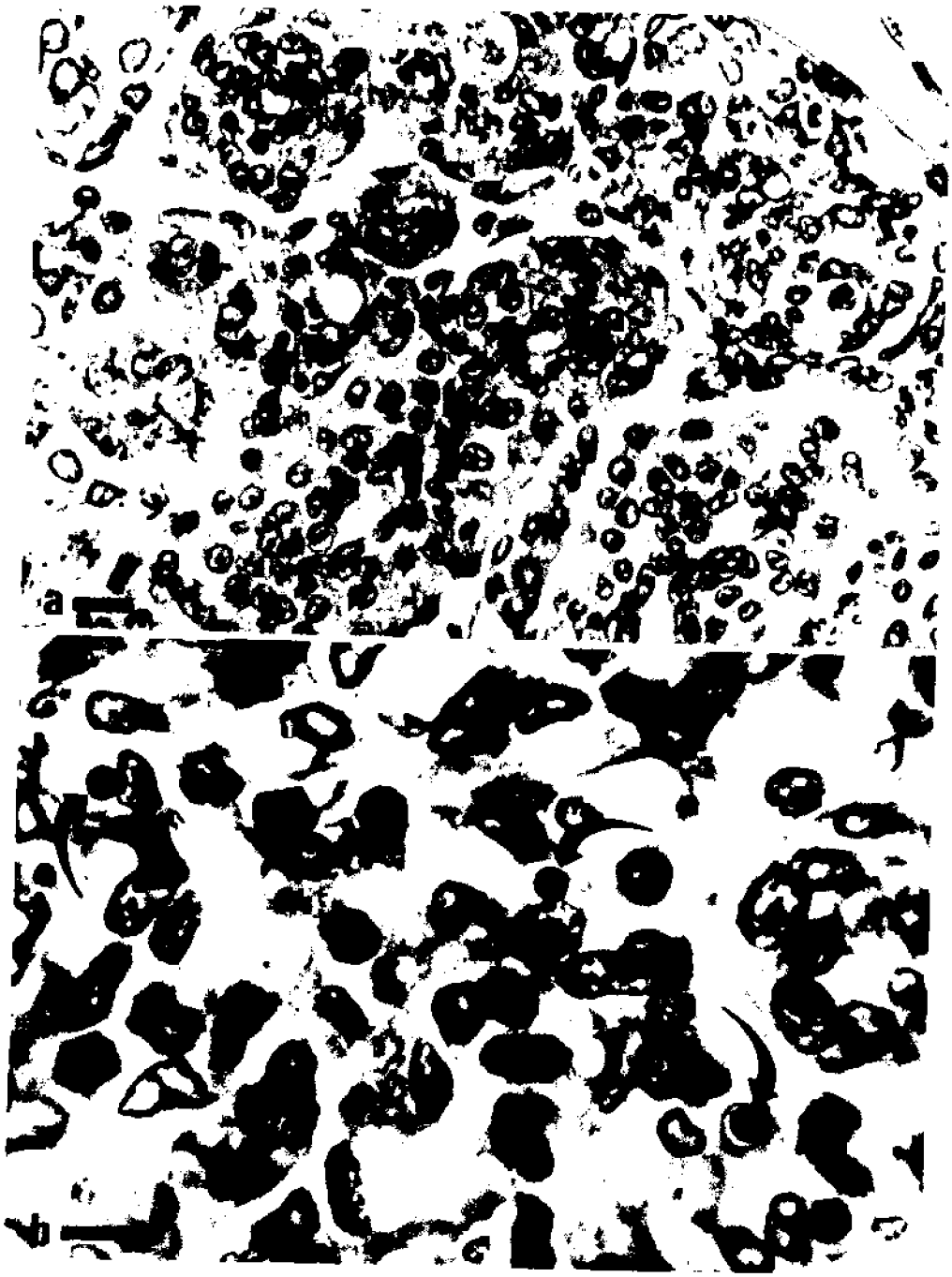


Figure 23. Light microscope photographs of H&E-stained sections of lymphoid organ tissue from a "yellow-head" shrimp. (a) Low magnification showing vacuolated cells and hypertrophic nuclei accompanied by densely staining, basophilic cytoplasmic inclusions marked by curved arrows (bar = 10 μm). (b) Higher magnification (bar = 5 μm).

Figure 24. Light microscope photographs of H&E-stained sections from the shrimp in Figure 23. Tissues other than those of the lymphoid organ contain cells with densely staining cytoplasmic inclusions (DCI). (a) Outer rim of the hepatopancreas showing normal tubule epithelial cells but showing DCI (curved arrows) in the interstitial tissue (bar = 10 μm). (b) High magnification of interstitial tissue from the hepatopancreas showing DCI (curved arrows) (bar = 5 μm). (c) Section of the midgut showing normal columnar epithelial cells to the extreme left but showing DCI (curved arrows) in the underlying connective tissue (bar = 5 μm). (d) Cardiac tissue section showing DCI (curved arrows) (bar = 5 μm). (e) Lymphopoietic tissue showing DCI (curved arrows) (bar = 5 μm).

only greed and a total disregard for our neighbor can prevent us from reaching the goal.

Literature Cited

Adams, A. 1991. Response of penaeid shrimp to exposure to *Vibrio* species. 1: 59-90.

Adams, J.R. 1991. Introduction and classification of viruses of invertebrates. *In*: J.R. Adams and J.R. Bonami (Eds.). Atlas of Invertebrate Viruses. CRC Press, Boca Raton. pp. 1-8.

Adams, J.R. and J.T. McClintock. 1991. Baculoviridae. Nuclear polyhedrosis viruses. Part I. Nuclear polyhedrosis viruses of insects. *In*: J.R. Adams and J.R. Bonami (Eds.). Atlas of Invertebrate Viruses. CRC Press, Boca Raton. pp. 87-204.

Agnihorti, N.P., H.K. Jain and V.T. Gajbhiye. 1986. Persistence of some synthetic pyrethroid insecticides in soil, water and sediment. Part I. J. Entomol. Res. 10: 147-151.

Aoki, T. 1992. Chemotherapy and drug resistance in fish farms in Japan. Proceedings to the First Symposium on Diseases in Asian Aquaculture, Fish Health Section, Asian Fisheries Society, Bali, Indonesia. 26-29 Nov. 1990.

Armstrong, D.A., D.V. Buchanan, M.H. Mallon, R.S. Caldwell and R.E. Millemann. 1976. Toxicity of the insecticide methoxychlor to the Dungeness crab *Cancer magister*. Mar. Biol. 38: 239-252.

Baticados, M.C.L., C.R. Lavilla-Pitogo, E.R. Cruz-Lacierda, L.D. de la Peña and N.A. Suñaz. 1990. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. Dis. Aquat. Org. 9: 133-139.

Bell, T.A. and D.V. Lightner. 1988. A Handbook of Normal Shrimp Histology. Special Publication No. 1, World Aquaculture Society, Baton Rouge, LA. 114 p.

Bonami, J.R. and D.V. Lightner. 1991. Unclassified viruses of crustacea. *In*: J.R. Adams and J.R. Bonami (Eds.). Atlas of Invertebrate Viruses. CRC Press, Boca Raton. pp. 597-622.

Bourquin, A.W., R.L. Garnas, P.H. Pritchard, F.G. Wilkes, C.R. Cripe and N.I. Rubinstein. 1979. Interdependent microcosms for the assessment of pollutants in the marine environment. Int. J. Environ. Stud.. 13: 131-140.

Bradbury, S.J. 1973a. The effect of parathion on crustacean skeletal muscle - I. The mechanical threshold and dependence on Ca^{++} ions. Comp. Biochem. Physiol. 44A: 1021-1032.

Bradbury, S.J. 1973b. The effect of parathion on crustacean skeletal muscle - II. Disruption of excitation-contraction coupling. Comp. Biochem. Physiol. 44A: 1033-1046.

Brock, J.A. 1991. An overview of diseases of cultured crustaceans in the Asia-Pacific region. ADB/NACA, Fish Health Management in Asia-Pacific. ADB Agriculture Dept. Report Series No. 1. NACA, Bangkok, Thailand. pp. 347-395.

Chalmers, A.E. and M.P. Osborne. 1986. The crayfish stretch receptor organ: a useful model system for investigating the effects of neuroactive substances. I. The effect of DDT and pyrethroids. Pestic. Biochem. Physiol. 26: 128-138.

Clark, J.R., L.R. Goodman, P.W. Borthwick, J.M. Patrick, Jr., G.M. Cripe, P.M. Moody, J.C. Moore and E.M. Lores. 1989. Toxicity of pyrethroids to marine invertebrates and fish: a literature review and test results with sediment-sorbed chemicals. Environ. Toxicol. Chem. 8: 393-401.

Cripe, G.M, A. Ingley-Guezou, L.R. Goodman and J. Forester. 1989. Effect of food availability on the acute toxicity of four chemicals to *Mysidopsis bahia* (Mysidacea) in static exposures. Environ. Toxicol. Chem. 8: 333-338.

Davis, B.D., R. Dulbecco, H.N. Eisen and H.S. Ginsberg. 1990. Microbiology. 4th ed. p. 927.

Day, K.E. 1989. Acute, chronic and sublethal effects of synthetic pyrethroids on freshwater zooplankton. Environ. Toxicol. Chem. 8: 411-416.

Dixon, B.A., J. Yamashita and F. Evelyn. 1990. Antibiotic resistance of *Aeromonas* spp. isolated from tropical fish imported from Singapore. J. Aquatic Animal Health. 2: 295-297.

Eisler, R. 1969. Acute toxicities of insecticides to marine decapod crustaceans. Crustaceana. 16: 302-310.

Elmalouk, T. and T. Giessner. 1976. Species difference in metabolism of parathion; apparent inability of hepatopancreas fractions to produce paraoxon. Comp. Biochem. Physiol. C. Comp. Pharmacol. 53: 19-24.

Fegan, D.F., T.W. Flegel, S. Sriurairatana and M. Waiyakruttha. 1991. The occurrence, development and histopathology of monodon

baculovirus in *Penaeus monodon* in southern Thailand. Aquaculture. 96: 205-217.

Flegel, T.W., D.F. Fegan, M. Guerin, S. Boonyaratpalin and S. Sriurairatana. 1992. High mortality of black tiger prawns from cotton shrimp disease in Thailand. *In*: M. Shariff, R.P. Subasinghe and J.R. Arthur (Eds.). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Soc. pp. 181-197.

Fukuyo, Y., K. Yoshida, T. Ogata, T. Ishimaru, M. Kodama, P. Pholpunthin, S. Wisessang, V. Phanichyakarn and T. Piyakarnchana. 1989. Suspected causative dinoflagellates of paralytic shellfish poisoning in the Gulf of Thailand. *In*: T. Okaichi, D.M. Anderson and T. Nemoto (Eds.). Red Tides. Biology, Environmental Science, and Toxicology. Elsevier, N.Y. pp. 403-406.

Gammon, D.W. and G. Sander. 1985. Two mechanisms of pyrethroid action: electrophysiological and pharmacological evidence. NeuroToxicology. 6: 63-86.

Gil-Turnes, M.S., M.E. Hay and W. Fenical. 1989. Symbiotic marine bacteria chemically defend crustacean embryos from a pathogenic fungus. Science. 246: 116-118.

Hatakeyama, S. and Y. Sugaya. 1989. A freshwater shrimp (*Paratya compressa improvisa*) as a sensitive test organism to pesticides. Environ. Pollut. 59: 235-336.

Hill, I.A. 1985. Effects on non-target organisms in terrestrial and aquatic environments. *In*: J.P. Leahey (Ed.). The Pyrethroid Insecticides. Taylor and Francis, London, England. pp. 151-262.

Itami, T., Y. Takahashi and Y. Nakamura. 1989. Efficacy of vaccination against vibriosis in cultured kuruma prawns *Penaeus japonicus*. J. Aquatic An. Hlth. 1: 232-242.

Joia, B.S., G.R.B. Webster and S.R. Loschiavo. 1985. Cypermethrin and fenvalerate residues in stored wheat and milled fractions. J. Agric. Food Chem. 33: 618-622.

Kodama, M., T. Ogata and S. Sato. 1989. Saxitoxin-producing bacterium isolated from *Protogonyaulax tamarensis*. *In*: T. Okaichi, D.M. Anderson and T. Nemoto (Eds.). Red Tides. Biology, Environmental Science, and Toxicology. Elsevier, N.Y. pp. 363-366.

Kongsom, S. 1991. Study of monodon baculovirus (MBV) and bacterial infection in juvenile tiger shrimp, *Penaeus monodon* Fabricius. M.Sc. thesis, Kasetsart University, Bangkaen, Bangkok, Thailand.

Kuhn, R., M. Pattard, K.D. Pernak and A. Winter. 1989. Results of the harmful effects of water pollutants to *Daphnia magna* in the 21 day reproduction test. Wat. Res. 23: 501-510.

Liao, I.C., M.S. Su, C.F. Chang and K.F. Liu. 1990. Experiments on producing *Penaeus monodon* postlarvae free from *Penaeus monodon* baculovirus (MBV). Proceedings of the ROC-Japan Symposium on Fish Diseases, Tungkang Marine Laboratory Contribution No. 95, Tungkang Marine Laboratory, Pingtung 92804, Taiwan, R.O.C. pp. 155-162.

Lightner, D.V., T.A. Bell and R.M. Redman. 1990. A review of the known hosts, geographic range and current diagnostic procedures for the virus diseases of cultured penaeid shrimp. Actes de Colloque. 9: 113-126.

Limsuwan, C. 1988. Bacterial melanization disease in the tiger prawn. Thai Fisheries Gazette. 41: 517-524. (In Thai with English abstract).

Limsuwan, C. 1989. How to solve one month mortality syndrome. His regular column in Tarn Setakit weekly newspaper, 20-25 March, 1989. (In Thai).

Limsuwan, C. 1991. Handbook for cultivation of black tiger prawns. Tansetakit Co. Ltd., 222 Tansetakit Building, Wipawadee-Rangsit Road, Ladyaw, Chatujak, Bangkok 10900, Thailand. 202. p

McLeese, D.W., C.D. Metcalfe and V. Ziko. 1980. Lethality of permethrin, cypermethrin and fenvalerate to salmon, lobster and shrimp. Bull. Environ. Contam. Toxicol. 25: 950-955.

Menasveta, P. 1987. Effects of water pollution on aquaculture development in Thailand. Water Qual. Bull. 12: 116-118.

Megharaj, M., K. Venkateswarlu and A.S. Rao. 1987. Influence of cypermethrin and fenvalerate on a green alga and three cyanobacteria isolated from soil. Ecotoxicol. Environ. Saf., 14: 142-146.

Mulla, M.S., H.A. Darwazeh and L. Ede. 1982. Evaluation of new pyrethroids against immature mosquitoes and their effects on non-target organisms. Mosquitoe News. 42: 583-590.

Nash, G., S. Chinabut and C. Limsuwan. 1987. Idiopathic muscle necrosis in the freshwater prawn, *Macrobrachium rosenbergii* de Man, cultured in Thailand. J. Fish Dis. 10: 109-120.

New, M.B., H. de Saram and T. Singh (Eds.). 1990. Technical and Economic Aspects of Shrimp Farming. Infofish, Kuala Lumpur. pp. 207-222.

Nikl, L., L.J. Albright and T.P.T Evelyn. The influence of several immunostimulants on the immune response of coho salmon to *Aeromonas salmonicida*. (prepublication manuscript) In press.

Owens, L., S. De Beer and J. Smith. 1991. Lymphoidal parvovirus-like particles in Australian penaeid prawns. Dis. Aquat. Org. 11: 129-134.

Raa, J., G. Roerstad, R. Engstad and B. Robertsen. 1992. The use of immunostimulants to increase resistance of aquatic organisms to microbial infections. *In*: M. Shariff, R.P. Subasinghe and J.R. Arthur (Eds.). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Soc. pp. 39-50.

Rashatwar, S.S. and F. Matsumura. 1985. Interaction of DDT and pyrethroids with calmodulin and its significance in the expression of enzyme activities of phosphodiesterase. Biochem. Pharmacol. 34: 1689-1694.

Reddy, P.S., A. Bhagyalakshmi and R. Ramamurthi. 1986a. Carbohydrate metabolism in tissue of fresh water crab (*Oziotelphusa senex senex*) exposed to methyl parathion. Bull. Environ. Contam. Toxicol. 36: 204-210.

Reddy, P.S., A. Bhagyalakshmi and R. Ramamurthi. 1986b. Changes in acid phosphatase activity in tissues of crab, *Oziotelphusa senex senex*, following exposure to methyl parathion. Bull. Environ. Contam. Toxicol. 37: 106-112.

Reddy, P.S., K.V. Ramana Rao and B.N. Murthi. 1988. Changes in nitrogen metabolism of penaeid prawn, *Penaeus indicus*, during sublethal phosphamidon and methyl parathion-induced stress. Bull. Environ. Contam. Toxicol. 41: 344-351.

Robertsen, B., G. Rorstad, R. Engstad and J. Raa. 1990. Enhancement of non-specific disease resistance in Atlantic salmon, *Salmo salar* L., by a glucan from *Saccharomyces cerevisiae* cell walls. J. Fish Dis. 13: 391-400.

Ruangpan, L. and T. Kitao. 1991. *Vibrio* bacteria isolated from black tiger shrimp, *Penaeus monodon* Fabricius. J. Fish Dis. 14: 383-388.

Ruangpan, L. and D. Sae-Oui. 1988. Melanosis in black tiger prawn, *Penaeus monodon*. Seminar on shrimp disease and their control, 7 December, 1988 (Abstracts). National Inland Fisheries Institute, Bangkok, Thailand. p. 20. (In Thai).

Schimmel, S.C., R.L. Garnas, J.M. Patrick, Jr., and J.C. Moore. 1983. Acute toxicity, bioconversion, and persistence of AC 222,705, benthocarb, chlorpyrifos, fenvalerate, methyl parathion and permethrin in the estuarine environment. J. Agric. Food Chem. 31: 104-113.

Sindermann, C.J. and D.V. Lightner (Eds.). 1988. Disease Diagnosis and Control in North American Marine Aquaculture. Elsevier, N.Y. 431 p.

Summonok, J. 1990. Shrimp die from pollution. Livestock Production Magazine. 7: 62-66. (In Thai).

Song, Y.L. and H.H. Sung. 1990. Enhancement of growth in tiger shrimp (*Penaeus monodon*) by bacterin prepared from *Vibrio vulnificus*. Bull. Eur. Ass. Fish Pathol. 10: 98-99.

Sung, H.H., Y.L. Song and G.H. Kou. 1991. Potential uses of bacterin to prevent shrimp vibriosis. Fish Shellfish Immunol. 1: 311-312.

Suvapepun, S. 1989. Occurrences of red tide in the Gulf of Thailand. *In*: T. Okaichi, D.M. Anderson and T. Nemoto (Eds.). Red Tides. Biology, Environmental Science, and Toxicology. Elsevier, N.Y. pp. 41-44.

Tamplin, M.L. 1990. A bacterial source of tetrodotoxins and saxitoxins. *In*: Marine Toxins: Origin, Structure and Molecular Pharmacology. American Chemical Soc. pp. 78-86.

Tijssen, P. and M. Arella. 1991. Parvoviridae. Structure and reproduction of densonucleosis viruses. *In*: J.R. Adams and J.R. Bonami (Eds.). Atlas of Invertebrate Viruses. CRC Press, Boca Raton. pp. 41-53.

Tonguthai, K., and P. Chanratchakool. 1992. The use of chemotherapeutic agents in aquaculture in Thailand. *In*: M. Shariff, R.P. Subasinghe and J.R. Arthur (Eds.). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Soc. pp. 555-566.

Vickers, J.E., P.B. Spradbrow, R.J.G. Lester and J.M. Pemberton. 1990. Detection of baculoviral DNA in infected prawns using polymerase chain reaction. Abstract. Symposium on Diseases in Asian Aquaculture, 26-29 November, 1990, Bali, Indonesia, Asian Fisheries Society. p. 104.

Vogt, G. 1987. Monitoring of environmental pollutants such as pesticides in prawn aquaculture by histological diagnosis. Aquaculture. 67: 157-164.

Ware, G.W. 1986. Fundamentals of Pesticides. Thompson Publications, Fresno, California.

Yu, C.C. and J.R. Sanborn. 1975. The fate of parathion in a model ecosystem. Bull. Environ. Contam. Toxicol. 13: 543-550.

Diseases of *Penaeus monodon* in Taiwan: A Review from 1977 to 1991

I-Chiu Liao
Taiwan Fisheries Research Institute
199 Hou-Ih Road, Keelung, Taiwan

and

Mao-Sen Su and Cheng-Fang Chang
Tungkang Marine Laboratory
Taiwan Fisheries Research Institute
Tungkang, Pingtung, Taiwan

Abstract

Penaeus monodon, the grass prawn, is an economically important prawn species in Taiwan. Production increased steadily from 1968, when artificial propagation techniques were established, through 1987. In the late 1980s, Taiwan became the world leader in cultured prawn production, with a peak production, for *P. monodon* alone, of 95,000 MT in 1987. A year later, however, an unexpected mass mortality occurred, displacing not only many prawn farms but also several sub-businesses in the prawn culture industry. The collapse of the industry was attributed to both pathogenic and nonpathogenic factors. This review describes some grass prawn diseases and disease syndromes reported in Taiwan from 1977 to 1991, including the pathogenic factors that most likely precipitated the 1988 crisis, and the treatments being used.

Introduction

Penaeus monodon, the grass prawn, is one of the most valuable indigenous aquatic species in Taiwan. Its culture history in Taiwan spans only a little more than two decades, beginning in 1968 when this species was first artificially propagated (Liao et al., 1969) and the first hatchery was established. Grass prawn culture soon became very popular because of the species' many favorable characteristics. *Penaeus monodon* exhibits the highest growth rate of all cultured penaeids (Liao and Chao, 1983). It is eurythermal and euryhaline. It is omnivorous rather than carnivorous and, therefore, requires a lower amount of protein in its feed (Liao and Liu, 1989). This translates into lower production costs. Grass prawns also require simple culture facilities such as clay bottom ponds for growout; hence, investment costs, especially the con-

Table 1. Production and export of *Penaeus monodon* in Taiwan, 1968 - 1989 (Liao, 1989).

Year	Production (MT)	Amount Exported (MT)
1968	61	
1969	69	
1970	73	
1971	76	
1972	112	
1973	119	
1974	140	
1975	150	
1976	270	
1977	1,100	
1978	1,600	
1979	4,100	
1980	5,000	
1981	6,000	69
1982	8,000	1,400
1983	15,000	6,100
1984	18,000	9,500
1985	30,000	14,000
1986	60,000	29,000
1987	95,000	42,000
1988	30,000	8,500

struction budget and maintenance costs, are relatively low (Liao, 1989).

These and other factors contributed to the rapid growth of *P. monodon* culture in Taiwan, especially in the late 1980s when Taiwan became the world leader in cultured prawn production. Peak production figures were registered in 1987 when production volume for *P. monodon* alone climbed to 95,000 MT (Liao, 1989) (Table 1). In 1988, however, mass mortality struck Taiwanese *P. monodon* farms. Production dropped by 70%, resulting in substantial losses to many farms and other businesses such as feed manufacturers, harvesters, and food processors.

A combination of pathogenic and non-pathogenic factors were attributed to the collapse of the industry. Of these, the pathogenic factors were prominent. No major problems were encountered under the traditional polyculture and extensive culture systems, with the exception of natural disasters and the presence of predators and competitors. As the culture systems shifted to semi-intensive and intensive styles, stocking densities were raised to 40 - 60 ind./m^2 and formulated feeds were used. Water quality and the general culture environment became harder to manage, and the culture species became more susceptible to diseases. Eventually, a variety of diseases, such as fouling by protozoan epicommensals and ectozoic algae, black gill disease, gill decay, telson damage, body cramp, and red discoloration were reported to afflict the prawn (Liao et al., 1977; Chen and Hwang, 1979; Liao, 1985; Liao et al., 1985).

As long as culture conditions are optimal, *P. monodon* appears to tolerate light to moderate infections (Lightner et al., 1987). However, poor management of the culture environment by farmers has, in many cases, most likely caused outbreaks predisposed by stressors, such as poor water quality, deteriorating environmental conditions and poor nutrition.

From 1977 to 1984, the study of prawn diseases was a minor concern in Tai-

wan. Since 1985, however, diseases have become a major issue, especially after the 1988 crisis. Scientists and prawn farmers have been doing their best to restore the industry by looking into the factors that caused the crisis. This review deals with the diseases and disease syndromes of *P. monodon* reported in Taiwan from 1977 to 1991, with emphasis on developments after the 1988 crisis, and the various measures that are being taken or proposed to recover from these diseases.

Diseases and Disease Syndromes

Epicommensal Infestations

Pathogens and Symptoms

Epicommensal organisms are apparently ubiquitous in prawn culture facilities (Lightner, 1985). All life stages may be affected, but the most serious losses are encountered in juvenile and adult stages, when the gills of the host become heavily fouled by epicommensal organisms such as filamentous bacteria, peritrich protozoans and pinnate diatoms, resulting in various forms of gill disease (Lightner, 1985). Among the more serious epicommensal diseases of cultured prawns, those caused by protozoans cause the most harm to grass prawns (Liao et al., 1977, 1985; Cheng and Liu, 1986). If there is too much organic matter in the pond, large numbers of protozoa readily attach to the body surfaces, appendages, or gills (Figs. 1-3).

Prawns infected by epicommensals are characterized by rough body surfaces, gill diseases or both. Protozoan epicommensals have not been observed growing internally in the gills or in other tissues (Fig. 4) but, when abundant on the body surfaces, appendages or gills, can cause difficulties in locomotion, feeding, molting, and respiration, resulting in mortalities (Couch, 1978; Johnson, 1978; Lightner, 1983, 1985; Chen et al., 1989b).

The most commonly reported protozoans include the peritrich ciliates *Zoothamnium* spp. and *Epistylis* spp. and the suctorian *Acineta* spp. Shigueno (1975), Johnson (1978), Liao et al. (1977, 1985), Chen (1978), Tareen (1982), Liao and Chao (1983), Lightner (1983, 1985), and Liao (1984) reported that *Epistylis* spp. infect prawn culture ponds, in general. Tung et al. (1991) studied *P. monodon* diseases in southern Taiwan and found that the rate of epicommensal infection was 6.9%, with the infection rate of *Zoothamnium* sp. at 80%. When this species attaches to *P. monodon*, the prawn turns blackish-brown and its body surfaces become fouled, movement is impaired, activity is reduced and, in serious cases, heavy mortalities result (Johnson et al., 1973; Johnson, 1978; Liao et al., 1985; Lightner, 1985; Cheng and Liu, 1986; Chang and Su, 1990). Table 2 lists the commonly reported epicommensal protozoans in Taiwan and proposed prevention or control methods.

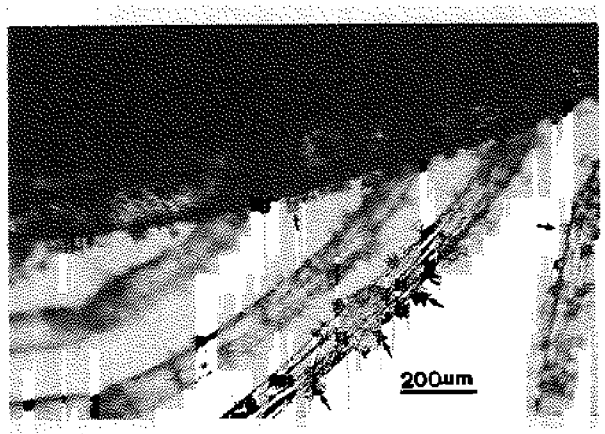


Figure 1

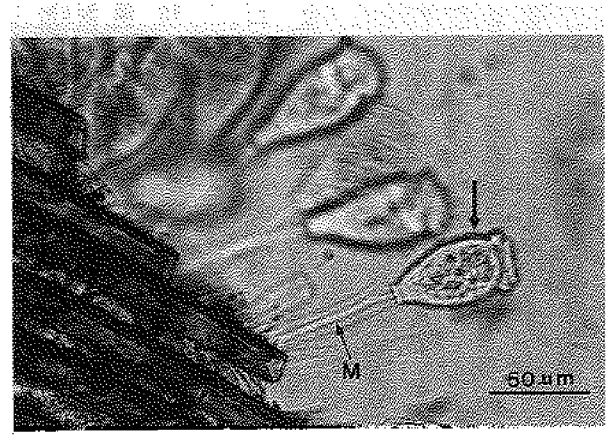


Figure 2

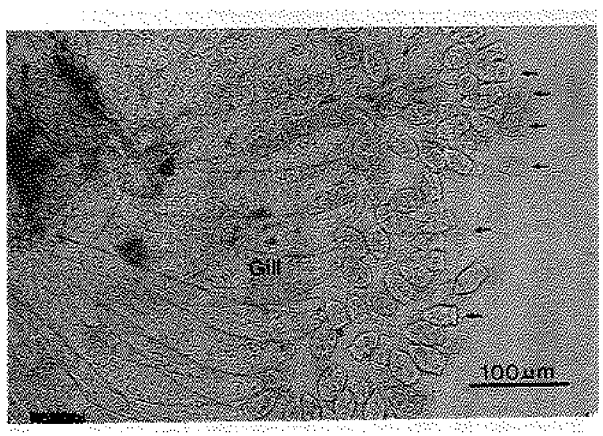


Figure 3

Figure 4

Figures 1 - 4. Epicommensal infestations of Penaeus monodon. *Fig. 1: Microscopic view of the walking legs with numerous* Acineta *sp. (arrows). Fig. 2: Microscopic view of the uropod edge with numerous* Zoothamnium *sp. (bold arrow).* ***M****: Myoneme. Fig. 3: Microscopic view of the gill. Heavy infection of the gill filament with* Zoothamnium *sp. Fig. 4: Histological section of the shell. Numerous* Epistylis *sp. (arrows) attached to the shell and the cuticle (Cut) is intact. H&E stain.*

Table 2. Epicommensal protozoan diseases of *Penaeus monodon* in Taiwan and suggested treatments. (Includes those reported from 1977 to 1991).

Disease	Agent	Stages	Infection Rate (%)	Treatment	References
Ciliate infestation	*Epistylis*	All stages		Formalin, 25 ppm or teaseed cake with 10% saponin.	Liao et al., 1977
		Juvenile to adult		Formalin, 30 ppm	Chen, 1978
	Epistylis (*E*), *Zoothamnium* (*Z*) and *Acineta*	Juvenile to adult	1.5		Chen and Hwang, 1979
		Juvenile to adult	Heavy	Formalin, 25-30 ppm, 1 day or copper sulfate, 0.5 - 1 ppm.	Liao et al., 1985
		Larvae		Formalin, 15 - 20 ppm, 10 - 12 h; or copper sulfate, 0.3 - 0.5 ppm	Liao et al., 1985
		Juvenile to adult	78	Formalin, 30 ppm	Yu and Chang, 1988
			60.2		Tung, 1989
			50	Formalin, 30 ppm, 1 day	Chang and Su, 1990; Chang, 1989
			76.9		Tung et al., 1991
			Z (80%)		
			E (20%)		
Nematode	*Thymascar*	Juvenile to adult		Formalin, 25 - 30 ppm, 1 day or copper sulfate, 0.5 - 1 ppm, 10 - 12 h or potassium permanganate, 25 - 30 ppm, 30 - 60 min	Liao et al., 1985
		Juvenile to adult	Heavy		Chen, 1978
				Change water, zeolite, 100 - 150 kg/1,000 m^2	Chang, 1989
Gregarine	*Cephalolobus*	Juvenile to adult	80	Not harmful	Chang, 1989

Treatment

Prawns are first screened for infections, and the culture environment is improved before chemical treatment is used. If the infection is not serious, the water is replaced, stimulating molting. After the prawns have molted, the water is changed again once or twice. If the infection is serious, 10 - 15 ppm teaseed cake containing 10% saponin is applied to the whole pond to stimulate molting. After the prawns have molted, the water is replaced two to three times to eliminate the protozoans (Liao et al., 1977).

Formalin can also be used. Adult prawns are treated with a 25 - 30 ppm bath for one day (Johnson et al., 1973; Chang and Su, 1990); larvae are given a 15-ppm bath for one day; and juveniles receive 15 - 20 ppm for 10 - 12 h (Liao et al., 1985). Prawns are not fed during Formalin treatment and the water is drained after 24 h to remove any traces of the chemical. After treating with Formalin for one or two days, benzalkonium chloride (BKC) is applied at a rate of 0.5 - 1.0 ppm for one day to prevent secondary infection. If the pond bottom deteriorates and has a high organic content, i.e., high nutrient load and heavy siltation, 100 - 120 kg/1,000 m^2 zeolite (silicate hydrated alkalialuminum) is applied to improve the pond condition and to inhibit the growth of protozoans (Chang, 1989).

Yellow Gill Disease

Pathogens and Symptoms

Diseased prawns appear normal externally except for a slightly darker appearance and the presence of light to dark yellow inflamed lesions in the gills (Fig. 5). If the infection is not serious, prawn activity and feeding is normal; otherwise, activity and feeding is reduced. Microscopic observation of the gill filaments of diseased prawns has revealed the presence of diatoms (Fig. 6). Prawns may become so infested by diatoms for an extended period that molting may be seriously impaired or be incomplete, resulting in mortalities due to hypoxia.

Treatment

Strict water quality management is observed, including more frequent water exchange to maintain transparency at 30 - 40 cm.

Nematode Parasitic Disease

Pathogens and Symptoms

Thymascar sp. is the etiological agent of nematode parasitic disease. Tissues infected by this parasite include the gills. Diseased prawns have dark body surfaces and melanized hemocytic gill lesions. The nematode attaches to the gills and body surfaces, impairing molting and feeding. However, the disease is not serious; only a few mortalities have been recorded. Increases in the population of nematodes may occur because of deterioration of the condition of the pond, i.e., high organic matter content.

Two nematode parasites, *Thymascaris* sp. (Fig. 7) and *Spirocamallanus pereirai*, can be transmitted to prawns either directly or indirectly by copepod eggs or fresh diets (from infected fish which may serve as an intermediate host for the parasites) (Overstreet, 1973; Johnson, 1978; Liao et al., 1985; Chang and Su, 1990).

Treatment

One-half to two-thirds of the pond water is replaced and 150 kg/1,000 m^2 zeolite is applied to improve the pond bottom. A one-day 25- to 30-ppm Formalin bath or a 12-h 0.5- to 1-ppm copper sulfate bath can also be used

Figure 5

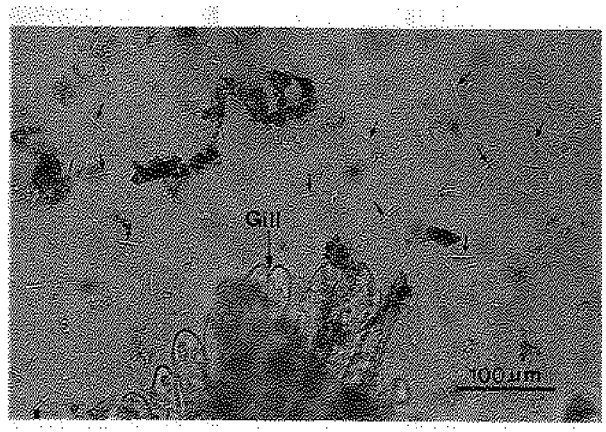


Figure 6

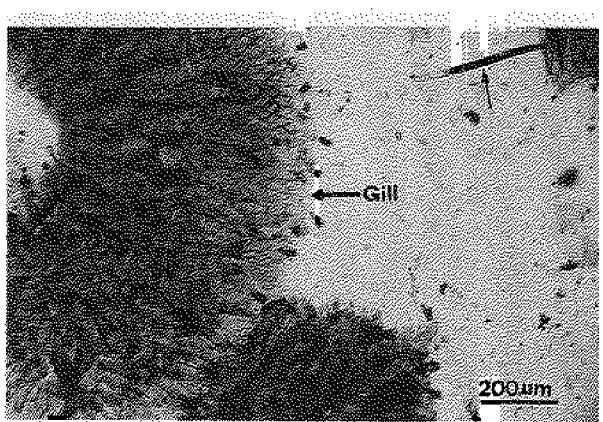


Figure 7

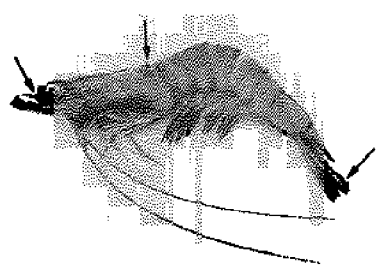


Figure 8

Figures 5 and 6. Yellow gill disease of Penaeus monodon. Fig. 5: *Specimen with well-developed gill lesions. Fig. 6: Microscopic view of the diatom* Nitzschia *sp. on the gills.*
Figure 7. Nematode parasitic disease: Microscopic view of Thymascaris *sp., coexisting with dirt, from the gills of* Penaeus monodon.
Figure 8. Ectozoic algal growth: Juvenile Penaeus monodon *showing grass-like material on the body surfaces (arrows).*

(Liao et al., 1985). After harvesting, the bottom silt is purged and 50-ppm sodium hypochlorite is applied for two weeks to kill the nematode eggs. Also, certain fishes are excluded from the ponds as they may serve as hosts for the parasites.

Ectozoic Algal Growth

Pathogens and Symptoms

Ectozoic algal growth occurs when few phytoplankton are present in the culture pond. Pond transparency is clear. The algae (e.g., *Enteromorpha* sp.) grow and attach to the shells of prawns (Fig. 8). Serious infestations may result in the formation of grass-like material on the body surfaces of the prawns. Infected prawns are lethargic or immobile, often observed lying by the side of the pond (Liao et al., 1977; Liao, 1984). Feeding is decreased or even absent. Dark pond sediments adhere to the shells because molting is impaired, affecting prawn movement. Large populations of algae also decrease dissolved oxygen levels at night, which harms the prawns (Liao et al., 1977, 1985; Baticados, 1988; Chang, 1989).

Treatment

Attention is given to water quality management by maintaining pond water transparency at 30 - 40 cm. If pond water is clean, it is fertilized with ammonium sulfate:calcium superphosphate:urea = 6:1:0.5, 10 kg/1,000 m^2. When prawns are infected with ectozoic algae, 10- to 20-ppm teaseed cake containing 10% saponin is applied for one-day to stimulate molting. If the infestation is serious, copper sulfate at 0.3 - 0.5 ppm is used, one-day dipping, to kill the algae. When the copper sulfate begins to take effect, water is exchanged more frequently.

Body Cramp

Pathogens and Symptoms

Body cramp usually occurs in the summer, when both air and water temperatures are high (Liao et al., 1977). Prawns exhibit a dorsal flexure of the abdomen that cannot be straightened, i.e., the whole body or certain parts appear cramped and curved, resulting in mortalities. This condition may occur when the temperatures of the upper and lower layers of the pond water and those of the air and pond water vary significantly, particularly during summer harvests or when prawns suddenly jump out of the water. In both instances, there is a rapid contraction of the prawn muscle. Affected prawns usually do not recover (Liao et al., 1977; Cheng and Liu, 1986; Chen and Lee, 1989) (Fig. 9). This condition is sometimes observed during hot nights, when a strong light is aimed at the pond, causing the prawns to jump and, thus, cramp. Besides temperature, poor nutrition, which affects neural transmission, may also contribute to body cramp (Chang, 1989).

Treatment

It is important to provide a nutritionally balanced fresh diet at frequent intervals. Harvesting and handling are

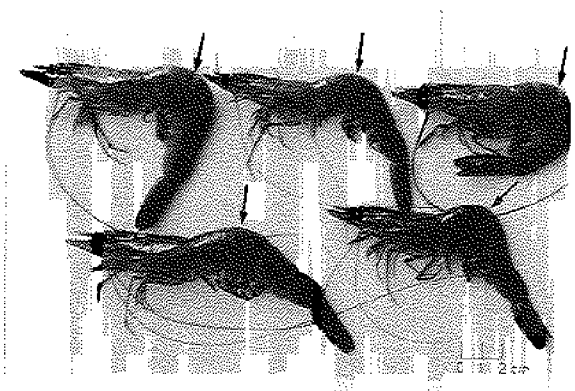


Figure 9

Figure 10

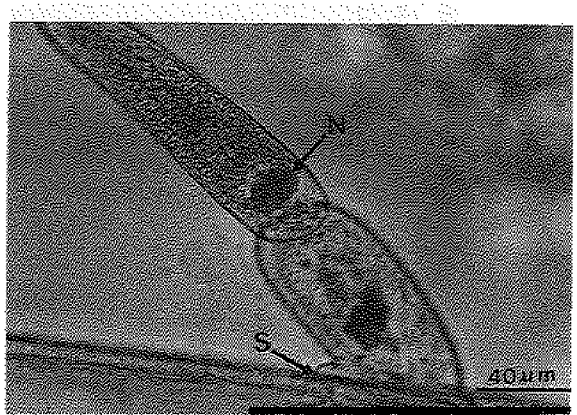


Figure 11

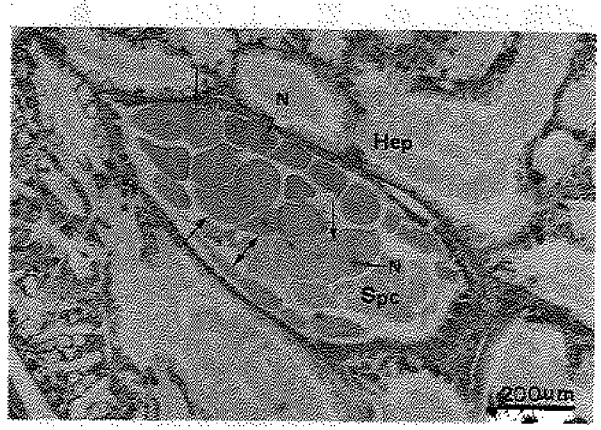


Figure 12

Figure 9. Body cramp: Various degrees of flexion in cramped Penaeus monodon *(Liao et al., 1977).*
Figures 10 - 12. Gregarine disease. Fig. 10: Stomach of an infected Penaeus monodon. *Numerous* Cephalolobus *sp. nearly fill the posterior chamber of the stomach. Fig. 11: Stomach of an infected* Penaeus monodon *showing a gregarine attached to the gastric sieves of the posterior chamber.* ***N****: Nucleus;* ***S****: Sucker. Fig. 12: Histological section of an infected* Penaeus monodon *showing unaffected tissues despite the presence of gregarines. H&E stain.* ***Hep****: Hepatopancreas,* ***Spc****: Posterior chamber of stomach,* ***N****: Nucleus.*

avoided during hot weather. Also, during hot periods, mechanical aeration is increased to maintain uniform water temperature.

Gregarine Disease

Pathogens and Symptoms

Gregarines are common inhabitants of the guts of wild and pond-reared prawns (Johnson, 1978; Overstreet, 1973; Couch, 1983; Lightner, 1985). In an investigation conducted by the authors on cultured prawn diseases in southern Taiwan in 1989, 80% of *P. monodon* were infected with gregarines (Fig. 10).

The gregarine *Cephalolobus* sp. is divided into large and small types; the large type is further divided into cylindrical and calabash shapes. Most of the large types have two or three segments, each of which has a nucleus. The last segment has a sucker that attaches to the gastric sieves (Fig. 11) of the posterior chamber of *P. monodon*'s stomach. The minimum body length of infected prawns was 1.5 - 1.7 cm, while the maximum was 9.8 - 10.2 cm. Seriously infected prawns were 2.5- to 5.5-cm long; *P. monodon* over 10.5-cm long appeared not to be affected by this disease (Chang and Su, 1991). Statistical analysis, investigation of culture ponds and histological observations of infected tissue showed that gregarine infections did not affect the growth of *P. monodon* (Fig. 12) (Chang and Su, 1991). Lightner (1985) reported that gregarines appear not to cause significant disease in penaeid prawns even when present in such large numbers as to occlude the midgut or hindgut lumen. Johnson (1978) also reported that absorption of food by the protozoa is perhaps detrimental, but does minor damage to the host prawn.

Treatment

Gregarines need a mollusc host to complete their life cycle; hence, one way to control the disease is to exclude this mollusc (Johnson, 1978; Baticados, 1988).

Black Gill Disease

Pathogens and Symptoms

Penaeus monodon suffering from black gill disease have brown or dark gills (Fig. 13). Seriously infected prawns exhibit soft shells, slow locomotion, decreased appetite and heavy surface fouling, resulting in mortalities. Table 3 lists some of the factors causing this disease. In Taiwan, black gill disease is caused by:

- Poor pond bottom conditions due to the culture system practiced, i.e., high prawn density and too much excrement in the pond, residual food or an overly long culture period, which cause organic matter to accumulate on the bottom (Chen and Hwang, 1979; Liao et al., 1985);

- Extended exposure to toxic pollutants such as cadmium, copper, potassium permanganate, zinc,

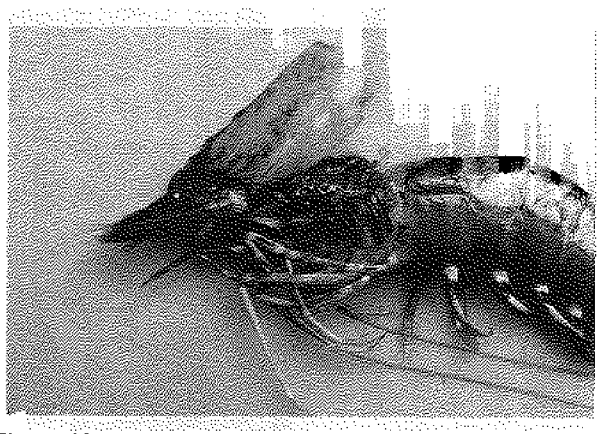


Figure 13

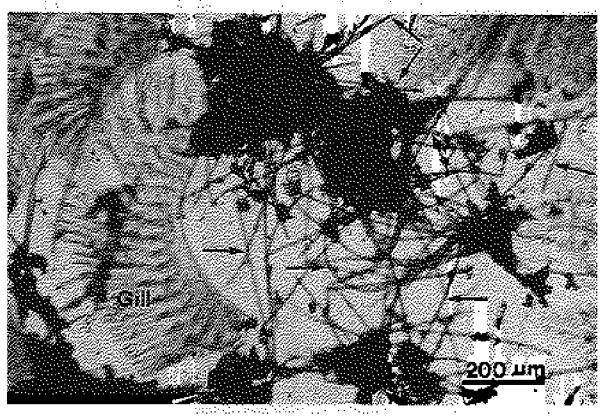


Figure 14

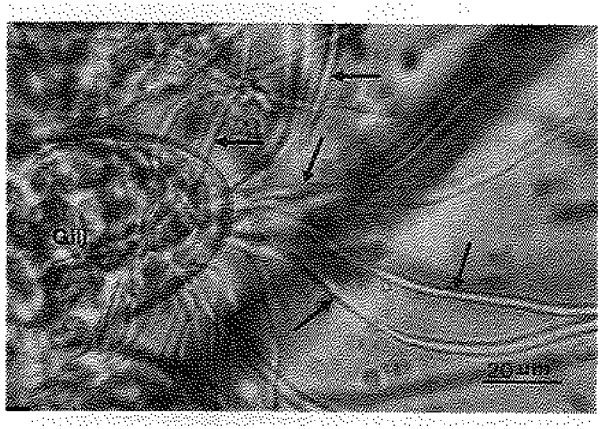


Figure 15

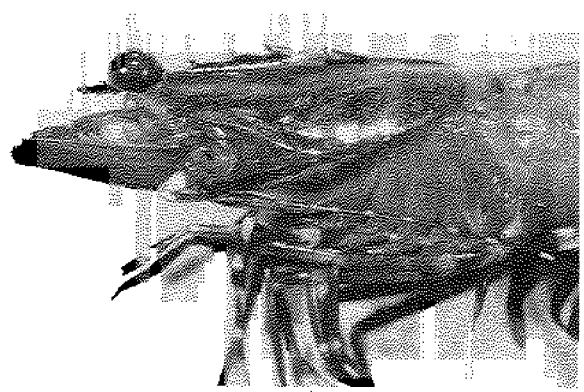


Figure 16

Figures 13 - 15. Black gill disease of Penaeus monodon. Fig. 13: *Heavy fouling of the gills (Liao et al., 1985). Fig. 14: Blue-green algae* (Oscillatoria *sp.) lesions on the gills. Fig. 15: Filamentous bacteria* (Leucothrix mucor) *on the gills.*
Figure 16. Red gill disease: Penaeus monodon *with well-developed lesions on the gills.*

Table 3. Black gill diseases of *Penaeus monodon* and their treatments in Taiwan.

Agent	Stages	Infection Rate (%)	Treatment	References
Ciliates (epizoa)	Juvenile to adult	15	Formalin, 30 ppm	Chen, 1978; Cheng and Liu, 1986 Chang and Su, 1990; Chang, 1989
Poor condition of pond bottom			Change water	Chen and Hwang, 1979; Liao et al., 1985
			Change water	Yu and Chang, 1988; Chang, 1989
Fungi	All	3	Malachite green, 0.5 - 0.8 ppm, or methylene blue, 8 - 10 ppm, 1 day	Liao et al., 1985; Chang, 1989 Chang and Su, 1990
Bacteria	Juvenile to adult			Cheng and Liu, 1986
Filamentous bacteria	All		Formalin, 30 ppm and change water	Chang and Su, 1990; Chang, 1989
Toxic pollutants	Juvenile to adult		Change water	Liao et al., 1985; Chang, 1989

ozone, ammonia and nitrite (Lightner, 1985) Baticados, 1988; Chen and Lee, 1989);

- Infestation of blue-green algae, e.g., *Oscillatoria* sp. (Fig. 14), *Spirulina* sp. and *Schizothrix* sp. (Lightner, 1985), which hinders water flow through the gill, stimulates the gill epithelial cells to proliferate, and causes soil particles to accumulate in the gills (Chang, 1989); and

- Filamentous bacteria (*Leucothrix mucor*) and epizootic infection (Fig. 15), which generally occur in sea water, in conjunction with heavy siltation, resulting in mortalities due to hypoxia or impaired molting (Sindermann, 1977; Lightner, 1983, 1985; Baticados, 1988; Chen and Lee, 1989). The same condition also occurs when numerous epizoa attaches to the gill (Baticados, 1988; Chang and Su, 1990).

Treatment

If the disease is caused by poor bottom conditions and algal attachment, the pond water is changed and zeolite at 100 kg/1,000 m^2 applied. If the disease is caused by epizoa and filamentous bacteria, Formalin at 25 to 30 ppm, one-day dipping, is applied. After Formalin treatment, water is replaced once or twice. Malachite green at 0.5 - 0.8 ppm or methylene blue at 8 - 10 ppm, one-day dipping, are also used (Liao et al., 1985). Malachite green and methylene

blue may remain in prawn tissue for up to one month; therefore, prawns treated with these chemicals are not harvested until about one month after treatment has been halted because the prawns can be carcinogenic.

Red Gill Disease

Pathogens and Symptoms

In the early stages of the infection, the gills appear light red. External body color, feeding and activity are normal. As the infection worsens, the gill becomes dark red, (Fig. 16) and the prawns stop feeding, choosing instead to lie on the pond side where they can be easily caught by hand. The prawns gradually weaken further and eventually die. Mortalities occur about two to three weeks after the onset of disease.

Red gill disease is caused by poor culture conditions, i.e., poor water quality and low oxygen levels. Microscopic observation shows that the dark-red gill filaments are filled with red-radiation line like capillaries (Fig. 17). *Vibrio parahaemolyticus* and *V. anguillarum* were sometimes isolated from the gill tissue and hemolymph of diseased individuals (Liao et al., 1985), but *V. damsela* and *V. harveyi* have also been reported to cause this disease (Huang, 1989). Broodstock are susceptible to red gill disease.

Treatment

To treat this disease, the culture environment is improved, prawns are moved to other ponds, or the water is changed frequently. At regular intervals of every 3 to 4 weeks, 50% BKC, 50% hyamine (benzethonium chloride) at 1 - 2 ppm (Liao et al., 1985) or 20% furazolidone at 10 - 20 ppm, one-day dipping, is used to prevent the spread of the bacteria. If the disease is caused by poor bottom conditions, zeolite at 100 kg/1,000 m^2 is also applied.

Red Discoloration

Pathogens and Symptoms

In the early stages of the infection, the prawn body changes from dark green to yellow green. Prawn behavior and activity are normal. As the infection gradually becomes serious, the body turns light red, red, and finally, dark red (Fig. 18). When the infection is serious, this disease syndrome may be accompanied by red gill disease. Diseased prawns have respiratory difficulties and the amount of fluid in the cephalothorax increases and becomes smelly; also, the hepatopancreas becomes pale (Liao et al., 1977; Sindermann, 1977; Cheng and Liu, 1986). The value of affected prawns may be low if marketed at this time, and when transported, the prawns often die. If red discoloration and red gill disease occur at the same time, heavy mortalities can result.

According to Liao et al. (1977), red discoloration is caused by rancid and spoiled diets or pond detritus that is rich in organic matter. It is not a bacterial infection. Cheng and Liu (1986) reported that this disease occurs when

Figure 17

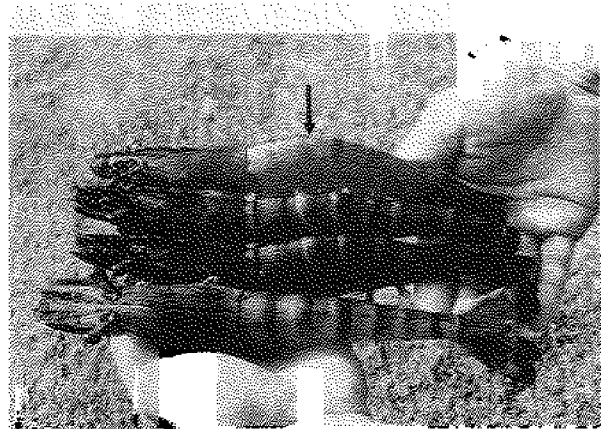


Figure 18

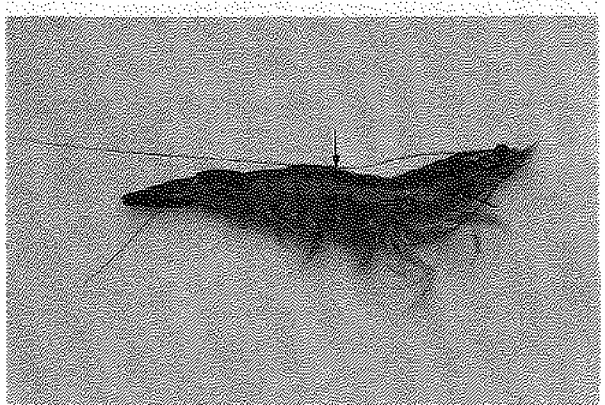


Figure 19

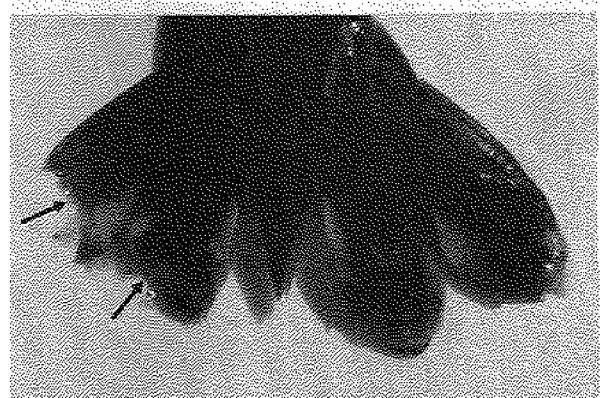


Figure 20

Figure 17. Red gill disease: Penaeus monodon *with red-radiation line on the gill filament.*
Figure 18. Red discoloration: Penaeus monodon *showing different stages of discoloration.*
Figure 19. Shell disease: Penaeus monodon *showing some black spots on the abdominal segment.*
Figure 20. Tail rot: Penaeus monodon *showing shredded tail extremities (arrows).*

the water quality is poor, when there is a high organic matter content and when the reduction layer of the pond bottom is thick. Significant histopathological changes do not accompany the disease. Lightner and Redman (1985) suggested that when the hepatopancreas atrophied and necrosis occurred, stored ß-carotene and other carotenoids were released into the hemolymph, spreading into the whole body.

Treatment

A fresh diet with a high protein content is given at increased rates (Liao et al., 1977). The culture environment is improved and water exchange is more frequent (Cheng and Liu, 1986), and 50% BKC or 50% hyamine at 1 - 2 ppm are used regularly.

Shell Disease

Pathogens and Symptoms

The exoskeletons of diseased individuals have black spots (Fig. 19) at random locations. In general, the cephalothorax, abdominal segments and uropod are easily infected. The black spots gradually cover the entire exoskeleton, with melanin accumulating around the spots. Diseased individuals lose their smoothness; this decreases their market value. Liao et al. (1985) reported that stressors such as excessive handling during transfers, crowding and injuries sustained from other prawns after molting result in external lesions that are secondarily infected with chitinolytic bacteria.

Cheng and Liu (1986) reported that shell disease occurs from April to October. In addition to environmental stress, *Vibrio* spp. can also cause this disease. According to Sindermann (1977), Lightner (1983, 1985) and Chen et al. (1989b), many kinds of production of lipoprotein, chitinolytic bacteria and *Vibrio* sp., *Aeromonas* sp., *Spirillium* sp. and *Flavobacterium* sp. are associated with shell disease.

Treatment

At the early stages of infection, exoskeletal breaks are not yet evident. The water is changed two or three times, or teaseed cake containing 10% saponin at 20 ppm, one-day dipping, (to stimulate molting) are used to treat this disease. Formalin at 20 ppm and malachite green at 0.3 ppm, at the same time, dipping for one day (Liao et al., 1985), are also applied. When infection is serious, BKC at 0.5 - 1 ppm is applied, one-day dipping, and reapplied two or three times once every five to seven days. If a bacterial infection is diagnosed, oxytetracycline or tetracycline at 40 - 60 ppm or furacin, nitrofurans, and furanace at 1 ppm with dipping is applied (Chen et al., 1989b).

Tail Rot

Pathogens and Symptoms

Tail rot has the same manifestations as shell disease, i.e., it is caused by environmental stress, like high density, excessive use of drugs or poor water quality; injuries due to collisions with other prawns; or tail injuries due to

incomplete molting. If secondary infection with chitinolytic and other types of bacteria occurs, necrosis of the tail develops, resulting in tail rot (Liao et al., 1985). The early stage of infection is characterized by swelling of the tail, especially near the margins; feeding and movement are still normal. In serious infections, tail extremities have necrosis with some portions torn into shreds (Fig. 20). If the shredding is two-thirds of the tail, prawn mobility is impaired and mortalities result.

Treatments

Following diagnosis, the culture environment is ameliorated first. Water changes are made or the pond bottom is improved to reduce stress. BKC at 1 ppm or furazolidone at 10 - 20 ppm, one-day dipping, is applied (Liao et al., 1985). If epicommensals are detected, Formalin at 20 ppm and malachite green at 0.3 ppm are applied concurrently, one-day dipping.

Penaeus Monodon Baculovirus (MBV) Disease

Pathogens and Symptoms

Infected prawns have no significant external signs of disease; they may, however, exhibit decreased feeding rates, slow growth, double (two-layered) shell and have an atrophied hepatopancreas (Fig. 21). Hispathological observations indicate that the hepatopancreatic tissue is not significantly damaged, but there are eosinophilic intranuclear occlusion bodies in the glandular epithelial cells (Chen et al., 1989c,d) (Fig. 22).

This type of virus spreads very quickly, resulting in high larval mortalities. Damage to adults is less severe. In serious infections, the virus ruptures hepatopancreatic cells, allowing occlusion bodies to pass through the midgut and to be excreted in the feces (Figs. 23 and 24). At this stage, the prawn is weak, exhibiting reduced feeding and activity. The body surfaces and gills are easily fouled with diatoms, epicommensal protozoa and filamentous bacteria, making the prawn susceptible to more serious harm (Anderson and Shariff, 1987; Cheng and Liu, 1986; Chang, 1989; Chen and Chang, 1989; Chen and Lee, 1989; Chen et al., 1989b; Lightner and Redman, 1981).

Chen et al. (1989a,d) reported that in Taiwan, MBV infected *P. monodon*, *P. penicillatus* and *Metapenaeus ensis*; *P. monodon* contracted the most serious infections (infection rate: 1984-1986, 18%; 1987-1988, 80%). According to Liao et al. (1990), the MBV infection rate in female broodstock taken from the coastal waters of Taiwan was only 33% in 1987. The infection rate jumped to 100% in October and December of 1988 and October 1989. The average over the entire year was 85%. MBV prevalence among imported female broodstock was only 40% (Fig. 25). Results of a number of MBV studies are shown in Table 4.

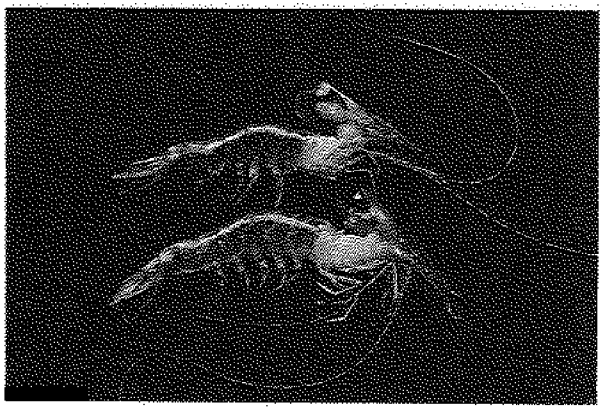


Figure 21

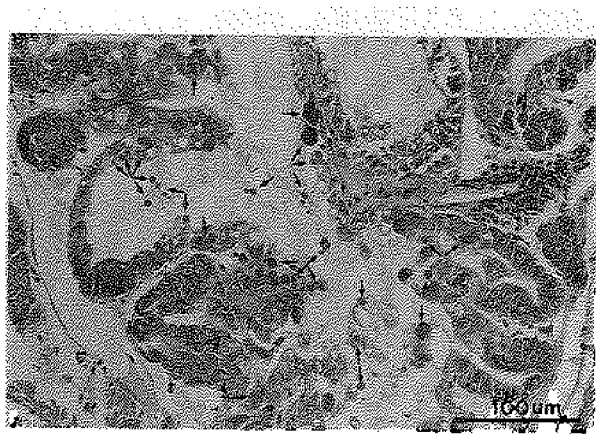


Figure 22

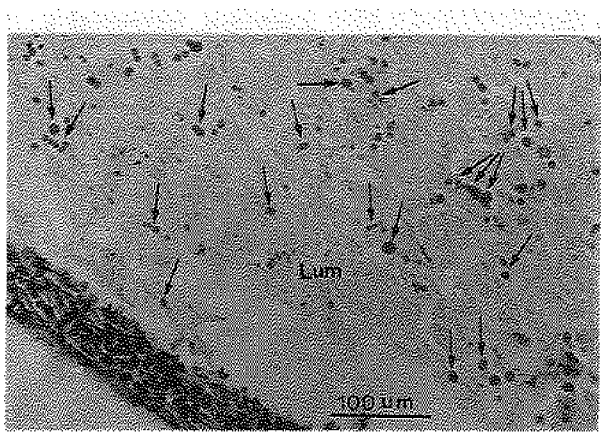


Figure 23

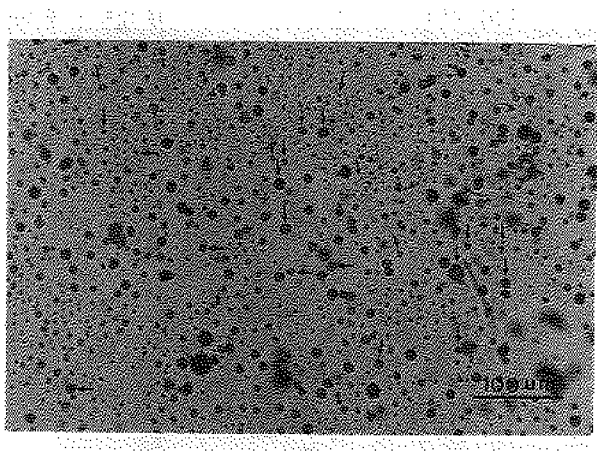


Figure 24

Figures 21-24. MBV in Penaeus monodon. Fig. 21: *Hepatopancreas (top) infected with MBV showing atrophy and whitening. Bottom: normal prawn. Fig. 22: Histological section of hepatopancreas heavily infected with MBV showing large numbers of eosinophilic occlusion bodies in the nuclei of glandular epithelial cells and tube of hepatopancreatic gland. H&E stain. Fig. 23: Histological section of midgut showing numerous occlusion bodies in the lumen. H&E stain.* **Lum**: *Lumen. Fig. 24: MBV occlusion body from feces. 0.05% Malachite green stain.*

Treatments

There is no treatment for MBV disease, but it does not affect healthy prawns. The disease agent is a virus that is latent even through the adult stages. If the prawn is unhealthy, or if a stress such as crowding is present, disease may occur. Therefore, good management is necessary. A density of 30 ind./m^2 is maintained, feeding is controlled, a fresh diet is provided and the protein and vitamin C content in artificial feed are increased. If MBV occurs with bacterial infection, BKC at 1 ppm or 20% furazolidone at 10 ppm, one-day dipping, is applied. The treatment is repeated five to seven days later, two to three times. Oxytetracycline and other antibiotics can also be used, 50 - 100 g/MT prawn weight, mixed in the feed for 1 week. The drugs are added to the feed, e.g., oysters or chicken eggs, using a blender for uniform mixing, then the mixture is exposed to air to dry. According to Liao et al. (1990), when culturing larvae, the eggs or nauplii should be washed with clean water to reduce the infection rate of MBV (Table 5).

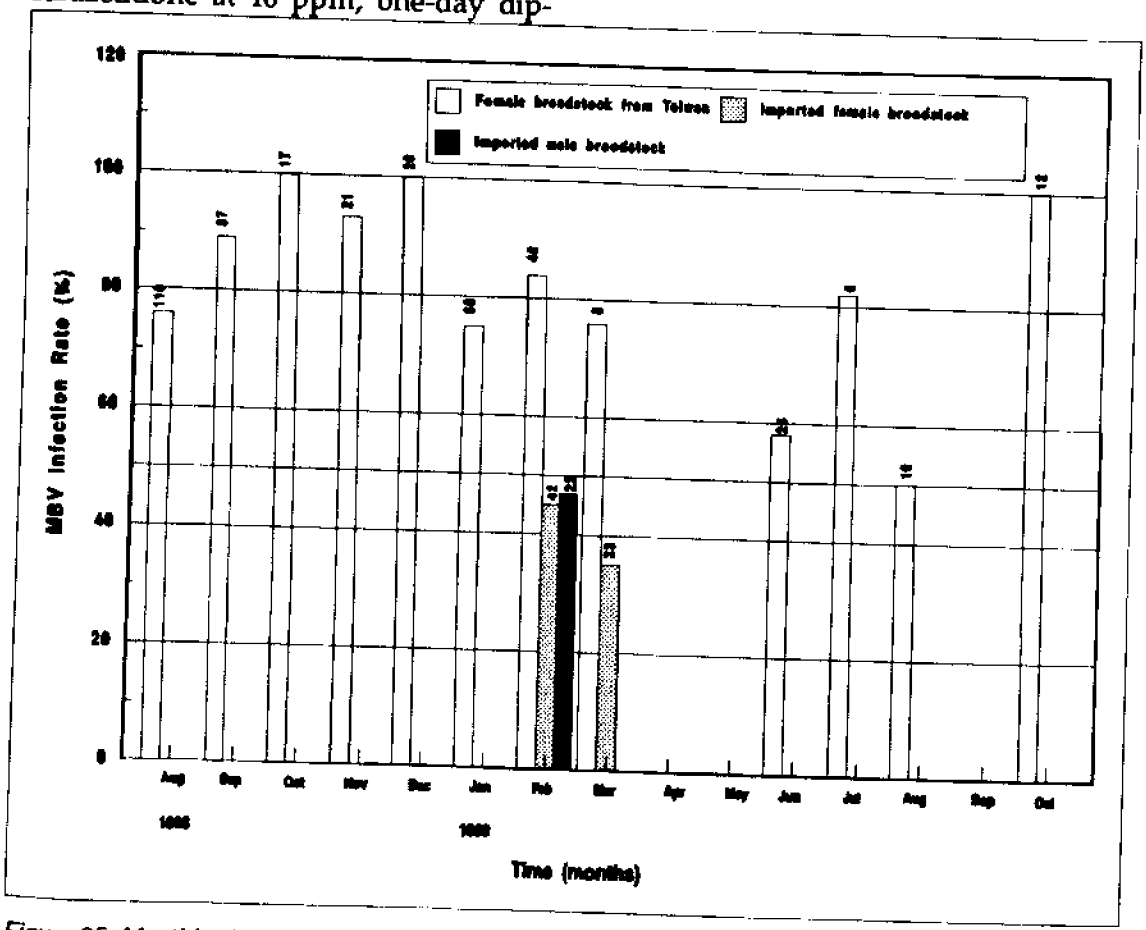


Figure 25. Monthly changes in the MBV infection rate in spawners of Penaeus monodon *caught from the coastal waters of Taiwan or imported from Southeast Asian countries. The numbers in the figure indicate sample sizes.*

Table 4. MBV infection in *Penaeus monodon* cultured in Taiwan.

Date	Stages	Infection Rate (%)	References
08-Nov-80	PL25	100	Lightner et al., 1983
16-Dec-80	PL63	71	
09-Jan-81	PL87	82	
18-Feb-81	PL127	60	
12-May-81	PL210	0	
04-June-81	PL233	7	
84-86	Postlarvae	17	Chen et al., 1989a
85-86	Juvenile	15	
1987	Juvenile	80	
1987	Adult (from hatchery)	52	
1987	Adult (from open sea)	17	
1988	Juvenile	82	
1988	Postlarvae	85	
1988	Adults (from open sea)	51	
87-88	Postlarvae	90	Tung, 1989
	Female spawners (Imported)	69	
	Female spawners (Local)	33	
	Juvenile to adult	41.8	
88-89	Female spawners (Imported)	35.5	Liao et al., 1990
	Male spawners (Imported)	22	
	Female spawners (Local)	85	

Table 5. MBV infection rate (%) in *Penaeus monodon* larvae reared from fertilized eggs or nauplii under different treatments (from Liao et al.,1990).

Date (1989)	Growth Stage[1]	Treatments[2]				
		A	B	C	D	E
13 June	PL1	0	0	0	0	0
20 June	PL8	0	0	0	0	0
26 June	PL14	0	5.0	0	0	0
08 July	PL26	0.5	11.0	0.5	0.5	0
13 July	PL31	1.5	31.0	0.5	1.0	1.5
20 July	PL38	3.0	49.0	2.0	1.5	2.0

[1]Fertilized eggs to PL8 in indoor tank; PL8 - PL38 in outdoor tanks.

[2]A: From MBV-free spawner. B: From spawner infected with MBV; fertilized eggs and nauplii were not washed. C: From spawner infected with MBV; fertilized eggs and nauplii were washed. D: From spawner infected with MBV; fertilized eggs were washed. E: From spawner infected with MBV; nauplii were washed.

Table 6. Bacterial diseases of *Penaeus monodon* in Taiwan and their treatments, 1977 - 1991.

Disease	Agent	Stages	Infection rate (%)	Treatment	Reference
Red gill	*Vibrio parahaemolyticus* and *V. anguillarum*	Juvenile to adult		BKC or hyamine, 1 - 2 ppm, 1 day or furazolidone (20%), 10 - 20 ppm, 1 day	Liao et al., 1985 Chang, 1989
Black spot Tail rot Red discoloration	Bacteria (secondary infection)			BKC or hyamine, 1 - 2 ppm, 1 day	Liao et al., 1985 Chang, 1989 Huang, 1989
Shell	*Vibrio* sp. *Aeromonas* sp. *Spirillum* sp. *Flavobacterium* sp.			Furacin or furanace, 1 ppm; chloramphenicol, 1 - 10 ppm or oxytetracycline, 40 - 60 ppm, 1 day or add in feed; oxytetracycline, 0.04 g/kg shrimp weight	Chen et al., 1989b Huang, 1989 Cheng and Liu, 1986
Necrotic hepatopancreas	*Vibrio* sp. *Pseudomonas* sp. *Aeromonas* sp.			EDTA, 10 - 50 ppm or BKC, 1.3 - 1 ppm or terramycin, 10 ppm; furacin or furanace, 2 ppm, fed OTC, 3 - 15 g/kg shrimp weight	Chen et al., 1989b Huang, 1989 Chang, 1989
Necrotic enteritis	Bacteria	Juvenile to adult			Chen and Liu, 1986
Vibrio	*Vibrio* sp.		76.9 41.9 38.5		Tung, et al., 1991 Tung, 1989 Chang and Su, 1990

Bacterial Diseases

Many kinds of bacteria may cause disease in *P. monodon*, especially in postlarvae and juveniles (Johnson, 1978; Lightner, 1983). Only a few kinds of bacteria, however, cause primary infection. Most incidences result from environmental factors or stress; then secondary bacterial infection can cause heavy mortalities. Bacterial diseases of *Penaeus monodon* are either body surface or internal infections. Tail rot and shell disease are external infections. The main internal infections occur in the hepatopancreas, hemolymph, gastric cavity, heart, muscle and gills; hepatopancreas and hemolymph infections are more serious, resulting in granulomatosis of the whole body (Cheng and Liu, 1986; Chang, 1989; Chen and Lee, 1989; Chen et al., 1989b; Huang, 1989; Liu,1990). The most common bacterial diseases in Taiwan are listed in Table 6.

Pathogens and Symptoms

Diseased prawns are covered with mud-like material and are lethargic and moribund, lying on the pond side and

occasionally surfacing quickly to the water surface. After a short period of time, they die. In serious infections, all the prawns in a pond stop eating, resulting in heavy mortalities. Externally, the diseased prawn has no significant symptoms, but has a double shell in the cephalothorax region, and the hepatopancreas atrophies (Figs. 26 and 27) or swells (Figs. 28 and 29) and granulomas or necrosis occur within hepatopancreatic cells. Diseased prawns are lethargic and have difficulty molting. If teaseed cake containing 10% saponin or frequent water changes are applied to stimulate molting, they may cause incomplete molting or molting that results in a soft shell, causing heavy mortalities.

Treatment

Zeolite (100 kg/1,000 m^2) is used to improve the condition of the pond bottom, but little pond water is replaced. If water is to be changed, the change should be gradual. BKC at 1 ppm or 20% furazolidone at 10 ppm, one-day dipping, may be used; treatments are repeated every five to seven days, two or three times. Chen et al. (1989b) recommended the use of furacin or furanace, 1 ppm; or chloramphenicol, 1 - 10 ppm; oxytetracycline, 60 - 250 ppm, 1- or 2- days dipping, or addition of 500 - 1,000 mg of oxytetracycline or 100 - 500 mg/kg of the prawn diet NF-180. Increasing the amount of fresh feed in the diet and adding high doses of vitamin C may increase the resistance of prawns to the disease.

Other Diseases

This section briefly describes some common diseases, such as associated isopods (Liao et al., 1985), muscle necrosis (Liao et al., 1985; Lightner, 1985; Baticados, 1988; Chen and Lee, 1989), chronic soft-shell syndrome (Baticados, 1988; Chen and Lee, 1989), blue disease (Chen and Lee, 1989), siderosis (Cheng and Liu, 1986) and gas-bubble disease (Chen and Lee, 1989). The pathogens and symptoms are as follows:

Associated isopods. Isopods attach to the prawn's gills, often on just one side; the attachment site appears swollen.

Muscle necrosis. Either the whole body, or a part of it turns white; muscle color is white and translucent. This disease may be caused by chronic stressors or a sudden injury.

Chronic soft-shell syndrome. The diseased prawn's shell is soft and thin; this makes it weak. Death results from impaired molting or cannibalism. This disease may be due to a nutritional deficiency, such as inadequate amounts of calcium and phosphate. The disease is controlled through proper formulation of artificial feeds and an improved culture environment.

Blue-disease. Sick individuals are pale or light blue. We call affected prawns "sky prawns." Blue disease is caused by a nutritional deficiency or poor water quality. The disease may be controlled by proper nutrition and man-

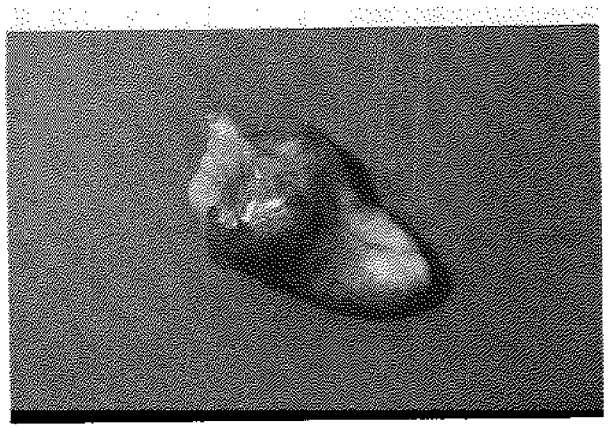


Figure 26

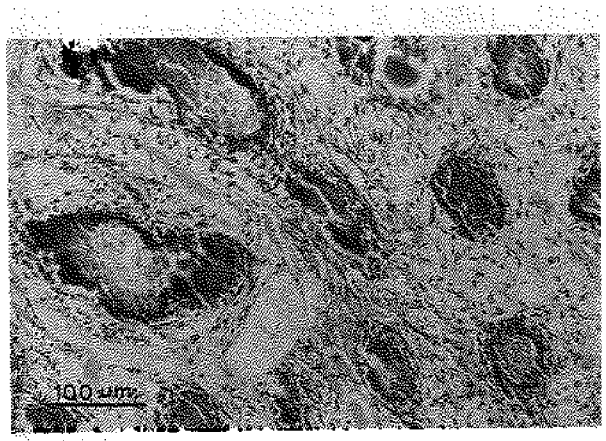


Figure 27

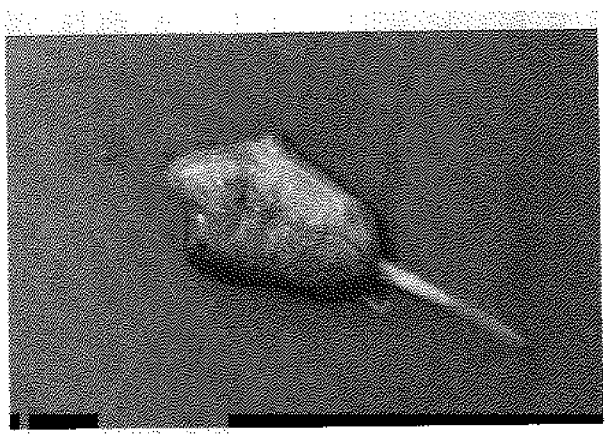


Figure 28

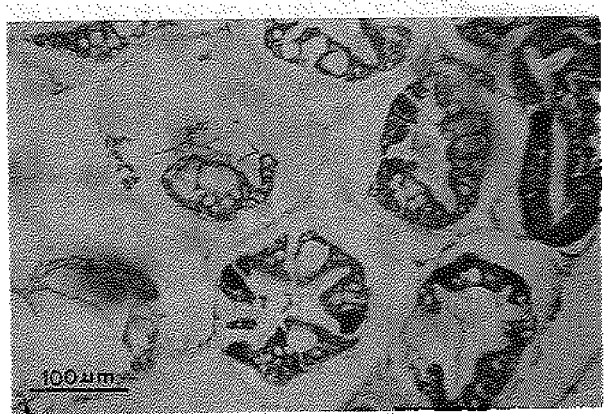


Figure 29

Figures 26-29. Bacterial diseases of Penaeus monodon. *Fig. 26: Hepatopancreas infected with bacteria showing atrophy, whitening and hardening. Fig. 27: Histological section of same atrophied hepatopancreas showing marked granulomatous lesions. No normal structure of the gland is seen in this picture. H&E stain. Fig. 28: Bacterial-infected hepatopancreas showing swelling. Fig. 29: Histological section of swollen hepatopancreas showing liquefactive necrosis.*

agement, i.e., the fresh feed component of the diet is increased. Alternatively, live prawns may be transferred to another pond.

Siderosis. It occurs in ponds using underground water with a high iron content. The body and gills of affected prawns are covered with dirt. If the condition is serious, the gill filament will exhibit foreign body inflammatory reaction.

Gas-bubble disease. This is caused by the supersaturation of water with dissolved oxygen or other gases. The cephalothorax and gills of sick individuals contain gas bubbles that may impede respiration, causing death. Prevention consists of maintaining transparency at 30 - 40 cm, and providing mechanical aeration on sunny days to reduce the concentration of atmospheric gases in the water.

Conclusions

Like other aquatic species, prawns are susceptible to many pathogens. Many diseases are caused by organisms that are part of the normal microflora; these opportunistic pathogens cause diseases only under stressful conditions that favor them over the host. Stress either lowers the resistance of the host or enhances the pathogenicity of pathogens. It increases the prevalence or severity of prawn diseases.

In Taiwan, stocking densities were raised to 40 - 60 ind./m^2 or more and the use of formulated feeds also increased. These practices degraded the culture environment, increasing *P. monodon*'s susceptibility to diseases. Because it is better to prevent, rather than treat diseases, we advocate the use of well-designed ponds, especially those with better water inlets and outlets, adequate pond preparation, optimum stocking densities, proper feeding methods and feed quality, and crop rotation. It is also important to improve methods of disease prevention, control or chemotherapy for many diseases, and to regulate the drugs and chemicals used as pesticides and chemotherapeutants that may pose a health risk to humans. These and other areas of study will reinforce the sound development of the prawn culture industry.

Literature Cited

Anderson, I.G. and M. Shariff. 1987. Mortalities of juvenile shrimp, *Penaeus monodon*, associated with *Penaeus monodon* baculovirus, cytoplasmic reo-like virus, and rickettsial and bacterial infections, from Malaysian brackishwater ponds. Asian Fisheries Science. 1(1): 47-64.

Baticados, M.C.L. 1988. Chapter 6: Diseases. *In*: Biology and Culture of *Penaeus monodon*. SEAFDEC Aquaculture Dept. Iloilo, Philippines.

Chang, C.F. 1989. Diseases of cultured prawns in Taiwan. Chapter 4. *In:* I.C. Liao., C.Z. Shyu., C.Y. Liu., S.C. Chou., W.Y. Liu and C.F. Chang (Eds.). Extension Handbook on Prawn Disease Prevention, Taiwan Fisheries Research Institute, Keelung, Taiwan. pp. 14-30. (In Chinese).

Chang, C.F. and M.S. Su. 1990. Studies on the investigation and prevention of fish and prawn diseases in South Taiwan. Unpublished research report. Tungkang Marine Laboratory, Taiwan Fisheries Research Institute, Tungkang, Pingtung, Taiwan. (In Chinese with English abstract).

Chang, C.F. and M.S. Su. 1991. Studies on the stomach parasite-gregarine (*Cephalolobus* sp.)

in *Penaeus monodon*. Unpublished research report. Tungkang Marine Laboratory, Taiwan Fisheries Research Institute, Tungkang, Pingtung, Taiwan. (In Chinese with English abstract).

Chen, H.P. 1978. Diagnosis and control of disease of *Penaeus monodon* cultured from overwintered juveniles. China Fisheries Monthly. 309: 3-11. (In Chinese with English abstract).

Chen, H.C. and B. Hwang. 1979. Investigation on the diseases of pond-reared shrimps in Ilan. China Fisheries Monthly. 317: 3-8. (In Chinese with English abstract).

Chen, S.N. and P.S. Chang. 1989. Applied and diagnostic method of *Penaeus monodon* Baculovirus infection in cultured prawns. Fish World. 4: 42-52. (In Chinese).

Chen, S.N. and W.C. Lee. 1989. Diseases of cultured shrimp. Fish World. 4: 24-41. (In Chinese).

Chen, S.N., P.S. Chang and G.H. Kou. 1989a. Observation on pathogenicity and epizootiology of *Penaeus monodon* Baculovirus (MBV) in cultured shrimp in Taiwan. Fish Pathology. 24(4): 189-195.

Chen, S.N., P.S. Chang, S.L. Huang and G.H. Kou. 1989b. Cures and drugs used in shrimp diseases. Fisheries Extension Technical 26A. Taiwan Fisheries Bureau. (In Chinese).

Chen, S.N., P.S. Chang, G.H. Kou and I.C. Liao. 1989c. Rapid and histopathological diagnosis of *Penaeus monodon* baculovirus infection in cultured prawn. Chapter 2. *In:* I.C. Liao, C.Z. Shyu, C.Y. Liu, S.C. Chou, W.Y. Liu and C.F. Chang (Eds.). Extension Handbook on Prawn Disease Prevention, Keelung, Taiwan. pp. 35-39. (In Chinese).

Chen, S.N., P.S. Chang, G.H. Kou and D.V. Lightner. 1989d. Studies on virogenesis and cytopathology of *Penaeus monodon* baculovirus (MBV) in the giant tiger prawn (*Penaeus monodon*) and the red tail prawn (*Penaeus penicillatus*). Fish Pathology. 24(2): 89-100.

Cheng, T.J. and C.I. Liu. 1986. Pathological study on major diseases of cultured grass shrimps (*Penaeus monodon* Fabricius). COA Fisheries Series No. 8, Reports on Fish Disease Research (VIII), Council of Agriculture, Taipei, Taiwan, R.O.C. pp. 75-86. (In Chinese with English abstract.)

Couch, J.A. 1978. Diseases, parasites and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic coasts of North America. Fish. Bull. 76(1):1-44.

Couch, J.A. 1983. Diseases caused by protozoa. *In:* Anthony J. Provenzano, Jr. (Ed.). The Biology of Crustacea, Vol. 6. Academic Press, New York, U.S.A. pp. 79-111.

Huang, S.L. 1989. Bacterial infection in cultured black tiger prawn (*Penaeus monodon*). Unpublished MS thesis, National Taiwan University, Taipei, Taiwan. (In Chinese with English abstract).

Johnson, S.K. 1978. Handbook of shrimp diseases. Texas A&M Sea Grant College Program, Texas A&M University, College Station, TX. 23 p.

Johnson, S.K., J.C. Parker and W.H. Holcomb. 1973. Control of *Zoothamnium* on penaeid shrimp. Proc. 4th Ann. Workshop, World Mariculture Society, U.S.A. pp. 321-331.

Liao, I.C. 1984. Status and problems of grass prawn culture in Taiwan. *In:* I.C. Liao and R. Hirano (Eds.). Proceedings of the R.O.C-Japan Symposium on Mariculture (TML Conference Proceedings 1), Tungkang Marine Laboratory, Tungkang, Pingtung, Taiwan, R.O.C. pp. 81-98.

Liao, I.C. 1985. A brief review of the larval rearing techniques of penaeid prawns. *In:* Y. Taki, J.H. Primavera and J.A. Llobrera (Eds.). Proc. First Int. Conf. Cult. Penaeid Prawns/Shrimps, Aquaculture Department, Southeast Asian Fisheries Development Center, Iloilo City, Philippines. pp. 65-78.

Liao, I.C. 1989. *Penaeus monodon* culture in Taiwan: Through two decades of growth. Int. J. Aq. Fish. Technol. 1(1): 16-24.

Liao, I.C. and N.H. Chao. 1983. Development of prawn culture and its related studies in Taiwan. *In:* G.L. Rogers, R. Day and A. Lim (Eds.). Proceedings of the First International Conference of Warm Water Aquaculture-Crustacea, Brigham Young University, Hawaii, U.S.A. pp. 127-142.

Liao, I.C. and F.G. Liu. 1989. A brief review of nutritional studies for *Penaeus monodon*. Aquacop. IFREMER. Actes de Colloque. 9: 355-380.

Liao, I.C., T.L. Huang and K.Katsutani. 1969. Summary of a preliminary report on artificial propagation of *Penaeus monodon* Fabricius. Chinese-American Joint Commission on Rural Reconstruction, Fisheries Series. 8:67-71. (In Chinese with English abstract).

Liao, I.C., F.L. Yang and S.W. Lou. 1977. Preliminary report on some diseases of cultured prawns and their control methods. JCRR Fish. Ser. 29: 28-33. (In Chinese with English abstract).

Liao, I.C., G.H. Kou, S.N. Chen and J.Y. Lai. 1985. Preliminary investigation on the diseases of cultured prawns in the Pingtung

area. COA Fisheries Series No. 4, Fish Disease Research (VII), Council of Agriculture, Taipei, Taiwan, R.O.C. pp. 86-94. (In Chinese with English abstract).

Liao, I.C., M.S. Su., C.F. Chang and K.F. Liu. 1990. Experiments on producing *Penaeus monodon* postlarvae free from *Penaeus monodon* baculovirus (MBV). *In:* G.H. Kou, H. Wakabayashi, I.C. Liao, S.N. Chen, and C.F. Lo (Eds.). Proc. R.O.C.-Japan Symp. Fish Diseases, National Science Council, Taipei, Taiwan, R.O.C. pp. 155-162.

Lightner, D.V. 1983. Diseases of cultured penaeid shrimp. *In:* J.P. McVey (Ed.). CRC Handbook of Mariculture, CRC Press, Inc., Boca Raton, Florida. pp. 289-320.

Lightner, D.V. 1985. A review of the diseases of cultured penaeid shrimps and prawns with emphasis on recent discoveries and developments. *In:* Y. Taki, J.H. Primavera and J.A. Llobrera (Eds.). Proc. First Int. Conf. Cult. Penaeid Prawns/Shrimps, Aquaculture Department, Southeast Asian Fisheries Development Center, Iloilo City, Philippines. pp. 79-103.

Lightner, D.V. and R.M. Redman. 1981. A baculovirus caused disease of the penaeid shrimp, *Penaeus monodon*. J. Invertebr. Pathol. 38: 299-302.

Lightner, D.V. and R.M. Redman. 1985. Necrosis of the hepatopancreas in *Penaeus monodon* and *Penaeus stylirostris* (Arthropoda, Decapoda) with red disease. J. Fish Dis. 8: 181-188.

Lightner, D.V., R.M. Redman and T.A. Bell. 1983. Observations on the geographic distribution, pathogenesis and morphology of the baculovirus from *Penaeus monodon* Fabricius. Aquaculture. 32: 209-233.

Lightner, D.V., R.P. Hedick, J.L. Fryer, S.N. Chen, I.C. Liao and G.H. Kou. 1987. A survey of cultured penaeid shrimp in Taiwan for viral and other important diseases. Fish Pathol. 22(3): 127-140.

Liu, C.I. 1990. The diseases of cultured *Penaeus monodon* with emphasis on recent discoveries in Taiwan. *In:* G.H. Kou, H. Wakabayashi, I.C. Liao, S.N. Chen, and C.F. Lo (Eds.). Proc. R.O.C.-Japan Symp. Fish Diseases, National Science Council, Taipei, Taiwan, R.O.C. pp. 180-201.

Overstreet, R.M. 1973. Parasites of some penaeid shrimps with emphasis on reared hosts. Aquaculture. 2: 105-140.

Shigueno, K. 1975. Shrimp Culture in Japan. Association for International Technical Promotion, Tokyo, Japan. 153 pp.

Sindermann, C.J. 1977. Disease diagnosis and control in North American marine aquaculture. Developments in Aquaculture and Fisheries Science. 6: 82-97.

Tareen, I.U. 1982. Control of diseases in the cultured population of penaeid shrimp, *Penaeus semisulcatus* (dde Haan). J. World Maricul. Soc. 13: 157-161.

Tung, M. C. 1989. Treatment of *Penaeus monodon* diseases and pond disinfectant. Chapter 3. *In:* I.C. Liao, C.Z. Shyu, C.Y. Liu, S.C. Chou, W.Y. Liu and C.F. Chang (Eds.). Extension Handbook on Prawn Disease Prevention, Taiwan Fisheries Research Institute, Keelung, Taiwan. pp. 40-43. (In Chinese).

Tung, M.C., S.S. Juang, A.K. Cheng, C.S. Lee, C.S. Tsai, S.C. Chen and R.S. Chern. 1991. Study on shrimp diseases under the different systems of the cultured growout ponds in Pingtung area. COA Fisheries Series No. 28, Environmental Survey and Its Improvement on Shrimp Grow-out Pond, Council of Agriculture, Taipei, Taiwan. pp. 327-355. (In Chinese with English abstract).

Yu, T.C. and C.F. Chang. 1988. Disease diagnosis and control in central Taiwan. Fisheries Extension. 18:13-18. (In Chinese).

Prevalence and Geographic Distribution of MBV and Other Diseases in Cultured Giant Tiger Prawns (*Penaeus monodon*) in the Philippines

José M. Natividad
BFAR-IDRC Fish Health Project
Bureau of Fisheries and Aquatic Resources
4th Floor Estuar Building, 880 Quezon Avenue
Quezon City, Philippines

and

Donald V. Lightner
Department of Veterinary Science
University of Arizona
Tucson, AZ 85721, U.S.A.

Abstract

Penaeus monodon baculovirus (MBV) was the most prevalent disease in hatchery-reared and pond-cultured *Penaeus monodon* in the Philippines. The incidence of MBV increased with increasing age. Furthermore, MBV was diagnosed in *P. monodon* in all sampling areas (provinces) every month throughout the sampling period. There was a low correlation between the occurrence of MBV and time (month); therefore, MBV epizootics are basically hatchery and/or pond management problems and do not relate to certain places or seasons.

A presumed type C baculovirus and red disease were also consistently diagnosed in MBV-infected *P. monodon*. This presumed type C baculovirus is the first case record in the Philippines. The following diseases were also diagnosed: fouling with the protozoans *Zoothamnium* sp. and *Vorticella* sp., larval mycosis, gill necrosis and hepatopancreatic vibriosis. A few cases of blue shrimp syndrome, filamentous bacterial disease and bacterial enteritis were also recorded.

Introduction

Maintaining a healthy shrimp population in a culture system requires a basic understanding of the etiology of the diseases that occur in the system. Unfortunately, limited information is available on shrimp diseases in the Philippines and Asia as a whole. This has frequently resulted in misdiagnosis and subsequent mistreatment and rampant abuse of antibiotics and other therapeu-

tants. The indiscriminate use of antibacterial drugs has resulted in the development of resistant strains of bacteria, making the problems even worse. The classical example of this problem is the emergence of some resistant strains of luminous bacteria (*Vibrio harveyi*) in some parts of the Philippines where chloramphenicol, penicillin, erythromycin, kanamycin, oxytetracycline, and sulfa drugs are used regularly.

Because it is so common in hatcheries and growout ponds in the Philippines, *Penaeus monodon* baculovirus (MBV) is of considerable importance. Histologically, the disease is characterized by prominent, multiple, eosinophilic (H&E stain) occlusion bodies within hypertrophied nuclei of the midgut or hepatopancreatic tubule epithelial cells. MBV can cause 70% cumulative mortality among juvenile and adult populations; hence, infection by this virus is nonselective as far as the life stages of the hosts are concerned (Lightner et al., 1983a; Sindermann and Lightner, 1988).

MBV was originally diagnosed in 1977 by Lightner and Redman (1981) in a population of laboratory-reared, juvenile *P. monodon*. Ironically, these MBV-infected *P. monodon* were obtained from a quarantined population in Mexico, but the postlarvae originated in Taiwan (Lightner and Redman, 1981; Lightner et al., 1983a). The first case of MBV in the Philippines was reported in 1981 by Lightner et al. (1983a) from a population of postlarval (PL5) *P. monodon*.

Some of the most important diseases of cultured and wild penaeid shrimp are discussed in the works of Johnson (1978), Overstreet (1978) and Lightner (1983, 1985). Thorough reviews of important viral, bacterial, fungal, parasitic and other diseases of cultured penaeid shrimp are presented by Lightner (1975), Lightner et al. (1984a, 1984b), Sindermann and Lightner (1988) and Lightner et al. (1989a). Overstreet (1978) has performed extensive studies on the parasitic and microbial diseases of wild shrimp in the Gulf of Mexico, while Fontaine (1985) did similar studies in the West Galveston Bay, Texas.

Lightner and Redman (1985a) and Lightner et al. (1987a) have identified several important viral and microbial shrimp diseases in Southeast Asia. Similar studies were also conducted in Malaysia by Anderson (1988) and Nash et al. (1988).

Research Objectives

Knowing the host and geographic distributions of diseases of cultured penaeid shrimp is important in terms of the interregional movement of shrimp stocks. The Philippines is composed of about 7,200 islands, and stocks are moved between islands regularly. Mapping the geographic distribution of penaeid shrimp diseases in the Philippines may help us predict and/or prevent some shrimp diseases on a regional scale.

This study was designed to document the incidence and geographic distribution of MBV and other diseases of

hatchery-reared and pond-cultured *P. monodon* populations in the Philippines, and to generate baseline information for the study of their epizootiology. This epizootiological data will be a valuable tool in formulating rational measures for the prevention and control of MBV and other shrimp diseases.

Specifically, the objectives of this study were:

- To determine the seasonal occurrence of MBV in *P. monodon* in the Philippines;
- To assess the geographic distribution of MBV and other *P. monodon* diseases in the Philippines;
- To determine the relationship of age as a host factor in the prevalence of MBV; and
- To identify other diseases of *P. monodon* and analyze their association with MBV-infected populations.

All data in this study came from diagnostic cases received at the BFAR-IDRC Fish Health Laboratory in Quezon City, Metro Manila or from samples collected during routine field trips.

There are three methods currently used to diagnose MBV infection in *P. monodon*: 1) direct examination, 2) enhancement of infection and 3) bioassay. The newest technique for diagnosing MBV employs an epifluorescent light microscope to examine phloxine-stained material (Thurman et al., 1990). The routine examination by histological (Bell and Lightner, 1988) and wet mount methods (Lightner et al., 1983a; Sindermann and Lightner, 1988) were used throughout this study because of their simplicity and speed.

Literature Review

Most diseases of cultured penaeid shrimp in the Philippines are associated with high-density culture, e.g., 100,000-450,000 postlarvae/ha. Under these crowded conditions, disease has become one of the major problems of the industry.

Generally, there are three factors that affect penaeid shrimp health: physical factors (e.g., mechanical injuries), chemical factors (e.g., toxins) and biological factors (e.g., viruses, protozoans, bacteria, and fungi). These three elements cause two major categories of disease — noninfectious diseases, which can be caused by physical, nutritional and chemical agents, and infectious diseases, which are caused by biological agents.

Of the six penaeid shrimp viruses, three are known to infect *P. monodon* in the Philippines — MBV, hepatopancreatic parvo-like virus (HPV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV). MBV is widespread in *P. monodon* hatcheries, where it causes moderate to high cumulative mortalities in postlarval populations and poor growth in pond-cultured stocks.

Another important penaeid virus is HPV. Its target organ is the hepatopancreas. This virus was first described by Lightner and Redman (1985b) from *P. chinensis* postlarvae, *P. merguiensis* juveniles, *P. semisulcatus* juveniles and *P. monodon* from the People's Republic of China, Singapore, Kuwait and the Philippines. Microscopically, pathognomonic signs of HPV infection are single basophilic (H&E stain), Feulgen-positive inclusion bodies in hypertrophied nuclei of epithelial cells of hepatopancreatic tubules.

IHHNV is probably the most investigated shrimp virus. It was most recently reported in the Philippines in 1989 from a population of *P. monodon* from Asturias, Cebu by Lightner (unpublished diagnostic case). IHHN disease was first recognized as virus-caused in a population of blue shrimp, *P. stylirostris*, and white shrimp, *P. vannamei* (Lightner et al., 1984a) at the University of Arizona's shrimp culture facility on Oahu, Hawaii.

Infectivity and pathogenicity studies (Bell and Lightner, 1984; 1987) showed that the target organs for IHHNV are tissues of ectodermal origin (e.g., epidermis, hypodermal epithelium of the foregut and midgut, nerve cord and the nerve cord ganglia) and tissues of mesodermal origin (e.g., hematopoietic organs, antennal gland tubule epithelium, mandibular organ, connective tissue and striated muscles). Microscopically, IHHN virus can be demonstrated by the presence of prominent eosinophilic (H&E stain), usually Feulgen-negative inclusion bodies in hypertrophied nuclei with marginated chromatin (following fixation in Davidson's AFA or Bouin's). IHHNV infects postlarvae and juveniles and may cause 80 to 90% cumulative mortality.

REO, the most recent addition to the list of penaeid viruses, was discovered from a population of juvenile *P. japonicus* in France by Tsing and Bonami in 1984 (Lightner et al., 1989a). This virus is best diagnosed by transmission electron microscopy because this procedure can distinctly demonstrate the eosinophilic to magenta staining (H&E) cytoplasmic inclusion bodies, which cannot be seen well by light microscopy (Sindermann and Lightner, 1988). A cytoplasmic reo-like virus was also described from a population of *P. monodon* with rickettsia-associated disease, although histologically, the virus did not appear to cause any tissue damage (Anderson, 1988).

Bacterial diseases of penaeid shrimp are also considered to be major problems in both hatchery-reared and imported stocks. Bacterial infections in penaeid shrimp can manifest themselves as pits in the cuticle, sometimes called "shell disease," or as localized infections within the body, which may lead to septicemia (Lightner, 1983). In the Philippines, the most serious bacterial disease is "luminous bacterial disease" (Pitogo et al., 1990). Although the etiologic agents of this disease are known — *Vibrio harveyi* and *V. splendicus* — the exact epizootiology and pathology of this disease remain unknown. These

bacteria have been isolated from shrimp eggs and larvae, and from sea water and pond sediments, where they characteristically glow in the dark.

Studies on other penaeid *Vibrio* infections have been performed by Lewis (1973), Lewis and Lawrence (1983), Takahashi et al. (1985), Egusa et al. (1988) and Itami et al. (1989). A septicemic form of bacterial disease was described by Lightner and Lewis (1975) from hatchery-reared white shrimp, *P. setiferus*. Most penaeid bacterial infections involve motile, Gram-negative and oxidase-positive bacteria such as *V. alginolyticus, V. parahaemolyticus, Pseudomonas* sp., *Aeromonas* sp. and *Flavobacterium* sp. (Lewis, 1973; Lightner, 1975; Lightner, 1983; Lightner et al., 1985).

Bacterial epicommensals are also very common in the estuarine environment and have been known to cause problems in pond-reared penaeids (McKee and Lightner, 1982). An example is *Leucothrix mucor*, which normally attaches to the gills of the host shrimp, impairing respiration and resulting in death (Lightner et al., 1975). Other bacterial epicommensals have also been described (Johnson, 1978; Lightner, 1983, 1985; Sindermann and Lightner, 1988).

The most important fungal pathogens causing mortalities in penaeid shrimp are *Fusarium solani* and *Lagenidium* sp. (Lightner and Fontaine, 1975; Hose et al., 1984; Bland et al., 1976). In the Philippines, *Lagenidium* sp. was isolated from a larval population of *P. monodon* (Lio-Po et al., 1982). Other fungi found in the Philippines include *Halipthoros philippinensis* (Lio-Po et al., 1985) and *Sirolpidium* sp. Most fungal diseases affect eggs, larvae and postlarvae. The ability of *Lagenidium* sp. and *Sirolpidium* sp. to exist as free-living saprophytes and their wide host distribution suggest that they may have a worldwide distribution (Lightner, 1985).

Noninfectious diseases are also common problems in pond-reared shrimp and in hatcheries. Examples include gas-bubble disease (Lightner et al., 1974; Suplee and Lightner, 1976), and intoxication with pollutants such as cadmium (Nimmo et al., 1977) and aflatoxin (Wiseman et al., 1982). Substances toxic for shrimp are also produced by some marine organisms such as blue-green algae (Lightner, 1978; 1982).

Materials and Methods

Sample Sources

All *P. monodon* examined came from two major sources. One group was obtained from regular sampling sites including preselected hatcheries and growout ponds from 11 locations in the Philippines (Fig. 1). The second source was comprised of samples submitted to the BFAR-IDRC Fish Health Laboratory in Quezon City, Metro Manila, by hatchery and growout pond operators.

When shrimp in a particular sampling area were experiencing mortalities or any form of disease at the time of the

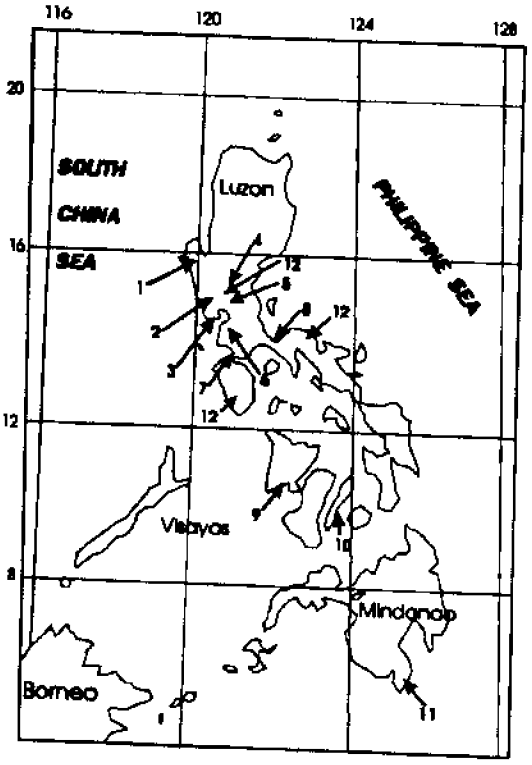


Figure 1. Map of the Philippines showing the sources of Penaeus monodon *samples. (1 = Pangasinan; 2 = Zambales; 3 = Bataan; 4 = Bulacan; 5 = Metro Manila; 6 = Cavite; 7 = Batangas; 8 = Quezon; 9 = Iloilo; 10 = Cebu; 11 = Cotabato; 12 = Other provinces which include Camarines Norte, Pampanga, Mindoro).*

sampling, selective sampling was done to include abnormal animals such as those exhibiting discoloration or other types of morbidity. No dead shrimp were collected if the time of death could not be ascertained. Farm history records were obtained using Farm History Sheets (Fig. 2.)

Processing Samples

All samples were immediately fixed in Davidson's fixative (Bell and Lightner, 1988) either by direct immersion (in the case of postlarvae) or both intrahepatopancreatic and intramuscular injection of the fixative (in the case of juveniles and adults). For larger shrimp, a slit was made in the cuticle, extending from the sixth abdominal segment to the base of the rostrum, enhancing the entry of the fixative into the specimen.

Postlarvae were fixed for 24 h, while juveniles and adults were fixed for 48 h. After fixation, shrimp were stored in 50% alcohol until they were processed for routine histology (Bell and Lightner, 1988; Humason, 1967) and examined using light microscopy. The "gut-gill panorama" technique developed by Bell and Lightner (1988) was adopted in the preparation for embedding the tissue samples. The tissues were prepared for light microscopy using the routine paraffin technique (Luna, 1968; Humason, 1967) and stained with modified Mayer's Hematoxylin and Phloxine/Eosine stain (H&E) (Bell and Lightner, 1988). In cases where the farmers wanted immediate results, the samples were examined for MBV infections using a "wet mount" technique (Lightner et. al., 1983a), which is locally called "MBV Rapid Test."

Method of MBV Diagnosis

Diagnosis of MBV infection was accomplished by histological demonstration of prominently hypertrophied nuclei, with spherical eosinophilic occlusion bodies in the hepatopancreatic cells and anterior midgut of the shrimp (Lightner and Redman, 1981; Lightner et al., 1983a). In some samples where the "wet mount" technique was used, MBV was

Case No.:	Date:	Farm Area:
Farm Location and Address:		
Type of Operation:	Hatchery	Growout
Culture Method:	Intensive	Extensive
Species Cultured		
Stocking Density:		Culture Period:
Water Management:	Tidal:	Pump:
	Aeration:	No Aeration:
Frequency of Water Changes:		
Source of PL:	Hatchery-reared:	Wild
Source of Broodstock	Wild	Imported
	Pond-reared	Country
Feed Type:	Natural Fertilization	
	Commercial Feeds	
	Others	
Feeding Frequency:	Time:	% Body Weight:
Name/Brand of Chemicals Used Routinely:		
History of Disease	Yes:	None:
	When:	
Life Stages Affected		
Mortality:	Acute	Chronic
Gross Signs Observed:		
Remarks:		

Figure 2. Farm history record sheet used to collect background information from the hatchery/farm where Penaeus monodon *samples were collected.*

detected by the presence of intensely green (due to the 0.1% aqueous malachite green stain) occlusion bodies that were distinct from the secretory granules and lipid droplets present in the tissue squash.

Diagnosis of other diseases was also accomplished by routine histopathology. Some protozoan and fungal infections were diagnosed using the wet mount method.

Statistical Analysis

The statistical methods used in this study were mainly correlation analyses such as the correlation of MBV prevalence with variables such as age, geographic distribution and monthly distribution, and correlation analysis between the occurrence of MBV and other diseases. All analyses were done using NCSS (Version 4.1 developed by Dr. Jerry L. Hintze, Kaysville, UT).

Table 1. Incidence of *Penaeus monodon* diseases and disease-causing organisms diagnosed between October, 1989 and December, 1990.

Disease	No. of Cases	Percentage
Penaeus monodon baculovirus	249	66.9
Type C baculovirus	7	1.9
Zoothamnium	43	11.6
Vorticella	14	3.8
Larval mycosis	18	4.8
Blue shrimp syndrome	3	0.8
Gill necrosis	14	3.8
Filamentous bacteria	3	0.8
Bacterial enteritis	6	1.6
HP vibriosis	11	2.9
Red disease	4	1.1
	372	100.0

Estimation of MBV prevalence was computed using the formula below:

$$Pr = \frac{MBVp}{n} \times 100$$

where:

Pr = Prevalence

$MBVp$ = No. of MBV-positive samples, and

n = Total number of samples

Results

Eleven major types of organisms/diseases were diagnosed between October, 1989 and December, 1990 in *P. monodon*. MBV was the most prevalent disease agent, accounting for 249 cases (66.9%) out of the total of 372 cases (Table 1).

Overall Prevalence of MBV. Out of a total of 372 cases from 12 major prawn-farming provinces in the Philippines, 249 were diagnosed positive for MBV. Overall, the prevalence of MBV was 66.9%. However, there was a very low correlation (r = 0.4) between the number of MBV cases and provinces where the cases were documented (Table 2).

In terms of the total number of *P. monodon* samples examined, 5,085 were MBV-positive and 4,025 were MBV-negative; hence, the overall prevalence of MBV infections in terms of the total number of shrimp examined was 55.8%. There was a low correlation (r = 0.3) between the number of MBV-infected animals and their location (Fig. 3).

Prevalence of MBV in Different Postlarval Stages. There was a high correlation (r = 0.92) between host age and occurrence of MBV. The occurrence of

Table 2. Overall incidence of MBV in *Penaeus monodon* based on the total number of cases examined from October, 1989 to December, 1990.

Province	No. Cases	MBV+	(%)	MBV-	(%)
Pangasinan	60	35	58.3	25	41.7
Zambales	110	67	60.9	43	39.1
Bataan	15	12	80.0	3	20.0
Bulacan	4	4	100.0	0	0.0
Metro Manila	10	3	30.0	7	70.0
Cavite	6	5	83.3	1	16.7
Batangas	96	65	67.7	31	32.3
Quezon	31	25	80.6	6	19.4
Iloilo	9	7	77.8	2	22.2
Cebu	14	9	64.3	5	35.7
Cotabato	5	5	100.0	0	0.0
Others[1]	12	12	100.0	0	0.0
	372	249	66.9	123	33.1

[1]Includes the provinces of Camarines Norte, Negros, Pampanga, Mindoro and some unknown sources

MBV in the different life stages of *P. monodon* in terms of the total number of cases received is shown in Table 3. With the exception of one MBV-positive PL3, all PL1 to PL6 samples were negative for MBV (Fig. 4).

Distribution of MBV. All the diagnostic cases came from 12 major provinces in the Philippines. Nine of these provinces were from Luzon Islands, two were from the Visayan Islands and one from Mindanao Island.

Among the provinces with less than ten cases of MBV, samples from Bulacan and Cotabato had 100% MBV prevalences; 4/4 and 5/5, respectively. Among the provinces with more than ten documented cases of MBV, Quezon had the highest rate of MBV infection; 80.6% (25/31). The provinces of Bataan and Batangas were second and third, re-

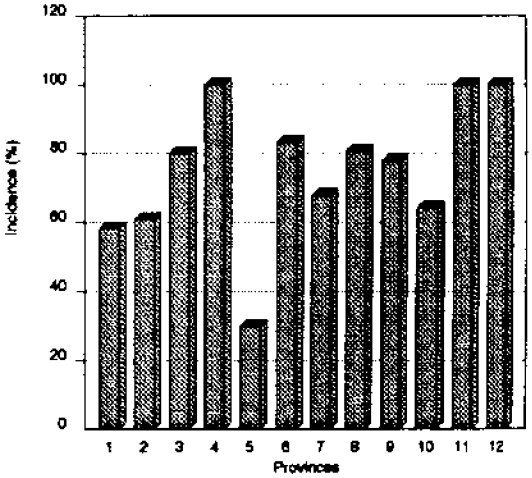


Figure 3. Overall incidence of MBV from October, 1989 to December, 1990 based on the total number of Penaeus monodon *samples from 12 provinces where the samples were collected, indicating that MBV was distributed throughout the country. (1 = Pangasinan; 2 = Zambales; 3 = Bataan; 4 = Bulacan; 5 = Metro Manila; 6 = Cavite; 7 = Batangas; 8 = Quezon; 9 = Iloilo; 10 = Cebu; 11 = Cotabato; 12 = Other provinces which include Camarines Norte, Pampanga, Mindoro).*

Table 3. Prevalence of MBV in different postlarval stages of *Penaeus monodon* based on the total number of cases from October, 1989 to December, 1990.

PL Stage	No. of Cases	MBV+	(%)	MBV-	(%)
01	1	0	0.0	1	100.0
02	2	0	0.0	2	100.0
03	3	1	33.3	2	66.7
04	4	0	0.0	4	100.0
05	6	0	0.0	6	100.0
06	6	0	0.0	6	100.0
07	11	2	18.2	9	81.8
08	17	8	47.1	9	52.9
09	18	6	33.3	12	66.7
10	26	14	53.8	12	46.2
11	26	17	65.4	8	30.8
12	23	18	78.3	5	21.7
13	10	6	60.0	4	40.0
14	20	14	70.0	6	30.0
15	38	30	78.9	6	15.8
16	32	27	84.4	5	15.6
17	19	14	73.7	5	26.3
18	28	24	85.7	4	14.3
19	14	12	85.7	2	14.3
20	22	16	72.7	6	27.3
21	2	2	100.0	0	0.0
22	9	7	77.8	2	22.2
23	2	2	100.0	0	0.0
24	3	3	100.0	0	0.0
25 - 35	12	10	83.3	2	16.7
42 - 45	7	6	85.7	1	14.3
50 - 60	7	7	100.0	0	0.0
62 - 180	4	3	75.0	1	25.0
	372	249	66.9	120	32.2

spectively, where 80% (12/15) and 67.7% (65/96) of the total number of cases were positive for MBV (Fig. 3).

In terms of the percentage MBV-positive animals, the province of Iloilo had the highest prevalence, 84.2% (176/209), followed by the provinces of Bulacan and Cavite where 83.6% (51/61) and 78.7% (122/155) of the total number of animals examined had MBV. Metro Manila had the lowest MBV incidence rate; 14.7% (36/245).

Monthly Incidence of MBV. The correlation between the incidence of MBV and time (month) was very low ($r = 0.3$). In terms of the total number of

Table 4. Monthly prevalence of MBV in *Penaeus monodon* based on the total number of cases examined from October, 1989 to December, 1990.

MONTH	No. of Cases	MBV+	(%)	MBV-	(%)
October	1	1	100.0	0	0.0
November	19	11	57.9	8	42.1
December	16	12	75.0	4	25.0
January	34	24	70.6	10	29.4
February	12	7	58.3	5	41.7
March	12	9	75.0	3	25.0
April	22	17	77.3	5	22.7
May	23	18	78.3	5	21.7
June	45	29	64.4	16	35.6
July	24	18	75.0	6	25.0
August	27	13	48.1	14	51.9
September	41	34	82.9	7	17.1
October	21	11	52.4	10	47.6
November	29	13	44.8	16	55.2
December	46	32	69.6	14	30.4
	372	249	66.9	123	33.1

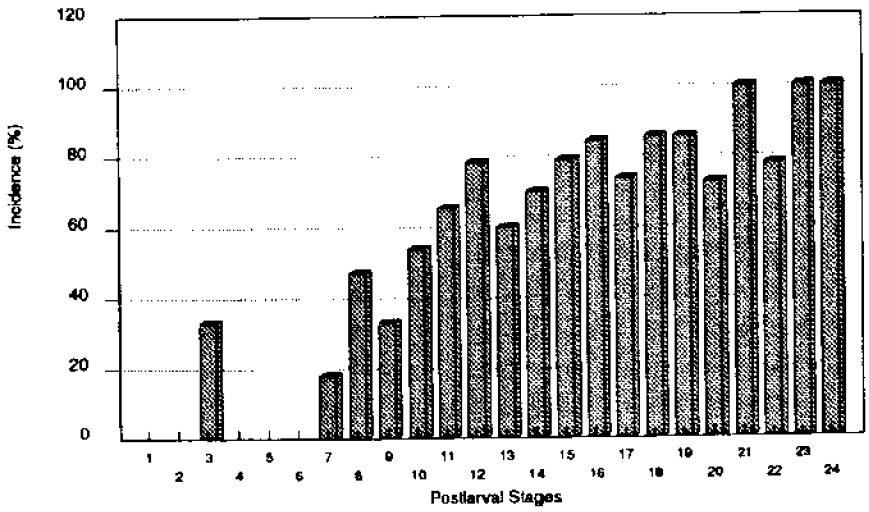


Figure 4. Prevalence of MBV in the different postlarval stages of Penaeus monodon *based on the total number of samples examined from October, 1989 to December, 1990. There is a high correlation between the age of the samples and the prevalence of MBV. Furthermore, note that the 33.3% incidence of MBV in PL3 constituted only one case.*

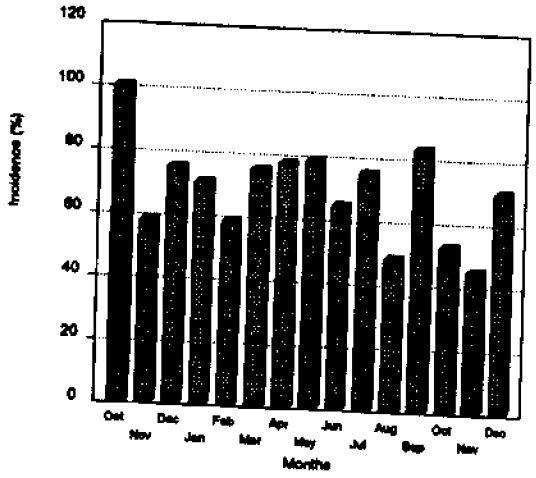


Figure 5. Monthly incidence of MBV based on the total number of Penaeus monodon *samples examined from October, 1989 to December, 1990. There is a low correlation between the incidence of MBV and months, indicating that MBV was prevalent in all months.*

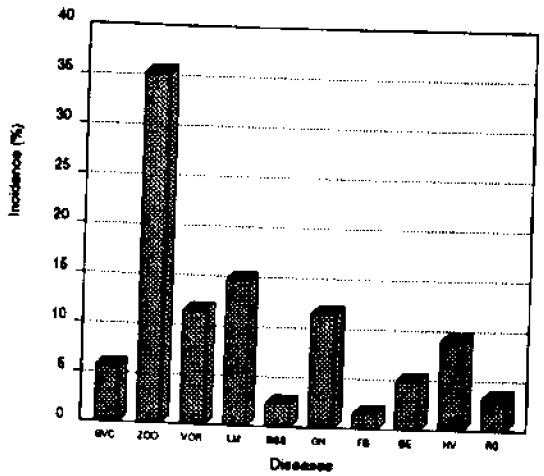


Figure 6. Incidence of other Penaeus monodon *diseases diagnosed from October, 1989 to December, 1990 based on the total number of samples examined. Note that these values do not include MBV. (BVC = Type C baculovirus; Zoo =* Zoothamnium*; Vor =* Vorticella*, LM = Larval mycosis; BSS = Blue shrimp syndrome; GN = gill necrosis; FB = Filamentous bacteria; BE = Bacterial enteritis; HV = Hepatopancreatic vibriosis; RD = Red disease.*

cases received, the month of September had the highest occurrence of MBV at 82.9% (34/41), while the month of May was second with 78.3% (18/23). November had the lowest MBV prevalence, 44.8% (13/29) (Table 4) and October actually had the highest prevalence (100%); however, only one case was documented during this period.

Based on the total number of **animals** examined, the prevalence of MBV was high in September (71.1%; 587/826), and July (61%; 431/706). MBV appeared to be least common in November; it was found in only 34.7% (167/481) of the animals sampled. However, there was a very low correlation (r = 0.2) between the number of MBV-infected shrimp and their monthly prevalence (Fig. 5).

Other Diseases. Ten major diseases or pathogens were diagnosed from 122 samples (Figs. 6, 7). There was a high correlation (r = 0.99) between the occurrence of these diseases and MBV (Table 5). One of the most significant findings was the discovery of a type C baculovirus (a nonoccluded baculovirus) from a population of postlarval *P. monodon*. This virus was diagnosed as a mixed infection with MBV in postlarvae, and is the first type C baculovirus reported in *P. monodon* from the Philippines.

Discussion

MBV was the most prevalent disease of *P. monodon*, accounting for 249 disease cases (66.9%). These figures are alarm-

ing to both hatchery and growout farmers.

The prevalence and distribution of MBV in *P. monodon* populations is a function of a complex interaction of several variables, including age, feeding habits and the extent of cannibalism. On the other hand, understanding MBV and its mode of transmission, virulence, persistence in the environment and ultrastructure are necessary to the understanding of this host-pathogen relationship.

Host age is one of the most important variables in the study of baculoviruses (Martin et al., 1987; Watanabe, 1987). In this study, the prevalence of MBV was higher among the older stages of postlarval *P. monodon*, whereas younger animals (e.g., PL1 to PL7) were not as susceptible to MBV infection. This finding is consistent with the results of previous work conducted by the authors in which a susceptibility study to MBV was performed on the different larval and postlarval stages of *P. monodon*. However, these findings disagree with those of Momoyama and Sano (1989) who studied BMNV infection in kuruma shrimp (*P. japonicus*). The authors found a strong negative correlation between BMNV infection and host age, indicating that the infectivity of BMNV in *P. japonicus* decreases with age.

Another important finding of this study was the association of other viral, protozoan, bacterial and fungal infections with MBV in *P. monodon* postlarvae. It is possible that these co-infecting microorganisms exerted synergistic effects with MBV, acting as biological stressors and enhancing the overall susceptibility of the host to infection. In fact, surface and gill fouling by epicommensals such as *Leucothrix mucor* and *Zoothamnium* sp. are suspected to compromise the respiratory system of MBV-infected *P. monodon*, causing significant mortalities (Lightner et al., 1983a). This kind of relationship has also been demonstrated in silkworm larvae. Individuals exposed to *Pseudomonas* sp. were more susceptible to viral infections (Watanabe, 1987).

There was a very low correlation between the prevalence of MBV and the geographic origin of the samples. In addition, there was no significant statistical correlation between the occurrence of MBV and time of year. This is in contrast to findings that the distribution of several insect baculoviruses is influenced by geography and season due to the migration patterns of insects (Tanada and Fuxa, 1987; Weiser, 1987). Hatchery-reared or pond-cultured tropical aquatic animals such as *Penaeus monodon* are confined to tanks and ponds and are subjected to very limited seasonal variation.

In Australia, Lester and Paynter (1989) reported an MBV-like virus from populations of *P. plebejus*, *P. monodon* and *P. merguiensis*. Until serological studies can be made comparing this baculovirus to MBV, it can not be ascertained if this is a new strain of MBV or a distinct species (Lightner et al., 1989a). It is, therefore, imperative to examine

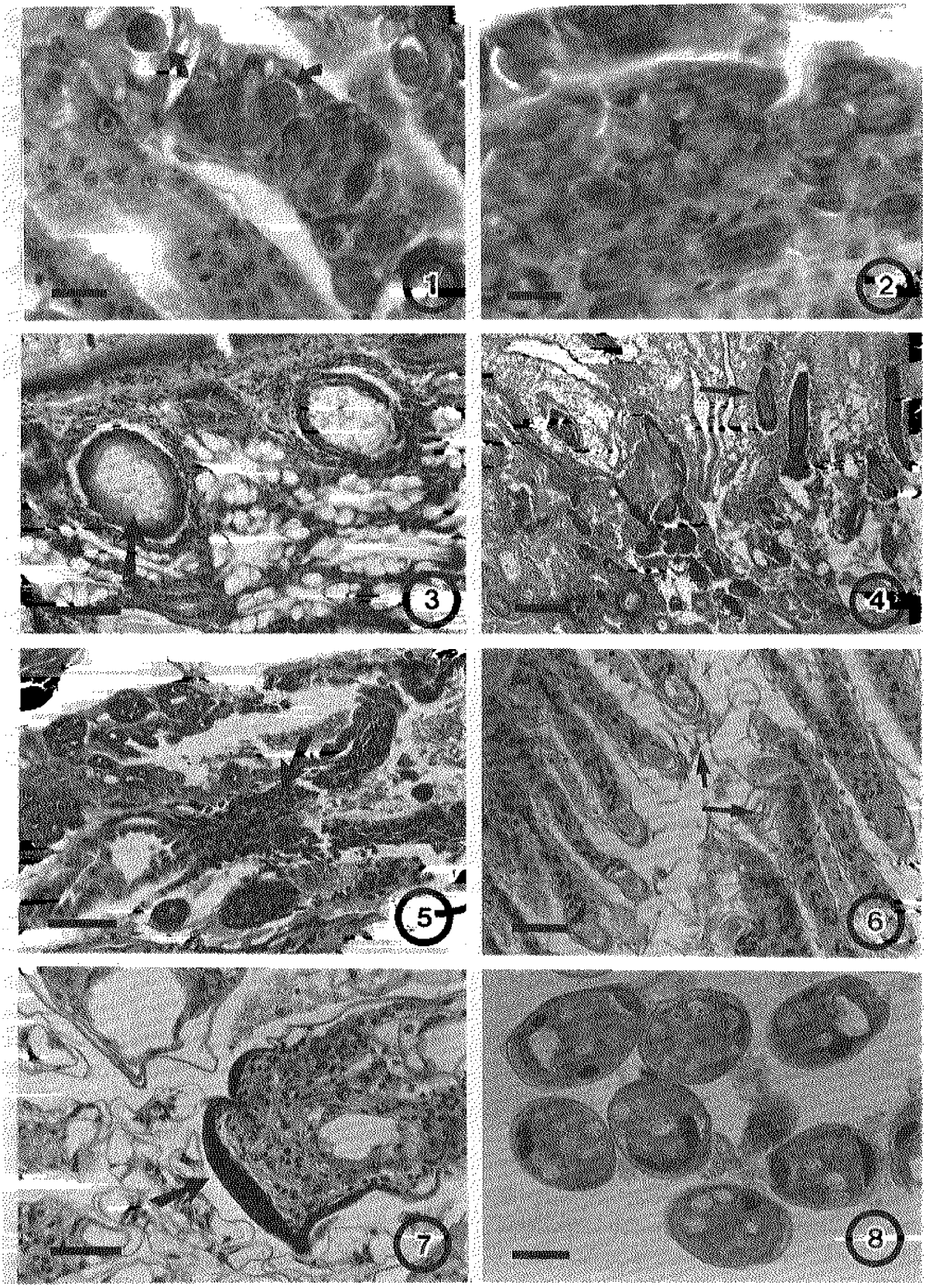

1
2
3
4
5
6
7
8

Table 5. Other diseases of *Penaeus monodon* based on the total number of cases received from October, 1989 to December, 1990.

Disease Diagnosed	No. of Cases	MBV+ Cases	Percentage
Type C baculovirus	7	7	100.0
Zoothamnium sp.	43	39	90.7
Vorticella sp.	14	13	92.9
Larval mycosis	18	16	88.9
Blue shrimp syndrome	3	2	66.7
Gill necrosis	14	11	78.6
Filamentous bacteria	2	0	0.0
Bacterial enteritis	6	5	83.3
HP vibriosis	11	8	72.7
Red disease	4	4	100.0
Total	122	105	

the problems underlying the prevalence of MBV at the hatchery and pond levels relative to existing hatchery and pond management practices and biotic factors. Lightner et al. (1983a) concluded that MBV is enzootic in Southeast Asia and cited as proof the high prevalence of MBV in the regions where farms use wild *P. monodon* broodstock in the mass production of

Figure 7. Photomicrograph of the different pathological conditions and disease-causing organisms in Penaeus monodon *populations.*

1. Penaeus monodon *baculovirus (MBV). The pathognomonic signs of MBV infection are the multiple, intranuclear spherical occlusion bodies (arrows) within the hypertrophied nuclei of the hepatopancreatic tissues of* P. monodon *postlarva. H&E staining. (Bar = 20 µm).*

2. Type C baculovirus. Histological section of the hepatopancreas of P. monodon *postlarva showing the hypertrophied nuclei (arrow) of the hepatopancreatic tubule epithelial cells. Note the absence of viral occlusions, which is the main characteristic of this type of baculovirus. H&E staining (Bar = 20 µm).*

3. HP necrosis. Histological section of the hepatopancreatic tissue of P. monodon *postlarva showing large masses of necrotic areas surrounded by hemocytes (arrow). This pathological condition is probably similar to vibriosis. H&E staining. (Bar = 100 µm).*

4. Red disease. Histological section of the hepatopancreas of juvenile P. monodon *with advanced red disease. Note the extensive hemocytic encapsulation and melanized areas which contain masses of necrotic tissue debris (arrows). H&E staining. (Bar = 200 µm).*

5. Bacterial enteritis. Histological section of the anterior midgut of an infected P. monodon *postlarva showing the necrotic areas and hemocytic infiltration in the affected area (arrow). This pathological condition is believed to be caused by bacteria. H&E staining. (Bar = 50 µm)*

6. Filamentous bacterial disease. Histological section of the gill filaments of P. monodon *juvenile showing heavy infection of filamentous bacteria, possibly* Leucothrix mucor *(arrows). H&E staining. (Bar = 20 µm)*

7. Gill necrosis. Histological section of a necrotic and melanized gill from a P. monodon *postlarva (arrow). H&E staining. (Bar = 50 µm)*

8. Zoothamnium *sp. This protozoan causes extensive damage to the gills and appendages in infected* P. monodon *postlarval, juvenile or adult populations. H&E staining. (Bar = 20 µm).*

postlarvae. MBV is present in most hatcheries and growout ponds in Southeast Asian countries such as Malaysia (Lightner et al., 1985; Anderson, 1988; Johnson and Lightner, 1988), Singapore (Brock et al., 1983; Lightner et al., 1985), Taiwan (Lightner et al., 1983a, 1987a; Johnson and Lightner, 1988; Lightner and Redman, 1991; Chen et al., 1989a), Indonesia (Nash et al., 1988; Lightner et al., 1992), Thailand (Natividad, BFAR diagnostic data from imported postlarvae) and the Philippines (Lightner, 1983; Johnson and Lightner, 1988; Lightner et al., 1992). MBV-infected spawners may continuously excrete feces contaminated with free MBV virions and occlusion bodies during spawning. These virions and occlusion bodies could easily remain associated with the shrimp larvae and infect them when they begin feeding.

In hatchery-reared *P. monodon* postlarval populations, MBV acts as a density-dependent disease that infects more hosts as host density is increased. The rate of MBV infection in postlarval *P. monodon* populations may be related to crowding stress (Lightner et al., 1983a; Sindermann and Lightner, 1988). In high-density stocking, MBV infection is easily enhanced through cannibalism. The designs of most hatcheries in the Philippines should also be reviewed. The fine mist generated by vigorous aeration systems in most hatcheries may facilitate the aerosol spread of MBV. Aerosol contamination between aquaria several meters apart has been demonstrated using the experimentally introduced spore-forming bacterium *Bacillus sphaericus* (Lightner, pers. comm.).

At present, there are no known treatments with which to cure MBV, nor are there any that can eliminate MBV from the culture environment. Most farmers in the Visayan region have reported some degree of success from incorporating 1,000 mg vitamin C/kg of feed given to pond-cultured *P. monodon* with chronic MBV infections. Although there have been no studies to substantiate this claim, it is possible that increased dietary vitamin C increases hemocyte count and activity, and improves the collagen integrity of the animals, thus improving their general resistance to diseases such as MBV. Support for this theory was provided by Pristavko and Dovzhenok (1974) with larvae of the codling moth (*Laspevresia pomonella*) that were fed different concentrations of vitamin C. When the amount of vitamin C was decreased, the hemocyte count of the larvae decreased and their susceptibility to *Beauveria bassiana* increased.

Among the other *P. monodon* diseases diagnosed, the most important was a **nonoccluded (Type C) baculovirus** that was found in seven cases (1.9%), mostly from the provinces of Zambales, Pangasinan and Quezon. Pathologically, the presumed type C baculovirus was diagnosed by the presence of hypertrophied nuclei, with marginated chromatin, a laterally displaced or disassociated nucleolus within infected hepatopancreatic tubule epithelial cells, but without occlusion bodies (Lightner

et al., 1989a; Momoyama and Sano, 1989; Sano et al., 1985). This presumed type C baculovirus is the first reported in *P. monodon* in the Philippines. It is possible that type C baculovirus may also be present in *P. monodon* from Australia and Indonesia (Lightner, unpublished data). The only extensively studied type C baculovirus in penaeid shrimp is baculoviral midgut gland necrosis virus (BMNV), which is common in *P. japonicus* hatcheries and ponds in Japan (Lightner, 1985; Sano et al., 1981; Sano et al., 1985; Sindermann and Lightner, 1988; Lightner et al., 1989a; Momoyama and Sano, 1989).

Some of the frequently diagnosed diseases in this study include gill and body surface fouling caused by two protozoans, ***Zoothamnium* sp.** and ***Vorticella* sp.**, and by an unidentified form of **filamentous bacteria**. However, elevated levels of these organisms result from water management problems; hence, they are usually regarded as noninfectious epicommensals. These organisms are common in hatcheries and ponds and are apparently ubiquitous in shrimp culture facilities (Lightner, 1985; Anderson, 1988). This study showed that postlarvae, juveniles and adults are all potential targets of these epicommensals. Epicommensal organisms can cause gill obstruction, leading to impaired respiration and, in severe cases, infections result in heavy mortalities due to hypoxia and impaired locomotion and molting (Sindermann and Lightner, 1988; Anderson, 1988). One filamentous bacteria, *Leucothrix mucor*, has been known to cause extensive fouling of host shrimp gills, blocking the diffusion of gases across the gill cuticle (Lightner et al., 1975; Couch, 1978).

Larval mycosis was diagnosed (via wet mount method) in 18 samples (4.9%). Although the etiological agents were not identified, diagnosis of mycosis in infected postlarvae was based on the characteristic branching fungal hyphae protruding from body surfaces, especially the appendages, cephalothorax and abdominal regions. Penaeid diseases of fungal etiology are very common in the Philippines (Hatai et al., 1980; Lio-Po et al., 1978; 1982; 1985). Although the fungal diseases of penaeids are believed to be ubiquitous in shrimp facilities (Lightner, 1985), fungal infections associated with *Haliphthoros philippinensis* have been reported to cause serious mortalities in hatcheries only in the Philippines (Hatai et al., 1980). Anderson (1988) claimed that there is a close association between antibiotic use and the occurrence of larval mycosis in Malaysia. He speculated that this may be due to the removal of bacterial epibiont competitors. Although Anderson's theory has not been proven experimentally, it is supported by the observations of one hatchery operator in Batangas province (Mr. Willy Espejo of SS Marine Resources Corp.) where the use of oxytetracycline to treat luminous bacterial disease in *P. monodon* mysis preceded a high incidence of serious larval mycosis infections.

Fourteen cases (3.8%) of **gill necrosis** were diagnosed in the *P. monodon* sampled. It is believed that this disease is

of bacterial etiology. It is grossly characterized by brownish discoloration of the gills and disintegration of the carapace. Histologically, the disease is characterized by the presence of multifocal melanized cuticular lesions in the gill filaments.

Bacterial enteritis was diagnosed in six cases (1.6%). It is characterized by septic lesions of the midgut epithelial cells and is normally accompanied by mild to severe necrosis and sloughing of the midgut epithelial lining. This disease is similar to hemocytic enteritis described by Lightner (1983) and Sindermann and Lightner (1988). Because hemocytic enteritis is often associated with certain types of filamentous blue-green algae, midgut lesions are believed to be caused by endotoxins derived from the algae (Lightner et al., 1987a).

Septic, multifocal necrosis and massive hemocytic inflammation of the hepatopancreatic tissues similar to HP vibriosis was another disease we found that may have a bacterial etiology (14 cases). *Vibrio* sp. is the etiologic agent of vibriosis and this disease syndrome is one of the most studied penaeid shrimp diseases (Lewis and Lawrence, 1983; Takahashi et al., 1985; Takahashi et al., 1985; Sindermann and Lightner, 1988; Itami et al., 1989). Similar types of lesions were also described by Egusa et al. (1988) from kuruma prawns (*P. japonicus*).

The pathological signs described for vibriosis in *P. japonicus* (Egusa et al., 1988) did not include the associated hepatopancreatic atrophy and gross signs of red coloration that characterize red disease (Lightner and Redman, 1989a). Four cases of **red disease** were diagnosed in this study; all were also infected with MBV. This disease is characterized by the presence of multifocal necrosis, often accompanied by massive hemocytic inflammation and marked atrophy of the hepatopancreas.

Although red disease is common in pond-cultured *P. monodon* populations in Taiwan and the Philippines, its etiology remains unknown. Lightner and Redman (1985) suggested that the red coloration of affected *P. monodon* is caused by the deposition of beta carotene and other carotenoids into the hemolymph. This author, however, has seen reddish shrimp that were pathologically negative for red disease. Interestingly, the aforementioned shrimp exhibited normal hepatopancreatic tissue, having no apparent morphological signs of hepatopancreatic atrophy.

Under these circumstances, it is possible that the red discoloration exhibited by some shrimp could be attributed to several factors, including season (Lightner and Redman, 1985a), feed type, a nutritional deficiency, and stressful environmental parameters such as highly acidic water. The latter has been demonstrated in samples from farms in Binmaley, Pangasinan and Calatagan, Batangas, where the pH of pond water in the farms were 4.5 and 4.9, respectively. Although these observations may have been circumstantial, the relationship between environmental pa-

rameters and red disease merits further study.

Three cases (0.8%) of **blue shrimp syndrome** were observed in this study. Since this is a relatively new disease syndrome, several etiologies have been suggested, including nutritional deficiency and/or viral infection (Baticados, 1987), and poor water quality and poor pond bottom condition. Two of the three cases in this study were infected with MBV. Bluish coloration is one of the gross signs of MBV infection in shrimp (Sindermann and Lightner, 1988).

It is interesting that IHHN and HPV, previously reported from *P. monodon* populations in the Philippines by Lightner (1985) and Lightner et al. (1989a), were not documented in this study. This indicates that either IHHN and HPV are not widely distributed in the Philippines, or that routine histological diagnostic procedures are not sensitive enough to diagnose infections by these agents in subacute cases.

Acknowledgments

This research was funded by the International Development Research Centre's Fish Diseases (Philippines) Project: 3-P-88-0022. We wish to express our sincerest gratitude to Mr. Ronaldo P. Ramos of the BFAR for his assistance in the histological processing of the majority of the samples.

Literature Cited

Anderson, I.G. 1988. Disease problems faced by marine prawn farms and hatcheries. *In*: T. Singh and A.K. Lee (Eds.). Marine Prawn Farming in Malaysia. Malaysian Fisheries Soc. Publ. # 1. pp. 67-83.

Baticados, M.C.L. 1987. Introduction to prawn health. Proceedings of the Technical Considerations for the Management and Operation of Intensive Prawn Farms. Nov. 16-21, Iloilo City, Philippines.

Bell, T.A. and D.V. Lightner. 1984. IHHN virus: Infectivity and pathogenicity studies in *Penaeus stylirostris* and *Penaeus vannamei*. Aquaculture. 38: 185-194.

Bell, T.A. and D.V. Lightner. 1987. IHHN disease of *Penaeus stylirostris*: effects of shrimp size on disease expression. J. Fish Dis. 10: 165-170.

Bell, T.A. and D.V. Lightner. 1988. A Handbook of Normal Penaeid Shrimp Histology. World Aquaculture Society. Baton Rouge, Louisiana. 114 p.

Bland, C.E., D.G. Ruch, B.R. Salser and D.V. Lightner. 1976. Chemical control of *Lagenidium*, a fungal pathogen of marine crustacea. Proc. World Maricul. Soc. 7: 445-472.

Brock, J.A., D.V. Lightner and T.A. Bell. 1983. A review of four virus (BP, MBV, BMN and IHHNV) diseases of penaeid shrimp with particular reference to clinical significance, diagnosis and control in shrimp aquaculture. Intl. Council for the Exploration of the Sea, C.M. 1983/Gen: 10/Mini-Symposium.

Chen, S.N., P.S. Chang and G.H. Kou. 1989a. Observation on pathogenicity and epizootiology of *Penaeus monodon* Baculovirus (MBV) in cultured shrimp in Taiwan. Fish Pathol. 24(4): 189-195.

Couch, J.A. 1978. Diseases, parasites and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic Coast of North America. Fish. Bull. 76: 1-44.

Egusa, S., Y. Takahashi, T. Itami and K. Momoyama. 1988. Histopathology of Vibriosis in the kuruma prawn, *Penaeus japonicus* Bate. Fish Pathol. 23(1): 59-65.

Fontaine, C.T. 1985. A survey of potential disease-causing organisms in bait shrimp from West Galveston Bay, Texas. NOAA Technical Memorandum NMFS-SEFC-169.

Hatai, K., B.Z. Bian, M.C.L. Baticados and S. Egusa. 1980. Studies on the fungal diseases of crustaceans. II. *Halipthoros philippinensis*

sp. nov. isolated from cultivated larvae of the jumbo tiger prawn *Penaeus monodon*. Transaction of the Mycological Society of Japan. 21: 47-55.

Hose, J.E., D.V. Lightner, R.M. Redman and D.A. Danald. 1984. Observations on the pathogenesis of the imperfect fungus *Fusarium solani* in the Californian brown shrimp, *Penaeus californiensis*. J. Invertebr. Pathol. 44: 292-303.

Humason, G.L. 1967. Animals Tissue Techniques. W.H. Freeman and Co., San Francisco, CA, 432. p.

Itami, T., Y. Takahashi and Y. Nakamura. 1989. Efficacy of vaccination against Vibriosis in cultured kuruma prawns *Penaeus japonicus*. J. Aquat. Animal Health. 1: 238-242.

Johnson, P.T. and D.V. Lightner 1988. The rod-shaped nuclear viruses of crustaceans: gut-infecting species. Diseases of Aquatic Animals; Dis. Aquat. Org. 5: 123-141.

Johnson, S.K. 1978. Handbook of Shrimp Diseases. Sea Grant Publication No. TAMU-SG-75-603. Texas A&M University, College Station, Texas. 23 p.

Lester, R.J.G. and J.L. Paynter. 1989. Diseases of cultured prawns in Australia. *In*: Advances in Tropical Aquaculture. AQUACOP IFREMER. pp. 97-101.

Lewis, D.H. 1973. Response of brown shrimp to infection with *Vibrio* sp. Proc. World Maricul. Soc. 4: 333-338.

Lewis, D.H. and A.L. Lawrence. 1983. Immunoprophylaxis to *Vibrio* sp. in pond reared shrimp. Proceedings of the First International Conference on Warmwater Aquaculture: Crustacea. Brigham Young University, Hawaii.

Liao, I.C. 1985. A brief review of the larval rearing techniques of penaeid prawns. *In*: Y. Taki, J.H. Primavera and A. Llobrera (Eds.). Proc. First International Conference on the Culture of Penaeid Prawns/Shrimps. Aquaculture Dept., Southeast Asian Fisheries Development Center, Iloilo, Philippines. pp. 65-78.

Lightner, D.V. 1975. Some potentially serious disease problems in the culture of penaeid shrimp in North America. *In*: Proceedings of the Third US-Japan Meeting on Aquaculture, US-Japan Cooperative Program in Natural Resources. Special Publications of the Fishery Agency, Japanese Government and Japan Sea Regional Fisheries Laboratory, Nigata, Japan. pp. 95-97.

Lightner, D.V. 1978. Possible toxic effects of the marine blue-green alga, *Spirulina subsalsa* in the blue shrimp, *Penaeus stylirostris*. J. Invertebr. Pathol. 32: 139-150.

Lightner, D.V. 1982. Toxic effect of certain marine blue-green algae to penaeid shrimp. Final Report. Sea Grant No. NA 79AA-D-0024.

Lightner, D.V. 1983. Diseases of cultured penaeid shrimp. *In*: J.P. McVey (Ed.). Handbook of Mariculture. CRC Press, Boca Raton, Florida. pp. 289-320.

Lightner, D.V. 1985. A review of the diseases of cultured penaeid shrimp and prawns with emphasis on recent discoveries and developments. *In*: Y. Taki, J.H. Primavera and J.A. Llobrera (Eds.). Proc. First International Conference on the Culture of Penaeid Prawns/Shrimps. Aquaculture Dept., Southeast Asian Fisheries Development Center, Iloilo, Philippines. pp. 79-103.

Lightner, D.V., T.A. Bell and R.M. Redman. 1989a. A review of the known hosts, geographical range and current diagnostic procedures for the virus diseases of cultured penaeid shrimp. AQUACOP IFREMER. pp. 113-126.

Lightner, D.V., T.A. Bell, R.M. Redman, L.M. Mohney, J.J. Natividad, A. Rukyani and A. Poernomo. 1992. A review of major diseases of economic significance in penaeid prawns/shrimps of the Americas and Indo-Pacific. *In*: M. Shariff, R.P. Subasinghe and J.F. Arthur (Eds.). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila, Philippines. pp. 57-80.

Lightner, D.V. and C.T. Fontaine. 1975. A mycosis of the American lobster, *Homarus americanus* caused by *Fusarium* sp. J. Invertebr. Pathol. 25: 239-245.

Lightner, D.V., C.T. Fontaine and K. Hanks. 1975. Some forms of gill disease in penaeid shrimp. Proc. World Maricul. Soc. 6: 347-365.

Lightner, D.V., R.P. Hedrick, J. L. Fryer, S.N. Chen, I.C. Liao and G.H. Kou. 1987a. A survey of cultured penaeid shrimp in Taiwan for viral and other important diseases. Fish Pathol. 22(3): 127-140.

Lightner, D.V. and D.H. Lewis. 1975. A septicemic bacterial disease syndrome of penaeid shrimp. Mar. Fish. Rev. 37: 25-28.

Lightner, D.V., and R. Redman. 1981. A baculovirus-caused disease of the penaeid shrimp, *Penaeus monodon*. J. Invertebr. Pathol. 38: 299-302.

Lightner, D.V., and R.M. Redman. 1985a. Necrosis of the hepatopancreas in *Penaeus mono-*

don and *Penaeus stylirostris* (Arthropoda: Decapoda) with red disease. J. Fish Dis. 8: 181-188.

Lightner, D.V. and R.M. Redman. 1985b. A parvo-like virus disease of penaeid shrimp. J. Invertebr. Pathol. 45: 47-53.

Lightner, D.V. and R.M. Redman. 1991. Hosts, geographic range and diagnostic procedures for the penaeid virus diseases of concern to shrimp culturists in the Americas. *In*: P. DeLoach, W.J. Dougherty and M.A. Davidson (Eds.). Frontiers in Shrimp Research. pp. 173-196.

Lightner, D.V., R.M. Redman and T.A. Bell. 1983a. Observations on the geographic distribution, pathogenesis and morphology of the baculovirus from *Penaeus monodon* Fabricius. Aquaculture. 32: 209-233.

Lightner, D.V., R.M. Redman, D.A. Danald, R.R. Williams and L.A. Perez. 1984a. Major diseases encountered in controlled environment culture of penaeid shrimp at Puerto Penasco, Sonora, Mexico. *In*: C.J. Sindermann (Ed.). Proc. 9th and 10th US-Japan Meetings on Aquaculture, NOAA Technical Report NMFS. 16: 25-33.

Lightner, D.V., R.M. Redman, T.A. Bell and J. Brock. 1984b. An idiopathic proliferative disease of the midgut and ventral nerve in the Kuruma prawn, *Penaeus japonicus* Bate, cultured in Hawaii. J. Fish. Dis. 7: 183-192.

Lightner, D.V., R.M. Redman, R.R. Williams, L.L. Mohney, J.P.M. Clerx, T.A. Bell and J.A. Brock. 1985. Recent advances in penaeid virus disease investigations. J. World Maric. Soc. 16: 267-274.

Lightner, D.V., B.R. Salser and R.S. Wheeler. 1974. Gas bubble disease in the brown shrimp, *Penaeus aztecus*. Aquaculture. 4: 81-84.

Lio-Po, G.D., C.R. Lavilla and A.T. Llobrera. 1978. Toxicity of malachite green to the larvae of *Penaeus monodon*. Kalikasan. Philippine Journal of Biology. 7:338-346.

Lio-Po, G.D., M.E.G. Sanvictores, M.C.L. Baticados and C.R. Lavilla. 1982. *In vitro* effects of fungicides on hyphal growth and sporogenesis of *Lagenidium* sp. isolated from *Penaeus monodon* larvae and *Scylla serrata* eggs. J. Fish Dis. 5: 97-112.

Lio-Po, G.D., M.C.L. Baticados, C.L. Lavilla and M.E.G. Sanvictores. 1985. *In vitro* effects of fungicides on the fungus *Halipthoros philippinensis*. J. Fish Dis. 8: 359-365.

Luna, L.G. (Ed.). 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. McGraw Hill, New York. 259 p.

Martin, S.W., A.H. Meek and P. Willeberg. 1987. Veterinary Epidemiology. Iowa State University Press, Ames, Iowa. 343 p.

McKee, C. and D.V. Lightner. 1982. Effects of several algicides and surfactants on filamentous bacterium *Leucothrix mucor* Oersted. Appl. Environ. Microbiol. 43: 715-718.

Momoyama, K. and T. Sano. 1989. Developmental stages of Kuruma shrimp, *Penaeus japonicus* Bate, susceptible to baculoviral mid-gut gland necrosis (BMN) virus. J. Fish Dis. 12: 585-589.

Nash, G.I., I.A. Anderson and M. Shariff. 1988. Pathological changes in the tiger prawn *Penaeus monodon* Fabricius, associated with culture in brackishwater ponds developed from potentially acid sulphate soils. J. Fish. Dis. 11: 113-123.

Nimmo, D.R., D.V. Lightner and L.H. Bahner. 1977. Effects of cadmium on the shrimp, *Penaeus duorarum, Paleomonetes pugio* and *Paleomonetes vulgaris*. *In*: J.F. Vernberg, A. Calabrese, F.D. Thurberg and W.B. Vernberg (Eds.). Physiological Responses of Marine Biota to Pollutants. Academic Press, New York. pp. 131-183.

Overstreet, R.M. 1978. Marine maladies? Worms, germs and other symbionts from the Northern Gulf of Mexico. Mississippi-Alabama Sea Grant Consortium, MASGP-78-021-1978.

Pitogo, C.L., L.J. Albright, M.G. Pañer and N.A. Suñaz. 1990. Studies on the sources of luminescent *Vibrio harveyi* in *Penaeus monodon* hatcheries. *In:* M. Shariff, R.P. Subasinghe and J.R. Arthur (Eds.). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila, Philippines. pp. 157-164.

Pristavko, V.P. and N.V. Dovzhenok. 1974. Ascorbic acid influence on larval blood cell number and susceptibility to bacterial and fungal infection in the codling moth *Laspeyresia pomonella*. J. Invertebr. Pathol. 24: 165.

Sano, T., T. Nishimura, K. Oguma, K. Momoyama and N. Takeno. 1981. Baculovirus infection of cultured Kuruma shrimp *Penaeus japonicus* in Japan. Fish Pathol. 15: 185-191.

Sano, T., T. Nishimura, H. Fukuda, T. Hayashida and K. Momoyama. 1985. Baculoviral infectivity trials on kuruma shrimp larvae, *Penaeus japonicus*, of different ages. *In*: A.E. Ellis (Ed.). Fish and Shellfish Pathology. Academic Press, London. pp. 397-403.

Sindermann, C.J. and D.V. Lightner (Eds.). 1988. Disease Diagnosis and Control in North American Marine Aquaculture. Elsevier, New York. 431 p.

Suplee, V.C. and D.V. Lightner. 1976. Gas-bubble disease due to oxygen supersaturation in raceway-reared brown shrimp. Prog. Fish Cult. 38: 158-159.

Takahashi, Y., Y. Shomoyama and K. Momoyama. 1985. Pathogenicity and characteristics of *Vibrio* sp. isolated from cultured kuruma prawn *Penaeus japonicus* Bate. Bull. Jap. Soc. Sci. Fish. 51(5): 721-730.

Tanada, Y. and J.F. Fuxa. 1987. The pathogen population. *In*: J.R. Fuxa and Y. Tanada (Eds.). Epizootiology of Insect Diseases. John Wiley and Sons, New York. 555 p.

Thurman, R.B., T.A. Bell and D.V. Lightner. 1990. Unique physico-chemical properties of the occluded penaeid shrimp baculoviruses and their use in diagnosis of infections. J. Aquat. Animal Health. 2: 128-131.

Watanabe, H. 1987. The host population. *In*: J.R. Fuxa and Y. Tanada (Eds.). Epizootiology of Insect Diseases. John Wiley and Sons, New York. 555 p.

Weiser, J. 1987. Patterns over place and time. *In*: J.R. Fuxa and Y. Tanada (Eds.). Epizootiology of Insect Diseases. John Wiley and Sons, New York. 555 p.

Wiseman, M.O., R.L. Price, D.V. Lightner and R.R. Williams. 1982. Toxicity of aflatoxin B1 to penaeid shrimp. Appl. Env. Microbiol. 44: 1479-1481.

The Status of Culture and Diseases of Penaeid Shrimp In Korea

Myoung Ae Park
National Fisheries Research & Development Agency
65-3 Shirang-ri, Kijang-up, Yangsan-gun
Kyong-Nam 626-900, Republic of Korea

Abstract

This paper reviews the status of cultured *Penaeus japonicus* and *P. chinensis*, and describes the diseases known to affect cultured shrimp in Korea.

Introduction

Public institutes and private companies first attempted to culture penaeid shrimp in the late 1970s, but commercial-scale culture was not successful in Korea until the early 1980s. Since that time, the development of semi-intensive shrimp culture in Korea has been accompanied by disease outbreaks. Unfortunately, few studies have been conducted on shrimp diseases and there is an urgent need for more research.

The Status of Penaeid Shrimp Culture In Korea

Culture Areas

The primary species of shrimp grown in Korea are the fleshy prawn, *Penaeus chinensis*, on the western coast of the Korean Peninsula, and the kuruma prawn, *P. japonicus*, along the southern coast. There are approximately 30 shrimp farms in Korea. Fifteen are concentrated near Chungnam province on the Yellow Sea; these are responsible for more than 80% of total production of cultured shrimp.

Penaeid shrimp seed are produced at three government institutes under the purview of the National Fisheries Research and Development Agency (NFRDA). *Penaeus japonicus* postlarvae have been produced and released at the Koje hatchery on the southern coast, and *P. chinensis* seed are cultured and released at Puan and Poryong hatcheries on the western coast (Fig. 1.).

Artificial Seed Production

From 1983 to the present, 33,565,000 *P. japonicus* and 23,790,000 *P. chinensis* postlarvae were produced by NFRDA

Table 1. Production of *Penaeus japonicus* and *P. chinensis* seed in government institutes (Units: thousands).

Year	*P. japonicus*		*P. chinensis*			Total
	Production	No. released	Production	No. released	Sales	
1983	325	325	–	–	–	325
1984	1,540	1,540	–	–	–	1,540
1985	2,200	2,200	–	–	–	2,200
1986	3,700	3,700	–	–	–	3,700
1987	4,800	4,800	1,070	500	500	5,870
1988	4,500	4,500	1,650	750	900	6,150
1989	5,000	5,000	3,320	1,500	1,820	8,320
1990	6,200	6,200	6,850	2,200	4,650	13,050
1991	5,300	5,300	10,900	7,300	3,600	16,200
Total	33,565	33,565	23,790	12,250	11,470	57,355

for stock enhancement and to sell (Table 1). Private shrimp culture enterprises, by comparison, produced 160 million *P. japonicus* and *P. chinensis* postlarvae in 1991 alone (Table 2).

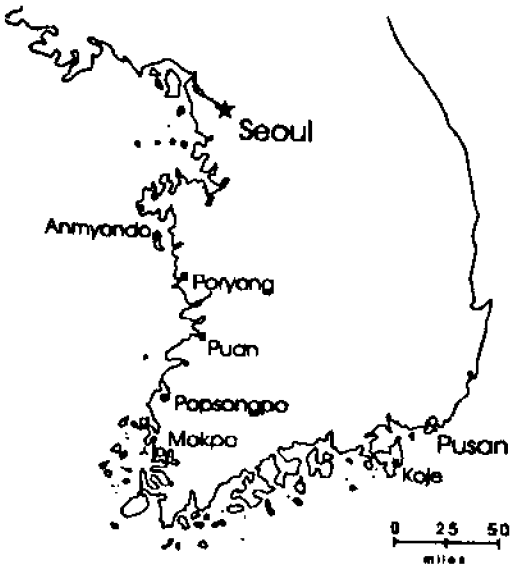


Figure 1. Penaeid shrimp culture areas in Korea.

Production of Shrimp

The total amount of shrimp harvested in 1990 was 3,775 MT; 312 MT from the culture fishery and 3,463 MT from the capture fishery. Production from Korea's culture fishery was still very low compared to the capture fishery — less than 10% of the total production (Table 3).

Diseases of Cultured Penaeid Shrimp In Korea

Several diseases afflict cultured penaeid shrimp in Korea; sometimes they cause serious damage. In general, little is known about penaeid shrimp diseases in Korea; therefore, few details regarding the overall status of diseases, diagnostic procedures and treatments are available at this time. The following diseases have been found in Korea.

Table 2. Production of *Penaeus japonicus* and *P. chinensis* seed in private companies (Units: millions).

Species	1983	1984	1985	1986	1987	1988	1989	1990	1991	Total
P. japonicus	10	10	15	18	18	25	22	45	65	228
P. chinensis	29	64	60	65	50	60	48	70	95	541
Total	39	74	75	83	68	85	70	115	160	769

Table 3. Total volume of penaeid shrimp harvested in Korea (Units: MT).

Year	Culture fishery		Capture fishery		Total
	P. japonicus	*P. chinensis*	*P. japonicus*	*P. chinensis*	
1986	51	82	864	1,503	2,500
1987	71	113	2,322	683	3,189
1988	79	102	4,481	1,080	5,742
1989	68	234	2,023	1,220	3,545
1990	55	257	2,397	1,066	3,775

Viral Diseases

HPV. Hepatopancreatic parvo-like virus (HPV) is known to infect *P. chinensis* (Lightner and Redman, 1985). Symptoms of HPV infection are poor growth rate, decreased feed consumption, slow movement and a propensity to aggregate in areas near the tank edge. Affected shrimp can also exhibit white patches on their abdomens. The means to prevent and treat HPV disease are unknown.

Histopathological observations funded by The Oceanic Institute's Asian Interchange Program in 1991 confirmed the presence of HPV-infected *P. chinensis* postlarvae at a government hatchery. This research will continue this year.

BMN. Baculoviral midgut gland necrosis (BMN) affects *P. japonicus* larvae, causing mass mortalities as a result of the destruction of the epidermal cells of the midgut and hepatopancreas (Sano et al., 1984; Lightner et al., 1989).

BMN-infected mysis and postlarvae stop feeding and swim with an abnormal posture near the surface of the water. Using histopathological methods, BMN was diagnosed in *P. japonicus* larvae in Korea in 1991.

Vibrio Disease

Vibrio disease has been observed in penaeid shrimp ranging in size from 1 g to adult. The most serious losses are incurred during growout.

Table 4 contains information on six strains of *Vibrio* sp. isolated from cultured *P. japonicus* that exhibited signs of disease (Kim and Chun, 1990). A bacterium was isolated from the heart, lymphoid organ and muscle of shrimp

Table 4. Sources of six strains of *Vibrio* sp. isolated from cultured kuruma prawns, *Penaeus japonicus*, with vibriosis.

Strain	Location	Date	Sea water temp.	Isolation media	Organ
N - 1					
N - 2	Namhae	9 Dec. 1989	22 - 24°C	BTB teepol	Lymphoid organ
N - 3					
A - 4					
A - 5	Anhung	10 Nov. 1989	19 - 21°C	BTB teepol	Lymphoid organ
A - 6					

from farms in Namhae and Anhung between August and October, 1989. The strains were then submitted for morphological, biochemical and physiological characterization (Fig. 2, Tables 5-7).

Several workers have reported effective therapy of bacterial diseases using antibiotics. Oxytetracycline is frequently used to treat Vibrio disease of penaeid shrimp in Korea.

Leucothrix Disease

Leucothrix mucor attaches to living and nonliving solid substrates, and it rapidly adheres to the body surfaces of penaeid shrimp. Affected individuals are dull in appearance and covered with muddy debris; if mortality occurs, it is usually due to hypoxia.

Leucothrix mucor may be managed with antibiotics or copper compounds (Lightner and Supplee, 1976).

Ciliate Disease

Zoothamnium sp. and *Epistylis* sp. also infest cultured penaeid shrimp. Serious outbreaks are prevented by keeping the culture water clean and by providing a high-quality diet. Formalin is reportedly effective in controlling these organisms on shrimp farms.

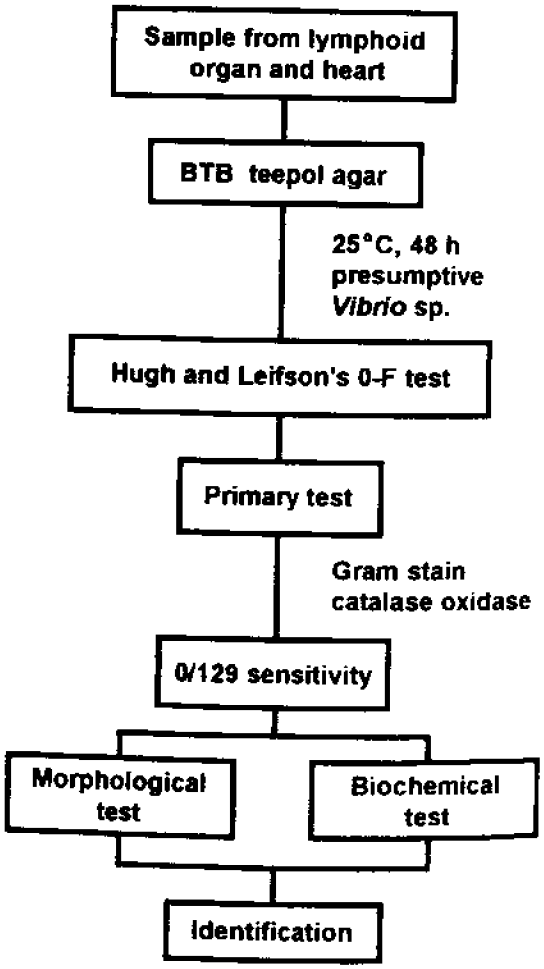


Figure 2. Scheme for detecting Vibrio *sp. in* P. japonicus.

Table 5. Biological characteristics of isolated strains.

Characteristics	N1	N2	N3	A1	A2	A3
Growth on inhibitory media:						
SS agar	-	-	-	-	-	-
2% NaCl BHI agar	+	+	+	+	+	+
BTB teepol agar	+	+	+	+	+	+
Brilliant agar	-	-	-	-	-	-
Growth at:						
5°C	-	-	-	-	-	-
10°C	(+)	(+)	(+)	(+)	(+)	(+)
25°C	+	+	+	+	+	+
30°C	+	+	+	+	+	+
37°C	-	-	-	-	-	-
NaCl tolerance:						
0%	-	-	-	-	-	-
1.0%	+	+	+	+	+	+
2.0%	+	+	+	+	+	+
3.0%	+	+	+	+	+	+
0.5%	-	-	-	-	-	-
Sensitivity to 0/129	+	+	+	+	+	+

Note (+): weak or delayed positive.

Problems and Countermeasures

Coastal Pollution

Pollution of coastal water with effluent from factories and with domestic sewage have caused a decline in the populations of plankton that were once available as feed for shrimp. Red tides are also more frequent as a result of agricultural runoff, especially during the rainy season. These factors have triggered shrimp diseases.

Government Support

A national center for shrimp disease diagnosis and control, with field units in several provinces, is needed. Also, the diagnostic capabilities for shrimp quarantine and research facilities need to be upgraded, particularly techniques for virus detection and isolation.

Other Needs

The following measures would help control the incidence of disease among cultured penaeid shrimp in Korea:

- Train professionals to conduct intensive research on shrimp diseases;
- Prevent infectious diseases through an early warning system for shrimp farms; and
- Increased international exchange of information on topics such as

Table 6. Main characteristics of the isolates and *Vibrio* sp. reported by several workers.

Characteristics	Strain						*Vibrio* sp.[1]	*Vibrio* sp.[2]	*Vibrio* sp.[3]	*Vibrio* sp.[4]	*Vibrio* sp.[5]
	N1	N2	N3	A4	A5	A6					
Single polar flagellum	+	+	+	+	+	+	+	+	+	+	+
Indole	+	+	+	+	+	+	+	+	+	+	+
MR	+	+	+	+	+	+	+	+	+	+	+
VP	−	−	−	−	−	−	−	−	−	−	−
Citrate utilization	+	+	+	+	+	+	+	+	+	+	+
0/129 sensitivity	+	+	+	+	+	+	+	+	+	+	+
Novobiocin	+	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+
Hydrogen sulfide	−	−	−	−	−	−	−	−	−	−	−
Acid from arabinose	−	−	−	−	−	−	−	−	−	−	−
mannose	+	+	+	+	+	+	+	+	+	+	+
sucrose	+	+	+	+	+	+	+	+	+	+	+
mannitol	−	−	−	−	−	−	−	−	−	−	−
inositol	−	−	−	−	−	−	−	−	−	−	−
salicin	−	−	−	−	−	−	−	−	−	−	−
0% NaCl	−	−	−	−	−	−	−	−	−	−	−
1%	+	+	+	+	+	+	+	+	+	+	+
3%	+	+	+	+	+	+	+	+	+	+	+
5%	+	+	+	+	+	+	+	+	+	+	+
7%	−	−	−	−	−	−	−	−	−	−	−
Growth at 5°C	−	−	−	−	−	−	−	−	−	−	−
25°C	+	+	+	+	+	+	+	+	+	+	+
37°C	−	−	−	−	−	−	−	−	−	−	−
42°C	−	−	−	−	−	−	−	−	−	−	−
Inhibitory media											
SS agar	−	−	−	−	−	−	−	−	−	−	−
BTB teepol agar	+	+	+	+	+	+	+	+	+	+	+
MacConkey agar	−	−	−	−	−	−	−	−	−	−	−
Brilliant agar	−	−	−	−	−	−	−	−	−	−	−

[1]Yasunaga and Yamamoto, 1977; [2]Muroga et al., 1979; [3]Takahashi et al., 1985; [4]Kusuda et al., 1979; [5]Ueki et al., 1988.

Table 7. Pathogenicity of the isolates to *P. japonicus*.

Group	Mean body weight of *P. japonicus* tested	No. viable cells/g (B.W.) inoculated (A4 strains)	No. challenged	No. deaths	Mortality (%)
1	2.3	2.5×10^7	6	6	100
2	2.4	2.5×10^6	6	6	100
3	2.3	2.5×10^5	6	5	83.3
4	2.5	2.5×10^4	6	4	66.6
5	2.4	2.5×10^3	6	4	66.6
Control	2.4	not inoculated	6	0	0

shrimp culture and the prevention and treatment of disease.

Literature Cited

Kim, J.H. and S.K. Chun. 1990. A vibriosis occurring in cultured kuruma prawn, *Penaeus japonicus*. J. Fish Pathol. 3(1): 1-9.

Kusuda, R., H. Sako and K. Kawa. 1979. Classification of *Vibrio* isolated from diseased fish 1. Fish Pathol. 13(3): 123-137.

Lightner, D., T. Bell and R. Redman. 1989. A review of the known hosts, geographical range and current diagnostic procedures for the virus diseases of cultured penaeid shrimp. Advances in Tropical Aquaculture, Tahiti, Feb. 20 - March 4, 1989, Aquacop. IFREMER, Actes de Colloque. 9: 113-126.

Lightner, D.V. and R.M. Redman. 1985. A parvo-like virus disease of penaeid shrimp. J. Invertebr. Pathol. 45: 47-53.

Lightner, D.V. and V.C. Supplee. 1976. A possible chemical control method for filamentous gill. Proc. World Maricul. Soc. 7: 473.

Muroga, K., S. Takahashi, H. Yamanoi and M. Nishibushi. 1979. Non-cholera *Vibrio* isolated from diseased Ayu. Bull. Jap. Soc. Sci. Fish. 45(7): 829-834.

Sano, T., T. Nishimura, H. Fukuda, T. Hayashida and K. Momoyama. 1984. Baculoviral mid-gut gland necrosis (BMN) of kuruma shrimp (*Penaeus japonicus*) larvae in Japanese intensive culture systems. Helgoländer Meeresunters. 37: 255-264.

Takahashi, Y., Y. Shimoyama and K. Momoyama. 1985. Pathogenicity and characteristics of *Vibrio* sp. isolated from cultured kuruma prawn. *Penaeus japonicus* BATE. Bull. Jap. Soc. Sci. Fish. 51(5): 721-730.

Ueki, N., T. Sugiyama and K. Mugora. 1988. *Vibrio* infection in the freshwater shrimp (*Palaemon paucidene*) predisposed by a parasitic isopod (*Tachea chinensis*) infestation. Fish Pathol. 23(3): 175-178.

Yasunaga, N. and N. Yamamoto. 1977. Characteristics of bacterial strains isolated from so-called Vibriosis of cultured red sea bream in the winter of 1977. Fish. Pathol. 12(3): 209-214.

Baculovirus Infection of Penaeid Shrimp in Japan

Tokuo Sano
Laboratory of Aquatic Pathology, Department of Aquatic Biosciences
Tokyo University of Fisheries, 4-5-7 Konan, Minato Ku
Tokyo 108, Japan

and

Kazuo Momoyama
Yamaguchi Prefectural Naikai Fisheries Experiment Station
Yamaguchi 754, Japan

Abstract

Baculoviral midgut gland necrosis (BMN) is a superacute viral disease, causing severe mortalities in kuruma shrimp larvae, *Penaeus japonicus*. With the goal of implementing realistic prophylactic measures, we developed diagnostic techniques for BMN, investigated the host range of BMNV, and tested the efficacy of a variety of chemical and physical agents as BMN virucides. The methods we developed can facilitate diagnosis in hatcheries. Washing fertilized eggs is the most effective prophylactic technique. In addition to *P. japonicus*, BMNV can infect *P. monodon*, *P. chinensis* and *P. semisulcatus* but not *Metapenaeus ensis* or *Portunus trituberculatus*.

Introduction

The OIE (Office Internationale des Epizooties) has dealt with fish disease matters since 1960 through the Fish Diseases Commission (FDC), which produces an annual report on the main developments regarding current diseases, new pathogens, and diagnostic and control methods used worldwide. The FDC has created a separate section for fish diseases in the Animal Health Code. This is currently being revised and will contain eight pathogens (six viral agents and two bacterial agents); all will be placed on List B. List B contains 89 communicable animal diseases considered to be of socioeconomic and/or public health importance within host countries, and are significant in the international trade of animals and animal products. Recently, the FDC has expanded to encompass diseases of molluscs and crustacea (De Kinkeline et al., 1990). Consequently, eleven pathogens were placed on List B, including *Penaeus monodon* baculovirus (MBV), *Baculovirus penaei* (BP) and baculoviral midgut gland necrosis (BMN) virus.

This indicates the importance of baculoviruses to penaeid shrimp culture.

List B agents cause no serious socioeconomic problems for terrestrial homeotherms, nor are they a public health consequence. However, the baculovirus particles released from victims of BMN, both moribund animals and latently infected adults, undoubtedly constitute an "aquatic biohazard." "Aquatic biohazard" may be defined as a hazard for aquatic animals such as fish and shellfish caused by aquatic pathogens in List B (Sano, 1992) as opposed to the pathogens in List A that are dangerous for humans and/or terrestrial homeothermal animals.

Examples of aquatic biohazards include IPN (infectious pancreatic necrosis), VHS (viral hemorrhagic septicemia), and EHN (epizootic hematopoietic necrosis). Aquatic pathogens, in general, have the potential to spread widely and rapidly, and furthermore, to be transmitted intergenerically. The resulting diseases prevail as enzootic or panzootic and cause serious socioeconomic consequences. The goal of our studies on BMN is the extermination of this shrimp disease. Toward that aim, we have studied three aspects: diagnosis of BMN, the host range of BMNV, and countermeasures for BMN. This paper describes our results.

Diagnostic Techniques for BMN

BMN is a superacute communicable disease of kuruma shrimp in Japan (Sano et al., 1981). The most important factor allowing for the prompt implementation of prophylactic measures for BMN is rapid, simple and accurate diagnosis, applicable *in situ* on shrimp seed production farms, if possible. The following two diagnostic techniques we have developed meet these criteria.

Fluorescent Antibody Diagnosis. The principle of this technique depends on visualizing the specific fluorescence to the antigen of the baculovirus in the affected midgut gland smear or in the intestinal epithelium. The fluorescent antibody (FA) staining of the smear or the organ reveals that the number of juveniles showing ubiquitously visible fluorescence tends to increase with time after inoculation with the virus. Visible fluorescence in the nuclei of the epithelial cells was recognized at 18 h postinoculation (Sano et al., 1985).

Dark Field Microscopic Diagnosis. This diagnosis confirms nuclear hypertrophy of the midgut gland epithelial cells, which is pathognomic for BMN virus infection, in fresh squash preparations under dark field illumination equipped with a wet-type condenser. Two to four days postinoculation at 25 - 30°C incubation temperature were considered to be satisfactory during the infectivity trial (Momoyama and Sano, 1988).

Host Range of BMNV

To determine the host range of BMNV, susceptibility trials were performed on

the larval stages of five species of crustaceans: giant tiger prawn (*Penaeus monodon*), fleshy prawn (*P. chinensis*), green tiger prawn (*P. semisulcatus*), greasyback shrimp (*Metapenaeus ensis*), and blue crab (*Portunus trituberculatus*). BMNV was inoculated in accordance with the water-borne method (Momoyama and Sano, 1988). Giant tiger prawns developed severe infections (mortality), both the fleshy prawns and green tiger prawns had temporary infections (no mortality), and both the greasyback shrimp and blue crabs were not infected with BMNV. Hence, the host range of BMN includes *Penaeus monodon*, *P. chinensis* and *P. semisulcatus*.

Countermeasures for BMN

In order to establish realistic countermeasures for BMN, the virucidal effects of chemical and physical agents were studied. The results obtained are as follows.

Virucidal Effects of Chemicals on BMNV. BMNV is inactivated by exposure for 10 min at 25°C with the following chemicals and concentrations: 5-ppm active principle concentrations of chlorine; 25-ppm active principle concentration of iodine; 100-ppm benzalkonium chloride; 100-ppm benzethonium chloride; 0.5% Formalin; and 30% ethanol. Also, the virus is inactivated with the following treatments: ethyl ether for 18 h, at 4°C; 25% NaCl solution within 10 h and 12.5% NaCl solution within 24 h (Momoyama, 1989a; 1989c).

Virucidal Effect of Physical Factors on BMNV. BMNV is inactivated with ultraviolet irradiation of 4.1×10^5 $\mu W \times s/cm^2$; summer sunlight exposure for 3 h at about 30°C; heating at 45°C for 120 min, at 50 and 55°C for 30 min, and at 60°C for 5 min; drying within 1.5 h at about 30°C (Momoyama, 1989b).

Sterilizing Effects of Egg-washing on BMNV. A prophylactic effect can be achieved by pouring enough sea water over the fertilized eggs of the shrimp or by soaking the eggs several times in an egg-washing tank. As a result of applying this measure *in situ* at a hatchery in Yamaguchi Prefecture, the incidence of BMN has dwindled year by year since 1985 (Table 1). Figure 1 illustrates the collecting procedure for the fertilized eggs of shrimp.

Discussion and Conclusions

The two diagnostic methods we developed are simple, rapid and inexpensive, facilitating the diagnosis of BMN at hatcheries or in the field. Using these techniques, the virucidal effects of chemical and physical agents for BMNV were elucidated, and the host range of BMNV was determined. Furthermore, a preventive method for BMN, egg-washing, was developed and successfully prevented BMN in hatcheries where it has been implemented. As shown in Table 1, BMN has not occurred since 1987. Consequently, we determined that it was unnecessary to develop highly sensitive diagnostics such as a baculoviral DNA probe.

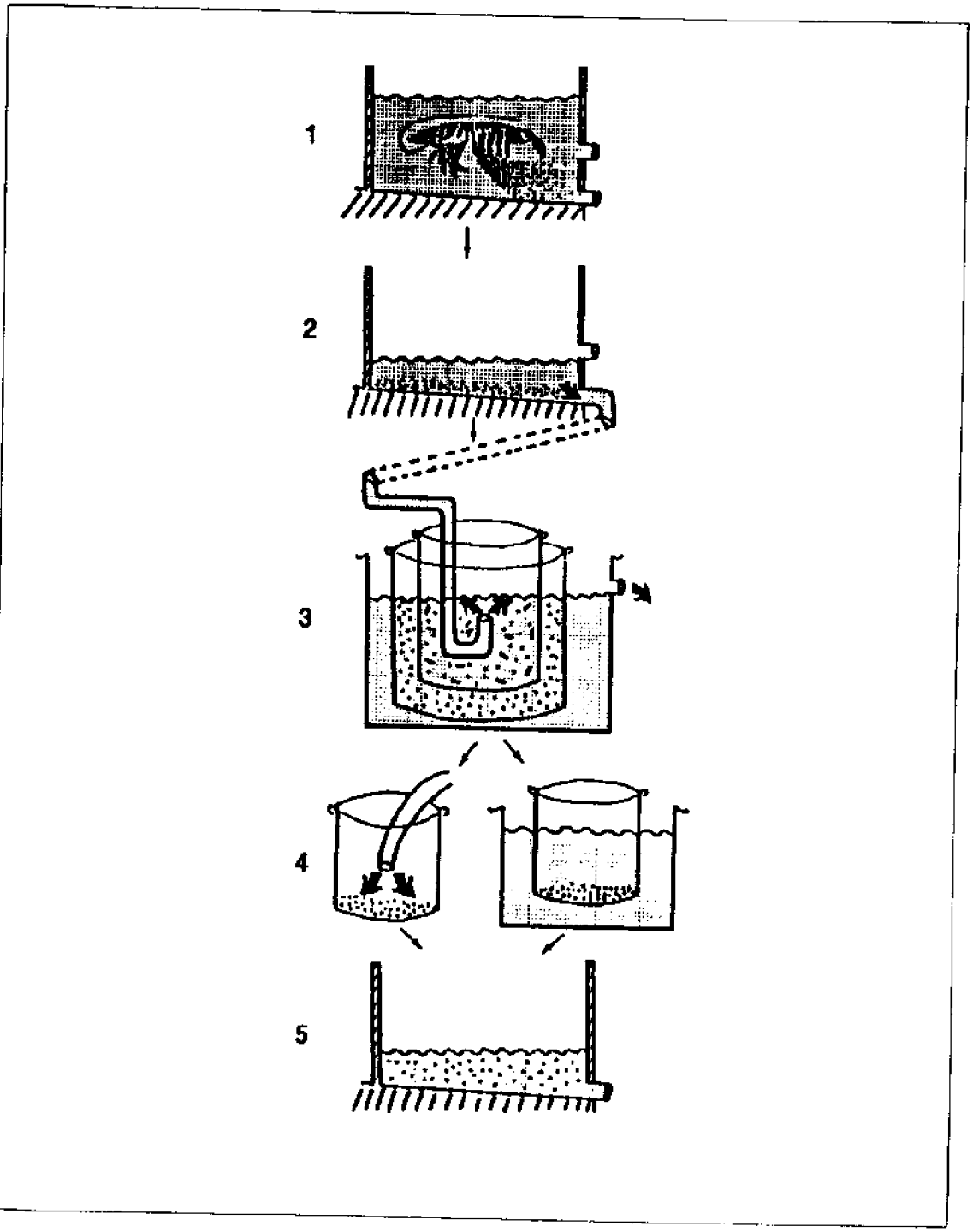


Figure 1. General procedure for collecting and washing shrimp eggs prior to incubation. (1) Broodstock spawn at night in spawning tank. The next day the spawner is removed, aeration is halted, and the eggs sink while the upper portion of sea water is drained. (2) The eggs are collected in a collection tank by means of a bottom drain pipe with gentle flowing sea water. (3) The eggs are collected with an outer 100 µm mesh net after they are first filtered through the inner 300 µm mesh net. (4) The eggs are washed with sterile sea water or by changing the water in the egg washing tank several times. The temperature of the rinsing water and the water in the egg washing tank should be the same as that in the spawning tank. (5) The eggs are incubated in a rearing tank with 40 - 50 cm-deep sea water that has been thermo-controlled and fertilized.

Table 1. Incidence of BMN in a kuruma shrimp hatchery in Yamaguchi Prefecture, 1982 to 1989.

Year	No. of tanks examined	No. of BMN cases	BMN incidence (%)
1982	42	28	66.7
1983	39	13	33.3
1984	39	7	17.9
1985	48	3	6.3
1986	49	4	8.2
1987	38	0	0
1988	23	0	0
1989	24	0	0

Baculoviral infection poses a danger to natural resources of penaeid shrimp. The most effective way to prevent the threat of BMNV is to exterminate the virus at the spot of a preliminary outbreak by virucidal measures. The recommended chemicals are chlorine and iodine, applied in the manner and doses indicated in this paper. Recently, Batts et al. (1991) reported that a 7.5-s exposure to a free iodine concentration of 0.14 mg/L inactivated 99.9% of infectious hematopoietic necrosis virus, suggesting that the water-borne route of virus transmission can be blocked by adding low iodine concentrations to the water supplies of hatcheries. However, whether these shorter exposure times and lower doses can also inactivate BMNV remains to be determined.

Literature Cited

Batts, W., M. Landolt and J.R. Winton. 1991. Inactivation of infectious hematopoietic necrosis virus by low levels of iodine. Appl. Environ. Microbiol. 57: 1379-1385.

De Kinkelin, P., T. Hastein, J. Krecek, S.N. Chen and B.J. Hill. 1990. The role of the OIE (Office Internationale des Epizooties) in improving awareness and control of international transfers of fish and shellfish diseases. Bull. Eur. Ass. Fish Pathol. 10: 4-6.

Momoyama, K. and T. Sano. 1988. A method of experimental infection of kuruma shrimp, *Penaeus japonicus* Bate, with baculoviral midgut gland necrosis (BMN) virus. J. Fish Dis. 11: 105-111.

Momoyama, K. 1989a. Virucidal effect of some disinfectants on baculoviral mid-gut gland necrosis (BMN) virus. Fish Pathol. 24: 47-49. (In Japanese with English abstract).

Momoyama, K. 1989b. Inactivation of baculoviral mid-gut gland necrosis (BMN) virus by ultraviolet irradiation, sunlight exposure, heating and drying. Fish Pathol. 24: 115-118. (In Japanese with English abstract.).

Momoyama, K. 1989c. Tolerance of baculoviral mid-gut gland necrosis virus (BMNV) to ether, NaCl concentration and pH. Fish Pathol. 24: 175-177. (In Japanese with English abstract.).

Sano, T., T. Nishimura, K. Oguma, K. Momoyama and N. Takeno. 1981. Baculovirus infection of kuruma shrimp, *Penaeus japonicus* in Japan. Fish Pathol. 15: 185-191.

Sano, T., T. Nishimura, H. Fukuda, T. Hayashida and K. Momoyama. 1984. Baculoviral mid-gut gland necrosis (BMN) of kuruma shrimp (*Penaeus japonicus*) larvae in Japanese intensive culture systems. Helgoländer Meeresunters. 37: 255-264.

Sano, T., T. Nishimura. H. Fukuda, T. Hayashida and K. Momoyama. 1985. Baculoviral infectivity trials on kuruma shrimp larvae, *Penaeus japonicus*, of different ages. *In*: A.K.

Ellis (Ed.). Fish and Shellfish Pathology. Academic Press, London. pp. 397-403.

Sano, T. 1992. Manual for Fish Virus Examination. FAO, Rome. (In Press).

Part II:

Contributed Papers -

Viral Diseases

Infection Route and Eradication of *Penaeus monodon* Baculovirus (MBV) in Larval Giant Tiger Prawns, *Penaeus monodon*

Chen, S.N., P.S. Chang and G.H. Kou
Department of Zoology, National Taiwan University
Taipei, Taiwan

Abstract

Our experiments showed that *Penaeus monodon* baculovirus (MBV) was transmitted orally. MBV may be eliminated from hatcheries by using MBV-negative broodstock or by washing nauplii or fertilized eggs several times in clean sea water, Formalin and iodophore.

Introduction

Of the six pathogenic viruses identified from penaeid shrimp, *Penaeus monodon* baculovirus (MBV) (Fig. 1), a nuclear polyhedrosis virus, is considered one of the most potentially serious pathogens to the larval stages of shrimp.

MBV has been found in shrimp from the Indo-Pacific and Pacific Coasts of Asia, Australia, Africa, and southern Europe and in North and South America (Anderson et al., 1987, Brock et al., 1983; Chen et al., 1989a; Lightner, 1983, 1988; Lightner et al., 1983, 1987). This virus infects a number of species of shrimp, including *P. vannamei, P. monodon, P. esculentus, P. semisulcatus, P. penicillatus, P. kerathurus, P. plebejus* and *Metapenaeus ensis* (Brock et al., 1983; Chen et al., 1989 a&b; Lester et al., 1987; Lightner and Redman, 1981; Lightner, 1983, 1988; Lightner et al., 1983, 1985, 1987, 1988). The most serious infections were found in cultured giant tiger shrimp, *P. monodon* (Chen et al., 1989c; Lightner, 1988).

Epizootiological studies on MBV in *P. monodon* revealed an incidence rate higher than 50% in postlarvae, juveniles and broodstock obtained from Taiwan (Chen et al., 1989c) and Southeastern Asia (Chen et al., 1990). Results obtained from a pathogenicity study showed that environmental stressors may significantly increase mortality in MBV- infected larvae in hatcheries (Chen et al., 1989c). A hatchery experiment also showed that MBV may initiate mortality and growth retardation in MBV-positive postlarvae

(Chen et al., 1989c; Chang and Chen, 1992). For this reason, MBV may result in variable larval production. To obtain a better quality of postlarval *P. monodon*, MBV should be eradicated from hatcheries.

The present paper attempts to describe the pathway of MBV infection. In addition, procedures for the eradication of MBV are also suggested.

Materials and Methods

To investigate the infection route of *Penaeus monodon*-type baculovirus (MBV) in *P. monodon*, nauplii and fertilized eggs derived from either MBV-positive or MBV-negative broodstock were used (Table 1). All experimental broodstock were collected from the open sea in southeastern Asia and imported into Taiwan.

For the broodstock, MBV infection was confirmed by the presence of occlusion bodies in shrimp feces. These were detected with the aid of 0.1% aqueous malachite green, 1% Gram's or Giemsa's staining solution and Olympus IM inverted and BH-2 light microscopes. Experimental larvae were maintained in hatchery ponds, with the exception of those used in Experiment 6. Each pond contained approximately 30 MT of 28- to 30-ppt sea water. Rearing temperatures ranged from 28 to 33°C.

Experiment 6 was conducted in plastic tanks. Each tank contained 5 - 10 MT of sea water and salinities and tempera-

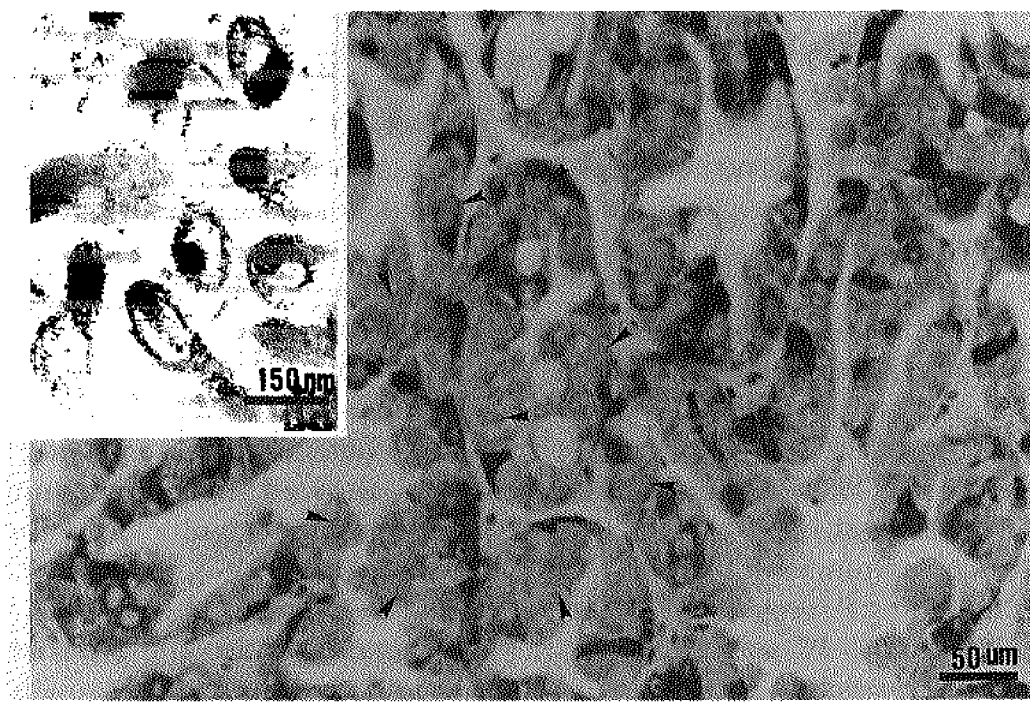


Figure 1a. Section of hepatopancreas of Penaeus monodon *infected by* Penaeus monodon *baculovirus (MBV). Note: Round occlusion bodies (arrows).*
Figure 1b. MBV particles.

Table 1. The incidence of MBV in larval *P. monodon* cultured under conditions indicated (in hatchery or nursery)*.

Exp. No.	Initial Stage	Water temp. range (°C)	Sample Source and Treatment[3]	MBV Infection Rate[2] (Positive/No. Examined)																					
				Z1	Z2	Z3	M1	M2	M3	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11**	P12	P15	P20	P30	P40
1a	N	28-30	MBV(-) broodstock	0/15	0/9	0/12	1/17	—	0/17	0/15	—	0/10	—	0/7	0/12	—	—	—	—	—	0/13	0/14	2/11	—	—
1b	N	28-33	MBV(+) broodstock	—	0/10	0/7	—	0/11	0/12	—	0/15	0/14	—	0/10	—	—	—	—	—	0/15	—	—	—	—	—
2a	N	28-30	MBV(-) broodstock +treatment	0/7	0/10	—	0/11	0/10	1/10	3/8	8/12	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2b	N	28-33		—	0/8	0/12	—	0/11	0/12	0/10	3/12	8/10	8/11	—	12/15	—	—	—	—	—	—	—	—	—	—	—
3a	N	28-30	MBV(+)broodstock +treatment	—	—	—	—	—	0/15	0/17	—	—	0/12	—	—	—	—	—	—	0/11	—	3/10	4/12	—	—
3b	N	28-30		—	—	—	—	—	0/15	0/17	—	—	0/12	—	—	—	—	—	—	0/11	—	3/10	4/12	—	—
3c	N	28-33		—	—	—	—	—	—	0/12	—	—	—	0/10	—	—	—	—	—	0/10	0/14	0/8	1/10	—	—
3d	N	28-33		—	—	—	—	—	—	0/14	—	—	—	0/15	—	—	—	—	—	0/12	0/9	1/11	3/11	—	—
3e[1]	N	28-33		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0/15	—	—	—	0/32	—
4a	N	28-30	MBV(+) broodstock + treatment	—	—	—	—	—	—	—	—	0/9	—	—	—	0/10	—	—	—	0/15	0/13	3/15	5/13	—	—
4b	N	28-30		—	—	—	—	—	—	—	—	0/17	—	—	—	1/13	—	—	—	0/17	2/12	3/15	5/13	—	—
4c	N	28-30		—	—	—	—	—	—	—	—	—	—	—	—	0/14	—	—	—	0/9	0/12	1/8	2/10	—	—
4d	N	28-33		—	—	—	—	—	—	—	—	—	—	0/8	—	—	—	—	—	0/12	1/8	2/10	—	—	—
4e	N	28-33		—	—	—	—	—	—	—	—	—	—	0/11	—	—	—	—	—	—	3/15	3/7	15/21	—	—
4f	N	28-33		—	—	—	—	—	—	—	—	—	—	0/16	—	—	—	—	—	0/17	0/10	1/10	3/13	—	—
4g	N	28-33		—	—	—	—	—	—	—	—	—	—	0/16	—	—	—	—	—	0/11	0/8	2/11	3/12	—	—
5a	N	28-30	MBV(-) broodstock +feces from broodstock	—	—	—	—	—	0/12	—	2/15	—	—	6/17	—	—	—	—	—	—	5/12	—	—	—	—
5b	N	28-33		—	—	—	0/10	—	0/9	—	1/11	—	—	5/13	—	—	—	—	—	—	7/14	—	—	—	—
6a[1]	E	28-30	MBV(+) broodstock+treatment	—	—	—	—	—	—	—	0/20	—	—	0/21	—	—	—	—	0/14	—	0/14	0/10	0/8	0/8	0/12
6b[1]	E	28-33	MBV(+) broodstock	—	—	—	—	—	—	—	0/12	—	—	0/12	—	—	—	—	0/5	—	—	0/7	—	0/6	0/10
6c[1]	E	28-33	MBV(+) broodstock	—	—	—	—	—	—	—	2/13	4/14	—	—	—	—	—	—	—	—	—	—	—	—	—
7	N	28-30	MBV(+) broodstock +washing with filtered sea water	—	—	—	—	—	—	—	—	—	1/20	0/20	—	—	—	—	—	—	—	—	—	—	—

*The experiments were performed in cement ponds in hatchery with approximately 30-40 MT of water, except those in plastic tanks.
**In every experiment, all the PL11 were moved to nursery ponds except those cultured in plastic tanks.
[1] The experiment was performed in a plastic tank with approximately 5-10 MT of water.
[2] The results were obtained from histopathological observations of hepatopancreata.
[3] The procedures for the treatment of nauplii and fertilized eggs are described in Table 3.

—: Not done
E: Fertilized egg stage N: Nauplius stage Z: Postzoeal stage M: Mysis stage P: Postlarval stage MBV(-): Free of MBV MBV(+): Positive for MBV

tures similar to those described above. Approximately 7,000 - 10,000 nauplii or fertilized eggs per ton were placed into each tank.

MBV infections in the hepatopancreata of developing stages of *P. monodon* larvae from zoea 1 to PL 40 were studied using tissue squashes and histopathological staining with 0.1% aqueous malachite green and hematoxylin and eosin (H&E), respectively, as described by Lightner (1983). For each stage, 5 - 32 randomly selected larvae were examined, and their MBV status was recorded.

To detect the mode of MBV transmission, nauplii derived from MBV-free broodstock were reared (in a similar tank) with the excrement from MBV-positive shrimp. The MBV status of these shrimp in the later stages was determined using the technique described above.

The eradication of MBV infection was achieved by the short-term washing of nauplii and fertilized eggs using filtered sea water with salinity of 28 - 30 ppt, 200 - 300 ppm Formalin and 20 - 30 ppm iodophore, as described in Figure 2.

During the course of larval rearing, aeration was continuous and the alga *Skeletonema costatum* was provided, as were artificial feeds (shrimp flake, plankton powder and micro-encapsulated feed, rotifers or brine shrimp larvae), to ensure a sufficient feed supply and complete growth of the experimental larvae. Feed debris and shrimp excrement were removed daily, and one-third of the sea water in each tank was exchanged at two-day intervals.

Results and Discussion

Pathway of MBV Infection

Penaeus monodon larvae produced from either MBV-positive or MBV-negative broodstock were examined histopathologically. The results in Table 1 and Figure 3 (Experiments 1 and 3) show that larvae produced from uninfected broodstock had no signs of infec-

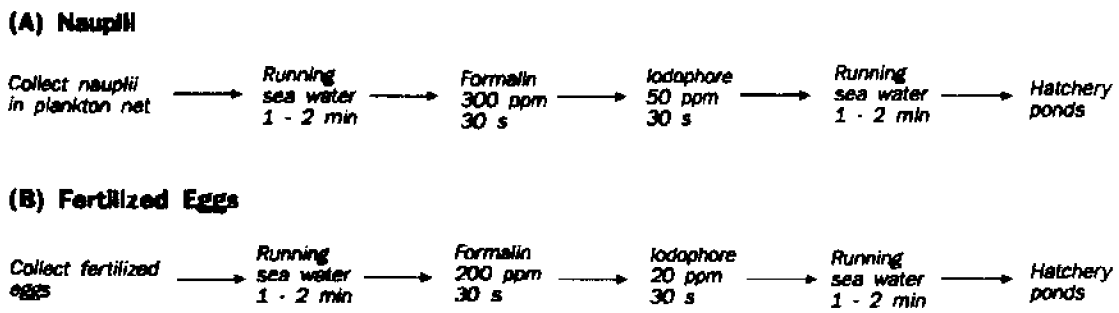


Figure 2. Procedures for the eradication of MBV in P. monodon *hatcheries. Note: In a hatchery, nauplii are much easier to collect than fertilized eggs. Furthermore, fertilized eggs are much more sensitive to Formalin than nauplii are.*

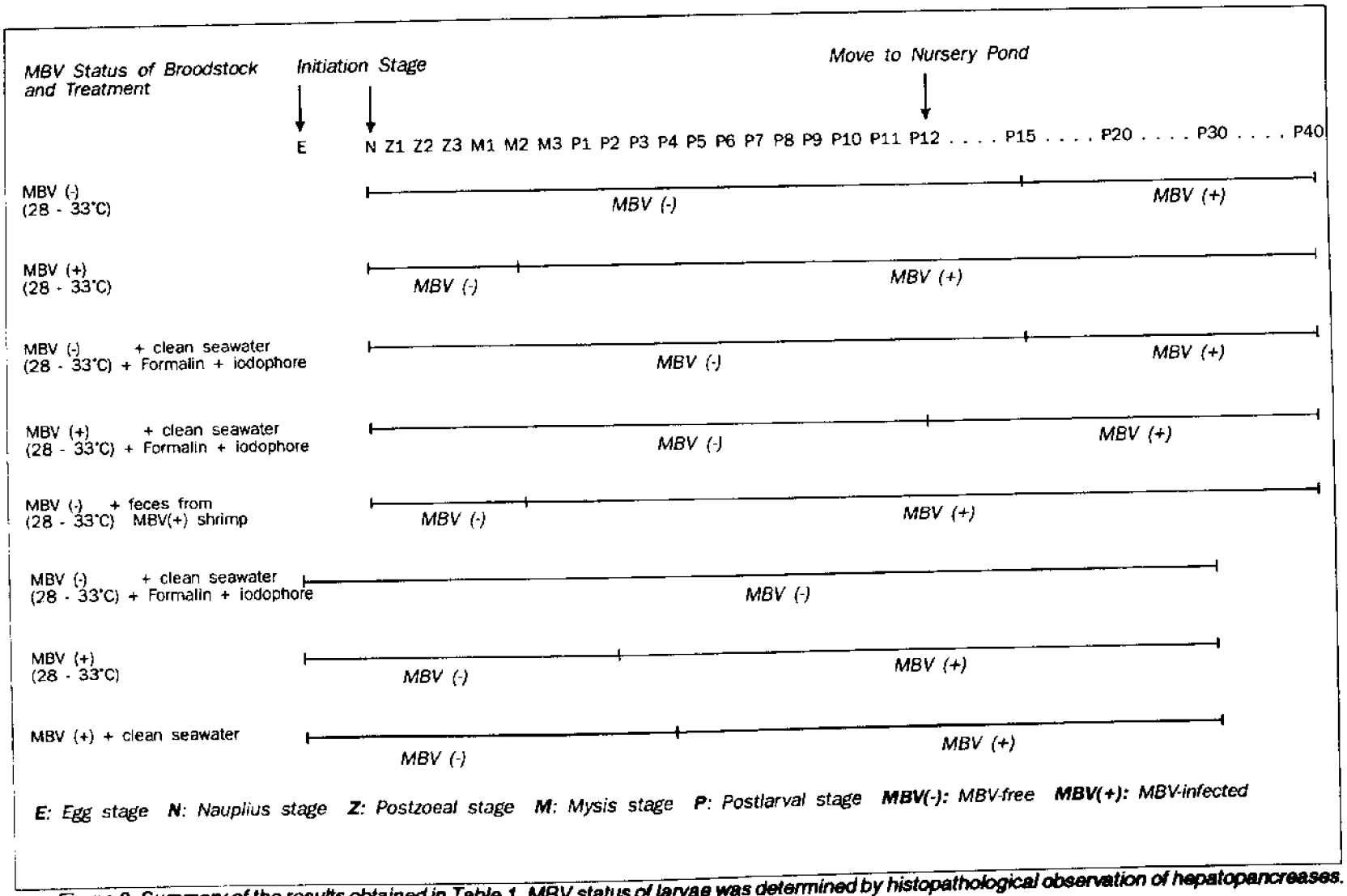


Figure 3. Summary of the results obtained in Table 1. MBV status of larvae was determined by histopathological observation of hepatopancreases. For broodstock, MBV status was determined by examination of feces.

tion while reared in the hatchery at 28 - 33°C. However, when these larvae were moved to a nursery pond, MBV-positive individuals were found (Table 1 and Fig. 3, Experiments 1 and 3). When nauplii or fertilized eggs derived from MBV-infected broodstock were reared in the hatchery, MBV-positive shrimp were discovered at the mysis or postlarval (PL) stage, respectively (Table 1 and Fig. 3, Experiment 2).

MBV-infected PL2 were also found when nauplii produced from MBV-positive broodstock were reared in plastic tanks (Table 1, Experiment 6c). However, larvae produced from MBV-free broodstock reared in plastic tanks containing filtered water revealed no sign of infection up to the PL40 stage (Experiments 6a and b).

When nauplii produced from MBV-free broodstock were exposed to MBV-positive feces, MBV was detected at the mysis stage (Table 1 and Fig. 3, Experiment 5).

Although no one has found evidence of vertical transmission of MBV in shrimp larvae, the present study shows that MBV may be transmitted by oral ingestion of occluded or free MBV virions. Baculoviruses were also found to be transmitted orally in *P. duorarum* (Couch, 1974) and *P. japonicus* (Momoyama, 1981; Momoyama and Sano, 1989).

The above experiments also suggest that oral ingestion of feces from MBV-positive shrimp is the main source of MBV infection. If this is true, eradication of MBV at the hatchery level may be feasible; hence, experiments were conducted to determine the best means of eliminating MBV from *P. monodon* hatcheries.

Eradication of MBV Infection

To eradicate MBV infection in larval shrimp, various washing procedures were tested. When nauplii and fertilized eggs obtained from MBV-infected broodstock were treated as described in Figure 2, no MBV-positive larvae were found in the hatchery pond (Table 1 and Fig. 3, Experiment 4). One day after the larvae were moved from the hatchery to the nursery pond, however, MBV was diagnosed in some of the larvae. In contrast, MBV was not detected in the nauplii derived from MBV-free broodstock.

These results also support the hypothesis that MBV was transmitted by oral ingestion of MBV virions. Furthermore, they reveal that in a controlled environment, MBV can be eliminated by washing nauplii or fertilized eggs thoroughly with filtered sea water, 200 - 300 ppm Formalin and 20 - 50 ppm iodophore.

Our results also showed that washing with filtered sea water alone may only reduce the rate of MBV infection rate in larval shrimp (Experiment 7). Since it is easier to collect nauplii than fertilized eggs, and because the latter are much more sensitive to the chemicals employed, commercial hatcheries should treat nauplii instead of fertilized eggs.

In conclusion, there are two ways to eliminate MBV from hatcheries, thereby significantly improving the quality of *P. monodon* larvae:

- Use only MBV-free broodstock, and
- Wash fertilized eggs or nauplii with filtered sea water, Formalin and iodophore.

Studies on baculoviral midgut gland necrosis (BMN) virus in *P. japonicus* also showed that viral infection may be eradicated by eliminating broodstock excrement followed by washing fertilized eggs with clean sea water (Momoyama, 1991).

Pathogenic studies showed that in *P. monodon*, MBV infection may initiate damage or loss of hepatopancreatic tubule and midgut tissue leading to dysfunction of these organs or tissues (Chen et al., 1989b; Lightner et al., 1983). Consequently, molting was delayed so that the MBV-infected postlarvae were irregular in body size (Chang et al., 1992). It was also noted that larval shrimp infected with MBV contain fewer hepatopancreatocytes than juvenile or adult shrimp; destruction of these cells may cause a serious disease or mortality. In comparison with control shrimp, MBV-infected larvae showed relatively high mortality rates (Chang et al., 1992; Chen et al., 1989c).

Our recent study also confirmed that juvenile and adult *P. monodon* are more resistant to MBV than larval shrimp (Chang, 1992). These results, when considered in light of the high incidence rate of MBV among cultured shrimp in the world (Chen et al., 1989c, 1990; Lightner et al., 1985) suggest that the eradication of MBV in hatchery-reared larvae and the production of MBV-free larvae are important. We have also reached the conclusion that to produce shrimp larvae of superior quality, a broodstock quarantine system for MBV infection should be established, and the development of eradication measures for MBV should be emphasized.

Acknowledgments

This work was supported by a grant from the U.S. Department of Agriculture (Grant No. FG-TA-111) and the Council of Agriculture and the National Science Council (Grant No. NSC-81-0209-B002-05) of the Republic of China.

Literature Cited

Anderson, I.G., M. Shariff, G. Nash and M. Nash. 1987. Mortalities of juvenile shrimp *Penaeus monodon*, associated with *Penaeus monodon* baculovirus, cytoplasmic reo-like virus and rickettsial and bacterial infections, from Malaysian brackishwater ponds. Asian Fisheries Science. 1:47-64.

Brock, J.A., D.V. Lightner and T.A. Bell. 1983. A review of four virus (BP, MBV, BMN and IHHN) diseases of penaeid shrimp with particular references to clinical significance, diagnosis and control in shrimp aquaculture. Proc. 71st Intl. Council for the Exploration of the Sea, C.M. 1983/Gen: 10/1-18.

Chang, P.S. 1992. Studies on the Biological and Biochemical Properties of *Penaeus monodon*-type baculovirus. Ph.D. Thesis, National Taiwan University, Taipei, Taiwan. 113 p.

Chang, P.S. and S.N. Chen. 1992. Effect of *Penaeus monodon*-type baculovirus (MBV) on

survival and growth of larval *Penaeus monodon*. Submitted for publication to Aquaculture.

Chen, S.N., P.S. Chang and G.H. Kou. 1990. Investigation of viral infection in cultured giant tiger prawn (*Penaeus monodon*) in Asia. *In*: G.H. Kou, H. Wakabayashi, I.C. Liao, S.N. Chen and C.F. Lo (Eds.). Proc. R.O.C.-Japan Symp. on Fish Diseases. National Science Council, Taipei, Taiwan. pp. 166-170.

Chen, S.N., P.S. Chang, G.H. Kou and D.V. Lightner. 1989b. Studies on virogenesis and cytopathology of *Penaeus monodon* baculovirus (MBV) in giant tiger prawn (*Penaeus monodon*) and the red tail prawn (*Penaeus penicillatus*). Fish Pathol. 24:89-100.

Chen, S.N., P.S. Chang and G.H. Kou. 1989c. Observation on pathogenicity and epizootiology of *Penaeus monodon* baculovirus (MBV) in cultured shrimps in Taiwan. Fish Pathol. 24: 189-195.

Chen, S.N., C.F. Lo, S.M. Liu and G.H. Kou. 1989a. The first identification of *Penaeus monodon* baculovirus (MBV) in cultured sand shrimp, *Metapenaeus ensis*. Bulletin of the European Association of Fish Pathologists. 9(3): 62-64.

Couch, J.A. 1974. An enzootic nuclear polyhedrosis virus of pink shrimp: ultrastructure, prevalence and enhancement. J. Invertebr. Pathol. 24: 311-331.

Lester, R.J.G., A. Doubrovsky, J.L. Paynter, S.K. Sambhi and J.G. Atherton. 1987. Light and electron microscope evidence of baculovirus infection in the prawn *Penaeus plebejus*. Dis. Aquat. Org. 3(2): 217-219.

Lightner, D.V. 1983. Diseases of cultured penaeid shrimp. *In*: J.P. McVey (Ed.). CRC Handbook of Mariculture. Vol. 1, Crustacean Aquaculture. CRC Press. Boca Raton, FL. pp. 289-320.

Lightner, D.V. 1988. Diseases of cultured penaeid shrimp and prawns. *In*: C.J. Sindermann and D.V. Lightner (Eds.). Disease Diagnosis and Control in North American Marine Aquaculture. Elsevier, Amsterdam. pp. 8-127.

Lightner, D.V., R.P. Hedrick, J.L. Fryer, S.N. Chen, I.C. Liao and G.H. Kou. 1987. A survey of cultured penaeid shrimp in Taiwan for viral and other important diseases. Fish Pathol. 22(3): 127-140.

Lightner, D.V. and R.M. Redman. 1981. A baculovirus-caused disease of the penaeid shrimp, *Penaeus monodon*. J. Invertebr. Pathol. 38: 299-302.

Lightner, D.V., R.M. Redman and T.A. Bell 1983. Observations on the geographic distribution, pathogenesis and morphology of the baculovirus from *Penaeus monodon* Fabricius. Aquaculture. 32(3/4): 209-233.

Lightner, D.V., R.M. Redman, R.R. Williams, L.L. Mohney, J.P.M. Clerx, T.A. Bell and J.A. Brock. 1985. Recent advances in penaeid virus disease investigations. J. World Maric. Soc. 16: 267-274.

Momoyama, K. 1981. Studies on infectious mid-gut gland necrosis of kuruma shrimp (*Penaeus japonicus* Bate)-Artificial infection. Bull. Yamaguchi Pref. Naikai Fish. Exp. St. 8: 1-11. (In Japanese).

Momoyama, K. 1991. Studies on baculoviral mid-gut gland necrosis (BMN) of kuruma shrimp, *Penaeus japonicus*. Bull. Yamaguchi Pref. Naikai Fish. Exper. St. 20: 1-91. (In Japanese).

Momoyama, K. and T. Sano. 1989.Developmental stages of kuruma shrimp, *Penaeus japonicus* Bate, susceptible to baculoviral mid-gut gland necrosis (BMN) virus. J. Fish Dis. 12: 585-589.

Viral Diseases of Cultured Penaeid Shrimp in Japan

Kazuo Momoyama
Yamaguchi Prefectural Naikai Fisheries Experiment Station
Yamaguchi 754, Japan

Abstract

About 900 million juveniles of approximately 20 crustacean species are produced for sea farming and pond culture every year in Japan. *Penaeus japonicus*, the kuruma shrimp, is the most important species, comprising approximately 90% of all juveniles and nearly 100% of pond cultured crustaceans produced annually. Two viral diseases, baculoviral midgut gland necrosis (BMN) of *P. japonicus* and *Penaeus monodon* baculovirus (MBV) infection of *P. monodon*, the grass shrimp, have been recorded from these species in Japan. BMN is a severe infectious disease causing high mortalities in hatcheries. MBV, by contrast, has been detected only once in postlarvae that were produced for experimental purposes from spawners imported from Taiwan. The research conducted in Japan on BMN and MBV is reviewed in this paper.

Introduction

Since the adoption of the 200-mile economic zone system in many nations, sea farming is now strongly encouraged to increase production of coastal fisheries resources in Japan. Every year about 700 million juvenile crustaceans belonging to approximately 20 species are produced at public sea farming centers for stocking into coastal waters (Japan Sea Farming Association, 1990). The main species produced are *Penaeus japonicus*, the kuruma shrimp (84%), *Portunus trituberculatus*, the blue crab (7%), and *Metapenaeus ensis*, the greasy-back shrimp (7%) (Table 1). Three other penaeid shrimp, *Penaeus semisulcatus*, the green tiger shrimp, *P. chinensis*, the fleshy shrimp and *P. latisulcatus*, the western king shrimp, are also produced for sea farming in certain regions.

Although juveniles of many crustacean species are produced for sea farming, *P. japonicus* is the only species reared to edible size in ponds in Japan. Since 1988, the number of seed *P. japonicus* imported from Taiwan for pond culture has increased substantially. Other penaeid shrimp, including non-native species, have never been cultured nor have they been imported, except for very rare experimental cases.

The annual production of pond-cultured *P. japonicus* from 1988 to 1990 was almost 3,000 MT, and about 200 million

Table 1. Crustacean species and number of juveniles produced for sea farming and pond culture in Japan in 1988.

Crustacean species	Sea farming		Pond culture	
	No. (million)	Percent	No. (million)	Percent
Shrimp				
Penaeus japonicus	557.1	83.8	194.2	99.9
Penaeus semisulcatus	9.4	1.4		
Penaeus chinensis	1.0	0.2		
Metapenaeus ensis	44.6	6.7		
Others (4 species)	0.3	0.0		
Crabs				
Portunus trituberculatus	48.5	7.3	0.2	0.1
Portunus pelagicus	2.3	0.3		
Others (6 species)	1.7	0.3		
Total	664.9	100	194.4	100

P. japonicus juveniles are produced annually at public and private hatcheries for pond culture.

Because it is the major crustacean species used for sea farming and pond culture in Japan, research on crustacean diseases has focused on *P. japonicus*. To date, two viruses have been recorded in crustaceans in Japan. One is baculoviral midgut gland necrosis virus (BMNV) in *P. japonicus* (Sano et al., 1981); the other is *Penaeus monodon* baculovirus (MBV) in *P. monodon* (Fukuda et al., 1988). For many years, BMNV caused serious losses during the production of juvenile *P. japonicus*.

MBV, first detected in Japan, is thought to have originated in a foreign country. After the initial discovery, MBV was never found again in Japan, probably because it is not infectious to postlarval *P. japonicus*, and very few *P. monodon* are cultured in Japan. One reason only two penaeid shrimp viruses have been found in Japan may be that Japan rarely imports live shrimp from foreign nations. Another factor is the lack of comprehensive viral disease surveys. The current practice of importing large numbers of seed *P. japonicus* without screening for viruses, however, will allow foreign viruses to spread easily in Japan. IHHN virus is the most dangerous of the potential viral introductions and also the most likely to be imported. It is highly pathogenic to *P. japonicus* (Lightner, 1985) and it exists in Taiwan (Lightner et al., 1987), from which many seed *P. japonicus* are imported.

Research on penaeid shrimp viruses in Japan has dealt almost entirely with BMN (Momoyama, 1991) — only one paper was published on MBV (Fukuda et al., 1988). This paper summarizes the results of the studies on BMN of *P. japonicus* and MBV infection of *P. monodon* in Japan.

Baculoviral Midgut Gland Necrosis (BMN)

Outbreaks and Mortalities

BMN was first noticed at a private hatchery in Yamaguchi Prefecture in 1971. In 1972, the disease affected most hatcheries, causing drastic mortalities in the district. Since then, BMN has repeatedly caused 90% or higher mortalities during the mass production of *P. japonicus* seed in Japan (Momoyama, 1981).

Recently, the frequency of BMN outbreaks has decreased significantly, probably as a result of preventive measures now employed at hatcheries (Momoyama, 1991). BMN is no longer a serious problem in the mass production of *P. japonicus* juveniles, although a few outbreaks are still reported every year.

Histopathology

Diseased shrimp lose their appetite, grow slowly, swim weakly, and develop soft and white turbid midguts at the advanced stage of infection. Shrimp dying of BMNV infection are usually less than 9.0 mm in body length (Momoyama, 1981).

Histological examinations confirmed that the midgut and the intestine are the target organs. Disarrangement and exfoliation of epithelial cells are remarkable in the midguts of diseased shrimp. Nuclear hypertrophy and chromatolysis of infected epithelial cells are the most characteristic cytopathological changes of BMN (Momoyama, 1981; Sano et al., 1981). Occlusion bodies are not formed in the hypertrophied nuclei, differentiating BMNV from other penaeid shrimp baculoviruses (Couch, 1974; Lightner, 1985; Lester et al., 1987; Johnson and Lightner, 1988).

Electron micrographs of the hypertrophied nuclei and the midgut lumen reveal numerous rod-shaped particles having outer and inner envelopes; these are baculovirus virions. The average length and diameter of the virions is 310 nm and 72 nm, respectively (Sano et al., 1981; Sano et al., 1984).

Vibrios often invade and grow in the midgut lumen of moribund shrimp (Momoyama, 1981). They must play an important role in killing BMNV-infected shrimp, but numerous hatchery trials with antibiotics were unsuccessful.

Diagnosis

Three diagnostic techniques have been developed for BMN. **Squash and stained preparation diagnosis** is used to detect the homogeneous hypertrophied nuclei in squashed and stained preparations of the affected midgut. The Feulgen reaction makes the difference between normal nuclei (about 10 μm in diameter) and infected hypertrophied nuclei (about 20 to 30 μm in diameter) clearer (Momoyama, 1983).

Dark field microscopic diagnosis is used to detect infected hypertrophied nuclei

in fresh squash preparations using a dark field microscope with a wet-type condenser. Infected nuclei are clearly seen in white against the dark background due to the increased reflected and diffracted rays produced by the numerous virus particles in the nuclei (Momoyama, 1983). Because this method has the advantages of simplicity, rapidity, precision and low cost, it is the only diagnostic method used in shrimp hatcheries.

Fluorescent antibody diagnosis is used to detect BMN-specific virus antigen in smears or sectioned preparations of the midgut. Sano et al. (1985) demonstrated BMNV infection in postlarvae 18 h after inoculation by detecting fluorescence in the nuclei of the midgut epithelial cells. The method was also used to demonstrate the presence of BMNV in the midguts of spawners latently infected with the virus (Momoyama, 1988).

Source of Infection

Epizootiological investigations have indicated that spawners with latent BMNV infections may be the main source of infection in hatchery epizootics. Histological examinations revealed nuclear hypertrophy of the midgut epithelial cells in three out of 70 spawners examined. Fluorescent antibody techniques revealed the presence of BMN-specific virus antigen in the hypertrophied nuclei of the spawners (Momoyama, 1988).

Furthermore, histological examination of the midguts of young *P. japonicus* that had recovered from BMN and were then cultured at a farm showed a high rate (31.4%) of BMNV infection (Momoyama, 1988). Regular or frequent BMN outbreaks on farms where BMN survivors are cultured suggest that these shrimp are a source of infection to larvae.

A Method of Experimental Infection

Since there is no cell line available for penaeid shrimp virus culture, a reliable method to experimentally infect larval *P. japonicus* with BMNV has been developed to facilitate studies on BMN. Water-borne inoculation using the filtrate of diseased postlarvae stored at -80°C is one means of infection. The virulence of material frozen at -80°C persists at almost the same level for at least seven years (Momoyama, 1989a). Demonstration of infection in test animals is accomplished by dark field microscopy four days postinoculation (Table 2) (Momoyama and Sano, 1988).

Susceptible Stages

The relationship between age and susceptibility to BMNV was studied using the infection method described above.

Fertilized eggs and nauplii were refractory to the virus, showing no evidence of infection on the final day of the infection challenge. The zoea, mysis larvae, PL2 (two day-old postlarvae) and PL4 were "highly susceptible" to infection with BMNV, exhibiting higher mortality and lower growth rates compared to control shrimp. PL6 were classified as "susceptible;" they grew only

Table 2. A method for experimentally infecting larval *P. japonicus* with BMNV.

Inoculum: 450 nm filtrate of BMNV-infected postlarvae (stored at -80°C)
Test shrimp: Mysis larvae (zoea to PL4 are also available)
Inoculation: Water-borne for two hours
Test period: Four days
Demonstration of infection: Dark field microscopic diagnosis
Rearing water temperature: 25 - 30°C
Food: Brine shrimp nauplii

slightly slower than controls. PL8 and PL10, by contrast, were refractory, exhibiting no mortality and no loss of growth, although some animals developed slight infections (Sano et al., 1985; Momoyama and Sano, 1989).

The route of infection with baculoviruses in shrimp is considered to be by oral ingestion of virus-contaminated sediments or by cannibalism of diseased shrimp (Couch, 1978; Lightner et al., 1983). Overstreet et al. (1988) established experimental infections in larval and postlarval *P. vannamei*, the whiteleg shrimp, with *Baculovirus penaei* (BP) by feeding virus-laden rotifers or brine shrimp. In the infection trials using *P. japonicus* and BMNV, food was not added to the water thus, the animals could not feed during the inoculation period. However, peristaltic movements, which are frequently observed in the esophageal region of shrimp, suggest the intake of sea water containing BMNV particles through the mouth. This hypothesis is supported by the observation that azocarmine G accumulated in the stomach and intestinal lumen of shrimp placed in sea water containing this dye (Momoyama and Sano, 1989). If the concentration of the virus in the water is high enough, virus-laden food may not be necessary to establish baculoviral infections.

Infection Cycle of BMNV

Based on the results obtained in the epizootiological and water-borne susceptibility studies, the following infection cycle was proposed (Fig. 1) (Momoyama, 1991). Some survivors recover from BMNV disease and reach maturity without entirely eliminating the virus from the body (A). BMNV grows in the nuclei of the midgut epithelial cells of these broodstock (B). Then the virus particles are excreted into the environmental water with feces and collapsing cells after being released into the lumen of the midgut tubules from the necrotic epithelial cells (C). Shrimp older than nauplius become infected with the virus by orally ingesting it (D). If the shrimp are between the zoea and PL6 stages (E), even if a few shrimp live normally by defeating the virus attack (F), most shrimp become diseased (G) and some will die (H). If the shrimp are older than PL6 (I), most shrimp live normally with a slight infection (J). Some of the recovered shrimp (K) as well as the slightly infected shrimp (F,

J) grow up to be the source of the next infection (L), completing the cycle (M). Some instances of BMN outbreaks were caused by contamination from other rearing tanks in the hatchery (N).

Inactivation and Survival of BMNV

Inactivation of BMNV by chemical and physical factors, and survival time of the virus in sea water at different temperatures were examined by means of water-borne infectivity experiments. BMNV was inactivated by 10-min exposure at 25°C to any of the following disinfectants: 5-ppm chlorine; 25-ppm iodine; 100-ppm benzalkonium chloride and benzethonium chloride; 30% ethyl alcohol; and 0.5% formalin (Momoyama, 1989b). The virus was also inactivated with the following chemical treatments: ethyl ether for 18 h at 4°C; NaCl, 25% solution within 10 h and 12.5% within 24 h; pH 1.0 within 10 min, pH 1.5 and 2.0 within 30 min, pH 2.5 within 60 min, and pH 3.0 and 4.0 within 180 min (Momoyama, 1989c).

With regards to physical factors, BMNV was inactivated by: ultraviolet irradiation of 4.1×10^5 μW x s/cm^2; summer sunlight exposure for 3 h; heating at 45°C within 120 min, 50 and 55°C within 30 min, and 60°C within 5 min (Momoyama, 1989d). In sea water, BMNV could not survive longer than 4 d at 30°C, 7 d at 25°C, 12 d at 20°C, and 20 d at 15°C (Momoyama, 1989a).

BMNV appears to be much more sensitive to chemicals and physical stresses than insect baculoviruses (Aruga, 1979),

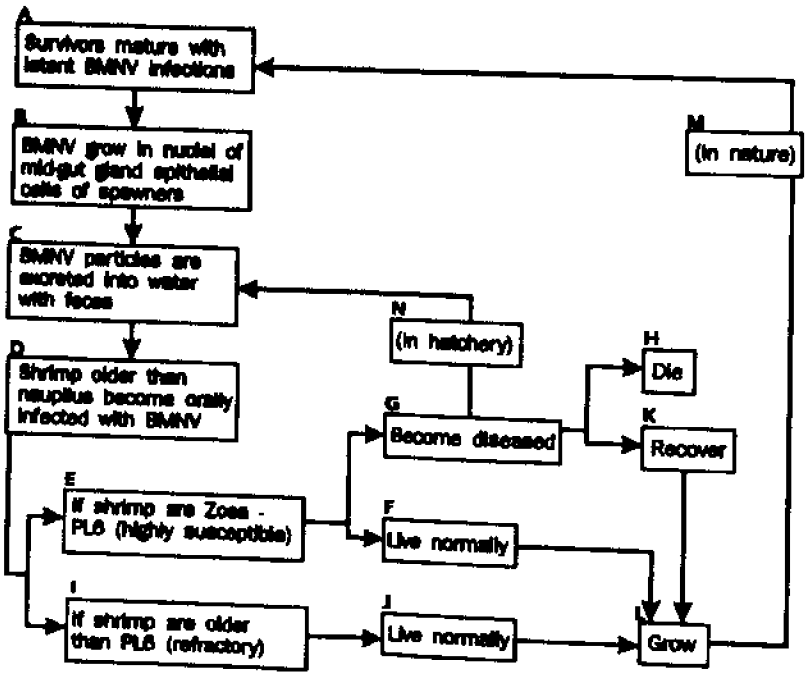


Figure 1. Infection cycle of BMNV.

probably because BMNV lacks occlusion bodies. In addition, BMNV presumably evolved in the marine environment, which is more constant than conditions on land.

Prevention

Referring to the proposed infection cycle for BMN, two countermeasures are used against BMN epizootics in Japan. One prevents transmission via broodstock feces by rinsing the fertilized eggs with virus-free sea water and transferring them to a disinfected rearing tank. The other prevents infection from previous batches of shrimp by addition of 20-ppm chlorine to the rearing tank to disinfect the water, etc. and to kill infected populations of shrimp. Since 1985, egg rinsing has been conducted on an industrial scale, and BMN has never occurred in hatcheries where this precaution is taken (Momoyama, 1991).

MBV Infection of *P. monodon*

Detection of MBV In Japan

In 1983, a *P. monodon* seed production experiment was conducted at a private hatchery in Yamaguchi Prefecture using broodstock imported from Taiwan. The resulting postlarvae appeared healthy and exhibited no external clinical signs. However, light and electron microscopy revealed the presence of MBV in the midgut epithelial cells of the postlarvae. Because of the prevalence of MBV in Taiwan (Lightner et al., 1987) and the scarcity of *P. monodon* along the coast of Japan (Hayashi, 1981), Fukuda et al. (1988) concluded that the virus had been introduced from Taiwan with the spawners.

Pathogenicity of MBV to Postlarval *P. japonicus*

The pathogenicity of MBV to *P. japonicus* PL1 was examined by water-borne and oral inoculation infectivity trials. Since postlarvae did not show any evidence of infection such as 1) the formation of nuclear occlusion bodies in the midgut epithelial cells, 2) lack of growth, or 3) significant mortality by either inoculation method, *P. japonicus* was judged to be refractory to MBV (Fukuda et al., 1988).

Literature Cited

Aruga, H. 1979. General Insect Pathology. Yokendo, Tokyo. pp. 648. (In Japanese).

Couch, J. A. 1974. An Enzootic Nuclear Polyhedrosis Virus of pink shrimp: ultrastructure, prevalence and enhancement. J. Invertebr. Pathol. 24: 311-331.

Couch, J.A. 1978. Diseases, parasites and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic coasts of North America. Fishery Bull. 76: 1-44.

Fukuda, H., K. Momoyama and T. Sano. 1988. First detection of monodon baculovirus in Japan. Nippon Suisan Gakkaishi. 54: 45-48. (In Japanese with English abstract).

Hayashi, K. 1981. Prawns, shrimps and lobsters from Japan (2), Family Penaeidae-Genus *Penaeus* 2. Aquabiology. 3: 452-455. (In Japanese).

Japan Sea Farming Association. 1990. Annual Seed Production and Stocking Record For Sea Farming 1988. 421. p. (In Japanese).

Johnson, P.T. and D.V. Lightner. 1988. Rod-shaped nuclear viruses of crustaceans: gut-infecting species. Dis. Aquat. Org. 5: 123-141.

Lester, R.G., A. Doubrovsky, J.L. Paynter, S.K. Sambhi and J.G. Atherton. 1987. Light and electron microscope evidence of baculovirus infection in the prawn, *Penaeus plebejus*. Dis. Aquat. Org. 3: 217-219.

Lightner, D.V. 1985. A review of the diseases of cultured penaeid shrimps and prawns with emphasis on recent discoveries and developments. *In*: Y. Taki, J.H. Primavera and J.A. Llobrera (Eds.). Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps. Iloilo City, Philippines, 4-7 December, 1984. pp. 79-103.

Lightner, D.V., R.P. Hedrick, J. L. Fryer, S.N. Chen, I.C. Liao and G.H. Kou. 1987. A survey of cultured penaeid shrimp in Taiwan for viral and other important diseases. Fish Pathol. 22: 127-140.

Lightner, D.V., R.M. Redman and T.A. Bell. 1983. Observations on the geographic distribution, pathogenesis and morphology of the baculovirus from *Penaeus monodon* Fabricius. Aquaculture. 32: 209-233.

Momoyama, K. 1981. Studies on infectious mid-gut gland necrosis of kuruma shrimp (*Penaeus japonicus*)-I, Occurrences and symptoms. Bull. Yamaguchi Pref. Naikai Fish. Exper. St. 8: 1-11. (In Japanese).

Momoyama, K. 1983. Studies on baculoviral mid-gut gland necrosis of kuruma shrimp (*Penaeus japonicus*)-III, Presumptive diagnostic techniques. Fish Pathol. 17: 263-268. (In Japanese with English abstract).

Momoyama, K. 1988. Infection source of baculoviral mid-gut gland necrosis in mass production of kuruma shrimp larvae, *Penaeus japonicus*. Fish Pathol. 23: 105-110. (In Japanese with English abstract).

Momoyama, K. 1989a. Survival of baculoviral mid-gut gland necrosis virus (BMNV) in infected tissues and in sea water. Fish Pathol. 24: 179-181. (In Japanese with English abstract).

Momoyama, K. 1989b. Virucidal effect of some disinfectants on baculoviral mid-gut gland necrosis (BMN) virus. Fish Pathol. 24: 47-49. (In Japanese with English abstract).

Momoyama, K. 1989c. Tolerance of baculoviral mid-gut gland necrosis virus (BMNV) to ether, NaCl concentration and pH. Fish Pathol. 24: 175-177. (In Japanese with English abstract).

Momoyama, K. 1989d. Inactivation of baculoviral mid-gut gland necrosis (BMN) virus by ultraviolet irradiation, sunlight exposure, heating and drying. Fish Pathol. 24: 115-118. (In Japanese with English abstract).

Momoyama, K. 1991. Studies on Baculoviral mid-gut gland necrosis (BMN) of kuruma shrimp, *Penaeus japonicus*. Bull. Yamaguchi Pref. Naikai Fish. Exper. St. 20: 1-91. (In Japanese).

Momoyama, K. and T. Sano. 1988. A method of experimental infection of kuruma shrimp larvae, *Penaeus japonicus* Bate, with baculoviral mid-gut gland necrosis (BMN) virus. J. Fish Dis. 11: 105-111.

Momoyama, K. and T. Sano. 1989. Developmental stages of kuruma shrimp, *Penaeus japonicus* Bate, susceptible to baculoviral mid-gut gland necrosis (BMN) virus. J. Fish Dis. 12: 585-589.

Overstreet, R.M., K.C. Stuck, R.A. Krol and W.E. Hawkins. 1988. Experimental infections with *Baculovirus penaei* in the white shrimp *Penaeus vannamei* (Crustacea: Decapoda) as a bioassay. J. World Aquaculture Soc. 19: 175-187.

Sano, T., T. Nishimura, H. Fukuda, T. Hayashida and K. Momoyama. 1984. Baculoviral mid-gut gland necrosis (BMN) of kuruma shrimp (*Penaeus japonicus*) larvae in Japanese intensive culture systems. Helgoländer Meersunters. 37: 255-264.

Sano, T., T. Nishimura, H. Fukuda, T. Hayashida and K. Momoyama. 1985. Baculoviral infectivity trials on kuruma shrimp larvae, *Penaeus japonicus*, of different ages. *In*: A.E. Ellis (Ed.). Fish and Shellfish Pathology. Academic Press, London. pp. 397-403.

Sano, T., T. Nishimura, K. Oguma, K. Momoyama and N. Takeno. 1981. Baculoviral infection of kuruma shrimp, *Penaeus japonicus* in Japan. Fish Pathol. 15:185-191.

Part II:

Contributed Papers -

Bacterial Diseases

Studies on the Epizootiology and Pathogenicity of Bacterial Infections in Cultured Giant Tiger Prawns, *Penaeus monodon*, in Taiwan

S.N. Chen, S.L. Huang and G.H. Kou
Department of Zoology, National Taiwan University
Taipei, Taiwan

Abstract

Studies on the epizootiology of bacterial infections in hepatopancreata of cultured giant tiger prawns, *Penaeus monodon*, in Taiwan during 1988-1990 showed that out of 357 and 274 bacterial isolates, 302 (84.6%) and 201 (73.4%), respectively, belonged to the genus *Vibrio*. *Vibrio harveyi*, *V. damsela*, *V. nereis*, *V. vulnificus* and *V. anguillarum* were most abundant in the hepatopancreata investigated.

Intramuscular or hemocoel inoculation of *V. harveyi* and *V. nereis* at concentrations ranging from 1.3×10^4 - 5.6×10^4 colony forming units (C.F.U.)/ml initiated mortality in experimental shrimp. Following inoculation of *V. harveyi*, histopathological changes were observed in the gills, lymphoid organ tissue, gut, heart, muscle and hepatopancreata.

Introduction

In the past decade, shrimp culture has become one of the most important worldwide agricultural industries. Recently, however, the intensive culture of penaeids has suffered catastrophic extinction in locations such as Taiwan and central Thailand as cultured shrimp are threatened by either serious diseases or mass mortality. Following integrative studies (Liao et al., 1989), it was found that "unsuitable environmental stress," "aging pond bottom," "incorrect management" and "inferior quality shrimp fry" can prompt disease-related shrimp mortalities. The above mentioned factors may weaken shrimp, allow invasion of opportunistic pathogens, and ultimately cause mass mortality.

Bacteria may play a major role in disease outbreaks of cultured giant tiger prawns, *Penaeus monodon* (Cheng, 1989; Huang, 1989). However, very little is known about the epizootiology and pathogenicity of bacterial diseases in cultured shrimp.

Song et al. (1990) reported that *Vibrio vulnificus* may initiate a pathogenic ef-

fect in *P. monodon*. Furthermore, investigation of the bacterial diseases of *P. aztecus* and *P. setiferus* revealed that *Vibrio, Aeromonas, Spirillum* and *Flavobacterium* are possible pathogens (Glen et al., 1980).

The present study attempts to identify bacteria isolated from hepatopancreata of diseased *P. monodon* cultured in Taiwan from 1988 to 1990. The pathogenicity of experimental infections of dominant species of bacteria is determined, and histopathological changes in the target tissues are also described.

Epizootiology

Methods

The investigation of pathogenic bacteria believed to be involved in mass mortalities was performed in Taiwan from 1988 to 1990. Giant tiger prawns, *P. monodon*, in several major culture areas in southern, middle, northern and eastern Taiwan were investigated. In each area, morbid shrimp were collected and bacteria were isolated from hepatopancreata. Prior to bacterial isolation, the shrimp were immersed in 70% alcohol for two to three minutes, then gills and opercula were removed, and hepatopancreata were exposed using sterile scissors. The bacteria were then isolated using TSA medium plus 1% NaCl. Subsequently, the shrimp were fixed in Davidson's fixative for 12 - 24 h and transferred to 70% alcohol for permanent preservation according to the procedures described by Lightner (1983).

The bacteria and hepatopancreata were brought back to the laboratory for species identification and histopathological observations. All bacteria were identified according to the procedures described in Bergy's Manual of Systematic Bacteriology (Baumann and Schubert, 1984; Brenner, 1984; Mannheim, 1984; Krieg et al., 1984) and the Colour Atlas and Textbook of Diagnostic Microbiology (Koneman et al., 1983). Most bacteria were *Vibrio* sp.; therefore, techniques described by Pacha and Kiehn (1969), Baumann et al. (1971, 1972, 1980, 1981) and Lee et al. (1981) were employed in conjunction with those in Bergy's Manual to classify *Vibrio* species.

Results

Only 19 (5.3%) of the bacterial isolates collected in 1988 were coccus (Table 1). Of 338 rod-shaped isolates, 302 were demonstrated to be *Vibrio*. One hundred thirty (43.1%) and 50 (16.6%) of the 302 strains were *V. harveyi* and *V. damsela*, respectively (Table 2). However, only 9.6% (29 isolates), 5.6% (17 isolates) and 5.6% (17 isolates) were *V. nereis, V. anguillarum* or *V. tubiashii*, respectively (Table 2). The remaining species (Table 2) were rare and considered to be less important contributors to the mass mortality.

Studies performed from 1989 to 1990 also showed that *Vibrio* spp. were the major bacteria isolated from the hepatopancreata of morbid *Penaeus monodon*

Table 1. Isolation of bacteria from hepatopancreata of morbid *Penaeus monodon* during 1988-1990.

Bacteria	1988		1989-1990	
	Total	%	Total	%
C (+) coccus	16	4.5	10	3.6
C (-) coccus	3	0.8	8	2.9
C (-) rods				
Nonfermentative	26	7.3	18	6.6
Enterobacter	6	1.7	12	4.4
Aeromonas	4	1.1	25	9.1
Vibrio	302	84.6	201	73.4
Total	357	100.0	274	100.0

in Taiwan. Out of 274 isolates, 201 were vibrios (Table 1). *Vibrio harveyi, V. damsela, V. nereis, V. vulnificus, V. tubiashii, V. anguillarum* and *V. parahaemolyticus* were most abundant in the hepatopancreata of cultured *P. monodon*.

Pathogenicity Study

Methods

Because *V. harveyi* and *V. nereis* were among the most abundant bacterial species, they were suspected of being associated with disease outbreaks. Therefore, these two bacteria were used in a pathogenicity study using artificial inoculation techniques. Immersion and inoculation techniques were employed to induce disease in experimental shrimp.

For the immersion experiment, shrimp weighing 10 g each were immersed in 2.8×10^7 colony forming units (C.F.U.)/ml of *V. harveyi* solution for 3 min. Ten similar-sized shrimp were immersed in 0.85% NaCl for 3 min as a control.

For the inoculation experiments, 0.02 ml of *V. harveyi* or *V. nereis* (concentration 2.8×10^6 or 1.3×10^6 C.F.U./ml, respectively) was injected with a G26 needle intramuscularly via the junction between the first and second abdominal segments. Similarly, 0.01 ml of each bacterial solution was injected into the hemocoel of experimental shrimp at the abdominal surface of the fifth segment. Forty shrimp were used in this study; there were ten shrimp in each group. Additionally, two groups of ten shrimp inoculated with 0.02 ml of 0.85% NaCl via muscle or hemocoel, were used as controls.

Results

Significant mortality was obtained in the experimental groups (Table 3). *Vibrio harveyi* or *V. nereis* injected at a concentration of 1.3×10^6 C.F.U./ml or more initiated significant mortality in experimental shrimp within four days after intramuscular or hemocoel injection. Cumulative mortality after hemocoel and intramuscular injection of *V. harveyi* is presented in Tables 4 and 5.

Table 2. Isolation of *Vibrio* sp. from hepatopancreata of morbid *Penaeus monodon* during 1988-1990.

Bacteria	1988		1989-1990	
	No.	%	No.	%
Vibrio anguillarum	17	5.6	8	4.0
V. cholerae	9	3.0	1	0.5
V. damsela	50	16.6	45	22.4
V. fluvialis	4	1.3	2	1.0
V. gazogenes	1	0.3	0	0.0
V. harveyi	130	43.1	54	26.9
V. logei	4	1.3	1	0.5
V. natriegens	10	3.3	2	1.0
V. nereis	29	9.6	25	12.4
V. ordalii	6	2.0	6	3.0
V. parahaemolyticus	0	0.0	12	6.0
V. pelagius (I)	2	0.7	1	0.5
V. pelagius (II)	1	0.3	1	0.5
V. splendidus (I)	3	1.0	2	1.0
V. splendidus (II)	1	0.3	1	0.5
V. tubiashii	21	7.0	14	7.0
V. vulnificus	4	1.3	17	8.4
V. sp.	10	3.3	9	4.5
Total	302	100.0	201	100.0

Table 3. Mortality in experimental and control groups after hemocoel and muscle inoculation of *Vibrio harveyi* (Strain No. 770713 A_3b) and *V. nereis* (Strain No. 770713 D_4b)[1]

Strain	Pathway (C.F.U./ml)	No. dead shrimp				Mortality (%)
		1 day	2 days	3 days	4 days	
770713 A_3b *V. harveyi*	hemocoel (2.8×10^6)	6	1	1	2	100
	muscle (5.6×10^6)	3	7	0	0	100
770713 D_4b *V. nereis*	hemocoel (1.3×10^6)	0	1	1	3	50
	muscle (2.6×10^6)	0	0	3	0	30
Control	hemocoel (saline)	0	0	0	0	0
	muscle (TSB)	0	0	0	0	0

[1]Shrimp weighed approximately 10 g. Those in experimental groups were inoculated with 0.02 ml of bacteria at the concentration indicated.

Table 4. Mortality of experimental *Penaeus monodon* after hemocoel inoculation of various dosages of *Vibrio harveyi*[1].

Dose (C.F.U./ml)	Mortality							Total
	1 day	2 days	3 days	4 days	5 days	10 days	21 days	
2.5×10^8	10							10
2.5×10^7	2	2	1	0	3	0	2	10
2.5×10^6	0	0	0	1	0	3	3	7
2.5×10^5	0	0	0	0	0	2	1	3
2.5×10^4	0	0	0	0	0	2	1	3
2.5×10^3	0	0	0	1	0	0	1	2
2.5×10^2	0	0	0	0	0	0	1	1
Control	0	0	0	0	0	0	0	0

[1]Shrimp weighed approximately 10 g. Those in experimental groups were inoculated with 0.01 ml of bacteria at the concentration indicated.

Table 5. Mortality of experimental *Penaeus monodon* after intramuscular injection of various concentrations of *Vibrio harveyi*[1].

Dose (C.F.U./ml)	Mortality							Total
	1 day	2 days	3 days	4 days	5 days	10 days	21 days	
3.8×10^8	10							10
3.8×10^7	3	2	2	2	1			10
3.8×10^6	1	0	0	1	0	2	1	5
3.8×10^5	0	1	1	0	0	0	3	5
3.8×10^4	0	0	0	0	0	2	0	2
3.8×10^3	0	0	1	0	0	1	0	2
3.8×10^2	0	0	0	0	0	1	1	2
Control	0	0	0	0	0	1[2]	0	1

[1]Shrimp weighed approximately 10 g. Those in experimental groups were inoculated with 0.01 ml of bacteria at the concentration indicated.

[2]Mortality due to molting.

Mortality also resulted when *V. harveyi* was inoculated at a concentration of 2.5×10^6 C.F.U./ml, and 100% mortality was obtained 24 h after intramuscular injection of *V. harveyi* at concentrations of 0.028×10^8 and 0.025×10^8 C.F.U./ml.

Ten days after immersion in 0.8×10^7 C.F.U./ml of *V. harveyi*, 50% mortality was observed. In contrast, no mortality was seen in the control group.

Histopathological Changes In Infected Shrimp

To investigate the histopathological changes initiated by experimental infection of *V. harveyi*, morbid experimental

shrimp were fixed in Davidson's fixative as described by Bell and Lightner (1988). *Vibrio harveyi* was recovered from all experimental shrimp; the histopathological changes were as follows:

Gills: Histopathological changes in gills were observed seven days after intramuscular or hemocoel inoculation of *V. harveyi*. However, significant gill abnormalities were observed among the shrimp immersed in *V. harveyi* suspension for four days.

The main pathological changes in the gills were swelling of secondary gill filament (Fig. 1) with an increase in the number of densely stained granules (Fig. 2). The invasion of bacteria was observed inside the secondary gill filament, which may initiate necrosis of the tissue (Fig. 3). Abnormal hemocytes and eosingranulocytes and damaged lymphoid tissue in the base of secondary gill filaments are common syndromes resulting from bacterial invasion of shrimp gill tissue.

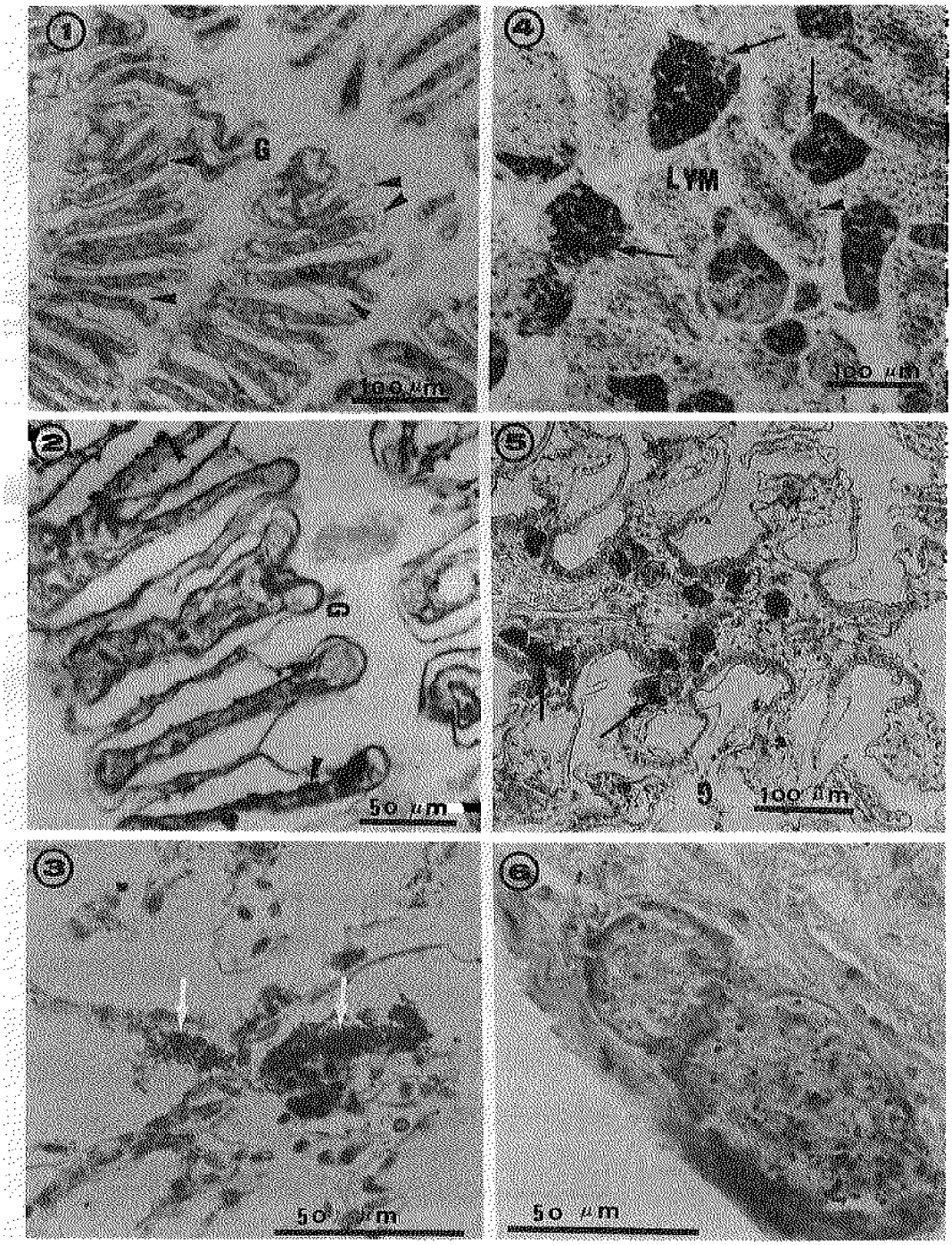
Lymphoid organ: Seven days after bacterial infection, either the Oka organ (Fig. 4) or lymphoid tissues in the gills (Fig. 5), submuscular layer of the stomach (Fig. 6), or subcuticular structures were seriously affected. Necrotic lymphoid nodules with mass aggregations of bacteria were found in the infected tissues of morbid shrimp (Figs. 4-6).

Stomach and midgut: Four days after inoculation of bacteria, the stomach and midgut exhibited signs of disease. Large numbers of densely stained hemocytes and bacteria were found in the spongy connective tissue of the stomach (Figs. 7-8).

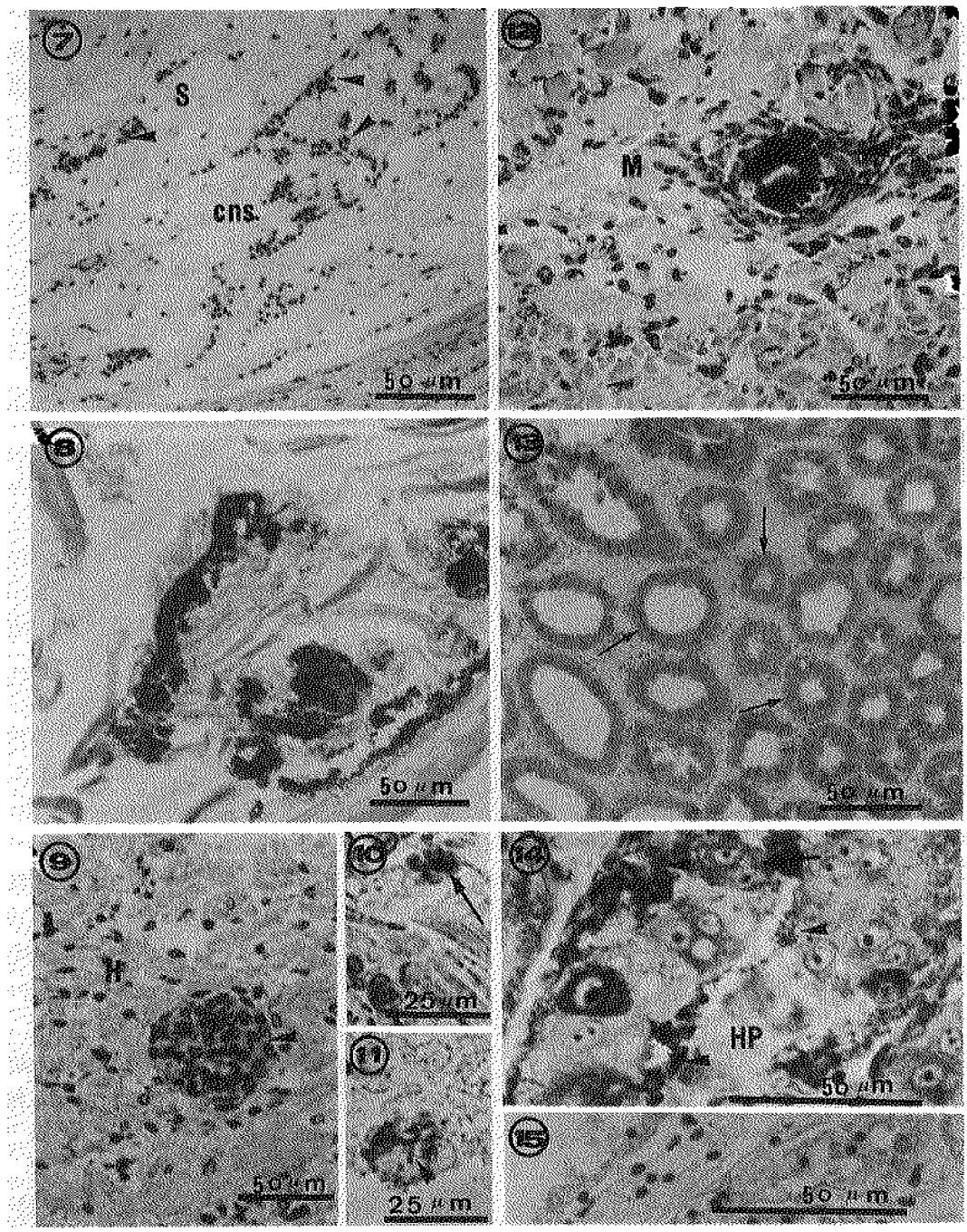
Heart: Histopathological changes in the heart tissue of infected shrimp were only observed in a few cases. Densely stained hemocytes, macrohemocytes and bacteria were found in the abnormal hearts (Figs. 9-11).

Muscle: Muscle necrosis and hemocyte encapsulated nodules were found at the injection sites (Fig. 12).

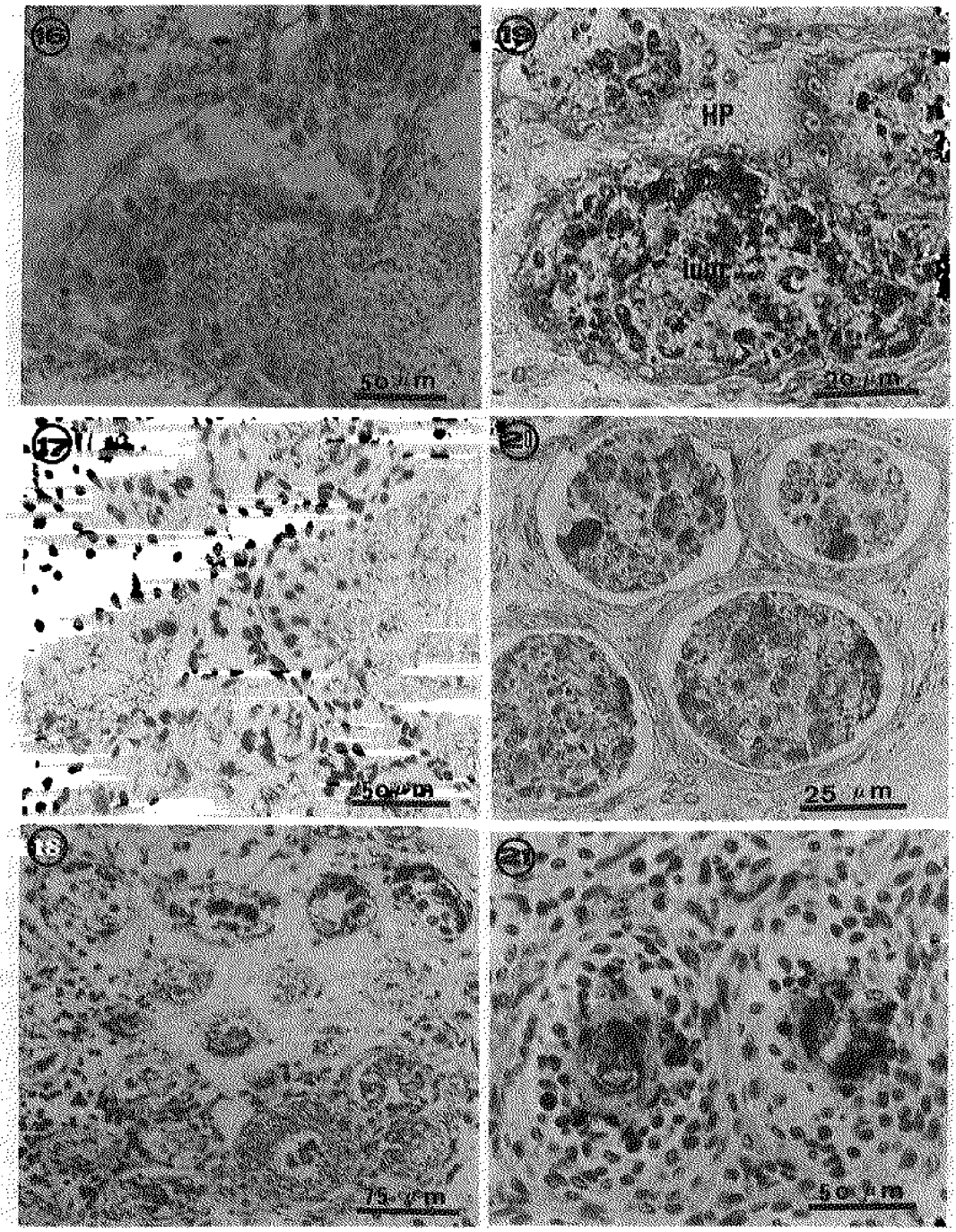
Hepatopancreata: The hepatopancreas was affected most severely after either immersion or injection of *V. harveyi*. Four days after infection, hepatopancreata became reddish and sinuses expanded significantly (Fig. 13). Multiplication of bacteria in hepatopancreatocytes was also observed in the histopathological sections (Fig. 14). Bacteria were also found in the basal membrane, sinus, and lumen; this could lead to degeneration or lysis of hepatopancreatocytes (Figs. 16-18). Densely stained hemocytes and bacteria were also found in the infected hepatopancreata 7 to 10 days after infection (Figs. 15-18). Hemocytes or bacteria aggregated in the necrotic areas of hepatopancreatocytes or in the hepatopancreatic lumen, where fibrella structures or granuloma-like structures were formed (Figs. 18-21). Fourteen to 21 days after infection with bacteria, the number of necrotic areas or granuloma-like structures in hepatopancreata increased significantly.



Figures 1-3. Histopathological changes in the gills of Penaeus monodon *seven days after artificial infection with* Vibrio harveyi. *Figure 1. Swelling of secondary gill filament. Note: dilation of basal membrane from epithelial layer (arrows). Figures 2-3. Densely stained granulocytes (arrows) and bacteria (Fig. 3; white arrow) in secondary gill filaments. G: Gill.*
Figure 4. Invasion of V. harveyi *into the lymphoid organ of* P. monodon. *Arrows indicate necrotic lumen, non-tailed arrows indicate normal lumen.*
Figures 5-6. Lymphoid tissues in gill (Fig. 5) and stomach (Fig. 6) of P. monodon *invaded by* V. harveyi.



Figures 7-8. Hemocytes (Fig. 7) and bacteria (Fig. 8) aggregate in the loose connective tissue of intestine (Fig. 7) and intestinal cavity (Fig. 8).
Figures 9-11. Histopathological observations of heart of P. monodon *infected by* V. harveyi. *Note: presence of hemocyte encapsulated nodule (Fig. 9, arrow), and necrotic area with aggregated bacteria (Fig 11) . Figure 10 shows phagocytosis in hemocytes.*
Figure 12. Hemocyte encapsulated nodule in muscle of bacteria at infection site (arrow).
Figures 13-14. Histopathological changes of P. monodon *hepatopancreata after artificial infection with* V. harveyi. *Note: Expansion of sinus (Fig. 13, arrows) and invasion of bacteria via hepatopancreatic lumens into hepatopancreatocyte (Fig. 14, arrows).*
Figure 15. Densely stained hemocytes in hepatopancreata of V. harveyi-*infected* P. monodon.



Figures 16-21. Various pathological changes in hepatopancreata of P. monodon *infected by* V. harveyi. *Note: aggregation of densely stained hemocytes.*
Figures 16-18. Obtained from a morbid shrimp; most hepatopancreatocytes have been damaged.
Figures 19-21. Obtained from live, active shrimp. Note: Formation of granuloma-like structure.

Discussion

After a survey of the diseases of cultured *P. monodon* in Taiwan, Lightner (1988) reported that the major bacteria isolated from morbid shrimp were *Vibrio* spp., *Pseudomonas* sp. and *Flavobacterium* sp. Our results also showed that *Vibrio* spp. are the dominant pathogenic bacteria in cultured giant tiger prawns in Taiwan. The results further suggest that *Vibrio* spp. play a major role in the initiation of mortality in cultured giant tiger prawns in Taiwan.

Lightner et al. (1988) also reported that *V. alginolyticus, V. anguillarum* and V. *parahaemolyticus* were the main species isolated from morbid shrimp collected in 1986. However, our results showed that *V. harveyi, V. damsela, V. nereis, V. tubiashii, V. anguillarum* and *V. parahaemolyticus* were the major species found in morbid shrimp collected from 1988 to 1990.

Although several factors may be involved in a mass mortality of cultured shrimp, our results, the epizootiological study incorporated with a pathogenicity test and histopathological observations, suggest that *V. nereis* and *V. harveyi* are the important *Vibrio* species contributing to the occurrence of mass mortality of cultured giant tiger prawns in Taiwan. *Vibrio harveyi* was also reported to significantly affect the survival of giant tiger prawn larvae in a hatchery in the Philippines (Pitogo, 1988).

In a pathogenicity study performed on *Penaeus japonicus*, Egusa et al. (1988) reported that 72 h after inoculation of *Vibrio* sp., the lymphoid tissues in various organs were seriously affected, but very little necrosis was found in lymphoid tissue. However, in our study of *P. monodon*, hemocyte encapsulated nodules as well as necrosis of internal organs were frequently found in infected shrimp. More hemocyte encapsulated nodules were found over time.

Serious hepatopancreatic necrosis may extend from the proximal area to the distal area of the organ. This suggests that the route of invasion may be from the stomach to the primary duct and the secondary duct, extending to the hepatopancreatic lumen. We also found that bacteria could multiply in the cells of various tissues.

This is the first report of the initiation of pathogenicity in *P. monodon* artificially exposed to *Vibrio* spp. Our results may have important implications for further studies of the bacterial diseases of cultured *P. monodon*, especially in areas such as immunology and chemotherapy. Work is in progress on the development of bacterial vaccines for cultured giant tiger prawns; those results will be presented elsewhere.

Acknowledgments

This work was supported by a grant from the U.S. Department of Agriculture (Grant No. FG-TA-111) and the Council of Agriculture and National Science Council of the Republic of China.

Literature Cited

Baumann P., L. Baumann and M. Mandel. 1971. Taxonomy of marine bacteria: the genus *Beneckea*. J. Bacteriol. 107: 268-294.

Baumann, L., P. Baumann, M. Medel and R.D. Allen. 1972. Taxonomy of aerobic marine eubacteria. J. Bacteriol. 110: 402-429.

Baumann, P., L. Baumann, S.S. Bang and M.J. Woolkalis. 1980. Reevaluation of the taxonomy of *Vibrio, Beneckea* and *Photobacterium*: abolition of the genus *Beneckea*. Curr. Microbiol. 4: 127-132.

Baumann, P., L. Baumann, S.S. Bang and M.J. Woolkalis. 1981. In list No. 6, validation of the publication of new names and combinations previously effectively published outside the IFSB. Int. J. Syst. Bacteriol. 31: 266-268.

Baumann, P. and R.H.W. Schubert. 1984. Bergy's manual of Systematic Bacteriology. Volume (I). Williams & Wilkins. Baltimore/London. pp. 516-550.

Bell, T.A. and D.V. Lightner. 1988. A Handbook of Normal Penaeid Shrimp Histology. Special Publication No. 1, World Aquaculture Society. Baton Rouge, LA. 114 p.

Brenner, D.J. 1984. Bergy's Manual of Systematic Bacteriology. Vol. (I). Williams & Wilkins. Baltimore/London. pp. 408-516.

Cheng, W. 1989. Isolation and characterization of *Vibrio damsela* infections for cultured shrimp in Taiwan. M.S. Thesis. National Taiwan University, Taipei, Taiwan. 96 p.

Egusa, S., Y. Takahashi, T. Itami and K. Momoyama. 1988. Histopathology of vibriosis in the kuruma prawn, *Penaeus japonicus* Bate. Fish Pathol. 23: 59-65.

Glen, R., R.S. Wheeler, and R.K. Sizemore. 1980. Characterization of brown spot disease of gulf coast shrimp. J. Invertebr. Pathol. 36: 255-263.

Huang, S.L. 1989. Bacterial infection in cultured black tiger prawn (*Penaeus monodon*) M.S. Thesis. National Taiwan University, Taipei, Taiwan.

Koneman, E.W., S.D. Allen., V.R. Dowell and H.M. Sommers. 1983. Colour Atlas and Textbook Diagnostic Microbiology. 2nd ed. J.B. Lippincott, New York.

Krieg, N.R., J.G. Holt, R.E.G. Murray, D.J. Brenner, M.P. Bryant, J.W. Moulder, N. Pfennig, P.H.A. Sneath and J.T. Staley (Eds.). 1984. Bergy's Manual of Systematic Bacteriology. Vol. (1) Williams & Wilkins. Baltimore/London. pp. 408-538.

Lee, J.V., P. Shrend, A.L. Furniss and T.N. Bryant. 1981. Taxonomy and description of *Vibrio fluvialis* sp. nov., (synonym group F vibrios, group EF6). J. Appl. Bacteriol. 50: 73-94.

Liao, I.C., C.E. Shyu, C.Y. Liu, S.C. Chou, Y.W. Liu and C.F. Chang (Eds.). 1989. Extension Handbook on Prawn Disease Prevention. Keelung, Taiwan. (In Chinese).

Lightner, D.V. 1983. Diseases of cultured penaeid shrimp. *In*: J.P. McVey (Ed.). CRC Handbook of Mariculture. Vol. 1, Crustacean Aquaculture. CRC Press, Boca Raton, Florida. pp. 289-320.

Lightner, D.V. 1988. Vibrio disease of penaeid shrimp. *In*: C.J. Sindermann and D.V. Lightner (Eds.). Disease Diagnosis and Control in North American Marine Aquaculture. 2nd ed. Elsevier, New York. pp. 42-47.

Mannheim, W. 1984. Bergy's Manual of Systematic Bacteriology. Vol. (I). Williams & Wilkins. Baltimore/London. pp. 550-575.

Pacha, R.E. and E.D. Kiehn. 1969. Characterization and relatedness of marine vibrios pathogenic to fish: physiology, serology and epidemiology. J. Bacteriol. 100: 1242-1247.

Pitogo, C.L. 1988. Isolation and identification of luminous bacteria causing mortalities in *Penaeus monodon* hatchery in Panay. SEAFDEC Asia Aquaculture. 10(1): 9.

Song, Y.L., W. Cheng, C.H. Shen, Y.C. Ou and H.H. Sung. 1990. Occurrence of *Vibrio vulnificus* infections in cultured shrimp and eel in Taiwan. Proceedings of ROC-Japan symposium on Fish Diseases. Taipei, Taiwan. pp. 172-179.

Part II:

Contributed Papers

Diagnostic Procedures

Current Diagnostic Methods for Agents and Diseases of Farmed Marine Shrimp

James A. Brock
Department of Land and Natural Resources
Aquaculture Development Program
335 Merchant Street, Rm. 348
Honolulu, Hawaii 96813, U.S.A.

Abstract

Shrimp farming is significantly impacted by disease episodes that are caused by a spectrum of biotic and abiotic agents. Clinical diseases on shrimp farms may be caused by multiple factors; yet, the interactions involving direct and indirect etiological factors are, for the most part, not well understood.

By and large, current diagnostics for shrimp diseases rely upon classic medical disciplines, including epidemiology, clinical medicine, pathology, microbiology, toxicology and molecular biology. Diagnoses are usually arrived at by detection of specific biotic or abiotic agents, principally in shrimp tissues, either on the basis of demonstration of the agent itself, or by recognition of pathognomonic lesions or structural changes that are highly suggestive for a specific agent. Sensitive chemical analyses are not in wide use for the detection of specific abiotic agents, thus, some of these factors may be more widespread than is presently realized. Rigorous scientific assessment of management, environment and nutritive factors is often lacking in etiological descriptions of shrimp diseases.

The study of shrimp viruses remains hindered by the lack of *in vitro* cell culture systems with which to culture crustacean viruses. This has resulted in the use of shrimp bioassay and other techniques to demonstrate live virus in substrates of unknown infection status. However, recent advances in the development of molecular methods will help diagnosticians achieve more rapid detection of at least some shrimp viruses. Molecular methods need to be developed for some nonviral biotic agents of shrimp diseases as well. Routine assessment of management and system limitations and the application of analyses for selected abiotic determinants may go a long way to avert clinical diseases on shrimp farms.

Introduction

In shrimp farming, the focus for disease control is usually either a tank, a pond, or groups of ponds, because options for disease management must be practical and economical at this level. Additionally, in recent years interest in Asia has emerged in managing aquatic animal diseases on the local (groups of farms in a given area), national, and regional levels (NACA, 1991).

The constraint of diseases on the productivity and economic viability of shrimp farming is one negative outcome of intensification (Lightner, 1983, 1988; Nash, 1990; Overstreet, 1990; Wyban and Sweeney, 1991). Where shrimp are cultured semi-intensively to intensively, epidemic and endemic diseases that result in mortality that approach or exceed 50% are commonplace. In one Asian country within the past five years, the shrimp farming industry, with an annual production valued at several hundred million dollars, virtually collapsed due to diseases (Lin, 1989) of apparently complex and, possibly, unclear cause. Furthermore, in most farming regions there is little indication that in the immediate future one can expect to see a significant reduction in disease-related losses on intensively managed shrimp farms.

Why is the shrimp farming industry in many areas faced with significant disease problems? Several contributing factors are:

- The widespread use of wild-caught shrimp, or offspring derived from wild-caught shrimp, for stocking hatcheries and farms. The natural suite of pathogens present in the wild stocks are continually introduced into hatcheries and farms.

- The lack of uniform standards for the transfer of shrimp between widely divergent geographical regions (see Sindermann, this volume). As Lightner (1990) has documented, the extensive movement of live shrimp within and between shrimp farming regions has resulted in the transfer of putative shrimp pathogens, mainly the shrimp viruses. Thus, cultured shrimp populations have been exposed to "new" pathogens, some of which may be capable of causing significant diseases in these new hosts.

- Although evaluation of stock quality is routine in many regions, there are no uniform standards for assessing animal quality (i.e., for pathogen and nonpathogen-related health) when stocks are transferred between the different culture phases (maturation-hatchery, hatchery-nursery, nursery-growout).

- The lack of uniform standards and routine monitoring protocols to assure nutritional quality of shrimp diets once the feeds are in the farmer's hands.

- Failure to apply routine monitoring protocols to assess pond water quality conditions in shrimp ponds.

- Overstocking of shrimp ponds relative to the ability of the system to successfully support growth of a high biomass of shrimp.

- The virtual indiscriminate use of drug additives in shrimp culture settings within some regions (Lin, 1989).

While it is quite apparent that diagnostic methods by themselves will not solve the disease problems facing shrimp farming, it is also obvious that methods of detecting biotic and abiotic agents will contribute to the process of implementing practical control strategies for many diseases faced by the shrimp farming industry today.

Historically, interest in the diseases of marine shrimp and pathogen detection methods preceded the development of shrimp aquaculture (Overstreet, 1973; Couch, 1978). At least in part, this was due to a considerable concern over the potential impact of disease on commercial shrimp fisheries. The development of shrimp farming has served to boost the interest in the diagnosis and control of shrimp diseases with economic considerations.

The purpose of this paper is to review the present methods used to detect the known biotic and abiotic agents associated with shrimp diseases, and to discuss selected issues concerning disease diagnosis as this pertains to shrimp farming.

Table 1. Causal factors associated with diseases of cultured penaeid shrimp.

Biotic	Abiotic
Viruses	Nutritional imbalances
Rickettsia	Toxicants
Bacteria	Environmental extremes
Fungi	Human activities
Protozoa	Genetic factors
Metazoa	

Disease Causation and General Issues Concerning Diagnostics

A variety of biotic and abiotic agents are associated as causal factors with shrimp diseases (Table 1). Interactions involving multiple factors (Overstreet, 1990), as illustrated in Table 2, are postulated, but studies to quantify these relationships have yet to be conducted or published. Nevertheless, knowledge gained through studies of diseases in terrestrial animal farming systems confirm that interactions are commonplace between causal factors (Martin et al., 1987), and that understanding contributing factors may lead to improved, more practical methods for disease control. Thus, it behooves the shrimp farming industry to recognize and understand complex etiologic associations for the diseases of farmed shrimp.

One interesting feature of penaeid shrimp is the rather common occurrence of multiple biotic agents (putative pathogens) within a given host or population unit. Some examples of this phenomenon are presented in Table 3. An impact of multiple pathogens on shrimp disease diagnosis is to complicate recognition of the most important causal agent for the disease. Illuminating these relationships in the context of the disease workings is important because without this knowledge, a pathogen of minor significance may be falsely attributed to be the cause of a major clinical disease outbreak.

Table 2. Examples of suggested direct and contributing factors for selected penaeid diseases.

Disease	Direct Factors	Contributing Factors	References
Vibriosis	Vibrio spp.	BP, HPV, MBV, vitamin C deficiency, algal toxins, crowding, transfer and handling, Fusarium infection	Lightner and Lewis, 1975; Lightner, 1978; Sparks, 1985; Lightner, 1988; Anderson, 1988; Nash, 1990
Hemocytic enteritis	Algal toxins	Low phytoplankton density in water	Lightner, 1978; Lightner, 1988
Muscle cramp	Cation imbalance	Capture, high water temperature	Lightner et al., 1988
Fusarium disease	*Fusarium solani*	REO,GNS, heavy metal poisoning, crowding, abrasions	Lightner, 1988
Larval mycosis	Phycomycete fungi	Excessive use of antibiotics	Boonyaratpalin, 1990

Note: BP = *Baculovirus penaei*, HPV = hepatopancreatic parvo-like virus, MBV = *Penaeus monodon* baculovirus, REO = reo-like virus, and GNS = gut and nerve syndrome.

Table 3. Examples of shrimp diseases with multiple biotic agents.

Species	Location	Agents	Reference
P. monodon	Malaysia, ponds	MBV, REO, Rickettsia, Gram-negative bacteria	Anderson et al., 1987
P. vannamei	Ecuador, ponds	IHHNV, BP, LOVV, HPV, Gram-negative bacteria, *Nematopsis* sp., peritrich protozoa	Brock, unpubl.
P. vannamei	Hawaii, ponds	IHHNV, BP, LOVV, Gram-negative bacteria, peritrich protozoa	Brock, unpubl.
P. chinensis	Hawaii, imported from Asia	IHHNV, HPV, LOVV, *Pleistophora* sp., cestode larvae	Brock, unpubl.

Note: IHHNV = infectious, hypodermal and hematopoietic necrosis virus, LOVV = lymphoid organ vacuolization virus

An approach used to clarify associations between biotic agents and clinical diseases is determination of infection prevalence in population samples, or quantitative estimates of the abundance of putative pathogens (severity index estimates) within target tissues of shrimp with disease signs. Often, the numbers of a particular pathogen cannot be determined directly, and structural changes (lesions), usually microscopic (i.e., viral inclusion bodies, hemocytic nodules surrounding bacterial colonies, etc.) considered as diagnostic indicators for the agent, may be counted to derive a severity estimate. Several examples of the application of infection prevalence or lesion severity estimates are listed in Table 4.

The detection of specific biotic agents in samples of shrimp tissues can be an intermediate step or an end point in a diagnostic process, depending on the purpose of the examination (see Table 5 for a list of some reasons to perform

Table 4. Prevalence and agent/lesion severity estimates for selected penaeid diseases.

Species	Disease/Agent	Prevalence/Severity Estimate	References
All penaeids	Gill fouling	Numerical grading of infestation levels of epicommensal bacteria, protozoa and algae	Lightner, 1983; Wyban and Sweeney, 1991
Penaeus vannamei	BP	Prevalence of polyhedra positive shrimp in a group or population sample	Couch, 1974; Overstreet et al., 1988
Penaeus stylirostris	IHHNV	Numerical grading of diagnostic cytopathology in target organs	Bell and Lightner, 1984
Penaeus californiensis	Fusarium	Number, size and gross appearance of the cuticle lesions	Hose et al., 1984
Penaeus vannamei	Runt-deformity syndrome (IHHNV)	Coefficient of variation (CV) and prevalence of deformed shrimp in population samples; numerical grading of diagnostic cytopathology	Kalagayan et al., 1991

diagnostic tests for shrimp diseases). It may also be illustrative to note that the agent recognized by a diagnostician is often the one he/she has been trained to see.

Current Detection Methods for Biotic Agents

Viruses

It is well documented that penaeid shrimp are hosts for a variety of intracellular prokaryotic agents, of which the most varied, widespread and significant as pathogens are the viruses.

Lightner (In press) lists 11 types of viruses that have been demonstrated from tissues of penaeid shrimp. Some of these viruses are reported to be the direct etiological agents of economically significant diseases in specific shrimp species. Although the detection methods for viral agents of shrimp have recently been reviewed comprehensively by Lightner and Redman (1991), a few points concerning these methods are discussed here.

Techniques currently used to demonstrate viral infections of penaeid shrimp include epidemiological features and grossly discernible structural changes, wet-mount microscopy with or without staining, histopathology, electron microscopy, specific antibody methods, a DNA probe and *in vitro* culture on a fish

Table 5. Some applications for diagnostic procedures developed for agents/diseases of farmed shrimp.

Detection of specific agents to guide prevention and treatment regimes for improved farm productivity 1. Surveillance/monitoring programs 2. Assess the efficacy of treatments 3. Identify reservoirs of infection or sources of contamination
Determine the cause(s) for episodes of clinical disease

Table 6. Some light microscopy methods for rapid detection of penaeid baculoviral infections in fresh tissue mounts (hepatopancreas or midgut feces).

Virus	Method	Reference
MBV	0.5% malachite green stain	Lightner, 1983
	Shorr's stain with hematoxylin	Nash, 1990
	Phase contrast microscopy	Nash, 1990
MBV and BP	Aqueous .001% phloxine stain and fluorescent microscopy	Thurman et al., 1990
BMNV	Feulgen stain	Momoyama, 1983
	Fluorescent antibody method	Sano et al., 1984
	Dark field microscopy	Momoyama and Sano, 1988

cell line. The majority of the penaeid virus infections are currently detected by light microscopy demonstration of structural changes (i.e., unique inclusion bodies within cells of specific target organs) characteristic for infection by the viral agent. While, for the most part, these detection methods allow for the recognition of the presence of viral disease because the abundance of the agent is probably quite high in the specific target tissue(s), these test methods may lack the sensitivity to detect latent or pre-patent carrier state infections. Furthermore, available methods for detecting shrimp viruses do not allow the diagnostician to discriminate between antigenically distinct strains, or discern virulence differences between geographically diverse populations of a particular virus.

For the majority of the hepatopancreatic baculoviruses, patent infection can be demonstrated rapidly in wet-mount impression smears of hepatopancreas tissues or fecal strands. The use of selected staining procedures has been reported to improve the visibility of the diagnostic viral occlusion (inclusion) bodies in hepatopancreas tissue preparations (Table 6). For baculoviral midgut gland necrosis virus (BMNV) infection of *Penaeus japonicus*, rapid demonstration of the pathognomonic hypertrophic nuclei of virus-infected hepatopancreas epithelium is reported using dark field optics (Momoyama and Sano, 1988). Additionally, direct demonstration of baculoviral antigens by a fluorescent antibody method is applied in shrimp hatcheries in Japan for the definitive diagnosis of BMNV (Sano et al., 1984).

An enzyme-linked immunosorbent assay (ELISA) technique was also reported for detection of penaeid baculoviruses (Lewis, 1986). The author indicated that detection of 10-ng viral protein/100 µL was possible using this ELISA method. However, further reports on the application of this convenient and useful technique have not been forthcoming.

Histopathology diagnosis is presently the most reliable measure for IHHNV and HPV infections (Lightner, 1988; Brock and Lightner, 1990; Lightner and Redman, 1991). Bell et al. (1990) described the application of a nonlethal biopsy/histopathology technique for detection of subclinical IHHNV infection of adult *P. vannamei*. More recently,

Table 7. Examples of chemical enhancement of the prevalence of BP infection in the shrimp, *Penaeus duorarum*. (After Couch, 1976).

Chemical/dosage	Duration of exposure (days)	# Shrimp evaluated	Prevalence of patent BP-infected shrimp (%)
Aroclor 1254 3 µg/L	30	20	60
Control	30	20	0
Mirex 0.01 - 0.23 µg/L	28	15	40
Control	28	15	6.7

DNA probes have been developed for IHHN and BP viruses (see Lightner et al., this volume).

While molecular and cell culture procedures are under development, methods that rely on conventional technology have been devised by researchers to improve the sensitivity of existing methods for detection of shrimp viruses. These procedures, applied for the demonstration of specific viral agents, include virus infection enhancement and amplification, conspecific shrimp challenge bioassay, indicator shrimp (use of a different shrimp species) challenge bioassay and *in vitro* growth in fish cell culture systems.

For shrimp viruses, enhancement and amplification methods are applied to detect latent or carrier state infections. Characteristically, this has been done in groups of shrimp introduced from distant geographic locations or targeted for transfer onto farms using specific pathogen-free (SPF) shrimp stocks.

Enhancement strategies generally involve prolonged holding of the test population in an isolation or quarantine facility with or without intentional crowding of the shrimp (Lightner and Redman, 1991). Also available, as an experimental approach, is infection enhancement through sublethal exposure to selected pesticides or heavy metals. An example of the effect of chemical enhancement on the prevalence of patent BP infection in *Penaeus duorarum* is given in Table 7.

Lightner et al. (1985) reported the application of indicator shrimp bioassay with the highly sensitive species *P. stylirostris* to detect IHHN virus in carrier state-infected *P. vannamei* populations. An example of a field application of an indicator shrimp IHHNV bioassay is given in Table 8. Results were negative for IHHNV infection by direct histological examination of tissues from *P. vannamei* that were stocked as SPF juveniles into ponds that had previously supported IHHNV-infected populations; but histological examination yielded IHHNV-positive results when *P. stylirostris* were examined after growout in similar ponds on this site.

Table 8. An example of the use of *P. stylirostris* indicator shrimp as a bioassay applied for the detection of IHHNV in shrimp ponds (Brock, unpubl.)[1]

	Penaeus vannamei	*Penaeus stylirostris*
No. ponds sampled	26	4
No. shrimp examined by histopathological methods	330	43
No. specimens with histopathological changes diagnostic for IHHNV	0	8 (19%)

[1]Shrimp used in this study were SPF for IHHNV when stocked into the test ponds. Shrimp specimens were sampled for histopathological examination when the ponds were harvested.

Conspecific shrimp bioassay systems for virus detection were developed to detect live virus in samples of shrimp tissue or other substrates. For example, Overstreet et al. (1988) described an experimental detection procedure for live BP in tissue samples. *Artemia* nauplii were incubated with tissue suspensions from samples of unknown infective BP status. The nauplii were subsequently fed to mysis stage *P. vannamei*, and after various incubation periods, samples of hepatopancreas tissue were examined microscopically for patent BP infection. These researchers later applied this test method to assess BP viability following exposure to a variety of physical and chemical agents selected for their potential use in the eradication of this virus from shrimp culture ponds and facilities (LeBlanc and Overstreet, 1991a, b).

Similarly, Momoyama and Sano (1988) reported transmission and spread of BMNV in larval *P. japonicus* via waterborne exposure to extracts of tissues containing infective BMNV. These studies demonstrated that a conspecific *P. japonicus* larval bioassay system could be used to assess the presence of live BMNV in tissue suspensions. This procedure differed from that of Overstreet et al. (1988) in that susceptible larval stages were subjected to baculovirus infection by the water-borne route rather than through bioencapsulation in live feeds.

Lewis et al. (1992) applied a novel technique to estimate the efficacy of shrimp baculovirus decontamination methods in shrimp farms by measuring, after exposure to various disinfection treatments, the viability in an insect cell culture assay system of an occluded insect baculovirus (*Autographa california*) impregnated onto filter paper strips.

Lu et al. (1991) screened tissues of farmed shrimp for viruses using a variety of fish cell lines. In tissue extracts from *P. stylirostris* and *P. vannamei*, these investigators successfully isolated a new rhabdovirus on an established fish cell line, epithelioma papulosum cyprini (EPC).

All of the above examples represent creative approaches that researchers have applied to the problem of virus

detection in shrimp tissues or shrimp farms. While there have been some gains reported in developing shrimp cell cultures for isolating shrimp viruses (Chen and Kou, 1989), these systems are not ready to be used for the routine detection of penaeid viruses. Thus, the need remains current for the use of shrimp bioassays and the other techniques for detecting shrimp viruses.

Detection is problematical for the reo-like viruses because these agents require direct visualization with a transmission electron microscope. Histopathological structural findings, the magenta-staining cytoplasmic inclusion bodies within hepatopancreas epithelium (Lightner and Redman, 1991) attributed to reo-like virus infection in shrimp, are subcellular lesions of unknown sensitivity and specificity for the detection of these viruses. Thus, determination that a group of shrimp are free of a penaeid reo-like virus cannot be presently assured in a timely or reliably sensitive fashion (see Lotz, this volume).

A similar enigma exists for a galaxy of viral agents that have recently been identified associated with histopathological lesions in the lymphoid organ of shrimp (Owens et al., 1991; Lightner, In press; Flegel et al., this volume). The cellular changes (foci of hyperplasia and hypertrophy with cytoplasmic vacuolation and various forms of inclusion bodies) of the lymphoid organ, which are suggestive of viral infection, have been frequently encountered (Brock, unpubl.) in clinically normal and abnormal cultured shrimp (*P. vannamei, P. stylirostris, P. monodon, P. chinensis, P. japonicus*). The histological changes that signal the presence of an infection of the lymphoid organ are easy to recognize microscopically. Precise microscopic descriptions of subtle differences in these structural changes are now being documented, but they may not allow diagnosis of specific viruses, due largely to the nonspecific nature of the shrimp host's response in the lymphoid organ to viral invasion. Thus, ultrastructural studies will be necessary to distinguish which virus or viruses may be present, or not present in pathological lymphoid organ tissues. Culture of the penaeid rhabdovirus in EPC cells is an effective approach for detecting this lymphoid organ agent (Lu et al., 1991). Further complicating the issue is the dearth of information concerning the lymphoid viruses as agents of disease. In this volume, detailed information is presented about several recently recognized lymphoid organ viruses of *P. monodon* cultured in Thailand (see Flegel et al., this volume).

The continued focus on developing improved diagnostic methodologies for penaeid viruses is a clear priority need. A reliable *in vitro* cell culture system is a top issue, as well as continued efforts to evolve highly sensitive molecular methods for detection of viral antigens or nucleic acid.

Rickettsia

Rickettsia-like and chlamydia-like agents are known from farmed shrimp in some

regions in Asia and the Americas (Chong and Loh, 1984; Lightner et al., 1985; Anderson et al., 1987; Brock, 1988; Krol et al., 1991; Lightner, In press). Economically significant syndromes of pond-reared *P. monodon* (Anderson et al., 1987) and pond-reared *P. vannamei* (Lightner, In press) have been attributed to infection by rickettsia-like agents.

The penaeid rickettsia-like agents have not been cultured *in vitro* on artificial media or in cell culture systems. Nor have molecular methods been developed for the identification of these agents in shrimp tissues. Histopathological methods are used to detect penaeid rickettsia-like agents, but the sensitivity of this procedure is limited to recognition of moderate to heavy infections for the agents that form microcolonies within the cytoplasm of specific target tissues, or by the characteristic cellular pathologic response, such as that which occurs in shrimp affected by the Texas necrotizing hepatopancreatitis syndrome. When the abundance of rickettsia-like organisms is low, histopathological determination is uncertain, and detection by transmission electron microscopy examination is the current method suggested for these infections (Krol et al., 1991). Special stains for bacteria, including tissue Gram stain or Giemsa methods, enhance the visibility of the penaeid rickettsia-like agents in histopathological preparations, and also in smears from infected tissues. Giemsa-stained impression smears of selected target tissues may have potential as a clinical technique for rapid detection of penaeid rickettsia-like organisms.

As a future research priority in penaeid diagnostics, development of immunologic reagents are needed for rapid detection of early or subclinical infections by the rickettsia-like agents of farmed shrimp.

Bacteria

Bacteria are associated with endemic to epidemic diseases of cultured shrimp (Sano and Fukuda, 1987; Liu, 1989). On the basis of morphological appearance, several forms of bacterial infection are recognized in farmed shrimp. These include filamentous and nonfilamentous cuticle fouling (Lightner et al., 1975); shell disease (Cipriani et al., 1980) and colonization of internal organs by bacteria that range from focal lesions to fulminating septicemia (Lightner, 1988). In many farming regions, diseases attributed to infection by *Vibrio* spp. are considered to be the most common and significant infectious problems impacting shrimp farming (Johnson, 1978; Lightner, 1983, 1985, 1988; Takahashi et al., 1985; Anderson et al., 1988; Baticados, 1988; Nash, 1988, 1990; Boonyaratpalin, 1990; Lavilla-Pitogo, 1990; Lin, 1989; Mohney et al., 1991).

Vibriosis is reported to be caused by a variety of *Vibrio* spp., but other Gram-negative genera have also been implicated as causative agents of septic syndromes of penaeid shrimp (Lightner, 1988). Other classes of biotic agents (viruses, rickettsia, fungi and/or protozoa); multiple species of bacteria recovered from moribund shrimp in a given

episode; nutritional deficiency (i.e., vitamin C deficiency); toxic syndromes (i.e., hemocytic enteritis); crowding; transfer; or handling may be concurrent features in shrimp populations affected by vibriosis. Thus, precise and timely etiologic diagnoses can be problematical as the recovery or demonstration of bacterial infection may not, in some cases, provide a comprehensive etiologic diagnosis. Moreover, subclinical nutritional deficiency and environmental conditions are suspected, but unproven, factors that may precede and lead to outbreaks of bacterial disease in cultured shrimp populations.

The presence of bacteria in disease episodes can be demonstrated rapidly by microscopic inspection of wet-mounts of whole larvae or tissue biopsy specimens (Lightner and Lewis, 1975; Lightner, 1983, 1988; Nash, 1990). Also, bacterial agents and host inflammation can be visually confirmed in histopathological preparations (Brock and Lightner, 1990). Standard microbiological methods are applied to detect bacterial agents in shrimp tissues (Lightner and Lewis, 1975; Lightner, 1983, 1985, 1988; Dempsey and Kitting, 1987; Lavilla-Pitogo, 1990). The majority of bacteria associated with shrimp diseases are nonfastidious and are identified, following *in vitro* isolation on artificial media, on the basis of their morphology, staining characteristics and biochemical test reactions (Lightner, 1983, 1988; Nash, 1990). Several of the pathogenic vibrios are bioluminescent. This aids recognition of this group of organisms, both in culture settings as well as once the organism is isolated on agar media (Pitogo, 1988; Mohney et al., 1991).

The filamentous bacterium *Leucothrix mucor* is a fastidious organism and requires a specialized media for *in vitro* culture (Lightner et al., 1975). Acid-fast bacterial infections are identified in histological preparations by special stains that demonstrate the acid-fast nature of the bacterial cell wall (Lightner and Redman, 1986; Krol et al., 1989).

Antibiotic sensitivity profiles are often determined for bacteria isolated from outbreaks of shrimp diseases to help shrimp culturists select the most appropriate drug for treatment. Standard procedures such as the Kirby-Bauer Method are characteristically used to make these determinations.

The development of specific immunologic reagents for detecting the common bacterial pathogens of shrimp would aid the speed and sensitivity of identification of these bacterial pathogens in cases of shrimp disease.

Fungi

Fungi are important pathogens of cultured shrimp. Of primary concern are members of several phycomycete families (*Lagenidium* sp., *Sirolpidium* sp. and *Haliphthoros* sp. are encountered most often) and the imperfect fungus genus, *Fusarium* spp., in particular, *Fusarium solani* (Egusa and Ueda, 1972; Lightner and Fontaine, 1973; Lightner, 1981). The disease of penaeids associated with

attack by the phycomycete fungi, termed larval mycosis, is a clinical problem limited to larval through postlarval stages. On the other hand, Fusarium disease is a clinical issue primarily of intensively cultured, older populations of certain sensitive penaeid species (i.e., *P. japonicus* and *P. stylirostris*) (Egusa and Ueda, 1972; Hose et al., 1984).

Diagnosis of larval mycosis is through microscopic demonstration of the vegetative hyphae, which are invariably abundant throughout larvae that have died or are dying from the disease. Identification of the fungus to genus can be determined directly in wet-mount preparations of infected larvae if the specialized sporangia, which differ between genera, can be found.

Fusarium solani infection results in large, variable sized, irregular shaped, heavily melanized ulcerated to nodular cuticular lesions (Hose et al., 1984). In susceptible penaeid species and the appropriate aged shrimp, clinical diagnosis of Fusarium disease can be made on the basis of the gross lesions. Confirmation through demonstration of branching, nonseptate mycelia and the canoe-shaped macroconidia, characteristic for the genus *Fusarium*, can often be made by microscopic examination of wet-mount impression smears of lesion material (Lightner et al., 1979). However, species identification of the fungus usually requires *in vitro* culture and isolation of the agent. Confirmation of the identification by submission of a representative isolate to a laboratory that routinely identifies members of the genus *Fusarium* is recommended.

Both the phycomycete fungi and *Fusarium* spp. can be readily isolated *in vitro* from clinical material on standard mycobiological media (Baticados et al., 1977; Colorni, 1989). Saboraud Dextrose Agar or PYG (peptone-yeast extract-glucose) Agar supplemented with 2.5% NaCl and penicillin-streptomycin are suitable for recovery of the agents of larval mycosis. Saboraud Dextrose Agar or Potato Dextrose Agar supplemented with 2.5% NaCl and penicillin-streptomycin are acceptable media for *in vitro* culture of *Fusarium* sp. from shrimp tissues.

Protozoa

Protozoa from several distinct orders are reported as pathogens of penaeid shrimp. Commonly encountered in shrimp farm environments are the sessile protozoans and others that colonize the cuticle surfaces, including the peritrichs *Zoothamnium* sp., *Epistylis* sp., *Vorticella* sp. and *Lagenophrys* sp.; the suctorians *Acineta* sp. and *Ephelota* sp.; various flagellates and an apostome ciliate (Overstreet, 1973, 1990; Couch, 1978, 1983; Johnson, 1978; Lightner, 1983, 1985).

Other groups include the gregarine genera *Nematopsis* and *Cephalolobus* (Lotz and Overstreet, 1990); the microsporidan genera *Agmasoma*, *Ameson* and *Pleistophora* (Lightner, 1988), one or more presently unclassified sporozoans considered to belong to the Or-

der Haplosporida (Dykova et al., 1988) and the ciliates *Parauronema* sp. and *Paranophrys* sp. (Couch, 1978).

Microscopic examination of wet-mounts or histological preparations of larvae or shrimp tissue biopsies are presently used to detect these organisms. Special stains such as the Giemsa Method improve visual recognition of sporozoan spores as well as the other protozoan parasites in impression smears or histological preparations of shrimp tissues.

Metazoa

In nature, penaeid shrimp are intermediate or final hosts in the life-cycles of a variety of metazoan parasites, including certain nematodes, cestodes, digenean trematodes and isopods (Overstreet, 1973; Lotz and Overstreet, 1990). Due in part to the complex life cycle requirements of these metazoans, infections of cultured shrimp are unusual unless both the parasites and the necessary alternate hosts coexist in the farm environment.

Detection of metazoan parasite infection is by demonstration of the organism during gross or microscopic examination of shrimp tissues. Taxonomic identification of helminths from penaeid shrimp requires familiarity and use of the pertinent descriptive literature on the parasites of shrimp. Diagnosis to species is best left to a trained parasitologist who has experience with the parasites of shrimp.

Current Diagnostic Methods for Abiotic Agents/Diseases

Abiotic determinants of diseases of farmed shrimp include nutritional, toxic, environmental, human and, possibly, genetic factors (Table 1). The detection methods utilized for abiotic agents of shrimp diseases are not unique, but parallel techniques that have been applied to identify these classes of etiologic agents of diseases of other groups of farmed animals. Approaches and procedures can be organized under the examination categories of epidemiological, clinical, pathology (gross and microscopic) and analytical chemistry methodologies.

For the abiotic factors of shrimp diseases, a complicating feature is that these agents are grossly or microscopically not directly visible in the tissues of shrimp. While diagnosis of states of clinical disease are practical based on a compatible history, and, for some determinants, identification of specific gross and/or microscopic structural changes (lesions) or for others analysis results; detection of subclinical states of disease that result from exposure to these abiotic factors, may, at times, go unrecognized. Specific, highly sensitive analytical procedures, which could point out when nutrient levels are marginal or detect the presence of toxic substances, have not been generally applied to diseases or syndromes of farmed shrimp; especially when biotic agents can be readily recovered from dying and dead shrimp. Thus, it is

quite possible that the significance of abiotic factors in shrimp disease causation is underestimated (Brock, 1991).

Nutritional Syndromes/Diseases

Nutritional syndromes/diseases of farmed shrimp are primarily problems encountered in groups of shrimp cultured indoors, in tanks that have a minimum of natural productivity and/or in intensive rearing conditions with a high biomass or standing crop of shrimp. Deficiency diseases/syndromes are much less likely to occur in extensively farmed shrimp. In part, this reflects contribution of natural productivity to the diet. Hence, as with the infectious diseases, the importance of dietary deficiencies closely parallels intensification of shrimp farming.

Nutritional diseases are not reported for shrimp larvae. This likely reflects our inability to recognize the early stages of deficiency diseases in larval populations, rather than the absolute absence of nutrient deficiency syndromes in hatchery populations. Once larvae lose condition, they rapidly fall prey to bacterial attack. Thus, it is possible that some bacterial disease outbreaks in penaeid hatcheries have their genesis as a deficiency problem. Hard data to support this speculation is presently lacking, however.

In growout farms, poor quality diet may result in reduced growth and increased feed conversion rate (FCR). These symptoms are vague, and may be caused by non-nutritional factors. Experimental growth trials can evaluate the competency of a diet to support shrimp growth to determine if reduced growth rate is diet-related. These trials are time-consuming and must be carried out with proper controls, but, if done correctly, will clearly identify or eliminate feed quality as the cause for the problem. Vogt et al. (1985, 1986) advocate histological or transmission electron microscope evaluation of the hepatopancreas for early recognition of dietary deficiency states in cultured shrimp. However, monitoring protocols such as those described by Vogt and co-authors are not apparently in wide use.

Four diseases are attributed to nutritional deficiency or nutrient imbalance of growout cultured penaeid shrimp, black death or ascorbic acid deficiency, body cramp, blue syndrome and soft-shell syndrome. The etiologic agents attributed to these diseases/syndromes are ascorbic acid deficiency (Lightner et al., 1977); tissue cation imbalance, particularly hypokalemia (low K^+) with hypercalcemia (high Ca^{++}) (Lightner et al., 1989); dietary deficiency of carotenoid pigments such as astaxanthin (Menasveta et al., 1990) and, possibly, low vitamin A (Lightner, In press); and dietary imbalance of Ca^{++} and P, or exposure to certain pesticides, including Aquatin, Gustathion A, Rotenone or Saponin (Baticados et al., 1986).

At present, presumptive diagnoses for the described nutritional diseases of farmed shrimp are made by demonstration of a compatible history and the

Table 9. Nutritional diseases and syndromes of cultured penaeid shrimp.

Disease	Compatible diagnostic findings	References
Ascorbic acid deficiency	Intensive culture; indoors; juvenile shrimp; high rate of growth; brown to black subcuticular lesions which histologically are hemocytic nodules that lack bacterial colonies centrally	Lightner et al., 1977; Lightner, 1988
Body cramp	Juvenile through adult shrimp fed primarily a processed feed; elevated water temperature; transfer and handling initiates onset; flexion of tail and cramping of the abdominal muscle	Johnson, 1978; Lightner, 1983, 1988
Chronic soft-shell syndrome	Juvenile through adult cultured *Penaeus monodon*; thin, flexible, rough or wrinkled exoskeleton	Baticados et al., 1987
Blue or pale color syndrome	Juvenile through subadult cultured shrimp; light blue coloration and reduced brown and grey-brown pigmentation of the exoskeleton	Menasveta et al., 1990

gross and microscopic structural changes (Table 9) that characterize shrimp suffering from prolonged exposure to diets lacking in the specific nutrient(s). Development and regular use of analytical protocols for monitoring selected nutrients in shrimp tissues or processed feeds would be helpful, along with quality control programs.

Toxicoses

Clinical syndromes and diseases are recognized in farmed shrimp to be caused by exposure to water-borne or orally ingested toxic substances. In hatchery systems, suspected toxic syndromes are, at best, poorly understood. For example, incidence of protozoea *P. vannamei* larvae with appendage deformities can be reduced by treatment of culture water with EDTA (Brock, 1991). While it is easy to recognize, microscopically, larvae with appendage deformities, the exact cause(s) for these lesions are not clearly known, but are presumed, largely on the basis of a favorable response to pretreatment of water with EDTA, to be due to the presence of "toxic" substances in the water.

Some poisons such DDT and PCBs are accumulated in the hepatopancreas of shrimp. Couch (1978) indicated shrimp exposed to 0.20-ppb DDT concentrated the chemical to 40 ppm. Thus, hepatopancreas tissue analysis for selected chemicals should provide a useful approach to detection of pesticides in suspected poisonings of farmed shrimp. Couch (1978) reported no histopathological changes in shrimp acutely poisoned with DDT, Dieldrin, Mirex and PCBs. Vogt (1987) reported histopathological changes in the hepatopancreas of *P. monodon*, prior to the onset of behavioral impacts, after exposed to 1-ppm dimethoate. Thus, histopathological examination has been suggested to be of some utility for diagnosing pesticide poisoning in cultured shrimp. However, the sensitivity and specificity, and, thus, usefulness of

Table 10. Diagnostic findings for some shrimp diseases caused by toxins.

Disease	Compatible history and findings suggestive or diagnostic for the disease/syndrome	Reference
Hemocytic enteritis	Runted, juvenile shrimp with an opaque, thickened anterior abdominal midgut intestine. Microscopically, midgut intestine has loss of mucosa and thick plaques of hyperplastic squamous cells covering exposed areas of the midgut basement membrane	Lightner, 1978
Aflatoxicosis	Subadult shrimp off feed; slow growth and variable mortality; red discoloration of the hepatopancreas present at times; histopathological changes in the lymphoid organ and hepatopancreas; demonstration of aflatoxin in the feed	Lightner, 1988
Black gill syndrome	Chronic course and variable signs; focal to diffuse black to brown discoloration of the gill lamellae; microscopically, the cuticle and deeper layers of lamellar tissue may be affected; biotic agents are often present and complicate diagnosis if primary abiotic factors are involved; rule-outs for possible toxic factors by analytical tests for heavy metals, ammonia, acid pH, etc.	Lightner, 1988
Toxic algae	Acute die-off of shrimp associated with an unusual algal bloom; no characteristic microscopic pathologic findings yet reported; pond water is acutely toxic to fish or shrimp; identification of known toxic species of algae, i.e., *Alexandrium tamarense*, in the pond water	Su et al., 1992

histopathological methods are likely to be low for the detection of most pesticide poisonings in shrimp.

Diagnosis of diseases or syndromes of known or suspected toxic cause are derived by demonstration of a compatible history, presence of characteristic structural changes, and, for selected agents, identification by analytical chemistry methods of the injurious agent in shrimp tissues, feeds or the culture environment. The more common toxic conditions of farmed shrimp and the current means for their recognition are listed in Table 10.

Environmental Extremes

Physical and chemical factors in the shrimp culture environment can be the direct or contributing causes of diseases of cultured shrimp. The more important of these factors include temperature or pH extremes; low dissolved oxygen; exposure to an abrupt, large change in salinity and dissolved gas supersaturation.

Diagnoses of diseases resulting from environmental factors are approached by interpretation of environmental monitoring findings, a compatible history, and specific structural changes found in affected groups of shrimp. The salient features for several physical and chemical factors relevant to shrimp disease causation are listed in Table 11.

Human Factors

Individuals formulate and implement husbandry procedures on shrimp farms. Decisions on stocking density, brands or types of feeds, feeding rates, frequency and scope of shrimp population and environmental parameter monitoring, water use, techniques ap-

Table 11. Diagnostic findings for some shrimp diseases/syndromes caused by environmental factors.

Disease/syndrome	Diagnostic findings suggestive of the disease/syndrome	Reference
Low temperature	Winter months, reduced growth and poor FCRs; no remarkable structural tissue changes; prolonged exposure to temperatures < 22 C	Brock, 1991
Acidic pH	Farm constructed in an area with acidic soils; slow growth and variable survival; brown to black gill lamellae; pH readings < 7	Lightner, 1988
Low dissolved oxygen	Die-off occurs at night and often just prior to daybreak; high standing crop of shrimp; abrupt onset following phytoplankton crash; dead and dying shrimp have opaque musculature; dissolved oxygen readings < 2 mg/L	Brock, 1991
Gas supersaturation	Tank or raceway cultured shrimp; gas bubbles in gill lamellae; total dissolved gas pressure > 110%	Lightner, 1988
Broken back syndrome	Tank-held shrimp that are recently transferred; dorsal separation of the pleural plates between the third and fourth segments; salinity of the water is extremely low	Couch, 1978

plied in transfers of shrimp stocks, etc. are tremendously important — misjudgments have the potential to significantly impact shrimp health and hatchery or farm productivity. Also, workers may make errors in carrying out procedures set up by hatchery/farm management. Implementation errors can directly influence disease occurrence, and survival or growth of shrimp. For example, on the management side, the farm manager who purchases a batch of processed feed that is months old because the cost has been reduced, may be buying a lot of trouble for the shrimp ponds. Importantly, it may be a month or more before shrimp fed the ration display clinical evidence of deficiency. On the worker side, if *Artemia* nauplii harvested from hatching tanks are not properly rinsed, high numbers of *Vibrio* spp. can be inoculated into larval rearing tanks, and, within a few days, larval vibriosis may occur. Depending on circumstances, the error can be frequent or sporadic; with other factors being equal, this will influence the pattern of occurrence of vibriosis in hatchery tanks.

Human decisional and implementation errors and the "disease" events they lead to may be separated in time by days to weeks. This time lag complicates recognition of cause and effect relationships between failures of husbandry procedures and disease outbreaks or other impacts on production. For example, during nursery transfers, prolonged out-of-water exposure of harvested juvenile shrimp may result in high post-stocking mortality, but low survival of the population may go unrecognized for several months until the pond is harvested.

Shrimp production loss due to employee and nonemployee theft is another aspect for consideration. A common pattern of presentation is

sporadic occurrence of apparently good survival and growth up to harvest, but reduced production due to lower-than-expected survival when ponds are harvested. The frequency of these unexpectedly low yield ponds may increase just before holidays; also, a good local market for the shrimp should be readily available.

Human error is seldom recognized as an element in disease causation and production deficiencies on shrimp hatcheries/farms. Indeed, while biotic agents remain widespread in cultured shrimp populations, this situation is not likely to change. Any direct or indirect factor that is not visible or measurable in the tissues of shrimp will be difficult to link causally to episodes of shrimp diseases or "mystery" losses of shrimp. Epidemiological and clinical methods are currently the tools available for definition of these problem areas.

Genetic Factors

Genetic factors are suspected to contribute as indirect factors in disease expression of farmed shrimp. Species susceptibility differences to infection by specific pathogens provide some examples of potential genetic variables as indirect factors of shrimp diseases. *Penaeus stylirostris* are highly susceptible to acute disease characterized by high morbidity and mortality from IHHN virus, while *P. vannamei* is much more resistant to disease impacts from this virus (Bell and Lightner, 1984). *Penaeus californiensis* and *P. japonicus* are highly sensitive to infection by *Fusarium solani*, while *P. vannamei* and *P. monodon* are much more resistant to infection.

With the development of captive breeding and the beginnings of domestication of penaeids (see Wyban, this volume), increased recognition is anticipated for the role of genetic factors in disease causation and disease control in farmed shrimp.

Assessing the Proficiency of Detection Methods

There are virtually no controlled study data with which to rate the proficiency of detection and diagnostic methods presently applied to identify agents and diagnose shrimp diseases. This is expected, considering the young age of the shrimp farming industry and the limited resources available worldwide for research on shrimp diseases. Nevertheless, some consideration of this topic, even at this point in time, seems worthwhile. In this regard, a list of seven assessment criteria are presented and briefly described in Table 12. This list was based on criteria used to evaluate human and veterinary medical diagnostic tests.

To most benefit producers, detection methods should give results rapidly, utilize reasonably priced and widely available equipment and reagents, and produce similar results when applied by different personnel trained in the use of the procedure.

Table 12. Some criteria for comparing disease diagnostic procedures and agent detection

Sensitivity	A measure of how well the test detects cases of the disease or can identify the presence of the agent of interest
Specificity	A measure of how well the test excludes subjects without the disease or those free of the agent of interest
Accuracy	A measure of the agreement of the test finding with a reference or "target" value
Precision	A measure of the reproducibility of the results when a given procedure is used to produce them
Speed	A measure of the time required to complete a test procedure
Availability of: – reagents – equipment – personnel	Consideration of the resources necessary to carry-out the test procedure
Cost	A measure of the cost involved to conduct the test procedure

Conclusions

Diseases are a major factor impacting the productivity of marine shrimp farming in regions where semi-intensive to intensive farming practices prevail. The causes of shrimp diseases are numerous. Possibly, and more often than is realized, disease outbreaks in farmed shrimp are the outcome of an interaction of multiple etiologic factors. In this regard, diseases of farmed shrimp parallel disease problems in intensive fish or land-based animal husbandry.

The diagnostic process can be thought of as comprised of two interactive categories: 1) agent detection, and 2) prioritization of agents as to their relative contribution to a given case or outbreak of disease. Obviously, if detection methods applied in a given case can only recognize certain types of determinants, the interpretation of the importance of these agents may be skewed, and, in some instances, misleading.

The detection methods for agents of shrimp diseases are similar to those used in other animal husbandry systems. However, molecular methods have only recently been developed for identifying biotic agents associated with diseases of farmed shrimp. These techniques will likely become increasingly available in the years to come. The lack of shrimp cell culture systems for isolating viruses has lead to the application of a range of shrimp bioassay techniques as interim methods for the study and/or the detection of shrimp viruses. Clearly, a priority research need is the development of shrimp cell culture systems.

Abiotic determinants have received less attention than their biotic counterparts in the study of shrimp diseases. Nevertheless, there are several significant clinical syndromes that have been linked to nutritional, environmental or toxic causes. It is possible that these represent the "tip of the iceberg" and the significance may be underestimated for the role of abiotic agents as subclini-

cal, contributing factors in disease outbreaks on shrimp farms. There is clearly a need to routinely incorporate nutritional and environmental parameter assessment when investigating outbreaks of shrimp diseases, particularly on intensive shrimp farms.

Acknowledgments

Support was provided by The Aquaculture Development Program, Department of Land and Natural Resources, State of Hawaii for the preparation of the manuscript. Mrs. Janet Yasamasu is thanked for assistance in obtaining references.

Literature Cited

Anderson, I.G., M. Shariff, G. Nash and M. Nash. 1987. Mortalities of juvenile shrimp, *Penaeus monodon*, associated with *Penaeus monodon* baculovirus, cytoplasmic reo-like virus, and rickettsial and bacterial infections, from Malaysian brackishwater ponds. Asian Fisheries Science. 1: 47-64.

Anderson, I.G. 1988. Disease problems faced by marine prawn farms and hatcheries. *In*: T. Singh and K.J. Ang (Eds.). Marine Prawn Farming in Malaysia — Present Problems, Future Prospects. Malaysian Fisheries Society Occasional Publication No. 1. pp. 67-83.

Anderson, I.G., M.N. Shamsudin, M. Shariff and G. Nash. 1988. Bacterial septicemia in juvenile tiger shrimp, *Penaeus monodon*, cultured in Malaysian brackishwater ponds. Asian Fisheries Science. 2: 93-108.

Baticados, M.C.L. 1988. Diseases of prawns in the Philippines. SEAFDEC Asian Aquaculture. 10(1): 1-8.

Baticados, M.C.L., G.D. Lio-Po, C.R. Lavilla and R.Q. Gucatan. 1977. Isolation and culture in artificial media of *Lagenidium*, from *Penaeus monodon* larvae. SEAFDEC Quarterly Research Report, Aquaculture Department. 1(4): 9-10.

Baticados, M.C.L., R.M. Coloso and R.C. Duremdez. 1986. Studies on the chronic soft-shell syndrome in the tiger prawn, *Penaeus monodon* Fabricius, from brackishwater ponds. Aquaculture. 56: 271-285.

Baticados, M.C.L., R.M. Coloso and R.C. Duremdez. 1987. Histopathology of the chronic soft-shell syndrome in the tiger prawn *Penaeus monodon*. Dis. Aquat. Org. 3: 13-28.

Bell, T.A. and D.V. Lightner. 1984. IHHN virus: Infectivity and pathogenicity studies in *Penaeus stylirostris* and *Penaeus vannamei*. Aquaculture. 38: 185-194.

Bell, T.A., D.V. Lightner and J.A. Brock. 1990. A biopsy procedure for the non-destructive determination of IHHN virus infection in *Penaeus vannamei*. J. Aquatic Animal Health. 2: 151-153.

Boonyaratpalin, S. 1990. Shrimp larval diseases. *In*: M.B. New, H. de Saram and T. Singh (Eds.). Technical and Economic Aspects of Shrimp Farming. Proceedings of the Aquatech '90 Conference, Kuala Lumpur, Malaysia, 11-14 June, 1990. pp. 158-163.

Brock, J.A. 1988. Rickettsial infection of penaeid shrimp. *In*: C.J. Sindermann and D.V. Lightner (Eds.). Disease Diagnosis and Control in North American Marine Aquaculture, 2nd ed., Elsevier Science, New York. pp. 38-41.

Brock, J.A. 1991. An overview of diseases of cultured crustaceans in the Asia-Pacific region. Fish Health Management in Asia-Pacific, Report on a Regional Study and Workshop on Fish Disease and Fish Health Management. ADB Agriculture Department Report Series No. 1. pp. 347-395.

Brock, J.A. and D.V. Lightner. 1990. Microbial pathogens and diseases of marine crustaceans. *In*: O. Kinne (Ed.). Diseases of Marine Animals. Vol. III, John Wiley & Sons, New York. pp. 245-349.

Chen, S.N. and G.H. Kou. 1989. Infection of cultured cells from the lymphoid organ of *Penaeus monodon* Fabricius by monodon-type baculovirus (MBV). J. Fish Dis. 12: 73-76.

Chong, Y.C. and H. Loh. 1984. Hepatopancreas chlamydial and parvoviral infections of farmed marine prawns in Singapore. Singapore Veterinary Journal. 9: 51-56.

Cipriani, G.R., R.S. Wheeler and R.K. Sizemore. 1980. Characterization of brown spot disease of Gulf Coast shrimp. J. Invertebr. Pathol. 36: 255-263.

Colorni, A. 1989. Fusariosis in the shrimp *Penaeus semisulcatus* cultured in Israel. Mycopathologia. 108: 145-147.

Couch, J.A. 1974. An enzootic nuclear polyhedrosis virus of pink shrimp: ultrastructure,

prevalence, and enhancement. J. Invertebr. Pathol. 24: 311-331.

Couch, J.A. 1976. Attempts to increase baculovirus prevalence in shrimp by chemical exposure. Progress in Experimental Tumor Research (Karger, Basel). 20: 304-314.

Couch, J.A. 1978. Diseases, parasites, and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic Coasts of North America. Fish. Bull. 76: 1-44.

Couch, J.A. 1983. Diseases caused by protozoa. *In*: A.J. Provenzano, Jr. (Ed.). The Biology of Crustacea, Vol. 6, Academic Press, N.Y. pp. 79-113.

Dempsey, A.C. and C.L. Kitting. 1987. Characteristics of bacteria isolated from penaeid shrimp. Crustaceana. 52(1): 90-94.

Dykova, I., J. Lom and E. Fajer. 1988. A new haplosporean infecting the hepatopancreas in the penaeid shrimp, *Penaeus vannamei*. J. Fish Dis. 11: 15-22.

Egusa, S. and T. Ueda. 1972. A *Fusarium* sp. associated with black gill disease of the Kuruma prawn, *Penaeus japonicus* Bate. Bull. Jap. Soc. Sci. Fish. 38: 1253-1260.

Hose, J.E., D.V. Lightner, R.M. Redman and D.A. Danald. 1984. Observations on the pathogenesis of the imperfect fungus, *Fusarium solani*, in the California brown shrimp, *Penaeus californiensis*. J. Invertebr. Pathol. 44: 292-303.

Johnson, S.K. 1978. Handbook of Shrimp Diseases. Texas A&M Sea Grant College Program, Texas A&M University, College Station, TX. 23 p.

Kalagayan, G., D. Godin, R. Kanna, G. Hagino, J. Sweeney, J. Wyban and J. Brock. 1991. IHHN virus as an etiological factor in runt-deformity syndrome of juvenile *Penaeus vannamei* cultured in Hawaii. J. World Aquaculture Soc. 22(4): 235-243.

Krol, R.M., W.E. Hawkins, W.K. Vogelbein and R.M. Overstreet. 1989. Histopathology and ultrastructure of the hemocytic response to an acid-fast bacterial infection in cultured *Penaeus vannamei*. J. Aquatic Animal Health. 1: 37-42.

Krol, R., W.E. Hawkins and R.M. Overstreet. 1991. Rickettsial and mollicute infections in hepatopancreatic cells of cultured Pacific white shrimp (*Penaeus vannamei*). J. Invertebr. Pathol. 57: 362-370.

Lavilla-Pitogo, C.R., M.C.L. Baticados, E.R. Cruz-Lacierda, L.D. de la Peña and N.A. Suñaz. 1990. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. Aquaculture. 91: 1-13.

LeBlanc, B. and R. Overstreet. 1991a. Effect of desiccation, pH, heat and ultraviolet irradiation on viability of *Baculovirus penaei*. J. Invertebr. Pathol. 57: 277-286.

LeBlanc, B. and R. Overstreet. 1991b. Efficacy of calcium hypochlorite as a disinfectant against the shrimp virus *Baculovirus penaei*. J. Aquatic Animal Health. 3: 141-145.

Lewis, D.H. 1986. An enzyme-linked immunosorbent assay (ELISA) for detecting penaeid baculovirus. J. Fish Dis. 9: 519-522.

Lewis, D.H., W.M. Charanza and M.T. Omran. 1992. Baculovirus-impregnated filter paper method for assessing disinfection protocols in shrimp mariculture facilities. J. Aquatic Animal Health. 4: 69-72.

Lightner, D.V. 1978. Possible toxic effects of the marine blue-green alga, *Spirulina subsalsa*, on the blue shrimp, *Penaeus stylirostris*. J. Invertebr. Pathol. 32: 139-150.

Lightner, D.V. 1981. Fungal diseases of marine crustacea. *In*: E.W. Davidson (Ed.). Pathogenesis of Invertebrate Microbial Diseases. Allanheld, Osmum, Totowa, New Jersey. pp. 451-484.

Lightner, D.V. 1983. Diseases of cultured penaeid shrimp. *In*: J. McVey (Ed.). CRC Handbook of Mariculture. Volume 1. Crustacean Aquaculture. CRC Press, Inc., Boca Raton. pp. 289-320.

Lightner, D.V. 1985. A review of the diseases of cultured penaeid shrimps and prawns with emphasis on recent discoveries and developments. *In*: Y. Taki, J.H. Primavera and J.A. Llobrera. Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps, Iloilo City, Philippines. pp. 79-103.

Lightner, D.V. 1988. Diseases in cultured penaeid shrimps and prawns. *In*: C. Sindermann and D. Lightner (Eds.). Disease Diagnosis and Control in North American Marine Aquaculture. Elsevier, Amsterdam. pp. 8-137.

Lightner, D.V. 1990. Viroses section: introductory remarks. *In*: F.O. Perkins and T.C. Cheng (Eds.). Pathology in Marine Science Academic Press. pp. 3-6.

Lightner, D.V., and C.T. Fontaine. 1973. A new fungus disease of the white shrimp *Penaeus setiferus*. J. Invertebr. Pathol. 22: 94-99.

Lightner, D.V., C.T. Fontaine and K. Hanks. 1975. Some forms of gill disease in penaeid shrimp. Proc. World Maricul. Soc. 6: 347-365.

Lightner, D.V. and D.H Lewis. 1975. A septicemic bacterial disease syndrome of

penaeid shrimp. Mar. Fish. Rev. 37(5-6): 25-28.

Lightner, D.V., L.B. Colvin, C. Brand and D.A. Danald. 1977. Black death, a disease syndrome related to a dietary deficiency of ascorbic acid. Proc. World Maricul. Soc. 8: 611-623.

Lightner, D.V., D. Moore and D.A. Danald. 1979. A mycotic disease of cultured penaeid shrimp caused by the fungus *Fusarium solani*. *In*: D.H. Lewis and J.K. Leong (Eds.). Proceedings of the Second Biennial Crustacean Health Workshop. Texas A&M University, College Station, Texas. pp. 137-156.

Lightner, D.V., R.M. Redman, R.R. Williams, L.L. Mohney, J.P.M. Clerx, T.A. Bell and J.A. Brock. 1985. Recent advances in penaeid virus disease investigations. J. World Maric. Soc. 16: 267-274.

Lightner, D.V. and R.M. Redman. 1986. A probable *Mycobacterium* sp. infection of the marine shrimp *Penaeus vannamei* (Crustacean: Decapoda). J. Fish Dis. 9: 357-359.

Lightner, D.V., R.M. Redman, R.R. Williams and T.A. Bell. 1989. Histopathology and clinical chemistry of muscle cramp syndrome in penaeid shrimp and its probable relationship to a dietary mineral imbalance. J. World Aquaculture Soc. 20(1): 50A (Abstract).

Lightner, D.V. and R.M. Redman. 1991. Hosts, geographic range and diagnostic procedures for the penaeid virus diseases of concern to shrimp culturists in the Americas. *In*: P. DeLoach, W.J. Dougherty and M.A. Davidson (Eds.). Frontiers of Shrimp Research. Elsevier, Amsterdam. pp. 173-196.

Lin, C.K. 1989. Prawn culture in Taiwan. What went wrong? World Aquaculture. 20(2): 19-20.

Liu, C.I. 1989. Shrimp disease, prevention and treatment. *In*: D.M. Akiyama (Ed.). Proceedings of the Southeast Asia Shrimp Farm Management Workshop. Published by the American Soybean Association, Singapore. pp. 64-74.

Lotz, J.M. and R.M. Overstreet. 1990. Parasites and predators. *In*: J.C. Chavez and N.O. Sosa (Eds.). The Aquaculture of Shrimp, Prawn and Crawfish in the World: Basics and Technologies. Midori Shobo Co., Ltd., Ikebukuro, Toshima-ku Tokyo, Japan. pp. 96-121.

Lu, Y., E.C.B. Nadala, J.A. Brock and P.C. Loh. 1991. A new virus isolate from infectious hypodermal and hematopoietic necrosis virus (IHHNV)-infected penaeid shrimps. J. Virological Methods. 31: 189-196.

Martin, S.W., A.H. Meek and P. Willeberg. 1987. Veterinary Epidemiology Principles and Methods. Iowa State University Press, Ames, Iowa. 343 p.

Menasveta, P., W. Worawattanamateekul, T. Latscha and J. Clark. 1990. Correction of giant tiger prawn (*Penaeus monodon* Fabricius) coloration by astaxanthin. World Aquaculture 90, Halifax, N.S., Canada. Abstract. 62 p.

Mohney, L.L., D.V. Lightner and T.A. Bell. 1991. An epizootic due to *Vibrio* spp. in pond-reared *Penaeus vannamei* in Ecuador. World Aquaculture 91, San Juan, Puerto Rico. Abstract. 45 p.

Momoyama, K. 1983. Studies on baculoviral mid-gut gland necrosis of kuruma shrimp (*Penaeus japonicus*)-III, Presumptive diagnostic techniques. Fish Pathol. 17: 263-268. (In Japanese with English abstract).

Momoyama, K. and T. Sano. 1988. A method of experimental infection of kuruma shrimp larvae, *Penaeus japonicus* Bate, with baculoviral mid-gut gland necrosis (BMN) virus. J. Fish Dis. 11: 105-111.

N.A.C.A. 1991. Fish Health Management in Asia-Pacific. ADB Agriculture Department Report Series No. 1. 627 p.

Nash, G.L. 1988. Diseases of shrimp and prawns. Fish Farming International. 15(8): 30-31.

Nash, G.L. 1990. *Penaeus monodon* grow-out diseases. *In*: M.B. New, H. de Saram and T. Singh (Eds.). Technical and Economic Aspects of Shrimp Farming. Proceedings of the AQUATECH '90 Conference (1990: Kuala Lumpur) Malaysia. pp. 172-190.

Overstreet, R.M. 1973. Parasites of some penaeid shrimps with emphasis on reared hosts. Aquaculture. 2: 105-140.

Overstreet, R.M. 1990. Antipodean aquaculture agents. Int. J. Parasitol. 20: 551-564.

Overstreet, R.M., K.C. Stuck, R.A. Krol and W.E. Hawkins. 1988. Experimental infection with *Baculovirus penaei* in the white shrimp *Penaeus vannamei* (Crustacea: Decapoda) as a Bioassay. J. World Aquacult. Soc. 19(4): 175-187.

Owens, L., S. De Beer and J. Smith. 1991. Lymphoidal parvo virus-like particles in Australian prawns. Dis. Aquat. Org. 11: 129-134.

Pitogo, C.L. 1988. Isolation and identification of luminous bacteria causing mortalities in *Penaeus monodon* hatcheries in Panay. SEAFDEC Asian Aquaculture. 10(1): 9-10.

Sano, T. and H. Fukuda. 1987. Principal microbial diseases of mariculture in Japan. Aquaculture. 67: 59-69.

Sano, T., T. Nishimura, H. Fukuda, T. Hayashida and K. Momoyama. 1984. Baculoviral mid-gut gland necrosis (BMN) of kuruma shrimp (*Penaeus japonicus*) larvae in Japanese intensive culture systems. Helgoländer Meersunters. 37: 255-264.

Sparks, A.K. 1985. Synopsis of Invertebrate Pathology Exclusive of Insects. Elsevier Science Publishers, Amsterdam. 423 p.

Su, H.M., I.C. Liao and Y.M. Chiang. 1992. Mass mortality of prawn caused by *Alexandrium tamarense* blooming in a culture pond in Southern Taiwan. *In*: T.J. Smayda and Y. Shimizu (Eds.). *In:* Toxic Blooms in the Sea. Elsevier, Amsterdam. In Press.

Takahashi, Y., Y. Shimoyama and K. Momoyama. 1985. Pathogenicity and characteristics of *Vibrio* sp. isolated from cultured kuruma prawn *Penaeus japonicus* Bate. Bull. Jap. Soc. Sci. Fish. 51: 721-730.

Thurman, R.B., T.A. Bell, D.V. Lightner and S. Hazanow. 1990. Unique physiochemical properties of the occluded penaeid shrimp baculoviruses and their use in diagnosis of infection. J. Aquatic Animal Health. 2: 128-131.

Vogt, G. 1987. Monitoring of environmental pollutants such as pesticides in prawn aquaculture by histological diagnosis. Aquaculture. 67: 157-164.

Vogt, G., V. Storch, E.T. Quinitio and F.P. Pascual. 1985. Midgut gland as monitor organ for the nutritional value of diets in *Penaeus monodon* (Decapoda). Aquaculture. 48: 1-12.

Vogt, G., E.T. Quinitio and F.P. Pascual. 1986. *Leucaena leucocephala* leaves in formulated feed for *Penaeus monodon*: a concrete example of the application of histology in nutrition research. Aquaculture. 59: 209-234.

Wyban, J.A. and J.N. Sweeney. 1991. Intensive Shrimp Production Technology: The Oceanic Institute Shrimp Manual. The Oceanic Institute, Honolulu, Hawaii. 158 p.

New Developments in Penaeid Virology: Application of Biotechnology in Research and Disease Diagnosis for Shrimp Viruses of Concern in the Americas

D.V. Lightner[1], B.T. Poulos, L. Bruce, R.M. Redman and J. Mari
Department of Veterinary Science
University of Arizona, Tucson, Arizona 85721, U.S.A.

and

J.R. Bonami
Laboratoire de Pathologie Comparee, INRA-CNRS
Universite Montpellier II, Sciences et Techniques du Languedoc
Place E. Bataillon, 34095 Montpellier Cedex 5, France

Abstract

Twenty years have elapsed since the first shrimp virus, *Baculovirus penaei* (BP), was described from Gulf of Mexico penaeids. Today, a dozen or more penaeid viruses are recognized, and all except BP have been "discovered" within the past 12 years. Many of these viruses were found because of their adverse effects on cultured shrimp. Despite the considerable economic importance of penaeid viruses to the world's aquaculture industry, relatively little is known about these pathogens. There are probably a number of geographic strains of viruses such as infectious hypodermal and hematopoietic necrosis virus (IHHNV), hepatopancreatic parvo-like virus (HPV), and the baculoviruses, *Penaeus monodon* baculovirus (MBV), BP, and baculoviral midgut gland necrosis virus (BMNV). However, until recently, no tools were available with which to investigate this question.

Likewise, until recently, the diagnostic methods available to shrimp pathologists were traditional procedures (which used light microscopy, histopathology, electron microscopy, direct serological methods, enhancement, and bioassays) that have been employed in other areas of animal and human pathology. Only recently have advanced biomedical methods been applied to the study of penaeid viruses and to the development of improved diagnostic procedures. Monoclonal antibodies for IHHNV and genomic probes for IHHNV and BP have been developed. These antibodies and genomic probes are the first of their kind developed as research tools and diagnostic reagents in crustacean aquaculture.

[1]Addressee for correspondence.

Table 1. Known and reported penaeid viruses (as of April 1992).

Abbreviation / Full name	Key reference
DNA VIRUSES	
Parvoviruses	
IHHNV = Infectious hypodermal and hematopoietic necrosis virus	Lightner et al., 1983a, b; Bonami et al., 1990
HPV = hepatopancreatic parvo-like virus	Lightner and Redman, 1985a
LOPV = lymphoidal parvo-like virus	Owens et al., 1991
RNA VIRUSES	
Baculoviruses	
BP-type = *Baculovirus penaei*-type viruses:	
BP from the Gulf of Mexico	Couch, 1974a, b
BP from Hawaii	Brock et al., 1986
BP from the Eastern Pacific	Lightner et al., 1985
MBV-type = *Penaeus monodon*-type baculoviruses	
MBV from Southeast Asia	Lightner et al., 1983c
MBV from Italy	Bovo, 1984 (in Lightner et al., 1985)
PBV = *Penaeus plebejus* baculovirus	Lester et al., 1987
BMN-type = baculoviral midgut gland necrosis-type viruses	Sano et al., 1981
BMN from *P. japonicus* in Japan	Brock and Lightner, 1990
TCBV = type C baculovirus of *P. monodon*	
HB = hemocyte-infecting nonoccluded baculovirus	Owens, 1991 (pers. comm.)
Reoviruses	
REO-III = "type III reo-like virus"	Tsing and Bonami, 1987
REO-IV = "type IV reo-like virus"	Bonami, Mari and Lightner, unpublished
Togavirus	
LOVV = lymphoid organ vacuolization virus	Bonami et al., In press

Introduction

At this time, nearly a dozen viral diseases of cultured and wild marine penaeid shrimp are known. Knowledge of these viruses and the diseases they cause ranges from "extremely scanty" to "considerable." Some of the penaeid viruses have been described solely on the basis of the diseases or disease syndromes in which they were found, on their location in host cells, and on their morphological characteristics. Many of the biochemical characteristics of a few of the penaeid viruses have been determined, permitting the taxonomic placement or tentative placement of these agents (Tables 1 and 2) (Couch, 1981, 1991; Mathews, 1982; Johnson and Lightner, 1988; Brock and Lightner, 1990; Lightner and Redman, 1991, 1992; and Adams and Bonami, 1991). Including the viruses in penaeids, more than 30 viral diseases are now known to occur in the Class Crustacea (Johnson, 1983, 1984; Bonami and Lightner, 1991; Adams and Bonami, 1991; Lightner and Redman, 1992). Major groups of viruses represented in the crustacea include the Re-

Table 2. Structure, morphology and probable classification of the penaeid shrimp viruses.

Virus	Approximate virion size (nm)	Nucleic acid	Probable classification
IHHNV	22	ssDNA	Parvo-like virus
HPV	22 - 24	ssDNA	Parvo-like virus
LOPV	25 - 30	ssDNA?	Parvo-like virus?
BP	55 - 75 x ~300	dsDNA	Baculovirus; occluded
MBV	~75 x 300	dsDNA	Baculovirus; occluded
BMN	~75 x 300	dsDNA	Baculovirus; nonoccluded
TCBV	? x ?	dsDNA	Baculovirus; nonoccluded
HB	? x ?	dsDNA	Baculovirus; nonoccluded
REO-III	50 - 70	dsRNA	Reo-like virus
REO-IV	50 - 70	dsRNA	Reo-like virus
LOVV	30 and 55	ssRNA?	Toga-like virus

oviridae, Picornaviridae, Parvoviridae, Togaviridae, Baculoviridae, Paramyxoviridae, Rhabdoviridae and Iridoviridae (Adams and Bonami, 1991). Hence, as the culture of penaeid shrimp increases, the discovery of other viral diseases from these groups (and possibly others) seems a virtual certainty.

Consistent with this prediction is the recent recognition that yet another disease syndrome of cultured penaeid shrimp, "yellow-head syndrome," is caused by a virus. The name of the disease syndrome reflects a consistently present clinical sign of a disease that has caused serious losses of cultured *Penaeus monodon* in Thailand. Yellow-head syndrome has been demonstrated experimentally to have a viral etiology in studies in which the disease was transmitted by injection of healthy shrimp with 0.22-µm filtrates of tissue homogenates prepared from affected shrimp (T. Flegel, Aquaculture Research Centre, Songkhla, Thailand, pers. comm., June 1992). However, no details are yet available on the nature of the "yellow-head syndrome" virus.

Several of the penaeid shrimp viruses infect several host species (Table 3) and have a vast geographic distribution. For example, BP is present in wild and cultured penaeid shrimp on both coasts of the Americas and in Hawaii. It seems inconceivable that the same virus type or strain could be on both sides of the American continents and in a native wild penaeid in Hawaii. Similar comments may be made about the host and geographic ranges of HPV and MBV in the Western Pacific and Indian Ocean. Hence, it is very likely that each of the known penaeid virus types is, in reality, composed of several distinct strains which may vary in their virulence attributes and in other ways. The development of molecular or serological methods for detection and differentiation of geographical strains of viruses like the BP group will advance the understanding and management capabilities for these pathogens.

Table 3. The penaeid viruses and their known natural and experimentally infected hosts.

Host Subgenus	Species[1]	Virus										
		BP	MBV	BMN	TCBV	HB	IHHNV	HPV	LOPV	REO-III	REO-IV	LOVV
Litopenaeus	*P. vannamei*	+++	+?				++	+		+		+
	P. stylirostris	++					+++	+				+?
	P. setiferus	+					(+)					
	P. schmitti	++						+				
Penaeus	*P. monodon*	++	+++		++	+	++	++	+	+		+?
	P. esculentus		+			+		++				
	P. semisulcatus		+				+	++				
Fenneropenaeus	*P. merguiensis*		++					++				
	P. indicus							+				
	P. chinensis						+	++			++	
	P. penicillatus	++	+					+				
Marsupenaeus	*P. japonicus*			+++			++			++		
Farfantepenaeus	*P. aztecus*	+++					(+)					
	P. duorarum	+++					(+)					
	P. brasiliensis	++										
	P. paulensis	++										
	P. subtilis	++										
	P. californiensis	+?					+					
Melicertus	*P. kerathurus*		+									
	P. marginatus	+++										
	P. plebejus		++									

+++ = very serious disease due to virus reported in one or more life stages of species.
++ = significant disease sometimes due to virus reported in one or more life stages of species.
+ = infections by virus known to occur in species, but with insignificant effects.
(+) = experimental infections achieved, but with insignificant effects.
[1]Classification according to Holthuis (1980)

The current diagnostic methods for the penaeid viral diseases have recently been reviewed (Lightner and Redman, 1992), and these methods have generally been dependent upon: 1) gross and clinical signs, and 2) light microscopic demonstration of unique cytopathology as demonstrated by direct microscopy of tissue impression smears, or by histopathology. Diagnosis of infection caused by a few of the viruses, most notably the togavirus and the reo-like viruses, is difficult, at best, using light microscopy. These are diagnosed with certainty only with transmission electron microscopy (TEM). Direct histopathology of Davidson's AFA-preserved tissues has been the method of choice for acute and most subacute forms of IHHN disease (Bell and Lightner, 1988; Lightner and Redman, 1992). However, for detection of asymptomatic IHHNV infections, enhancement and bioassay techniques were devel-

oped (Lightner et al., 1983b; Brock et al., 1983; Bell and Lightner, 1984, 1987; Lightner and Redman, 1992).

The purpose of the enhancement technique is to increase the prevalence and/or severity of infection within a captive population to increase the chances of a positive diagnosis in populations from which direct samples might otherwise provide a negative diagnosis. In the application of the enhancement technique for IHHNV, a population of postlarval shrimp (*P. stylirostris* or *P. vannamei*) are reared under relatively crowded and stressful conditions to about PL60. Samples for histopathology are taken at intervals throughout the 30- to 60-day enhancement period, and if IHHNV is present, its prevalence and/or severity of infection will be increased to diagnosable levels. Enhancement has, at best, only limited usefulness as a diagnostic method for demonstration of IHHNV infection in subadult or adult *P. stylirostris* or *P. vannamei* that are asymptomatic carriers of the virus.

Infection by IHHNV in asymptomatic carriers was made possible initially by use of a bioassay-based diagnostic method (Lightner et al., 1983b, 1987; Lightner and Redman, 1992). In the bioassay procedure, potential carriers of IHHNV are bioassayed with sensitive "indicator" shrimp, which are typically 0.05- to 2-g juvenile *P. stylirostris*. The "indicator" shrimp are most effectively exposed to the "test" shrimp in a bioassay for IHHNV by feeding minced, pooled carcasses or gnathothoracies of the "test" shrimp to the "indicator" shrimp over a 14-day period. If IHHNV is present in the "test" shrimp, the indicator shrimp typically display readily diagnosable IHHN disease in histopathological samples taken on or after day 14 of a typical 28-day bioassay.

The present diagnostic procedures for the penaeid viral diseases are largely dependent upon history, clinical signs, direct light microscopy of tissue or fecal squashes, or on histopathology (Table 4). Electron microscopy is important in some diagnostic applications. Techniques like enhancement and bioassays, when coupled to histopathology, add to the sensitivity of these procedures. The practical application of these procedures is sometimes difficult; however, and their sensitivity is often very limited. Examination of relatively small samples is one important limitation; the length of time and the amount of specialized equipment required to carry out routine histopathology and/or electron microscopy are other factors limiting their practical usefulness. Also, the cost of histopathology and electron microscopy, and of maintaining isolation labs for enhancement and bioassay add to the list of reasons why better, more rapid, more sensitive diagnostic procedures are needed.

Diagnostic procedures using tissue culture, serologic methods, and DNA probes, which have become state of the art in human and veterinary medicine, are being developed for some penaeid shrimp diseases (Table 4). This paper is

Table 4. Diagnostic methods for the penaeid viral diseases: the traditional methods from the literature and the status of research and development on new methods.

Method	IHHNV[1]	HPV[1]	LOPV[2]	MBV[1]	BMN[3]	BP[4]	HB[5]	REO-III[6]	REO-IV[6]	LOVV[7]
Direct BF LM	-	++	-	++	++	++	-	-	-	-
Phase LM	-	-	-	++	++	++	-	-	-	-
Dark field LM	-	-	-	++	++	++	+	-	-	-
Histopathology	++	++	+	++	++	++	+	+/?	+/?	+/?
Enhancement/ Histology	++	++	-	++	-	++	-	-	-	-
Bioassay/Histology	+++	-	-	-	+	+	-	-	-	-
Transmission EM	+	+	++	+	+	+	+	++	++	++
Scanning EM	-	-	-	-	-	+	-	-	-	-
Fluorescent Antibody	ntcd	-	-	-	++	+	-	-	-	-
ELISA with PAbs	-	-	-	-	-	+	-	-	-	-
ELISA with MAbs	ntcd	-	-	-	-	+	-	-	-	-
DNA Probes	+++/C	ntcd	-	ntcd	-	ntcd	-	-	-	-

*Definitions and key references for each virus:
- = no known or published application of technique.
+ = application of technique known or published.
++ = application of technique considered by authors of present paper to provide reasonable diagnostic sensitivity.
+++ = technique provides best available diagnostic sensitivity.
C = commercially available kits under consideration by a manufacturer of diagnostic kits.

[1]Lightner and Redman, 1992; Lightner, unpublished.
[2]Owens et al., 1991
[3]Momoyama, 1983, 1988, 1989a, b, c, d; Momoyama and Sano, 1988, 1989
[4]Couch, 1991; Lightner and Redman, 1992; Johnson, 1990; Overstreet et al., 1988
[5]Adams and Bonami, 1991; Tsing and Bonami, 1987; Anderson et al., 1987; Nash et al., 1988; Krol et al., 1990
[6]Adams and Bonami, 1991
[7]Bonami et al., In press

Note: BF = bright field examination of tissue impression smears and wet mounts, LM = light microscopy, EM = electron microscopy, ELISA = enzyme-linked immunosorbent assay, PAbs = polyclonal antibodies, MAbs = monoclonal antibodies.

a summary of recent efforts in our laboratory to apply biotechnological techniques using gene probes and monoclonal antibodies to develop new diagnostic methods and research tools for penaeid shrimp viruses.

Characterization of IHHNV

A single batch of adult *P. vannamei*, the progeny of cultured broodstock, was used as the source of IHHNV for characterization studies. Infection in this batch of broodstock was confirmed by direct histological examination of Davidson's preserved specimens and by bioassay tests using 0.5- to 0.1-g *P. stylirostris* as the indicator for IHHNV (Lightner et al., 1983b, 1987; Bonami et al., 1990). Using procedures outlined in Bonami et al. (1990), IHHNV was purified from homogenates of the gnathothoracies of the IHHNV-infected *P. vannamei* in a series of steps in which

differential centrifugation of homogenized tissue was followed by sucrose and CsCl density gradient centrifugation. IHHNV (full virions) formed a discrete band at a density of 1.405 g/ml in the final CsCl gradient. The virus was pelleted and utilized for subsequent studies in which we determined the virion size, the polypeptide composition of its capsid, and its nucleic acid type.

IHHNV was found to be most like members of the Parvoviridae in its characteristics. IHHNV is a nonenveloped icosahedral particle averaging 22 nm in diameter (Fig. 1); having a mean buoyant density of 1.405 g/ml in CsCl density gradient centrifugation; having a linear, single-stranded DNA (ssDNA), genome approximately 4.1 kbp in size, with the majority of its ssDNA strands being minus (-) strands that are incorporated into the capsids separately from the complementary plus (+) strands; and having four polypeptides, VP1 to VP4, with molecular weights of 74, 47, 39 and 37.5 K-daltons (Fig. 6), respectively, making up its capsid (Bonami et al., 1990; Mari et al., In prep. [a]).

Infectivity studies, using semipurified (from sucrose gradients) and purified (from CsCl gradients) IHHNV injected into juvenile *P. stylirostris*, confirmed that the 22-nm particles isolated from infected shrimp are the cause of IHHN disease. Further confirmation that IHHN is caused by the 22-nm virus particle was obtained by immunizing mice with highly purified preparations (from CsCl gradients) of IHHNV, and, subsequently, testing the resultant anti-IHHNV serum with tissues from known IHHNV-infected and known uninfected *P. stylirostris*. The hyperimmunized sera from these mice provided a clear distinction between IHHNV-infected and uninfected shrimp in fluorescent antibody (FAB) tests. This serologic test, the infectivity trials run with purified virus, and subsequent tests with a gene probe for IHHNV DNA (discussed later in this review) confirmed that the virus isolated and characterized was indeed IHHNV.

These findings contradict Lightner's earlier opinion on the taxonomic placement of IHHNV (Lightner, 1988), and findings reported by Lu et al. (1989) and Loh et al. (1990), in which the authors reported that IHHNV was a picornavirus or even a rhabdovirus (Lu et al., 1991). Inspection of the data presented in the papers by Lu et al. (1989) and Loh et al. (1990) revealed that the virus-like particles they isolated were 12 nm in diameter (not 19 nm as the authors reported), are morphologically identical to penaeid hemocyanin (van Bruggen, 1986), and that the chemical characteristics reported for the presumed virus particles (Lu et al., 1989) are those expected for hemocyanin (Bonami et al., 1990). These same authors claimed (Loh et al., 1990) to have cultured IHHNV in a fish cell line. However, subsequent studies showed that the virus grown in the EPC fish cells was a rhabdovirus (Lu et al., 1991). Likewise, infectivity studies run with both the 12-nm particle (Lu et al., 1989;

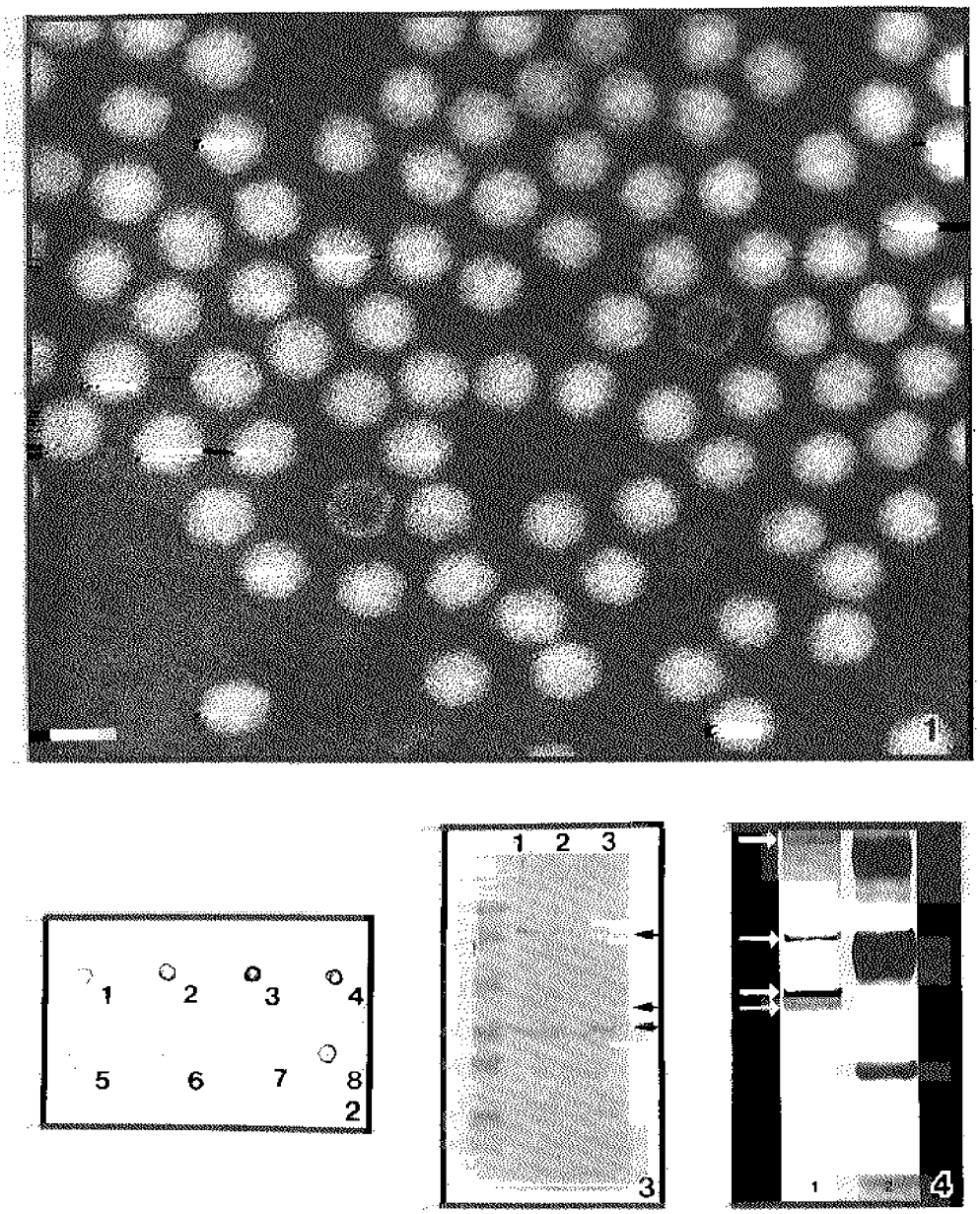
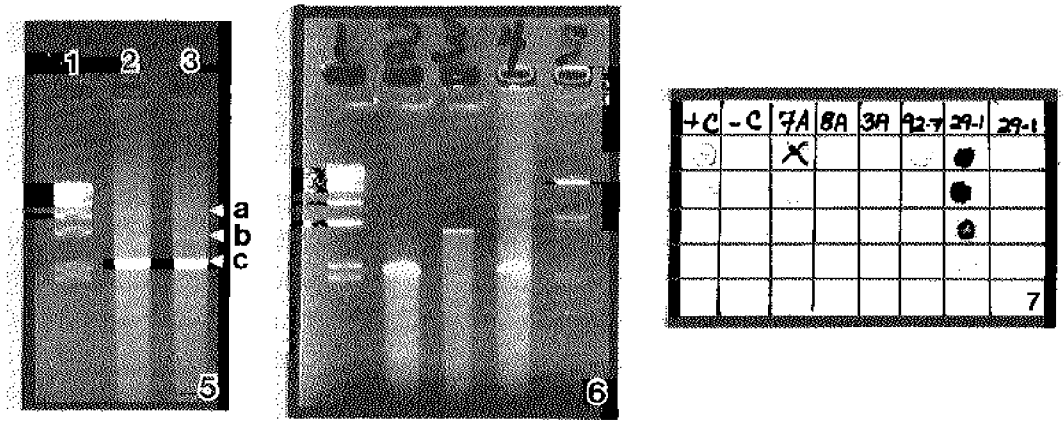


Figure 1. Transmission electron micrograph of purified IHHNV prepared from Penaeus vannamei *by density gradient ultracentrifugation. 2% PTA staining. Bar = 20 nm.*

Figure 2. Immunoblot of purified IHHNV reacted with monoclonal antibodies. Spots 1-6 are IgM class monoclonal antibodies; spot 7 is a mouse MAb made to a nonviral antigen; and spot 8 is a pool of MAbs 1-6. All single and pooled MAbs, except the MAb in spot 7, show a strong reaction with IHHNV.

Figure 3. Western blot of purified IHHNV reacted with L-5 MAb. Lane 1 contains molecular weight markers (placed with transfer for reference); lanes 2 and 3 contain IHHNV polypeptides V1-VP4 (arrowheads) that are demonstrated by their reaction to the L-5 MAb.

Figure 4. Lane 1: A silver-stained gel of IHHNV polypeptides. IHHNV has four polypeptides, VP1, VP2, VP3 and VP4 (arrowheads). Lane 2 contains molecular weight markers.



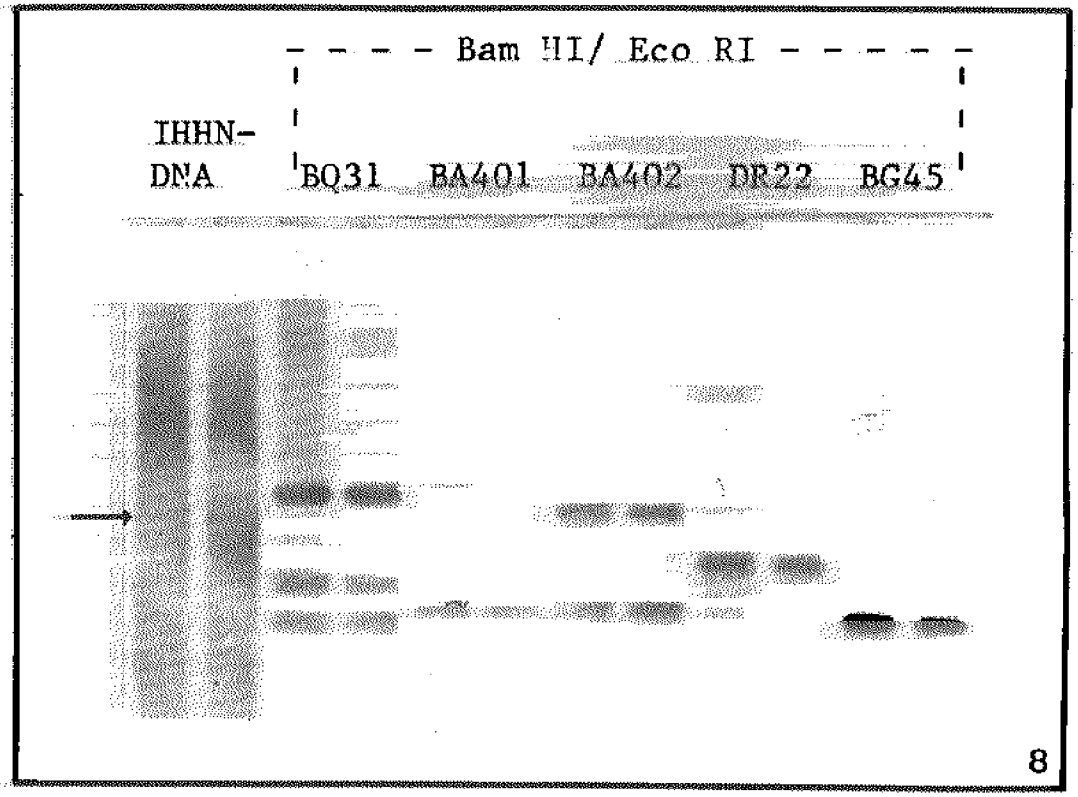


Figure 5. IHHNV DNA (lanes 2 and 3) compared to molecular weight markers (lane 1). Band a (located at about 6.5-7 kbp) corresponds to partially associated + and - ssDNA; band b (estimated at 4 kbp) is dsDNA; and diffuse band c (located at 2.6 - 1.5 kbp) is ssDNA. Ethidium bromide stain (0.5 g/ml) incorporated in the gel.

Figure 6. IHHNV DNA following treatment with RNase A (lane 4), Mung Bean nuclease (lane 3), heat treatment (5 min at 95°C and chilled quickly in an ice bath; lane 2), and separation by agarose gel electrophoresis. Bands visible in lanes 1 and 5 are molecular weight markers, lane 2 is ssDNA, lane 3 is dsDNA, and lane 3 shows no degradation by RNase. Ethidium bromide stain (0.5 g/ml) incorporated in the gel.

Figure 7. Dot blot hybridization with the DIG-labeled BS4.5 probe of Log dilutions of IHHNV, infected, uninfected homogenized shrimp tissues. Labeled rows are: +C = positive control; -C = negative control; 7A, 8A, 3A and 92-7 = + for IHHNV; and 29-1 (both rows) is a Log dilution of purified IHHNV preparation from 10^{0} to 10^{-9}. The first position in 7A and 3A contain no sample.

Figure 8. Hybridization of the DIG-labeled probe BS4.5 after Southern transfer from the agarose gel to IHHNV DNA bands (left two lanes; arrow indicates band c of ssDNA) and with restriction digests of itself (BQ31) and with other smaller IHHNV DNA inserts (BA401, Ba402, DR22, and BG45).

Loh et al., 1990) and with the rhabdovirus were reported as "inconclusive" in the challenged juvenile *P. stylirostris*. Hence, this series of contradictory reports by Lu et al. (1989 and 1991) and Loh et al. (1990) indicate that this group is not working with IHHNV.

Monoclonal Antibodies to IHHNV

To initiate production of murine monoclonal antibodies (MAbs) to IHHNV, highly purified virus was prepared according to previously described methods (Bonami et al., 1990). Specifically, IHHNV was purified from infected *P. stylirostris* collected from a commercial shrimp farm in Hawaii. Crude shrimp homogenate was clarified by a series of low-speed centrifugations and the virus was pelleted at 145,000 g. The virus suspension was treated with activated charcoal, filtered on a Celite-235 bed, extracted with freon, and the virus again pelleted at 145,000 g. The virus suspension was further purified on a 15 - 40% sucrose gradient followed by a 25 - 45% CsCl gradient. Fractions corresponding to a density of 1.405 g/ml contained intact virions as confirmed by transmission electron microscopy (TEM). BALB/cByJ strain mice (The Jackson Laboratory, Inc., Bar Harbor, Maine) were immunized intraperitoneally with purified IHHNV (first injection in Freund's Complete Adjuvant). Three days after a final intravenous immunization, the mouse with the highest titer was euthanized and its spleen removed. Spleen cells were maintained in culture in a conditioned medium designed to promote activated B-lymphocyte growth (Kowalski et al., 1990). Spleen cells were fused with SP2/0 myeloma cells after days 0, 1, 5, and 8 in culture. Cultures containing growing colonies were assayed for the presence of virus-specific antibody by indirect enzyme-linked immunosorbent assay (ELISA).

In the indirect ELISA tests, 96-well plates were incubated overnight at 4°C with crude supernatant fluids from IHHNV-infected or uninfected shrimp. After washing with phosphate-buffered saline with Tween-20 (PBS-Tween), wells were blocked with PBS-Tween containing 10% nonfat dry milk. Supernatant fluid from hybridoma cultures was added to the wells for 1 h at 37°C. Goat anti-mouse IgG, IgM, light chain antibody conjugated to horseradish peroxidase, and the substrate-color reagent ABTS (2,2-azino-bis-(3-ethyl benzthiazoline-6-sulfonic acid)) were used to develop the reactions. The optical densities were read at 405 nm in a BT 2000 Microkinetics plate reader (Fisher). Hybridomas found by ELISA to display high specificity and good intensity in their reaction to IHHNV-infected shrimp tissues (and a very faint or no reaction to uninfected shrimp tissues) were subcloned three or more times by limiting dilution to obtain monoclonal cultures. The clones from these hybridomas provided six "working" clones. The six MAbs selected were subsequently characterized and all were found to be of the mu-kappa (IgM) isotype (Table 5) (Poulos et al., In prep.).

Table 5. Characterization of monoclonal antibodies to IHHNV.

Monoclonal antibody[2]	No. limiting dilutions	Isotype	ELISA[1]	
			+Control[3]	-Control[4]
L-1	3	mu-kappa	0.738	0.001
L-2	3	mu-kappa	1.080	0.006
L-3	3	mu-kappa	1.161	0.002
L-4	3	mu-kappa	0.917	0.004
L-5	3	mu-kappa	1.341	0.005
L-6	4	mu-kappa	0.854	0.005

[1] Optical density at 405 nm.
[2] MAb obtained from hybridoma tissue culture supernatant fluid.
[3] Case #87-102; homogenate of IHHNV-infected *P. stylirostris*, confirmed by histology; collected in 1987 and stored at -70°C.
[4] Case #83-2448; homogenate of uninfected *P. stylirostris*, confirmed by histology; collected in 1983 and stored at -70°C.

To further characterize these MAbs, they were assayed with purified IHHNV in immunoblots and in Western blot assays. Immunoblots were produced by dotting purified IHHNV onto nitrocellulose membranes. Western blots were prepared by electro-transfer of SDS-PAGE gel bands onto nitrocellulose membranes. The membranes were baked at 37°C for 2 - 4 h, blocked for 1 h with PBS-Tween containing 10% nonfat dry milk, and baked again for 30 min. The membranes were incubated with hybridoma supernatant fluid, which was semipurified and concentrated by ultrafiltration for 2 h at 37°C. After washing in PBS-Tween, the membranes were incubated with goat anti-IgG, IgM, light chain antibody conjugated to horseradish peroxidase (HRP) for 1 h at 37°C. After washing in PBS-Tween, the reaction was amplified using the BLAST™ HRP Blotting Amplification System from NEN-DuPont Corp. Color development was achieved using 4 CN/H_2O_2 (4-chloro-1-naphthol /hydrogen peroxide). The six IgM MAb were found to react strongly with purified IHHNV in immunoblots and in Western blots (Poulos et al., In prep.) (Figs. 2 and 3).

The six MAbs were used successfully in indirect ELISA to distinguish between IHHNV-infected and uninfected shrimp of known disease status in archived samples (samples that had been stored at -70°C for more than one year) (Table 6). However, with fresh tissue samples of known IHHNV status (from history, histopathology or bioassay), more variable results were obtained. It was determined that fresh clinical specimens of uninfected shrimp may elicit a nonspecific response of variable intensity in the indirect ELISA. This finding indicates that there are substances in fresh, crude, shrimp extracts that bind with murine antibodies non-specifically, but that are not found in archived samples after long-term frozen storage (Poulos et al., In prep.).

Table 6. Comparison of clinical results using different diagnostic methods for the detection of IHHNV infections in samples of *P. vannamei* and *P. stylirostris*.

Sample number	ELISA[1] (MAb)	Histology	DNA hybridization[2] (using probe BS4.5)
87-102	+++	+	+++
87-85	+	+	+
88-25	+	+	+++
91-32b	++++	+	+++
91-42-86A	+	+	+
91-135-7	+++	+	+
91-139-A3	+	+	+
92-26D	+	NT	+++
83-2448	–	–	–
21-88	–	–	–
91-27	–	–	–
09-88	–	+	+++
91-18	–	+	+
91-33	–	+	+++
91-84-3N6A	+++	–	–
91-139-C1	+	–	–
92-24-LT4	+	–	–
92-26C	+	NT	–
92-7 virions[3]	+	NA	+++
90-149 virions	+	NA	+
87-103 virions	+++	NA	+++

[1] ELISA assay was run using MAb to IHHNV; Histology was read for presence of Cowdry A inclusion (CAI) bodies; DNA hybridization was performed with IHHNV clone BQ31.
[2] Mari et. al. Cloning of the IHHNV genome and the use of probes in diagnosis. Manuscript in preparation [a].
[3] Partially purified IHHNV virions from infected shrimp.

+ = relative intensities of positive colorimetric reactions. The number of +'s indicates positive reaction intensity.
– = negative colorimetric reaction.
NT = not tested.
NA = not applicable.

Preliminary studies into the nature of the substance(s) in fresh shrimp tissues that binds murine antibodies suggest that the substance(s) may be lectins, which are an important component of the "immune" or defense mechanism of crustacea (Olafsen, 1986; 1988). Lectins are large, glycoprotein molecules that bind to specific polysaccharides on other glycoproteins. Normal and IHHNV-immune mouse sera were found to produce a positive ELISA reaction when assayed against uninfected shrimp homogenates using a secondary antibody that detects all classes of mouse immunoglobulin, but not when using a secondary antibody specific only for the gamma chain of mouse IgG; this activity may be due to the binding of shrimp lectins on the carbohydrate side-chains of the IgM molecule (Poulos et al., In prep.) (Table 7).

Table 7. ELISA detection of nonspecific binding of murine immunoglobulins by substances in crude shrimp homogenates.

Primary antibody	Secondary detection mouse IgG, IgM (H&L)[1]		Antibody to: mouse IgG gamma[2]	
	+Shrimp	-Shrimp	+Shrimp	-Shrimp
Anti-IHHNV mouse serum, 1:1000	0.725	0.371	0.694	0.009
Normal mouse serum, 1:1000	0.228	0.053	0.013	0.014
L-6 supernatant, undiluted	0.776	0.006	0.009	0.009

[1] Antibody labeled with horseradish peroxidase; detects mouse IgG and IgM, heavy and light chains.
[2] Antibody labeled with horseradish peroxidase; detects only the gamma heavy chain of mouse IgG.

The application of ELISA with murine antibodies in shrimp disease diagnosis where tissue homogenates are used may be limited until a method of blocking the shrimp lectins is developed that does not destroy viral epitopes necessary for specific MAb detection of IHHNV in fresh clinical specimens. However, production of murine IgG monoclonal antibodies (which contain relatively less and different glycoproteins than do IgM antibody molecules) to IHHNV will provide an alternative ELISA reagent, and its use may circumvent nonspecific binding by lectins or other factors in fresh shrimp tissue homogenates.

Development of Gene Probes

IHHNV Gene Probes

A batch of 75 gnathothoracies from IHHNV-infected, 10-g, juvenile *P. stylirostris* was the source of IHHNV for the development of DNA probes. These shrimp had been stored at -70°C following collection from a commercial shrimp farm in Hawaii in August 1987. Purification of the virus, verification of its purity by TEM, and extraction of its DNA were accomplished as described previously (Bonami et al., 1990; Poulos et al., In prep.).

Once isolated from their capsids and suspended in buffer, complementary positive and negative strands of isolated IHHNV ssDNA tend to re-associate to form double-stranded DNA (dsDNA), as shown in Figures 5 and 6, in which bands representing ssDNA, partially re-associated, and fully annealed dsDNA, are shown following RNAase treatment and agarose gel electrophoresis. RNAase digestion did not affect the three bands, confirming that all three bands are DNA. After electrophoresis, DNA bands of interest were excised from the gel and the DNA was directly recovered using the GENECLEAN II Kit (Bio 101, Inc.). Isolated IHHNV DNA was digested with Mung Bean nuclease to remove the ssDNA, leaving only dsDNA. After phenol /chloroform extraction and ethanol precipitation, the dsDNA was resuspended in buffer and blunt-end ligated with T4 DNA ligase in the dephosphorylated

Sma I site of the pUC 18 vector. Transformation was done according to standard methods (Seidman, 1989) using competent *E. coli*-DH5 cells (Mari et al., In prep. [a]).

About 5,000 transformed *E. coli* (white colonies; unaltered colonies are blue in this cloning system) colonies were isolated, and, of these, 500 colonies were randomly selected for further analysis. Alkaline lysis minipreps (Birnboim, 1983) were used for screening transformed plasmids isolated from the white *E. coli* colonies. After digestion with different restriction enzymes, the size of the IHHNV dsDNA inserts was found in a large proportion of the transformed colonies to be less than 1 kbp; only 5% of them exhibited a size greater than 1 kbp; and only one clone, BQ31, had an insert of 4.5 - 4.7 kbp. Because the insert size of the clone BQ31 corresponded apparently to a full-size IHHNV genome, it was chosen for initial investigation. Other clones with inserts 2 kbp or larger were also selected for further studies. These clones had inserts of 2.3, 2.0, 3.2 and 2.2 kbp, and were designated as BG45, BA401, BA402, and DR22, respectively. After digestion with different restriction enzymes, the size of the insert of BQ31 was estimated to be 4.5 - 4.7 kbp, which is larger than the TEM-estimated size of the IHHNV genome, 4.1 kbp. This was confirmed by agarose gel electrophoresis: the B band of the viral dsDNA migrates a little farther than does the BamHI/SacI-digested insert of BQ31 (which is coded as BS4.5).

DNA inserts selected as potential probes for IHHNV detection were labeled using the nonradioactive Genius I Kit (Boehringer Mannheim, Inc.), which contains digoxygenin-11-dUTP as the DNA label and uses an ELISA-based system for final detection. To verify that selected inserts were derived from IHHNV DNA, Southern transfer (Maniatis et al., 1982) and dot-blot techniques were performed on nitrocellulose membranes BA85 (Schleicher & Schuell, Inc.). A Southern blot of the IHHNV DNA (showing the three different bands A, B and C) was probed with DIG-11-dUTP-labeled BS4.5. Figure 8 shows clearly the hybridization of the probe with the most visible (band C, ssDNA) of the three bands. This confirms that the cloned DNA corresponds to at least a part of the viral genome of IHHNV (Mari et al., In prep. [a]).

The specificity of the probe BS4.5 was further investigated by reacting the probe with a variety of insect and shrimp parvoviruses. BS4.5 was reacted with dot-blots prepared from extracted insect parvovirus DNA; with purified IHHNV DNA; purified IHHNV virions; homogenized tissues from known IHHNV-infected shrimp; healthy shrimp; and purified HPV (= hepatopancreatic parvo-like virus [Lightner and Redman, 1985a]); and tissues (hepatopancreas) of HPV-infected shrimp. The results (Fig. 7 and Table 8) indicate clearly that this probe has a high degree of specificity for IHHNV (no cross hybridization with the shrimp DNA from healthy animals or with HPV). The detection limit in

Table 8. Results of assays with the DNA probe BS4.5 for IHHNV against a variety of insect and shrimp parvoviruses including HPV and IHHNV.

Parvovirus	Host and/or sample	Hybridization results
Insect		
SCLA	*Aedes albopictus*	none
JcDNV	*Juonia caenia*	none
AdDNV	*Acheta domestica*	none
VIM	*Culex pipiens*	none
Shrimp		
IHHNV	purified DNA	+++
	purified virions	+++
	infected *P. vannamei*	+++
	infected *P. stylirostris*	+++
	uninfected *P. vannamei*	none
	uninfected *P. stylirostris*	none
HPV	purified virions (*P. chinensis*)	none
	infected *P. monodon*	none

these dot-blot assays with Log dilutions of IHHNV DNA was estimated to be about 0.1 pg of IHHNV DNA.

The BS4.5 probe was tested *in situ* on paraffin sections of IHHNV-infected and healthy shrimp. Infected and uninfected *P. stylirostris* and *P. vannamei* were fixed in Davidson's fixative, embedded, and sectioned at 5 µm using standard histological procedures (Bell and Lightner, 1988). Some sections were stained with H&E for use as a histological reference (Fig. 9). Others were used for *in situ* hybridization using DIG-11dUTP-labeled probes. No reaction was found in uninfected tissues. Conversely, a strong positive reaction was obtained in infected shrimp sections (Figs. 10-12). Known target tissues for IHHNV were positive at the cellular level, and exhibited labeled virus-containing areas in both the cytoplasm and nucleus of many cell types (Figs. 10-12).

Especially significant in the *in situ* hybridization tests is the intense reaction of the Cowdry type A inclusion bodies, which are the principal diagnostic histological characteristic of IHHN disease (Figs. 9-12). Histopathology of parallel H&E-stained sections from the same infected and uninfected tissues (Fig. 9) was in agreement with the previously published data on the disease (Lightner et al., 1983a; Bonami and Lightner, 1991; Lightner and Redman, 1991, 1992). Perhaps more important was the recognition by the probe of IHHNV-infected tissues that were not detected as readily in routine H&E-stained sections. The fact that the IHHNV probes tested react only with IHHNV-infected tissues, and not with insect parvovirus DNA, and, moreover, not with HPV-infected tissues, underlines their high specificity to IHHNV. This definitively resolves the controversy of the real causative agent of IHHN disease, in which another research group has contended that IHHNV might be a picor-

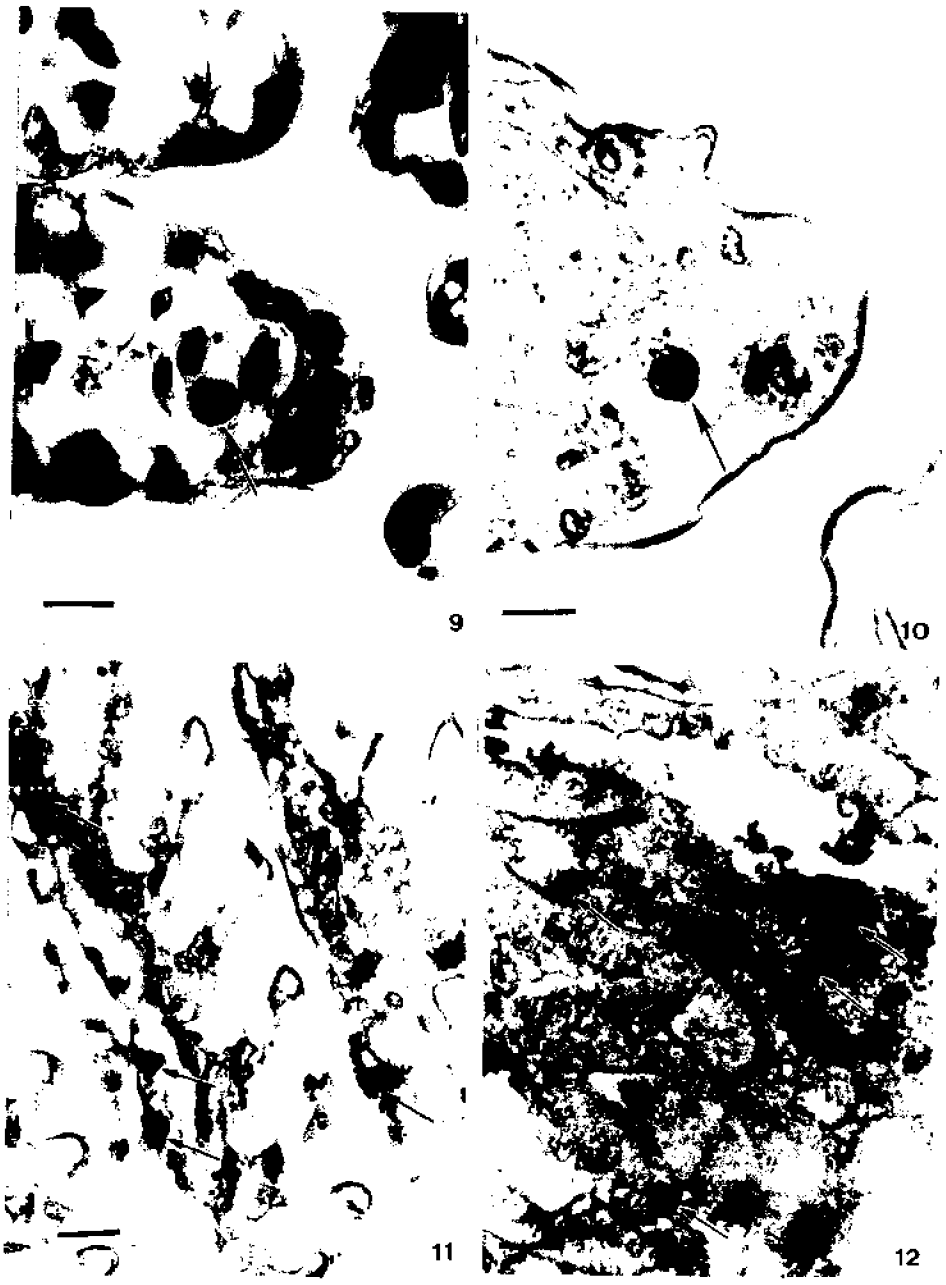


Figure 9. Light photomicrograph of an intranuclear, eosinophilic, Cowdry type A inclusion body (CAI; arrow) that is diagnostic for IHHNV infection. Section is of the gills of a juvenile Penaeus stylirostris. *H&E stain. Bar = 10 µm.*

Figure 10. Light photomicrograph of a similar CAI (arrow) in the gills of a juvenile P. stylirostris, *which is stained dark purple by the genomic probe BS4.5 to IHHNV. No stain. Bar = 10 µm.*

Figures 11 and 12. Histological sections of gills and antennal gland from a P. stylirostris *with a very heavy IHHNV infection. Many focal lesions are demonstrated by the BS4.5 probe that consists of CAIs (arrows), cytoplasmic masses of IHHNV, and virus-containing cell debris from necrotic cells (arrowheads). No stain. Bars = 20 µm.*

navirus or a rhabdovirus (Lu et al., 1989; Loh et al., 1990; Lu et al., 1991).

Baculovirus (BP and MBV) Gene Probes

BP is an important virus of the American penaeids *P. vannamei* and *P. stylirostris*, while MBV is its counterpart in Asian, African and Australian penaeid shrimp (Tables 1-3). The diagnostic procedures for detection of acute or subacute BP and MBV infections are simple and straightforward (Table 4). However, the current diagnostic procedures for BP and MBV do not provide diagnosis of latent carriers of these viruses, nor do they provide the needed research tools to determine how many virus types make up each of the BP and MBV groups. Furthermore, important research and disease management questions (i.e., latency, transovarial versus horizontal transmission, etc.) cannot be answered with the available methods for detecting these viruses. Hence, the development of specific DNA probes for these viruses has been initiated by our laboratory. Because BP is of more immediate interest to the shrimp culture industry of the Americas, most of our work has been concentrated it, and the results of that work will be reported here. The status of our work with MBV will be reported elsewhere (Mari et al., In prep. [b]).

BP Virus

BP DNA was obtained from virions purified from BP-infected juvenile P. vannamei obtained from pond-reared stock in Hawaii. Methods used for BP purification and DNA extraction have been described previously (Bruce et al., 1991; Mari et al., In prep. [b]). Confirmation of the purity of purified BP preparations was determined by UV spectrophotometer and TEM examination of 2% PTA-stained samples of BP from CsCl fractions after density gradient centrifugation.

Following extraction of DNA from purified BP virions, the dsDNA was digested with the restriction enzyme BamHI, and the resultant fragments were separated using agarose electrophoresis. Bands of the BamHI-digested DNA were extracted from the gel and ligated into the BamHI-digested and dephosphorylated vector pUC 18. The recombinant plasmids were then used to transform *E. coli* DH5 cells. Transformed cells were frozen back to create a library of BP DNA, and initial screening of the recombinant plasmids in the transformed cells was performed to begin restriction enzyme mapping of the BP DNA inserts. The larger BP DNA inserts were selected as potential gene probes, labeled with the Amersham nonradioactive enhanced chemiluminescence kit, and tested against known BP-infected shrimp samples and known negative controls. One 3.9-kbp insert of BP DNA (HQ-15) was selected as a promising BP gene probe. HQ-15 reacts well with the BP positive controls tested, and it does not display a reaction against negative control or uninfected shrimp tissues. These findings were verified using the same piece of DNA as a probe but labeling it with the Boehringer Mannheim Genius I system.

Table 9. Summary of results of trials in which the BP probe HQ-15 was tested with various samples.

Case No.	Species	Origin	Sample type	Test Number						
				1	2	3	4	5	6	7
91-7	*P. vannamei*	Hawaii	DNA	+	+	+				
91-7	*P. vannamei*	Hawaii	OBs	+	+	–	+	+	+	+
91-7	*P. vannamei*	Hawaii	HP	+	+	+	+/–	+		
92-14	*P. stylirostris*	Guam	HP	+	+	+	+	+/–	+	
91-5	*P. vannamei*	Guam	HP	+						
90-154	*P. vannamei*	Ecuador	HP	+	+	+	+/–	+	+	
Tu87-2	*P. vannamei*	Ecuador	feces	+	+	+	+	+		
90-186	*P. vannamei*	Miss.[1]	DNA	+	+	+	+			
91-19	*P. vannamei*	Miss.[1]	DNA	+						
91-12	*P. vannamei*	Brazil	HP	–	–	–				
91-83	*P. aztecus*	Florida	OBs	–	–	–	–	–	+/–	–
92-15	mix[2]	Mexico	HP	–	–	–	–			
		IHHNV	DNA	–	–	–	–	–	–	–
87-8/17	*P. stylirostris*	Hawaii	HP	–	–	–				
83-2448	*P. stylirostris*	Hawaii	HP	–	–	–	–	–		

[1]Larvae infected with BP isolated from shrimp from Ecuador.
[2]Mix = pooled mix of shrimp HP homogenates of wild *P. vannamei*, *P. stylirostris* and *P. californiensis* from Mexico.
Note: OBs = BP tetrahedral occlusion bodies, HP = hepatopancreas.

Samples of shrimp with known BP infections and with unknown BP status from different geographical regions were tested in clinical trials with the probe HQ-15 to determine the usefulness of the probe as a diagnostic reagent and to investigate the possible use of the probe to detect potentially different geographical strains of BP (Table 9). In preliminary trials, the probe displayed a positive reaction against DNA extracted from all BP components (i.e., purified BP DNA, purified nucleocapsids, purified polyhedra, and supernatant from a homogenized preparation) of the BP-infected *P. vannamei* from Hawaii, from which the probe was made (case no. 91-7 in Table 9). In the clinical tests, the HQ-15 probe displayed a positive reaction against Ecuadorian-derived, BP-infected, larval and juvenile *P. vannamei*, and with feces collected from infected *P. vannamei* from Ecuador. The probe exhibited no reaction against polyhedra purified from BP-infected wild *P. aztecus* from the Gulf of Mexico; with presumed BP-infected *P. vannamei* from Brazil; or with a pooled mix of shrimp hepatopancreas homogenates of wild *P. vannamei*, *P. stylirostris* and *P. californiensis* from Mexico. As expected, HQ-15 did not hybridize with IHHNV. However, HQ-15 did react with a sample of *P. stylirostris* from a population that had been reared in

Guam and which had no prior history of BP. Additionally, the probe exhibited no reaction against known, uninfected, homogenized hepatopancreas preparations, which served as negative controls (Table 9).

The initial results with the probe HQ-15 suggest that there may be differences in Atlantic and Pacific strains of BP, which are distinguished by this particular probe. Results also suggest that the probe may detect latent BP infections in populations like the Guam-reared *P. stylirostris* population, which had no prior history of patent BP infections.

Acknowledgments

Grant support for the shrimp disease studies reported was from the U.S. Department of Agriculture's Gulf Coast Research Laboratory Marine Shrimp Farming Consortium; Sea Grant, U.S. Department of Commerce; and Groupement de Cooperation Scientifique sur les Bases Biologiques de l'Aquaculture.

Literature Cited

Adams, J.R. and J.R. Bonami (Eds.). 1991. Atlas of Invertebrate Viruses. CRC Press, Boca Raton, FL.

Anderson, I.G., M. Shariff, G. Nash and M. Nash. 1987. Mortalities of juvenile shrimp, *Penaeus monodon*, associated with *Penaeus monodon* baculovirus, cytoplasmic reo-like virus, rickettsia and bacterial infections, from Malaysian brackish water ponds. Asian Fisheries Science. 1: 47-64.

Bell, T.A. and D.V. Lightner. 1984. IHHN virus: Infectivity and pathogenicity studies in *Penaeus stylirostris* and *Penaeus vannamei*. Aquaculture. 38: 185-194.

Bell, T.A. and D.V. Lightner. 1987. IHHN disease of *Penaeus stylirostris*: effects of shrimp size on disease expression. J. Fish Dis. 10: 165-170.

Bell, T.A. and D.V. Lightner. 1988. A Handbook of Normal Shrimp Histology. Special Publication No. 1, World Aquaculture Society, Baton Rouge, LA. 114 p.

Birnboim, H.C. 1983. A rapid alkaline extraction method for the isolation of plasmid DNA. Method Enzymol. 100: 243-255.

Bonami, J.R. and D.V. Lightner. 1991. Unclassified viruses of crustacea. *In:* J.R. Adams and J.R. Bonami (Eds.), Atlas of Invertebrate Viruses. CRC Press, Boca Raton, FL.

Bonami, J.R., M. Brehelin, J. Mari, B. Trumper and D.V. Lightner. 1990. Purification and characterization of IHHN virus of penaeid shrimps. J. General Virology. 71: 2657-2664.

Bonami, J.R., J. Mari, B.T. Poulos and D.V. Lightner. In press. Isolation, purification and characterization of a toga-like virus associated with histopathological changes of the lymphoid organ of penaeid shrimp. Dis. Aquat. Org.

Brock, J.A., D.V. Lightner and T.A. Bell. 1983. A review of four virus (BP, MBV, BMN, and IHHNV) diseases of penaeid shrimp with particular reference to clinical significance, diagnosis and control in shrimp aquaculture. Proc. 71st Intl. Council for the Exploration of the Sea, C.M. 1983/Gen: 10/1-18.

Brock, J.A., L. K. Nakagawa, H. Van Campen, T. Hayashi and S. Teruya. 1986. A record of *Baculovirus penaei* from *Penaeus marginatus* Randall in Hawaii. J. Fish Dis. 9: 353-355.

Brock, J.A. and D.V. Lightner. 1990. Diseases of Crustacea. Diseases caused by microorganisms. *In:* O. Kinne (ed.), Diseases of Marine Animals, Vol. 3. John Wiley & Sons, N.Y. pp. 245-349.

Bruce, L.D., B.B. Trumper and D.V. Lightner. 1991. Methods for viral isolation and DNA extraction for a penaeid shrimp baculovirus. J. Virological Methods. 34: 245-254.

Couch, J.A. 1974a. Free and occluded virus similar to Baculovirus in hepatopancreas of pink shrimp. Nature. 247: 229-231.

Couch, J.A. 1974b. An enzootic nuclear polyhedrosis virus of pink shrimp: ultrastructure, prevalence, and enhancement. J. Invertebr. Pathol. 24: 311-331.

Couch, J.A. 1981. Virus diseases of invertebrates other than insects. *In*: E.W. Davidson (Ed.), Pathogens of Invertebrate Microbial Diseases. Allanheld, Osmun Publishers, Totowa, New Jersey. pp. 125-160.

Couch, J.A. 1991. Baculoviruses of invertebrates other than insects. *In:* J.R. Adams and J.R. Bonami (Eds.), Atlas of Invertebrate Viruses. CRC Press, Boca Raton, FL.

Holthuis, L.B. 1980. Shrimps and Prawns of the World. FAO Species Catalog. Vol. 1. Food and Agriculture Organization of the United Nations, Rome. 271 p.

Johnson, P.T. 1983. Diseases caused by viruses, rickettsiae, bacteria, and fungi. pp. 1-78, *In*: A.J. Provenzano, Jr. (Ed.), The Biology of Crustacea, Vol. 6. Academic Press, N.Y.

Johnson, P.T. 1984. Viral diseases of marine invertebrates. Helgoländer Meeresunters. 37: 65-98.

Johnson, P.T. and D.V. Lightner. 1988. The rod-shaped nuclear viruses of crustaceans: gut-infecting species. Dis. Aquat. Org. 4: 123-141.

Johnson, S.K., 1990. Handbook of Shrimp Diseases. Sea Grant Publ. No. TAMU-SG-90-601, Texas A&M Univ. 25 p.

Kowalski, M., K. Hannig, G. Klock, P. Gessner, U. Zimmermann, G.A. Neil and D.W. Sammons. 1990. Electrofused mammalian cells analyzed by free-flow electrophoresis. BioTechniques. 9: 332-341.

Krol, R.M., W. Hawkins and R.M. Overstreet. 1990. Reo-like virus in white shrimp *Penaeus vannamei* (Crustacea: Decapoda): co-occurrence with *Baculovirus penaei* in experimental infections. Dis. Aquat. Org. 8: 45-49.

Lester, R.J.G., A. Doubrovsky, J.L. Paynter, S.K. Sambhi and J.G. Atherton. 1987. Light and electron microscope evidence of baculovirus infection in the prawn *Penaeus plebejus*. Dis. Aquat. Org. 3: 217-219.

Lightner, D.V. 1988. Diseases of Penaeid Shrimp. *In*: C.J. Sindermann and D.V. Lightner (Eds.), Disease Diagnosis and Control in North American Marine Aquaculture. Second Edition. Elsevier Scientific Publishing Co., Amsterdam. pp. 8-133.

Lightner, D.V., R.M. Redman and T.A. Bell. 1983a. Infectious hypodermal and hematopoietic necrosis (IHHN), a newly recognized virus disease of penaeid shrimp. J. Invertebr. Pathol. 42: 62-70.

Lightner, D.V., R.M. Redman, T.A. Bell and J.A. Brock. 1983b. Detection of IHHN virus in *Penaeus stylirostris* and *P. vannamei* imported into Hawaii. J. World Mariculture Soc. 14: 212-225.

Lightner, D.V., R.M. Redman and T.A. Bell. 1983c. Observations on the geographic distribution, pathogenesis and morphology of the baculovirus from *Penaeus monodon* Fabricius. Aquaculture. 32: 209-233.

Lightner, D.V. and R.M. Redman. 1985a. A parvo-like virus disease of penaeid shrimp. J. Invertebr. Pathol. 45: 47-53.

Lightner, D.V., R.M. Redman, R.R. Williams, L.L. Mohney, J.P.M. Clerx, T.A. Bell and J.A. Brock. 1985. Recent advances in penaeid virus disease investigations. J. World Maric. Soc. 16: 267-274.

Lightner, D.V., L.L. Mohney, R.R. Williams and R.M. Redman. 1987. Glycerol tolerance of IHHN virus of penaeid shrimp. J. World Aquacult. Soc. 18: 196-197.

Lightner, D.V. and R.M. Redman. 1991. Hosts, geographic range and diagnostic procedures for the penaeid virus diseases of concern to shrimp culturists in the Americas. *In*: P. DeLoach, W.J. Dougherty, and M.A. Davidson (Eds.) Frontiers of Shrimp Research. Elsevier, Amsterdam. pp. 173-196.

Lightner, D.V. and R.M. Redman. 1992. Geographic distribution, hosts, and diagnostic procedures for the penaeid virus diseases of concern to shrimp culturists in the Americas. *In*: A.W. Fast and L.J. Lester (Eds.) Culture of Marine Shrimp: Principles and Practices. Elsevier. pp. 573-592.

Loh, P.C., Y. Lu and J.A. Brock. 1990. Growth of the penaeid shrimp virus infectious hypodermal and hematopoietic necrosis virus in a fish cell line. J. Virological Methods. 28: 273-280.

Lu, Y., P.C. Loh and J.A. Brock. 1989. Isolation, purification and characterization of infectious hypodermal and hematopoietic necrosis virus (IHHNV) from penaeid shrimp. J. Virological Methods. 26: 339-344.

Lu, Y., E.C.B. Nadala, J.A. Brock and P.C. Loh. 1991. A new virus isolate from infectious hypodermal and hematopoietic necrosis virus (IHHNV)-infected penaeid shrimps. J. Virological Methods. 31: 189-196.

Maniatis, T., E.F. Fritsch and J. Sambrook. 1982. Molecular cloning. A laboratory manual. C.S.H., 545 p.

Mari, J., J.R. Bonami and D. Lightner. In preparation {a}. Structure and cloning of the genome of IHHNV, an unusual parvovirus pathogenic for penaeid shrimp. Diagnosis of the disease using a highly specific probe.

Mari, J., J.R. Bonami, B.T. Poulos and D.V. Lightner. In preparation {b}. Partial charac-

terization and cloning of the genome of the PmSNPV (MBV = monodon baculovirus) from *Penaeus monodon*.

Mathews, R.E.F. 1982. Classification and nomenclature of viruses. Intervirology. 17: 1-199.

Momoyama, K. 1983. Studies on baculoviral mid-gut gland necrosis of Kuruma shrimp (*Penaeus japonicus*)-III. Presumptive diagnostic techniques. Fish Pathol. 17: 263-268.

Momoyama, K. 1988. Infection source of baculoviral mid-gut gland necrosis (BMN) in mass production of Kuruma shrimp larvae, *Penaeus japonicus*. Fish Pathol. 23: 105-110.

Momoyama, K. 1989a. Virucidal effect of some disinfectants on baculoviral mid-gut gland necrosis virus (BMN). Fish Pathol. 24: 47-49.

Momoyama, K. 1989b. Tolerance of baculoviral mid-gut gland necrosis virus (BMNV) to ether, NaCl concentration and pH. Fish Pathol. 24: 175-177.

Momoyama, K. 1989c. Survival of baculoviral mid-gut gland necrosis virus (BMNV) in infected tissues and sea water. Fish Pathol. 24: 179-181.

Momoyama, K. 1989d. Inactivation of baculoviral mid-gut gland necrosis (BMN) virus by ultraviolet irradiation, sunlight exposure, heating and drying. Fish Pathol. 24: 115-118.

Momoyama, K. and T. Sano. 1988. A method of experimental infection of Kuruma shrimp larvae, *Penaeus japonicus* Bate, with baculoviral midgut gland necrosis (BMN) virus. J. Fish Dis. 11: 105-111.

Momoyama, K. and T. Sano. 1989. Developmental stages of Kuruma shrimp, *Penaeus japonicus* Bate, susceptible to baculoviral mid-gut gland necrosis (BMN) virus. J. Fish Dis. 12: 585-589.

Nash, M., G. Nash, I.G. Anderson and M. Shariff. 1988. A reo-like virus observed in the tiger prawn, *Penaeus monodon* Fabricius, from Malaysia. J. Fish Dis. 11: 531-535.

Olafsen, J.A. 1986. Invertebrate lectins: biochemical heterogeneity as a possible key to their biological function. Chapter 8. *In*: M. Brehelin (Ed.). Immunity in Invertebrates. Cells, Molecules, and Defense Reactions. Springer-Verlag, Berlin. pp. 94-111.

Olafsen, J.A. 1988. Role of lectins in invertebrate humoral defense. *In*: W.S. Fisher (Ed.). Disease processes in marine Bivalve Molluscs. Am. Fisheries Soc. Sp. Publ. 18: 189-205.

Overstreet, R.M., K.C. Stuck, R.A. Krol and W.E. Hawkins. 1988. Experimental infections with *Baculovirus penaei* in the white shrimp *Penaeus vannamei* as a bioassay. J. World Aquacult. Soc. 19: 175-187.

Owens, L., S. De Beer and J. Smith. 1991. Lymphoidal parvo-like virus in Australian prawns. Dis. Aquat. Org. 11: 129-134.

Poulos, B.T., B. Trumper, J.R. Bonami and D.V. Lightner. In preparation. Monoclonal antibodies to IHHNV of penaeid shrimp.

Sano, T., T. Nishimura, K. Oguma, K. Momoyama and N. Takeno. 1981. Baculovirus infection of cultured Kuruma shrimp *Penaeus japonicus* in Japan. Fish Pathol. 15: 185-191.

Seidman, C.E. 1989. Introduction of plasmid DNA into cells. *In*: F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl (Eds.). Current Protocols in Molecular Biology. Vol. 1. John Wiley & Sons, N.Y. pp. 1.8.1-1.8.8.

Tsing, A. and J.R. Bonami. 1987. A new viral disease of the shrimp, *Penaeus japonicus* Bate. J. Fish Dis. 10: 139-141.

van Bruggen, E.F.J. 1986. Electron microscopy of hemocyanins 1942-1986. Micron and Microscopica Acta. 17: 167-173.

Part II:

Contributed Papers

SPF Stocks

Selective Breeding of Specific Pathogen-Free (SPF) Shrimp for High Health and Increased Growth

James A. Wyban
The Oceanic Institute
Makapuu Point
Waimanalo, Hawaii 96795, U.S.A.

Abstract

World shrimp farming depends on seed that is either gathered from the wild or produced in hatcheries by wild-caught spawners. This approach is inherently unreliable. In contrast, great success has been achieved through selective breeding in meat production technologies such as poultry, cattle and swine. It is likely that shrimp farming's future will include selective breeding and domestication. Shrimp diseases have had a devastating effect on shrimp farming in both the United States and around the world. In other meat production industries, modern growers rely on certified virus-free stock to avoid virus-caused diseases. Continued expansion of the global shrimp industry will require reliable supplies of virus-free shrimp.

Based on these assumptions, the U.S. Marine Shrimp Farming Program initiated a project in 1989 to develop reliable supplies of specific pathogen-free (SPF) *P. vannamei* for the U.S. industry. The project includes a selective breeding program to improve the quality of the SPF stocks. This paper describes our approach and initial results in developing a selective breeding program for improving the SPF stock production performance.

Introduction

World shrimp farmers produced over 633,000 MT of penaeid shrimp in 1990 (Rosenberry, 1991). Worth more than $2.5 billion, farmed shrimp supplied over 25% of the world's demand. Nearly all of this production was derived from seed either gathered from the wild or produced from wild-caught spawners. Because of the inherent instability of this approach and the tremendous success of breeding programs in other meat production technologies, shrimp farming's future will likely include selective breeding and domestication. Shrimp farmers will use improved stocks, bred for optimal performance in culture.

In the last several years, shrimp diseases have had a devastating effect on U.S. and world shrimp farming. Diseases increase risk, deterring invest-

ment and commercial development. In addition, exotic diseases carried by non-native shrimp are perceived as environmental risks that, in the U.S., could preclude the use of non-native shrimp with the best commercial potential (such as *Penaeus vannamei* and *P. monodon*). Many of these problems are caused by viruses, for which there are no therapeutic cures (Kalagayan et al., 1991; Lightner, In press). In other meat production industries such as poultry, cattle and swine, modern growers rely on certified virus-free stocks to avoid virus-caused disease. Similarly, sustainable development of the global shrimp industry will require reliable supplies of virus-free shrimp.

Building on these assumptions, the U.S. Marine Shrimp Farming Program (USMSFP) initiated a project in 1989 to develop specific pathogen-free (SPF) *P. vannamei*. The goal of the USMSFP is to stimulate expansion of the shrimp farming industry in the United States. Therefore, the purpose of its SPF program is to establish reliable supplies of SPF *P. vannamei* for the U.S. industry. Following establishment of SPF shrimp stocks, the USMSFP planned to initiate a selective breeding program to improve the production quality of the SPF shrimp.

The establishment and performance of the SPF stock has been described by Wyban et al. (1992). This paper describes our approach and initial results in developing a selective breeding program for improving the SPF stock production performance.

In 1990, a collaboration with Dr. James Lannan, Oregon State University, was undertaken to develop a management plan for the SPF stock (Lannan and Wyban, 1990). Recognizing the genetic implications of breeding the small closed population of SPF shrimp, the plan was designed to avoid inbreeding and conserve the genetic integrity of the SPF stock. As the success of the SPF program emerged, opportunities to expand the broodstock management program into a breeding program for domestication and improvement of the SPF stock of *P. vannamei* increased.

A breeding advisory group was convened at The Oceanic Institute (OI) in July 1991. The group included breeding experts from the poultry, swine and salmon industries who were brought to Hawaii to help plan the breeding program for SPF shrimp. The establishment of founding stocks, quarantine and screening protocols, nuclear herd management, facility requirements, breeding strategies and commercial opportunities for the SPF breeding program were discussed. The breeding advisory group included: Drs. James Lannan, Oregon State University; Trygve Gjedrem, Institute of Aquaculture Research, Norway; Fred Shultz, Animal Breeding Consultant, California; and Mr. Andrew Coates, Pig Improvement Company, Kentucky.

Following several days of formal presentations and lively discussions, the breeding experts provided detailed recommendations on developing a breeding program for SPF shrimp. This

manuscript is a synthesis of the recommendations provided by the advisory group. It also describes progress to date toward establishing a selective breeding program for SPF *P. vannamei*.

Goals of the SPF Breeding Program

The goal of the breeding program is to develop a faster-growing cultured shrimp (*P. vannamei*) through selective breeding.

The specific objectives of the SPF breeding program are:

- To maintain the SPF status of stocks;
- To avoid inbreeding; and
- To improve shrimp growth and survival to market size (20 g).

Establishing the Founder SPF Stock

Building on the SPF concept developed for livestock industries, the SPF shrimp program was initiated in 1989 (Wyban et al., 1992). Since *P. vannamei* is the principal shrimp species cultured in the United States and the rest of the Western Hemisphere (Rosenberry, 1991), it was chosen as the target species for the program.

The definition of what constitutes an SPF shrimp population follows the guidelines developed by the International Council for the Exploration of the Sea for working with exotic species (ICES, 1988). Only disease-causing microbes that can be reliably diagnosed and physically excluded from a facility are considered. Diagnosable microbes that cause economically significant disease in *P. vannamei* and that can be excluded from a facility (specific pathogens) are listed in Table 1.

Table 1. A working list of excludable pathogens of *Penaeus vannamei*.

Group	Pathogen
Virus	Infectious hypodermal and hematopoietic necrosis virus (IHHNV)
Virus	*Baculovirus penaei* type-A baculovirus (BP)
Virus	Hepatopancreatic parvo-like virus (HPV)
Protozoan	Microsporidians
Protozoan	Gregarines
Protozoan	Haplosporidians
Metazoan parasites	Nematodes and cestodes

Development of a historical record through ongoing screening is necessary to insure SPF status. Throughout this manuscript, broodstock shrimp that have passed through this rigorous process are referred to as "SPF broodstock." When SPF broodstock are transferred to a commercial facility, they and the nauplii and postlarvae derived from those broodstock are referred to as "high health" shrimp, because their SPF status is no longer certain, and a new historical record for that facility must be established.

In June 1989, postlarval *P. vannamei* were imported from a hatchery in Mexico. Following preliminary SPF diagnosis by histology, these shrimp were shipped to Hawaii and maintained in

quarantine. Bioassays confirmed that these shrimp were IHHN-free and repeated histopathology examinations indicated they were also free of the other excludable pathogens. They were then shipped to OI's SPF shrimp quarantine facility (OI-Keahuolu) on the island of Hawaii. Postlarvae produced from these "tentative" SPF broodstock were diagnosed as SPF, thus confirming the stock's full SPF status. This founding group of SPF *P. vannamei* is referred to as Kona Population 1.

Addition of New Genetic Material

The original population of SPF shrimp probably represents a narrow genetic sampling of the species. To avoid detrimental founder effects, the breeding program was advised to start with the widest possible sampling of the species (Shultz, 1986; Gall, 1990). However, rigorous screening of new stock additions must be employed to protect the valuable SPF status of the first population.

Numerous attempts to acquire additional samples of SPF postlarvae from the wild to expand the gene pool of the SPF stock have been unsuccessful. IHHN virus is now widespread in wild stocks of *P. vannamei* throughout its range (Lightner et al., 1990; Pantoja-Morales and Lightner, 1991; Lotz et al., 1991). A new approach to developing SPF populations involving nondestructive individual broodstock screening using gene probe diagnostic procedures is currently being tested (Lightner, this volume).

A genetic diversity analysis comparing *P. vannamei* within its natural range to Kona Population 1 was recommended (Lannan, 1980) and has been initiated. Researchers from Tufts University and Worchester Polytechnical Institute are using molecular techniques (both nuclear and mitochondrial DNA) for this purpose. Similar techniques have been used in other marine invertebrates (Brown and Paynter, 1991). In addition to providing measures of diversity within the SPF population(s), the molecular techniques used for diversity analysis may also yield "marker genes" that can be used in the breeding program, if they can be correlated with growth.

A comparison of genetic variation in a farmed population of *P. vannamei* with three natural populations across the species range using allozyme techniques found very low levels of variation and heterozygosity in all four stocks, and very low levels of differentiation between the wild populations (Sunden and Davis, 1991). The authors concluded that allele frequencies among populations throughout the species range have little variation. However, traits of economic (breeding) importance may be under different selective pressures across the range, and differentiation between populations or locales could be significant.

Breeding Program Design

In maintaining captive broodstocks, there are two goals that impose conflicting requirements for management. The

first goal requires procedures that avoid detrimental inbreeding of the captive broodstock. The second goal requires genetic manipulation to improve production performance. To preclude conflict, the SPF breeding program consists of two levels: multiple discrete populations and multiple maternal lineage families within each population (Fig. 1). A similar two-level broodstock management program is used in Norway to breed Atlantic Salmon (Refstie, 1990).

Previous selective breeding efforts for reproductive quality indicated that *P. vannamei* responds to selection, and reproductive quality can be improved by selective breeding (Wyban et al., submitted). Realized heritability estimated for several quantitative variables including nauplii/spawn, spawning frequency and hatch rate were compared in two selected families against nonselected controls. Two different full-sib families from the two outstanding reproductive females with the highest hatch rates, mating/spawning frequency, and lifetime nauplii production were reared to adult size. Two independent experiments were conducted comparing the selected families against nonselected controls. Selected females far out-produced the nonselected controls in both experiments, indicating that *P. vannamei* will respond to selection for a quantitative character.

Because much of the fundamental knowledge about inheritance of economic traits in shrimp is unknown, a number of foundation experiments were recommended. Best opportunities to improve shrimp performance by selective breeding will be verified by systematic, scientific methods to optimize the effectiveness of the breeding program to improve economically important traits for shrimp culture.

The objectives of these foundation experiments are:

- To determine male and female contributions to the quantitative traits, growth and survival;
- To estimate variance components (additive and nonadditive genetic variation, and nongenetic variation) and phenotypic, genetic and environmental correlations.

If there is additive genetic variance for growth rate, individual selection will be applied. Responses from each generation will add to previous gains — as steps in a stairway. If there is heterosis for growth, it will be utilized to add to the effects of individual selection. While crossbreeding must be repeated each generation, it has the advantage that one's competitors cannot duplicate the specific crosses.

A "sub-line" system will be used in the breeding program. A population (resulting from one importation) will be subdivided into multiple, genetically isolated, maternal lineage families. The system is based on the theory that because of inbreeding, genetic variance (V) within a family will go to **zero** as the inbreeding coefficient (F) goes to 1. As the variance between families in-

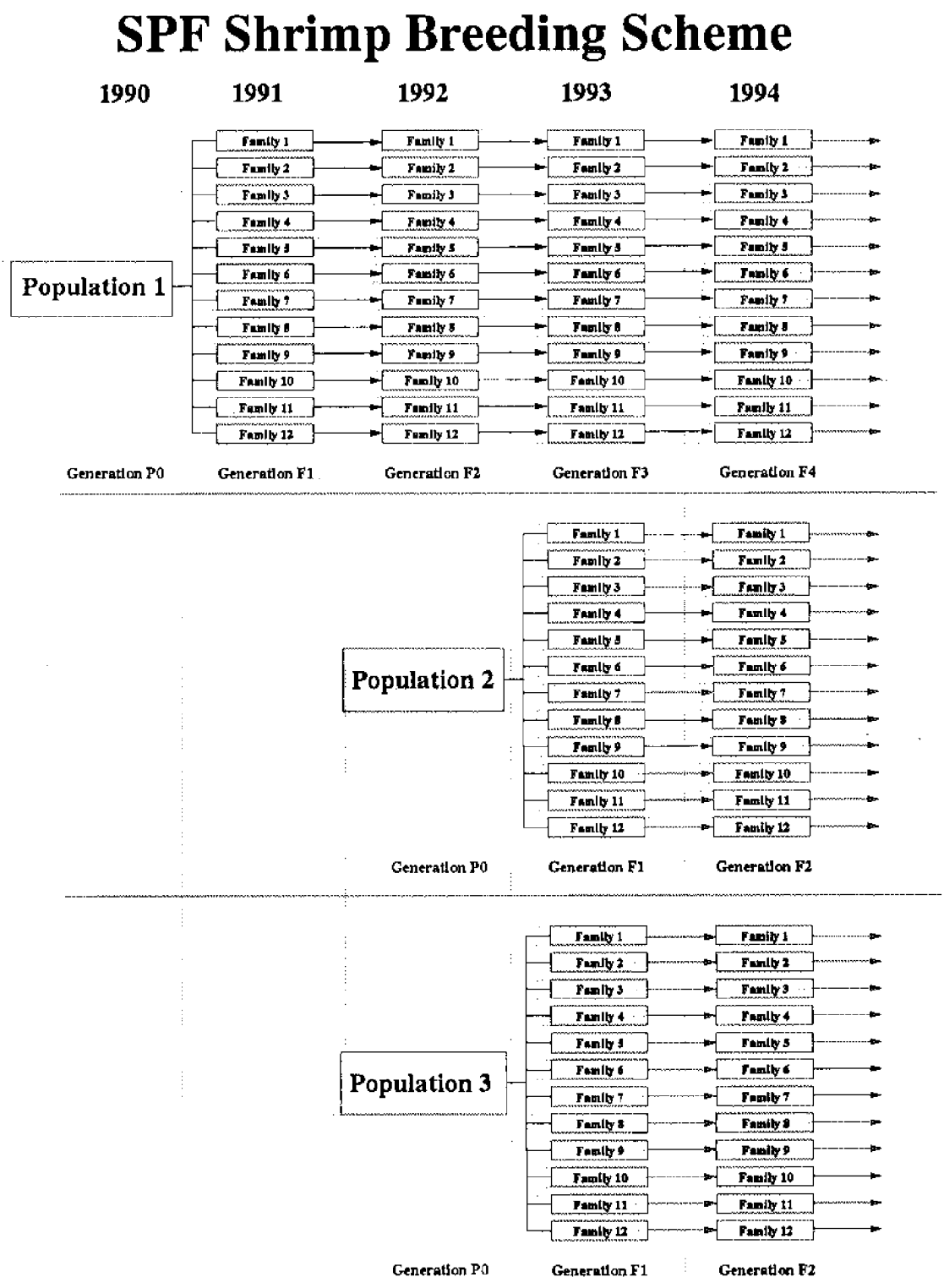


Figure 1. SPF shrimp breeding scheme. Genetically discrete populations are subdivided and maintained in 12 individual families.

creases, the total variance of the population increases to 2V. In reality, V and F never reach these extremes and selection plays an important modifying role.

SPF Broodstock Pedigrees

Current procedures permit accurate identification of the maternal parent of a cross, but the paternal parent is not determined. Therefore, pedigrees based on maternal families will be maintained for each SPF population. To ensure continuity of the pedigrees, the progeny resulting from the mating of each maternal line will be reared in separate tanks up to market size, when they are large enough to be individually tagged with bird bands. The families will then be combined and mass reared in broodstock ponds.

Mating Scheme

In each generation, two selected females from each maternal line family will be mated with two males chosen at random from the broodstock, and no male will be mated to more than one female. The following mating plan will ensure that the effective population size is maximized:

1. Stock four females from each of 12 families, and four males from each of 12 families into maturation tanks.

2. Collect all females mated during an active one-week period (to eliminate multiple matings by a single male); spawn at least one female from each family and stock individual larval-rearing systems.

3. For families that have not successfully reproduced, artificial insemination will be used with randomly chosen males to produce progeny from the remaining lines.

4. Nauplii from each family will be reared in separate 20-L buckets to PL10 stage. Each family will then be moved outdoors and reared separately in 5,000-L tanks up to 15 to 20 g, when individuals will be tagged with bird band eye tags.

5. At tagging size, the largest 20% of males and 20% of females in each family will be selected and tagged; smaller animals will be discarded.

Selection for Growth Rate

Improved growth rate to market size is the number one breeding priority for production cost reduction (Gjedrem, 1985). Sensitivity analysis of breakeven price to input costs and performance in intensive shrimp culture found that survival and growth rate had the greatest effects of all factors studied (Wyban et al., 1988). Thus, improvement of these two factors are the principal targets for the selective breeding program. The phenotypic correlation between weight at market age (average 20 g population) and adult weight (when selected for breeding) will be determined early in the program. If the correlation is high, shrimp will not

have to be weighed at market age, or both weights can be used for selection. If the correlation is low, selection will be at market age. Whole shrimp weight will be used as the criterion for selection.

Selection for disease resistance was not recommended by the advisory group. Knowledge of shrimp diseases to allow effective exposures or to adequately measure shrimp response is insufficient at this time. This technical problem has been described as it relates to testing efficacy in drugs for shrimp (Bell, this volume).

If genotype-environment interaction is important, separate stocks for different environments must be developed. If there is no interaction, ranking of strains or crosses for breeding value is the same in different environments, and the selection scheme can concentrate on developing the best strain for all environments. In Salmonid fishes and tilapia, genotype-environment interaction is negligible (Gjedrem, 1985). Early in the program, experiments will be conducted to determine whether genotype-environment interaction is important.

Facilities

uccessful implementation of the SPF reeding program requires a network f facilities. To introduce new popula-ons, a quarantine is required where ntested "tentative" SPF stock can be aintained and tested. When adults, e "tentative" stocks are moved to a quarantine reproduction facility and their resultant offspring are tested. If the offspring are SPF, then these new stocks can be introduced to the SPF nucleus breeding center.

Nucleus Breeding Centers

The nucleus breeding center (NBC) is where the SPF breeding program is implemented. It was unanimously recommended by the breeding advisory group that two SPF facilities at separate, isolated locations be established. Two or more facilities are required to reduce risk of losing broodstocks because of contamination with infectious agents. All of the selective breeding activities are conducted at the NBCs, which are operated under strict quarantine procedures. Each facility should be capable of producing and maintaining at least 48 families in four populations. Postlarvae from the best strains produced by the selection program will be distributed from the NBCs to broodstock multiplication centers (Fig. 2).

Multiplication Centers

The nucleus breeding centers are not designed to produce enough broodstock to satisfy industry demand. Regional multiplication centers to produce SPF broodstock for commercial distribution must be developed. This approach follows the model developed in Norway for breeding Atlantic Salmon (Refstie, 1990). These centers will obtain improved SPF postlarvae from the NBCs to be cultured to broodstock for commercial distribution. The multipli-

cation centers must have full quarantine capabilities in order to maintain high-health status and exclude pathogens throughout the one-year broodstock culture cycle. The resulting high-health broodstock will then be transferred to commercial hatcheries for seed production.

Progress Toward Goals

As of April 1992, Kona Population 1 has been subdivided into 12 separate full-sib families and two of these families reached the F2 generation. Performance data collected for each family are listed in Table 2.

Each family was spawned from a single female mated with a random male, and larvae were reared in separate 20-L buckets. At PL10, shrimp in each family were stocked into separate outdoor tanks (5,000 L). Nurseries were harvested at PL58-PL65. Three hundred fifty random shrimp from each family were restocked into growout, and biweekly growth rates were monitored during growout until harvest after 20

Table 2. Growth performance data recorded for each family in SPF breeding program.

Stage	Performance Data
Z2	Survival (%)
PL10	Survival (%)
PL30	Mean individual weight (g)
PL45	Mean individual weight (g)
PL45	Coefficient of variation (CV) in size (%)
PL45	Survival (%)
PL45	FCR
PL60	Mean weight (g)
PL60	CV (%)
PL60	Survival (%)
PL60	FCR
PL60 to 20 weeks	Biweekly individual weight (g)
20 weeks	Mean weight (g)
20 weeks	CV (%)
20 weeks	Survival (%)
20 weeks	FCR

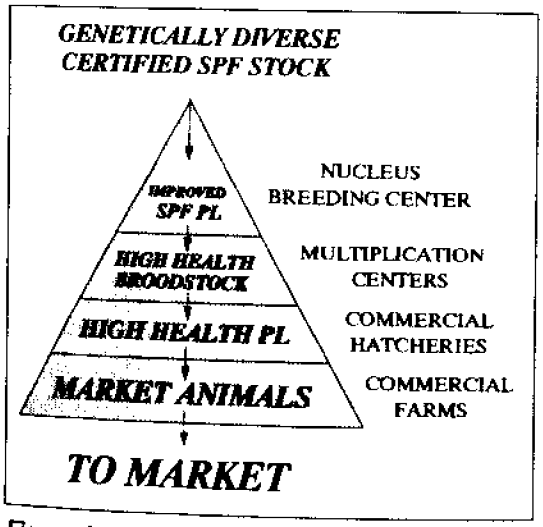


Figure 2. Production pyramid for high health, genetically improved shrimp. Shrimp only move down the pyramid. Shrimp products are listed inside the pyramid; facilities are listed to the right.

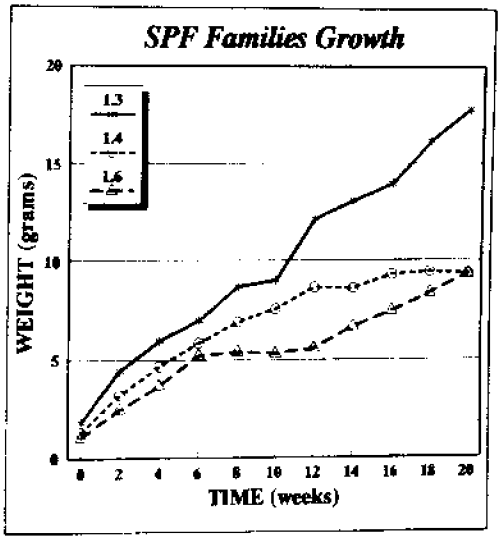


Figure 3. Growth of three SPF shrimp families of identical age cultured under identical conditions.

weeks. Figure 3 illustrates growth of three families spawned on the same day (same age) and grown under identical conditions. The difference in growth among the three families indicates there is significant diversity for growth among the families in Population 1.

As of April 1992, seven families that reached selection size (20 weeks in growout) have been harvested. Their relative production performance is plotted in Figure 4. Families were ranked by mean size at 20 weeks and coefficient of variation (CV), food conversion ratio (FCR), and survival were also plotted using the same family ranking. These seven families were not all reared simultaneously (as in Fig. 3), so environmental differences (temperature) could have contributed some of the variation in growth. Nonetheless, the substantial variability in growth among these families suggests there may be sufficient genetic variation to improve *P. vannamei* growth rate by selective breeding. In addition, the significant negative correlations of CV ($p < .01$) and FCR ($p < .05$) with growth suggest that the best family in terms of growth is also the best in other production criteria.

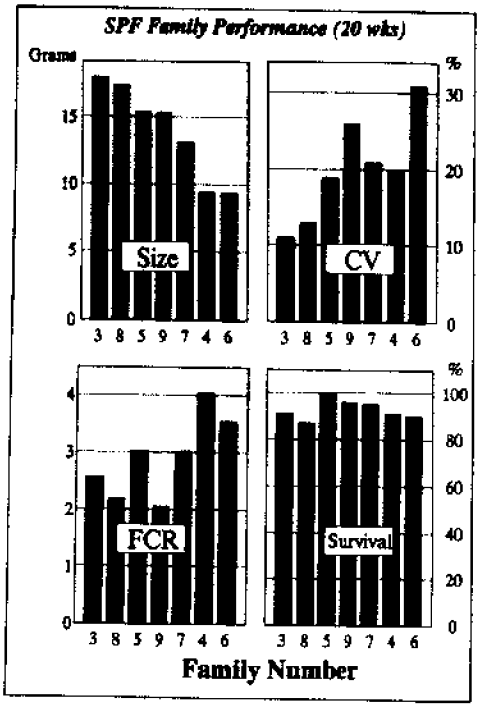


Figure 4. Growth performance of seven SPF families ranked by size at 20 weeks in growout. Using the same ranking, coefficient of variation (CV) in individual size, feed conversion ratio (FCR) and survival are also listed.

The breeding of SPF shrimp for high health and improved growth is only beginning. Based on these preliminary results and knowledge of the success enjoyed by breeders of other meat animals, significant opportunities to improve shrimp performance and advance the industry are waiting.

Conclusions

- A single population of SPF *P. vannamei* has been established in Hawaii and is called Kona Population 1.
- A breeding program designed to protect their SPF status, avoid unnecessary inbreeding, and improve growth and survival through selective breeding has been established.
- Kona Population 1 has been subdivided into 12 full-sib families.

- Significant variation in growth among families was observed.

- A strong correlation between growth to market size and size uniformity and feed conversion efficiency was observed.

Acknowledgments

The advice of the breeding advisory group is sincerely appreciated. It includes Drs. James Lannan, Fred Shultz and Trygve Gjedrem and Mr. Andrew Coates. Mrs. Betty Sonoda provided office support. Funding for this project from CSRS of the U.S. Department of Agriculture to the U.S. Marine Shrimp Farming Program Grant No. 91-38808-5851.

Literature Cited

Brown, B.L. and K.T. Paynter. 1991. Mitochondrial DNA analysis of native and selectively inbred Chesapeake Bay oysters, *Crassostrea virginica*. Mar. Biol. 110: 343-352.

Gall, G.A.E. 1990. Basis for evaluating breeding plans. Aquaculture. 85: 125-142.

Gjedrem, T. 1985. Improvement of productivity through breeding schemes. GeoJournal. 10(3): 233-241.

ICES. 1988. Code of practice to reduce the risks of adverse effects arising from introduction of non-indigenous marine species. Proceedings of the 1979 Statutory Meeting of the International Council for the Exploration of the Sea (ICES). *In*: C.J. Sindermann and D.V. Lightner, (Eds.). Disease Diagnosis and Control in Marine Aquaculture in the Americas. Elsevier, New York, N.Y.

Kalagayan, H., D. Godin, R. Kanna, G. Hagino, J. Sweeney, J. Wyban and J. Brock. 1991. IHHN virus as an etiological factor of runt deformity syndrome (RDS) in juvenile *Penaeus vannamei* cultured in Hawaii. J. World Aquaculture Soc. 22(4): 235-243.

Lannan, J. E. 1980. Broodstock management of *Crassostrea gigas* I. Genetic and environmental variation in survival in the larval rearing system. Aquaculture. 21: 323-336.

Lannan, J. E. and J. Wyban. 1990. Broodstock management program for SPF shrimp. Unpublished manuscript. The Oceanic Institute. 24 p.

Lightner, D.V. In press. Diseases of cultured penaeid shrimp. *In*: J.P. McVey (Ed.). CRC Handbook of Mariculture: Crustacean Aquaculture. CRC Press, Boca Raton, Fla.

Lightner, D.V., R.R. Williams, T.A. Bell, R.M. Redman and L.A. Perez. 1990. A collection of case histories documenting the introduction and spread of the virus disease IHHN in penaeid shrimp culture facilities in Northwestern Mexico. *In*: C.J. Sindermann (Ed.). Proceedings of the International Symposium on the Effects of Introductions and Transfers of Aquatic Species on Resources and Ecosystems. Special Publication of the World Aquaculture Society. pp. xx-xx.

Lotz, J.M., R.M. Overstreet, D.V. Lightner and R.M. Redman. 1991. Occurrence of IHHN virus in penaeid shrimp from wild populations of the eastern Pacific Ocean. 22nd Annual Meeting of World Aquaculture Society (Abstract).

Pantoja-Morales, C. and D.V. Lightner. 1991. Status of the presence of IHHN virus in wild penaeid shrimp from the coast of Somera Mexico. Society for Invertebrate Pathology. (Abstract)

Refstie, T. 1990. Application of breeding schemes. Aquaculture. 85: 163-169.

Rosenberry, R. 1991. World Shrimp Farming, 1990. Aquaculture Digest, San Diego, CA. 55 p.

Shultz, F.T. 1986. Developing a commercial breeding program. Aquaculture. 57: 65-76.

Sunden, S.L.F. and S.K. Davis. 1991. Evaluation of genetic variation in a domestic population of *Penaeus vannamei* (Boone): a comparison with three natural populations. Aquaculture. 97: 131-142.

Wyban, J.A., J.N. Sweeney and R.A. Kanna. 1988. Shrimp yields and economic potential of intensive round pond system. J. World Aquaculture Soc. 19(4): 210-217.

Wyban, J.A., J. Swingle, J.N. Sweeney and G.D. Pruder. 1992. Development and commercial performance of high health shrimp from SPF broodstock *Penaeus vannamei*. *In*: J. Wyban (Ed.). Proceedings of the Special

Session on Shrimp Farming, World Aquaculture Society, Baton Rouge, LA. pp. 254-260.

Wyban, J.A., R. Oyama and R. Deupree. Submitted. Response to selection for reproductive quality in the Pacific white shrimp, *P. vannamei*. Aquaculture.

Developing Specific Pathogen-Free (SPF) Animal Populations for Aquaculture: A Case Study for IHHN Virus of Penaeid Shrimp

Jeffrey M. Lotz
Gulf Coast Research Laboratory
P.O. Box 7000, 703 E. Beach Blvd.
Ocean Springs, Mississippi, U.S.A.

Abstract

To secure specific pathogen-free (SPF) broodstock for integration into nuclear breeding operations, the utmost care must be used to detect pathogens and to prevent the introduction of pathogens. Confidence in the SPF status of a founder population increases with the development of a lengthy history of negative diagnostic results and increases with the variety and sensitivity of the diagnostic methods employed. The relatively small number of animals needed and the finite time frame makes founder population acquisition easier than securing SPF seed. Wild stocks can be sources of SPF animals; however, aquaculture activities have spread pathogens to wild stocks, diminishing the supply of wild SPF animals. The procedures employed to secure SPF populations from wild stocks can, in principle, be extended to extract SPF individuals from contaminated populations.

Introduction

Since the discovery and subsequent description of infectious hypodermal and hematopoietic necrosis virus (IHHNV) (Lightner et al., 1983a), the virus has been a major force in determining the activities and directions of the U.S. shrimp farming industry. In fact, the widespread use of *Penaeus vannamei* rather than the faster growing *P. stylirostris* is largely because *P. vannamei* is more resistant to IHHNV-induced mortality.

IHHNV is a parvovirus (Bonami et al., 1990) and its course of infection in *P. stylirostris* has been well documented (Bell and Lightner, 1984; 1987). Juveniles with acute IHHN disease exhibit reduced feeding, mottling of the cuticle, unusual swimming behavior, and, ultimately, 80 - 95% mortality. The infection in *P. vannamei*, on the other hand, induces no clear signs of disease and no dramatic increase in mortality. Hence, *P. vannamei* was considered an asymptomatic carrier of the virus (Lightner et al., 1983b).

However, recently there has been evidence that IHHNV infections can cause disease syndromes in *P. vannamei* under typical aquaculture conditions. In 1989, an extensive epizootic of IHHNV infection in *P. vannamei* occurred throughout the U.S. shrimp farming industry as well as elsewhere in the Western Hemisphere. At the same time, there was an increase in reports of runting, deformities and decreased production on farms. In 1991, Kalagayan et al. linked IHHNV to runt-deformity syndrome (RDS) by demonstrating the presence of RDS in IHHNV-infected animals but not in IHHNV-free animals. Although the precise relationship between IHHNV and RDS has yet to be detailed, it is clear that IHHNV can have a severe negative impact on cultured *P. vannamei*. Concomitant with the demonstration that IHHNV can cause serious diseases in aquacultured penaeids, concern over the possible release of the exotic virus into U.S. coastal waters grew.

These two factors resulted in an effort within the United States to control the spread of IHHNV. The Gulf Coast Research Laboratory Consortium (GCRLC) is responsible for this effort. The GCRLC was formed in 1984 to accelerate the development of marine shrimp farming in the United States through research and technology transfer. It is comprised of six institutions working cooperatively (Fig. 1) and is funded by the United States Department of Agriculture.

In 1989, the six institutions comprising the GCRLC undertook to disinfect contaminated facilities at member institutions (four of the six had been contaminated with IHHNV), and restock them with IHHNV-free *P. vannamei*. A source of IHHNV-free *P. vannamei* had been located in Mexico by

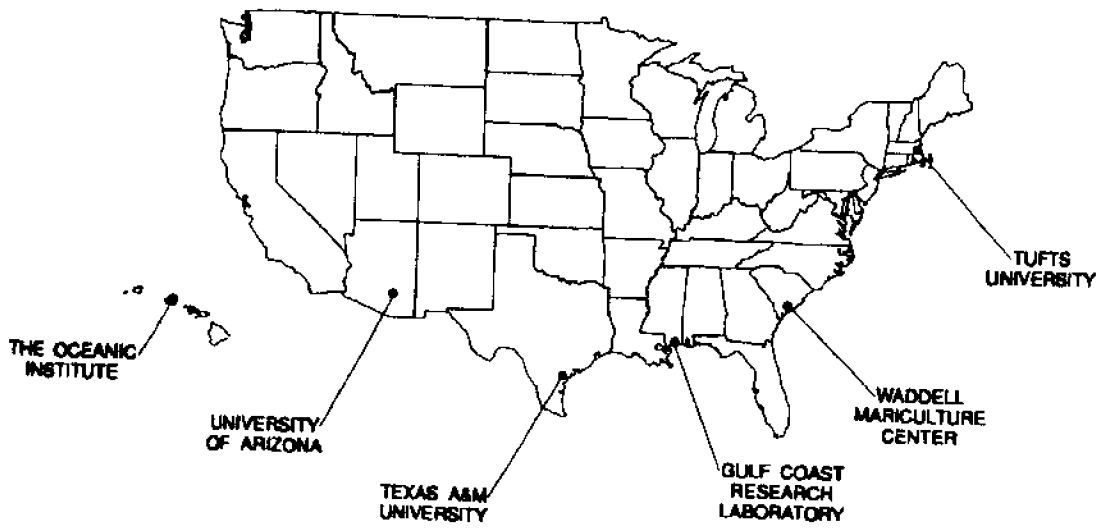


Figure 1. Institutional members of the Gulf Coast Research Laboratory Consortium.

researchers at the University of Arizona. The strategy was to relocate IHHNV-free postlarvae to an isolation facility, grow them to broodstock, and produce postlarvae. The IHHNV-free postlarvae could then be provided to member institutions. In the summer of 1989, the sources of IHHNV-free animals in Mexico were infected with IHHNV. Lightner et al. (In press) have documented the introduction and spread of IHHNV throughout the aquaculture industry in the northern half of Mexico. In addition, Pantoja-Morales and Lightner (1991) have confirmed the introduction of IHHNV into the wild stocks of *P. vannamei, P. stylirostris* and *P. californiensis* of the Gulf of California. Lightner (pers. comm.) has also determined the existence of IHHNV in wild *P. vannamei* and *P. occidentalis* from the coast of Ecuador. Finally, Lotz et al. (1991) also found IHHNV in wild *P. vannamei* and *P. stylirostris* postlarvae in the Gulf of California and throughout the Pacific coastal waters of Central America (Figs. 2 and 3).

No animals whose status relative to IHHNV is unknown are allowed into clean facilities. Hence, a domestic supply of certified IHHNV-free animals was needed. The shrimp would need to be subjected to acute quality control measures, and a lengthy history of aggressive and repeated diagnostic testing for IHHNV would need to be developed. Eventually, a plan was developed for obtaining and maintaining a central supply of IHHNV-free animals that would be protected from IHHNV

Figure 2. Map of Mexico, Central America, and northern South America showing the distribution of Penaeus vannamei *and* Penaeus stylirostris. *The two species have co-extensive distributions in the area shown.*

Figure 3. The distribution of IHHNV and BP in penaeid shrimp. The viruses have co-extensive distributions. Shaded areas represent the occurrence of IHHNV and BP. Unshaded areas represent regions which have not been surveyed.

as well as other pathogens and from the dangers of genetic inbreeding. The plan has been initiated by the GCRLC, and the resulting program may be viewed as the initial phase in the domestication of *P. vannamei*.

Founder Populations

The nucleus of the protected stocks is kept in strict isolation and managed under a protocol to maintain sufficient genetic diversity and allow for the judicious selection of desirable production traits. The operation of the nuclear breeding facility is detailed in Wyban (this volume) and will not be dealt with further here. Instead, I will focus on the efforts of the GCRLC to secure SPF populations for the start or enhancement of stocks in the nuclear breeding facility. Populations destined to be incorporated into the nuclear breeding facility are referred to as founder populations.

The goal of any founder population acquisition is to provide specific pathogen-free shrimp that can increase the genetic diversity of the stock held at the nuclear breeding facility. Therefore, it would be desirable to secure several founder populations of shrimp from distinct geographic locations throughout the natural range of *P. vannamei*. The more animals that are obtained from more sites, the greater the increase in genetic diversity at the nuclear breeding facility. However, one must also consider what can actually be acquired and subsequently maintained. Attention to the resources available, the biology of the shrimp, and the experiences of animal breeding programs in poultry, salmon, and swine caused us to seek approximately 12 separate male-female crosses from each of three to ten sites. Therefore, the total number of animals needed (if collected from ten sites) is 240 (120 males and 120 females). Once these animals are in place, no more importations are necessary.

The small number of animals to be secured and the finite number of founder populations necessary to accomplish the goal sets this type of activity apart from the more extensive and continuous import of animals for general seed acquisition or production. Supplying animals to found a nuclear breeding facility entails low volume, but necessitates high confidence in assuring that pathogens of interest are absent from all 240 animals.

Founder Population Acquisition

The initial step in securing founder populations is to determine which pathogens and potential pathogens should be excluded. The next step is to locate potential sources of founder populations, and the third step is collecting the potential founder populations. The fourth step is to ensure the candidate founder populations are free of all pathogens that are to be excluded.

Pathogens to be Excluded

It is essential to itemize the specific pathogens to be excluded from the

founder populations. Goals such as "disease-free" are too general to be useful. No animal populations can be maintained free of disease; the term "disease" includes maladies of unknown and unforeseen causes. It is not possible to exclude agents that are unknown. Because new agents are being described from penaeid shrimp regularly, it is probably unavoidable that a new agent will eventually be detected in established nuclear breeding stocks.

Further, no animal population can be maintained free of a pathogen that is undetectable or effectively undetectable. For example, the reo-like viruses presently are only detectable with the aid of a transmission electron microscope. To undertake the development of reo-free shrimp stocks would be premature. Nevertheless, it is of paramount importance that all pathogens in the stock be known, including those that may not actually be on the list of pathogens to be excluded. As the nuclear breeding stocks become more valuable to an industry, expensive measures may be justified to rid these stocks of a new agent.

Known obligate parasites of unknown effect should be excluded if possible. (In this paper, "parasite" is defined as any organism that uses another organism as its habitat. This includes bacteria and viruses). In general, it is not good practice to leave a parasite off the exclusion list simply because its effect is unknown; such an agent may become a problem in the future. A parasite that turns out to be a problem in a nuclear breeding stock is much more serious than the same agent in a farm pond. The investment in the nuclear breeding stock is too great to risk not excluding poorly understood parasites.

It is important that all pathogens on the exclusion list can be excluded from the populations. Attempting to exclude organisms such as *Vibrio* sp., facultative parasites of shrimp and humans that are ubiquitous in shrimp growout ponds and tanks, would be futile. However, it might be possible to exclude a serotype of a particular species of *Vibrio*, e.g., one of the human pathogenic *Vibrio cholera* biovars.

On the other hand, some pathogens can be easily excluded from a population while in quarantine. For example, tetraphyllidean cestode larvae are common in wild penaeids. The definitive hosts for these animals are elasmobranch fish; skates, for example. Since shrimp ponds do not have skates in them, the life cycle is not completed and the parasite is eliminated. Gregarines, which often use bivalves as intermediate hosts, are a potentially serious problem. While some intermediate hosts may be found in shrimp ponds, our experience with the gregarine *Nematopsis penaeus* from penaeid shrimp in the Gulf of Mexico suggests that the shrimp lose their infections several days after relocation to a quarantine tank.

The actual list of pathogens to be excluded also depends upon the species of focus and the geographic area under consideration for stock acquisition.

With the extensive movement of shrimp species around the world for aquaculture purposes, the geographic range of a particular pathogen may be less important than the species of shrimp under consideration.

Pathogens of *Penaeus vannamei*

In this section, I will outline the pathogens considered for the exclusion list by the GCRLC. More detailed accounts of the pathogens may be found in Lightner (1983) or Sindermann and Lightner (1988). Although viral pathogens are our primary concern, we considered many other disease agents. Despite our attention to numerous agents, however, prospective founder populations were invariably destroyed as a result of contamination with one of two viruses, IHHNV or *Baculovirus penaei* (BP).

Viruses. The main group of organisms targeted for exclusion from the founder populations are the viruses. Viruses are the most widespread of the serious pathogens of *P. vannamei*, and there are no proven therapeutics to eliminate viruses from infected animals. The initial focus was on IHHNV and BP because these are serious pathogens and they were likely to be encountered frequently. They were, in fact, the most common pathogens encountered. We also considered the other known baculoviruses (occluded and nonoccluded) and the parvo-like viruses to be unacceptable. However, with the possible exception of HPV (hepatopancreatic parvo-like virus), it was unlikely that we would find other known baculoviruses or parvo-like viruses in *P. vannamei*. The reo-like viruses (Krol et al., 1990) were not placed on the exclusion list because no reasonable diagnostic methods exist.

Bacteria. Most shrimp bacterial problems are caused by secondary invasion by free-living bacteria. Targeting these for exclusion would be fruitless. Furthermore, it is extremely difficult to distinguish between bacterial infections acquired in quarantine from those acquired in nature. Were bacteria to become a problem, it is likely that we could have eliminated them during quarantine by the judicious use of antibiotics.

The only bacterial agents we considered as potential primary parasites of shrimp are the rickettsia-like organisms. We therefore screened animals for hepatopancreatic granulomas that might have indicated an infection with rickettsia-like organisms (Krol et al., 1991). Founder populations containing rickettsia-like organisms would have been destroyed.

Fungi. Although fungi (e.g., *Fusarium solani*) are of concern in shrimp culture, they are not considered primary pathogens of shrimp (Lightner, 1988) and were, therefore, not to be excluded. However, the presence of a fungus in a sample may have precluded its incorporation into the nuclear breeding stock as it may have portended other stress-related problems.

Protozoa. The fouling protozoan ciliates (peritrichs such as *Zoothamnium*

sp., suctorians such as *Acineta* sp., and apostomes such as *Hyalophysa* sp.) were not listed for exclusion. However, a heavy infestation could indicate stress in the quarantine facility and might have precluded the inclusion of a sample into the nuclear stocks for general health considerations.

Microsporans (e.g., *Ameson* sp., *Agmasoma* sp., *Pleistophora* sp. and *Thelohania* sp.) were placed on the exclusion list. However, they occur at low prevalence in wild populations, and infected individuals are easily detected and removed from a population during quarantine. In addition, there is some evidence that a piscine primer host may be necessary to allow transmission from one shrimp host to another (Iversen and Kelley, 1976). Microsporans, therefore, could have been eliminated from a contaminated population during the quarantine period because transmission would have been prevented by the absence of fish. The removal of affected individuals would have prevented pathogens from leaving the quarantine facility.

Gregarine protozoans may be of concern in some aquaculture settings. However, they are apparently eliminated spontaneously from shrimp during the quarantine phase. The exclusion of intermediate hosts from quarantine facilities prevents transmission. Therefore, gregarines were not placed on the exclusion list.

Helminths. We did not consider that helminths (cestodes, trematodes, and nematodes) needed to be excluded. The exclusion of intermediate hosts from the quarantine and nuclear breeding facilities would prevent transmission and eliminate most helminths.

Crustaceans. Crustaceans were not placed on the list. Crustaceans such as bopyrid isopods could have been eliminated by removal of infected individuals. These parasites also utilize an intermediate host (e.g., copepods), making transmission more difficult in quarantine.

Locating Potential Sites for Acquisition

Once a list of pathogens to be excluded was developed, the process of screening possible sources of founder populations began. The two options were culture facilities and wild populations. Culture facilities are often contaminated with IHHNV as well as BP throughout the range of culture of *P. vannamei*. Therefore, we surveyed wild populations for the presence of the two agents of primary interest.

Detection of Viruses

All animals were screened for viruses by means of standard histological examination (Bell and Lightner, 1988). IHHNV was detected by the presence of Cowdry Type A intranuclear inclusions in several tissues of ectodermal or mesodermal origin (Lightner et al., 1983a). BP was detected by the presence of intranuclear polyhedral occlusion bodies in cells of the hepatopancreas (Couch, 1974).

The sampling protocol prescribed that initially a site be screened by collecting a small grab sample of animals from the area, fixing the animals for histological examination while still in the field, and subsequently examining them for the presence of IHHNV and BP. If a sample was not positive after the examination of ten to 20 animals, then a large batch of live animals was to be obtained and placed into quarantine. In practice, however, the grab samples and the live samples were taken simultaneously wherever possible. Therefore, samples for examination were a combination of direct samples of wild postlarvae and adults, and a large number of wild postlarvae held in quarantine for 30 to 60 days.

We used postlarvae instead of juveniles or adults for several reasons. First, postlarvae are easier to handle and maintain in quarantine, allowing us to screen a larger number of animals. Second, because animals must be sacrificed for examination, we needed a large enough sample to insure that we would have animals left over. Third, postlarvae or young juveniles often show diagnostic signs of the two viruses better than older juveniles or adults (Bell and Lightner, 1987; LeBlanc and Overstreet, 1990).

If IHHNV was not detected after 60 days in quarantine, a bioassay diagnosis for IHHNV was performed. An IHHNV bioassay diagnosis consists of feeding a sample of *P. vannamei* that is suspected of carrying IHHNV to the more susceptible *P. stylirostris* (Lightner et al., 1983b). After nine to 30 days, any infected *P. stylirostris* will show mortality and the Cowdry Type A intranuclear inclusions characteristic of IHHNV infection.

Assuming animals were negative for IHHNV after a "stylirostris-bioassay," the next step was to ship the remaining animals to a quarantine facility in Hawaii for further examination and clearing. From there, introduction into the nuclear breeding facility was to begin.

Direct integration of the founder population into the nuclear breeding facility is an option. However, in general, it is more prudent to grow the new population into broodstock and introduce tested offspring as families into the nuclear breeding facility.

The absence of a pathogen from a potential founder population can only be assured "to-the-best-of-our-abilities." Thus, for certification, it is necessary to specify the methods of detection used, the number of times the diagnosis was applied, the number of animals to which the diagnosis was applied, and the length of quarantine. Confidence in the absence of a pathogen increases with increased sensitivity of the diagnostic techniques, greater number of tests performed, greater number of animals checked, and longer periods of quarantine.

Quarantine Facilities and Procedures

The primary means of assuring that a pathogen is not present in a founder

population is to develop a lengthy history of negative diagnostic test results. Quarantine is the crucial step in developing an appropriate history.

Quarantine serves three functions. An individual may have been infected with the pathogen of interest only recently, and, therefore, may not have developed the signs of infection. In this case, the quarantine period should provide the time necessary for the shrimp to develop signs of infection. Second, some infectious agents such as BP are more likely to be found if an infected host is stressed (Couch, 1974). Quarantine is usually stressful and can provide such a stimulus. Third, the quarantine procedure can amplify a disease agent within the quarantined sample. Infectious agents present in a small number of animals will eventually be transmitted to uninfected individuals. The parasite becomes more prevalent and can be detected by examining fewer individuals.

The danger in quarantine is, of course, that the quarantined populations are open to exposure and subsequent infection with important pathogens. The use of quarantine requires extremely tight quality control and precautions to prevent contamination of samples.

Quarantine Facilities

The quarantine facility and procedures used at the Gulf Coast Research Laboratory (GCRL) in Ocean Springs, Mississippi, U.S.A., will be examined. The two main goals of the quarantine facilities are preventing the contamination of samples with pathogens from surrounding areas and animals, and preventing the contamination of surrounding grounds and waters with exotic species and their pathogens.

The quarantine facilities at GCRL consist of a large greenhouse (15 m x 9 m) sited on a concrete slab with an 8- cm high lip completely surrounding the slab (Fig. 4). The greenhouse contains 15 2,000-L circular tanks. Each 2,000-L tank has an in-tank biofilter and can be drained individually into effluent PVC drain pipes. The effluent pipes empty into septic tanks equipped with chlorinators. From the chlorinating septic tank, the effluent can be pumped to the municipal sewer line. Contamination between tanks is prevented by operational procedures and the quality of the maintenance crew. The crew is well trained in isolation, sanitation, and disinfection procedures.

The other facility used for quarantine is a 3 m x 5 m isolation room that can accommodate four 650-L circular tanks. Each tank is separated from the others by shower curtains and each tank has its own in-tank biofilter. The tanks are not drained during the holding period and the water is chlorinated at the end of the holding period. Each tank has its own maintenance equipment: thermometer, nets, beakers, etc. The room has a center floor drain that empties into an outside septic tank equipped with a chlorinator. From the chlorinating septic tank, the effluent can be pumped to the municipal sewer line.

Cross contamination is prevented by operational procedures.

We employ sentinel tanks containing uninfected animals whenever possible. For example, we presently use sentinel tanks and aquaria containing IHHNV-free *P. stylirostris* as a check against contamination. The sentinel tanks are treated as the other quarantine tanks and are subjected to the routine procedures employed for the other quarantine tanks.

Quarantine Procedures

To minimize the risk of releasing exotic organisms during quarantine activities, all effluent water is disinfected. Disinfection is accomplished with 100- to 200-ppm chlorine for 1 - 24 h prior to discharge into the municipal sewer lines that terminate at a landfill. All dead animals, molts, feces, etc. are disinfected with chlorine- or iodine-containing disinfectants or by autoclaving. Surfaces are cleaned with chlorine- or iodine-containing disinfectants.

To ensure that infectious diseases are not introduced into the quarantine facilities or transferred between tanks, we established routine sanitary work practices. These include restricted access, the use of foot baths at the entrances to all doors, regular cleaning and disinfecting of equipment and rooms, disinfection of shrimp waste and debris, and clean food preparation areas.

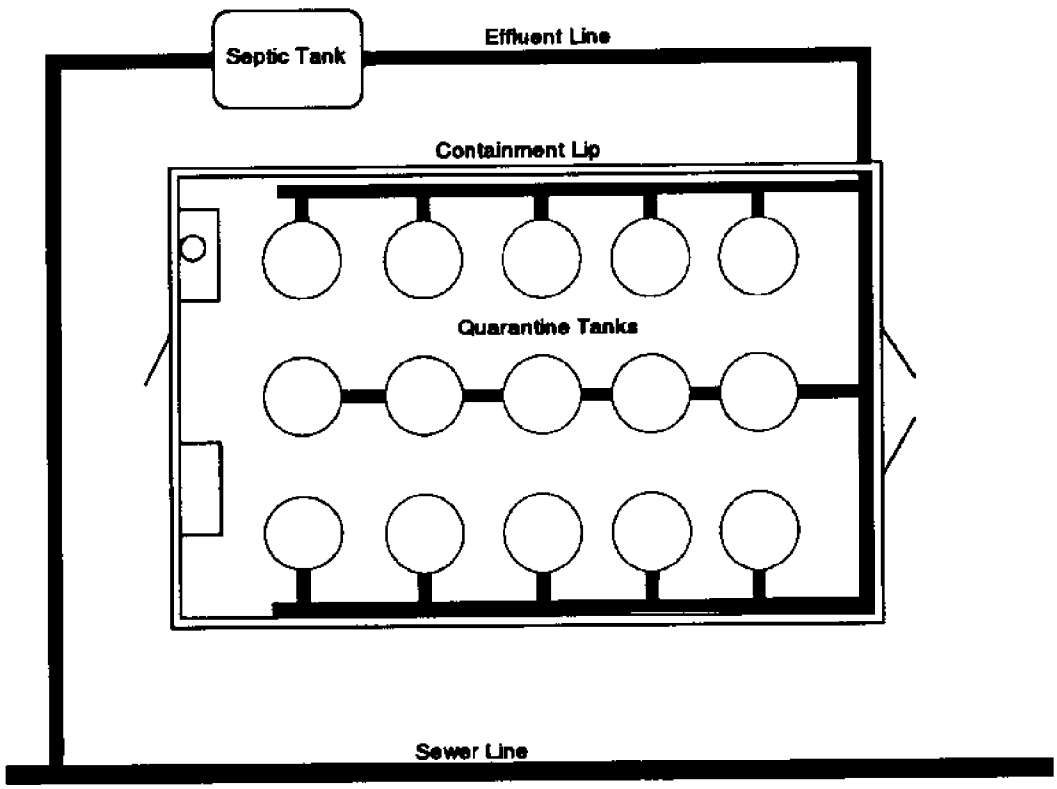


Figure 4. Floor plan of the greenhouse quarantine facility at the Gulf Coast Research Laboratory.

Most introductions and transfers of pathogens occur as a result of the transport of shrimp or shrimp parts. Therefore, special attention is paid to prevent the transfer of tank contents from one to another; similarly, equipment is segregated by tank with no overlap in use. Dry feed is kept away from shrimp and shrimp debris. Each tank has its own nets, and nets are disinfected after each use and allowed to dry completely between daily cleanings. Surgical gloves are used during routine tank cleaning, and gloves are disinfected and discarded after each tank is cleaned. Natural water is used in the facility; it is routinely settled, filtered and disinfected with chlorine.

The SPF Status of Wild *Penaeus vannamei*

The geographic range of *P. vannamei* extends from the northern Gulf of California to the northern portion of Peru (Fig. 2). Through the efforts of the GCRLC, the status of wild *P. vannamei* has been documented along most of its range (Lotz et al., 1990; Lightner et al., In press). The most widespread of the agents of interest is, unfortunately, the virus of most crucial interest: IHHNV.

Figure 3 shows the distribution of IHHNV in wild penaeids throughout the Pacific Coast of the Americas. The highest density of IHHN virus is in the Gulf of California, where all of the specimens in a sample of wild, adult *P. stylirostris* were found to be IHHNV-positive. It is significant that this area has one of the highest densities of *P. stylirostris* anywhere throughout its range. The range of *P. stylirostris* is coextensive with that of *P. vannamei*.

The Gulf of Panama also appears to have high levels of IHHNV, but lower than the Gulf of California. This area yielded fixed-in-the-field samples of *P. vannamei* postlarvae with IHHNV infections. The lowest density of IHHNV appeared to be in the northern portion of Central America. No positive fixed-in-the-field samples were found from Guatemala, Nicaragua, or northern Costa Rica; however, virus was detected in samples from Nicaragua and El Salvador after 30 to 60 days of quarantine. Southern Mexico has not been surveyed; its status is unknown. The waters surrounding Ecuador have yielded evidence that IHHNV is present in wild populations, but surveying has not been as extensive as that in Central America and Northern Mexico.

The survey of shrimp of the Pacific Ocean revealed the presence of numerous parasites in addition to the viruses. Peritrich and apostome ciliates were common on the gills. Gregarines were seen in the intestines. Finally, nematode, cestode, and trematode larvae were observed in the hepatopancreases, muscles and nerve cords of infected shrimp.

Acquiring SPF Stock From Contaminated Sources

Acquiring SPF animals from geographic areas free of the pathogens of interest is simply a matter of collecting

samples and subjecting them to the process of certification. However, if no pathogen-free sites are found, acquiring SPF animals is more complicated.

The data acquired from the survey throughout *P. vannamei*'s geographic distribution revealed that no area was unaffected by IHHNV. Since the widespread outbreak of IHHNV in 1989, no IHHNV-free potential founder populations have been found, despite extensive efforts and the quarantining of numerous samples of wild postlarvae. Therefore, our original objective of locating IHHNV-free geographic sites had to be reformulated. Instead of seeking IHHNV-free populations of shrimp, we now wish to secure a certain number of IHHNV-free individuals from populations of shrimp which are known to carry the virus.

When we conducted our survey using quarantine, it was desirable to collect as large a sample of shrimp as possible in order to be certain that the region was free of the virus. Our target for sampling postlarvae under the initial survey objective was to quarantine 10,000 to 20,000 animals for each sample. If one of those 10,000 to 20,000 animals was infected, then, in time, a large proportion would become infected and the virus would be easily detected after 60 days of quarantine. Presently, there is no way to select individual postlarvae from a contaminated sample. Consequently, if one animal in a sample is positive for the virus, the whole sample is destroyed.

Our inability to detect virus from some areas prior to 60 days in quarantine suggests that only a few animals were carrying the virus. If only a few animals from a given wild population carry the virus, a small sample containing 1,000 to 5,000 shrimp may be virus-free. If the prevalence of an agent in the wild is known, securing animals free of the agent is a statistical sampling problem. What is the optimum sample size to be certain no infected animals are present in a given sample?

The above problem is related to the problem fish inspectors face determining how many fish to examine from a particular batch to have a certain degree of confidence that they will find the pathogen. The American Fisheries Society "blue book" provides a table that recommends how many fish should be examined (Amos, 1985). For example, if a parasite is present in 10% of fish and the lot being evaluated contains 4,000 fish, 27 should be examined in order to be at least 95% confident that the pathogen will be detected, if present. The answer to the fish inspectors' problem is determined from the hypergeometric statistical distribution and applies to sampling small target populations.

When the target population is very large (e.g., a wild population of shrimp) then the related binomial distribution applies. Table 1 provides the maximum sample sizes that ensure at least a 50% chance that no animals in the sample are infected with the pathogen of interest. Once the maximum

Table 1. Sample size that ensures a 50% chance of a parasite being absent from a sample.

Prevalence in source population		Sample size
50.0%	1/2 infected	1
20.0%	1/5 infected	3
10.0%	1/10 infected	6
5.0%	1/20 infected	13
1.0%	1/100 infected	68
0.5%	1/200 infected	138
0.1%	1/1,000 infected	692
0.05%	1/2,000 infected	1,385
0.01%	1/10,000 infected	6,931

acceptable sample size is determined, how many separately packaged samples should be obtained to be 95% certain at least one sample is free of infection?

If there is a 50% chance (probability = 0.5) that an infected animal is present in a sample, the probability that it is present in both of two such samples is

$$0.5 \times 0.5 = 0.25 \ (25\%)$$

and, therefore,

$$100\% - 25\% = 75\%.$$

Hence, there is a 75% chance that the pathogen of interest is absent from either one or both samples. If three such samples are collected, the chances are that 87.5% of the time one of the three samples will have no infected individuals. Through this process, it is clear that if a parasite infects 50% of the packaged samples, one should collect five separately packaged samples to ensure that at least 95% (actually 96.8%) of the time an area is sampled, one of the five samples will be free of infected individuals. The likelihood of finding uncontaminated packages increases as more packages are collected.

Once the size of the sample and the number of samples to be collected is determined, then the collection of animals and the lengthy procedure of quarantine and subsequent development of the critical SPF history begins. Because shrimp will be selected from contaminated areas, the development of SPF histories is critical.

The ability to select animals from the wild rests on the assumption that not all animals from a contaminated wild population carry the pathogen. In principal, obtaining SPF individuals from contaminated culture facilities is the same as obtaining them from wild sources. However, the likelihood that there are specific pathogen-free individuals in a facility is reduced because the animals in culture are at higher densities than in nature. Hence, the

rate of transmission of pathogens is higher. In facilities with a history of IHHNV, the prevalence of infection can be 100%, and is typically higher than that in most wild stocks carrying IHHNV. Obtaining SPF animals from a contaminated facility would, therefore, probably necessitate screening individual shrimp.

Screening Individual Animals

If the technology to reliably screen individual animals was available, the necessity of clearing a whole sample of animals would be eliminated. In the batch quarantine method, an entire batch is rejected if one positive animal is found. However, screening individuals one at a time could yield a large number of negative animals, even if the prevalence of the virus is greater than 50% .

One problem associated with individual screening is the need to take a piece of the animal for histological evaluation. Postlarvae are too small and delicate to do this without sacrificing the animal; large juveniles and adults are more amenable to this procedure. Bell et al. (1990) compared diagnosis of IHHNV using periopods with diagnosis from a more complete histological examination. However, the ability to nondestructively screen large animals is offset by two factors. As animals age (become larger) the likelihood of detecting infections of either IHHNV or BP decreases. Additionally, removing a periopod on several occasions from a single individual increases the likelihood of mortality.

Recently, however, researchers at the University of Arizona have made substantial progress in the development of molecular diagnostic tools for IHHNV and BP (see Lightner et al., this volume). The advances may eventually allow large-scale screening of individual broodstock. The molecular tools are already being tested for their ability to reliably screen individual broodstock.

The possibility of selecting uninfected animals from contaminated facilities and populations rests on the assumption that not all animals exposed to IHHNV or other viruses develop an infection. This hypothesis has yet to be tested, but the prospects are very bright.

Acknowledgments

Support for this work was provided in part by the Cooperative State Research Service of the United States Department of Agriculture through grants 88-38808-3319 and 92-38808-6920.

Literature Cited

Amos, K.H. 1985. Procedures for the Detection and Identification of Certain Fish Pathogens. 3rd ed. Fish Health Section, American Fisheries Society, Corvallis, Oregon, U.S.A.

Bell, T.A. and D.V. Lightner. 1984. IHHN virus: infectivity and pathogenicity studies in *Penaeus stylirostris* and *Penaeus vannamei*. Aquaculture. 38: 185-194

Bell, T.A. and D.V. Lightner. 1987. IHHN disease of *Penaeus stylirostris*: effects of shrimp size on disease expression. J. Fish Dis. 10: 165-170.

Bell, T.A. and D.V. Lightner. 1988. A Handbook of Normal Penaeid Shrimp Histology. Special Publication No. 1. World Aquaculture Society, Baton Rouge, Louisiana, U.S.A.

Bell, T.A., D.V. Lightner and J.A. Brock. 1990. A biopsy procedure for the non-destructive determination of IHHN virus infection in *Penaeus vannamei*. J. Aquatic Animal Health. 2: 151-153.

Bonami, J. R., B. Trumper, J. Mari, M. Brehelin and D.V. Lightner. 1990. Purification and characterization of the Infectious Hypodermal and Haematopoietic Necrosis Virus of penaeid shrimps. J. General Virology. 71: 2657-2664.

Couch, J.A. 1974. An Enzootic Nuclear Polyhedrosis Virus of Pink Shrimp: Ultrastructure, Prevalence, and Enhancement. J. Invertebr. Pathol. 24: 311-331.

Iversen, E.S. and J.F. Kelly. 1976. Microsporidosis successfully transmitted experimentally in pink shrimp. J. Invertebr. Pathol. 27: 407-408

Kalagayan, H., D. Godin, R. Kanna, G. Hagino, J. Sweeney and J. Wyban. 1991. IHHN Virus as an etiological factor in Runt-Deformity Syndrome (RDS) of juvenile *Penaeus vannamei* cultured in Hawaii. J. World Aquacult. Soc. 22: 235-243.

Krol, R.M., W.D. Hawkins and R. M. Overstreet. 1990. Reo-like virus in white shrimp *Penaeus vannamei* (Crustacea: Decapoda): co-occurrence with *Baculovirus penaei* in experimental infections. Dis. Aquat. Org. 8: 45-49.

Krol, R.M., W.D. Hawkins and R.M. Overstreet. 1991. Rickettsial and mollicute infections in hepatopancreatic cells of cultured Pacific white shrimp (*Penaeus vannamei*). J. Invertebr. Pathol. 57: 362-370.

LeBlanc, B.D. and R.M. Overstreet. 1990. Prevalence of *Baculovirus penaei* in experimentally infected white shrimp (*Penaeus vannamei*) relative to age. Aquaculture. 87: 237-242.

Lightner, D.V. 1983. Diseases of cultured penaeid shrimp. *In*: J.P. McVey (Ed.) CRC Handbook of Mariculture. Volume I. Crustacean Aquaculture. CRC Press, Inc. Boca Raton, Florida, U.S.A.

Lightner, D.V. 1988. Fungus (*Fusarium*) disease of juvenile and adult penaeid shrimp. pp. 64-69. *In* : C.J. Sindermann and D.V. Lightner (Eds.). Disease Diagnosis and Control in North American Marine Aquaculture (2nd edition). Elsevier, Amsterdam, Netherlands.

Lightner, D.V., R.M. Redman and T.A. Bell. 1983a. Infectious hypodermal and hematopoietic necrosis (IHHN), a newly recognized virus disease of penaeid shrimp. J. Invertebr. Pathol. 42: 62-70.

Lightner, D.V., R.M. Redman, T.A. Bell and J.A. Brock. 1983b. Detection of IHHN virus in *Penaeus stylirostris* and *Penaeus vannamei* imported into Hawaii. J. World Maric. Soc. 14: 212-225.

Lightner, D.V., R.M. Redman, T.A. Bell and L.A. Perez. In press. A collection of case histories documenting the introduction and spread of the virus IHHN in penaeid shrimp culture facilities in northwestern Mexico, *In*: C.J. Sindermann (Ed.). Proceedings of the International Symposium on the Effects of Introductions and Transfers of Aquatic Species on Resources and Ecosystems. Special Publication of the World Aquaculture Society. Baton Rouge, Louisiana, U.S.A.

Lotz, J.M., R.M. Overstreet, D.V. Lightner and R.M. Redman. 1991. Occurrence of IHHN virus in penaeid shrimp from wild populations of the eastern Pacific Ocean. J. World Aquacult. Soc. 22: 37A.

Pantoja-Morales, C. and D.V. Lightner. 1991. Status of IHHN virus in wild penaeid shrimp from the coast of Sonora, Mexico. Program and Abstracts of the XXIV Annual Meeting of the Society for Invertebrate Pathology.

Sindermann, C.J. and D.V. Lightner (Eds.) Disease Diagnosis and Control in North American Marine Aquaculture (2nd edition). Elsevier, Amsterdam, Netherlands.

Growth and Survival of Virus-infected and SPF *Penaeus vannamei* on a Shrimp Farm in Hawaii

Nick Carpenter
Amorient Aquafarm, Inc., P.O. Box 131
Kahuku, Hawaii 96731, U.S.A.

and

James A. Brock
Department of Land and Natural Resources
Aquaculture Development Program
335 Merchant Street, Rm. 348
Honolulu, Hawaii 96813, U.S.A.

Abstract

In late 1982, Amorient Aquafarm initiated work with *Penaeus vannamei* at their maturation and hatchery site located in Kahuku on the island of Oahu, Hawaii. From 1983 to 1989, laboratory tests detected no known virus diseases or other obligate pathogens in shrimp cultured on the Amorient farm. In early 1989, however, infectious hypodermal and hematopoietic necrosis virus (IHHNV) was discovered in stocks of *P. vannamei* at the farm. The effect of IHHNV infection on shrimp production was dramatic and was expressed as a marked decrease in growth that is characteristic of runt-deformity syndrome (RDS) of *P. vannamei*. In the IHHNV-infected RDS groups, the coefficient of variation in size (CV) increased from 10 - 20% to 40%, and pond yields decreased accordingly. In mid-1990, *Baculovirus penaei* (BP) infections were also found at low prevalence and severity levels in samples of shrimp examined from Amorient ponds; however, no negative impact on production could be attributed to the BP infection. In January 1992, the farm was stocked with the progeny of SPF *P. vannamei* broodstock. This report considers the resulting disappearance of RDS and the production and yield improvements obtained when IHHNV-free shrimp were again cultured on the Amorient farm.

Introduction

Amorient Aquafarm, Inc. is a 175-acre (79.6-ha) shrimp and prawn farm located on the North Shore of Oahu, Hawaii. The farm was constructed in 1977 and was originally designed for the culture of the freshwater prawn, *Macrobrachium rosenbergii*. On the main farm site, there are 142 1-acre (0.46-ha) earthen ponds and one 0.5-acre (0.23-ha) concrete-sided round pond. There are also 10 0.25-acre (0.11-ha) broodstock ponds at an adjacent area 0.5

miles from the main farm site. The maturation/hatchery facility is located at a third site close to the broodstock pond area.

In late 1982, 78 adult, wild-caught *Penaeus vannamei* were shipped from Ecuador and introduced in quarantine at the Amorient Aquafarm maturation/hatchery site in Kahuku, Oahu, Hawaii. The intent of the introduction was for the Amorient staff in Hawaii to develop appropriate technology and gain experience in shrimp maturation and larval rearing, and to eventually transfer this knowledge to the company's 1,000-acre (455-ha) commercial shrimp farm in Ecuador.

In mid-1986, a subpopulation of adult *P. monodon* obtained from the Aquaculture Development Program, State of Hawaii, were stocked into the Amorient Aquafarm maturation facility. This group of shrimp originated as offspring from a wild-caught spawner collected in waters off Sabah, Malaysia. After introduction to Hawaii and repetitive direct histopathology and shrimp bioassay evaluation during the 18 months of quarantine following introduction, a small group of adult shrimp was transferred to the Amorient Company.

On the basis of initial success in reproduction, spawning, production of postlarvae and favorable results in growout with *P. vannamei*, the decision was made to engage 80% of the Kahuku farm in shrimp culture. Over the next several years, shrimp production was rather consistent, ranging between 2,400 and 3,000 lbs/acre/yr (2,400 - 3,000 kg/ha/yr). Starting in late 1986, *P. monodon* were also stocked for growout, but by early 1988 culture of this species was limited due to poor pond performance relative to *P. vannamei* under the environmental conditions and husbandry practices in use at that time on the Amorient farm.

In mid-1987, an outbreak of infectious hypodermal and hematopoietic necrosis virus (IHHNV) disease occurred on a shrimp farm near Amorient Aquafarm. The origin of the IHHNV in this outbreak is not determined. However, by late 1988, IHHNV was detected in samples of F6 generation shrimp collected from the Amorient maturation/hatchery area, and by mid-1990, the virus was widespread in growout ponds on the farm. Also, in August 1990, *Baculovirus penaei* (BP) infection was found in shrimp sampled from the Amorient farm.

In December 1990, The Oceanic Institute provided P1 generation, Mexican-derived, specific pathogen-free (SPF) *P. vannamei* broodstock to Amorient Aquafarm from which SPF postlarvae[1] were produced and stocked into growout ponds.

Materials and Methods

Comparison between non-SPF-derived and SPF shrimp pond production and

[1]The term "high health" animals is preferred by Wyban (this volume) when referring to animals that have been removed from an SPF quarantine facility.

Table 1. A summary of the IHHNV and BP histopathology results for *Penaeus vannamei* samples from Amorient Aquafarm ponds for the period of 1987 through 1991.

Year	Stock	# Ponds sampled	# Shrimp examined	# BP+	#IHHNV+
1987	Ecuador	25	155	0	0
1988	Ecuador	4	40	0	0
1989	Ecuador	8	150	0	70 (61%)
1990	Ecuador	6	60	12 (20%)	52 (87%)
1991	Mexico	24	270	11 (4%)	0

growth performance was done by determination of the total weight of shrimp harvested from ponds, percent survival, weekly growth rate, feed conversion ratio, and size distribution (mean, standard deviation and coefficient of variation) for shrimp weight from random samples (minimum N = 100) of different populations.

From 1983 onwards, diagnostic examination for the detection of known penaeid viruses and other obligate pathogens were periodically conducted on the offspring of *P. vannamei* and *P. monodon* cultured at the Amorient site, and on stocks produced from the Amorient maturation/hatchery facility that were distributed to other locations in Hawaii.

For disease monitoring, shrimp were sampled at various sizes/ages, including postlarvae (PL6-12), 0.5- to 1.5-g nursed juveniles, 4- to 10-g subadults from growout ponds, and >35-g broodstock. Specimens were either frozen for the *P. stylirostris* bioassay test (Lightner et al., 1985) or killed by injection and immersion in Davidson's fixative (Humason, 1979) for histopathology evaluation. Histological processing and slide preparation followed standard procedures. Tissue sections were stained with hematoxylin and eosin (Luna, 1968).

Prior to restocking the ponds in 1991 with the progeny of SPF broodstock, the ponds were dried for two weeks, and 800 lbs of agricultural limestone was spread over the pond bottoms. The bottom gravel of the round pond was dried and then partially filled to cover the substrate, which was treated with 10 mg/L of chlorine overnight before the pond was refilled.

In the growout trials, a 25% protein, locally produced pellet was fed to shrimp stocked into earthen ponds, and a 45% protein, imported pelleted ration was provided to the shrimp in the round pond.

Results and Discussion

The histopathology examination results for *P. vannamei* sampled from the Amorient farm and maturation/hatchery areas for the period from 1987 through 1991 are listed in Table 1. In addition, *P. stylirostris* bioassay trials were conducted on shrimp that originated from

the Amorient population up to mid-1987. For example, between January and May 1987, indicator shrimp bioassay tests were carried out on tissue samples from three groups (N = 50) of Amorient subadult to adult *P. vannamei*. Prior to 1989, infections by either BP or IHHN viruses were not detected in bioassays or by direct histopathology evaluation of shrimp from the Amorient farm. Direct histopathology tests conducted on juvenile through adult (N = 30) *P. monodon* sampled from several ponds in August 1987 were also negative for known obligate shrimp pathogens.

However, IHHN virus infection was detected histologically in samples of postlarvae collected from the Amorient hatchery in early 1989 (Fig. 1). As the year progressed, the frequency of IHHN virus-positive groups increased, and by May of that year, IHHNV was detected in 100% of the postlarval groups sampled. Within IHHNV-positive groups, the average number of individuals with histologically diagnosable IHHNV infection increased through 1989 and 1990. Average prevalence of infection in early 1990 was 26.2%, but during the summer of 1990, prevalence of infection increased to an average of 43.1%. Coincidentally, broodstock were replaced every four to six months, and the increased prevalence of infection may have reflected higher levels of IHHNV infection in the older broodstock.

In late 1990, The Oceanic Institute provided several groups of SPF nauplii (Mexico stock) to Amorient Aquafarm. The postlarvae produced with these nauplii tested negative by histological

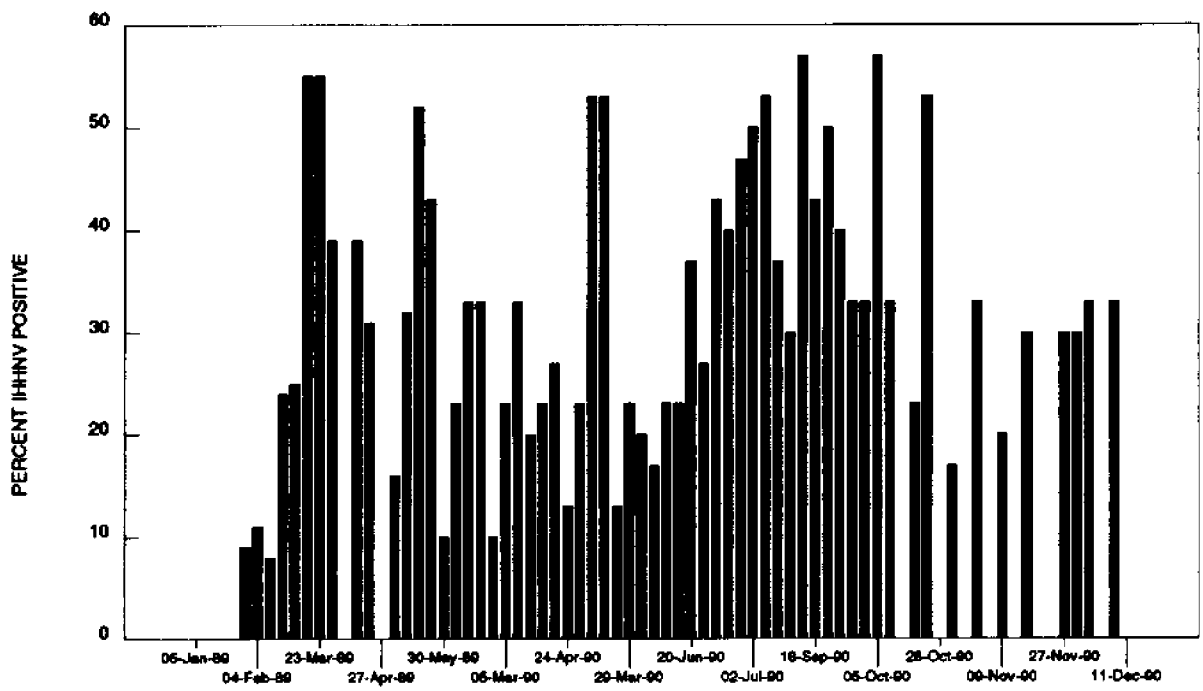


Figure 1. Percentage of IHHNV-positive individuals within groups of postlarvae tested for IHHNV.

criteria for IHHNV infection, whereas postlarvae produced in sister tanks using nauplii from IHHNV-infected broodstock (Amorient's Ecuador stock) continued to test positive for IHHNV (Fig. 1). At no point during this period did any of the postlarval populations (N = 71) harvested from the hatchery test positive for BP.

Unfortunately, histogram assessments were not carried out for shrimp cultured on the Amorient farm before IHHNV was detected, from 1982 through 1988. RDS was not apparent in early 1989; this is demonstrated by random histograms of populations from 1-acre earthen ponds in which the size coefficient of variation (CV) was only 17% (Fig. 2a). However, a nursery harvest at that time contained an unusually high number of "small" juveniles — a large percentage weighed 0.3 g or less (Fig 3a). As time passed, on average, the CV slowly increased, peaking at 46% in late 1990 (Fig. 2b). The increasing CV was also apparent in successive crops harvested from the intensive, 0.5-acre round pond (Figs. 4a, b). As a result of RDS, the average harvest size decreased from 11.9 g to 8.5 g in the 2 growout trials conducted during this period.

In late December 1990, Amorient Aquafarm received SPF *P. vannamei* broodstock. These shrimp were founder-generation stock collected by the U.S. Shrimp Consortium as postlarvae in Mexico and grown to broodstock at the quarantine facility of The Oceanic Institute in Hawaii.

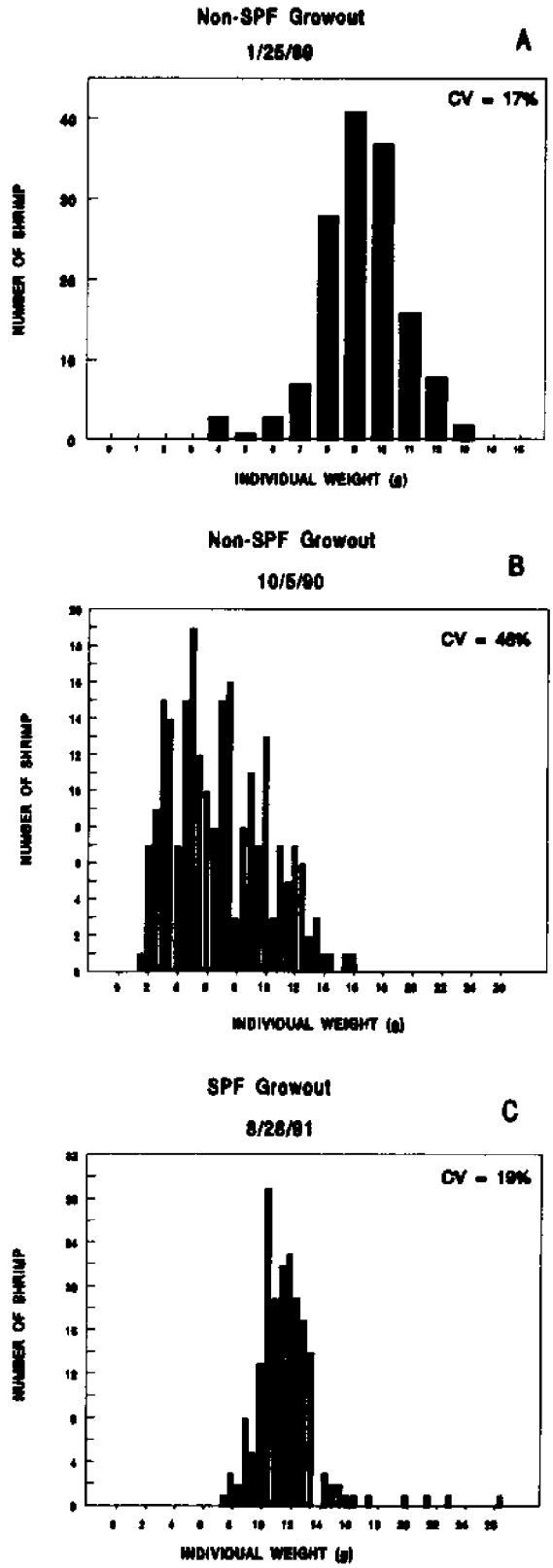
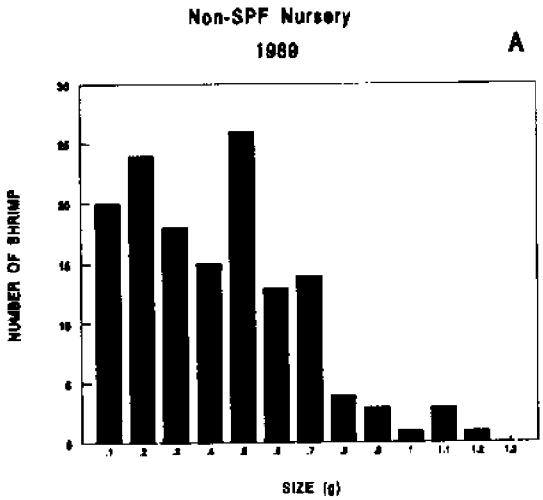
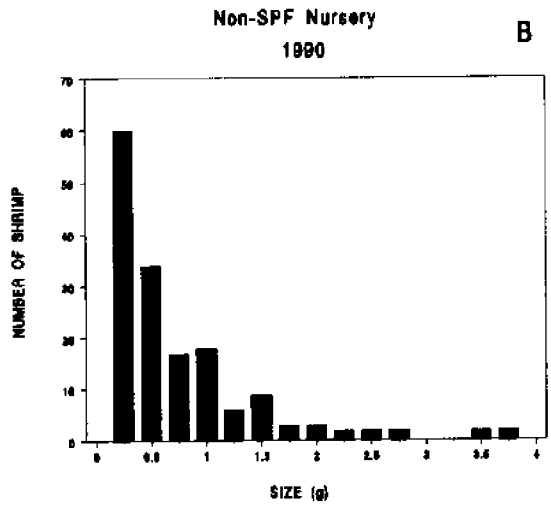


Figure 2. Size variation in Penaeus vannamei *during growout.*





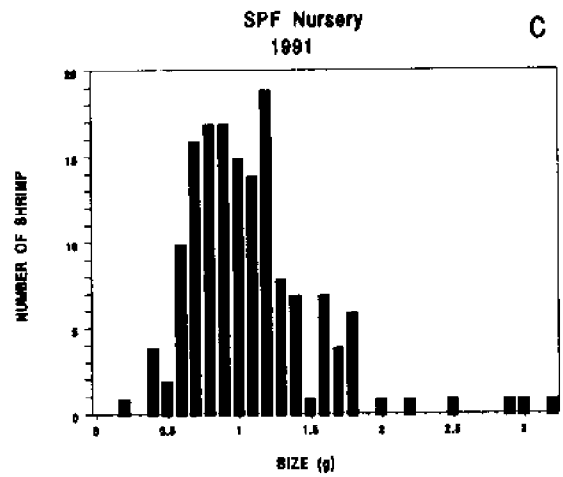
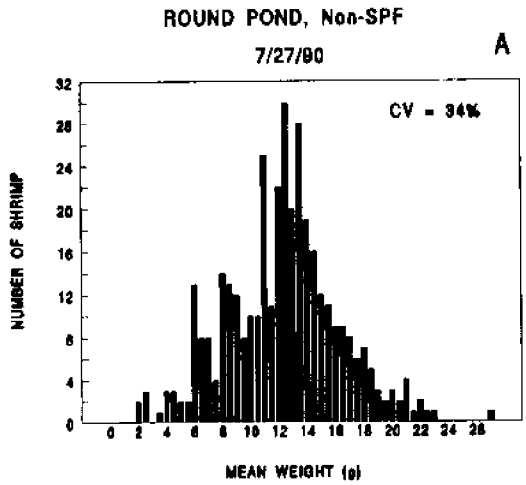
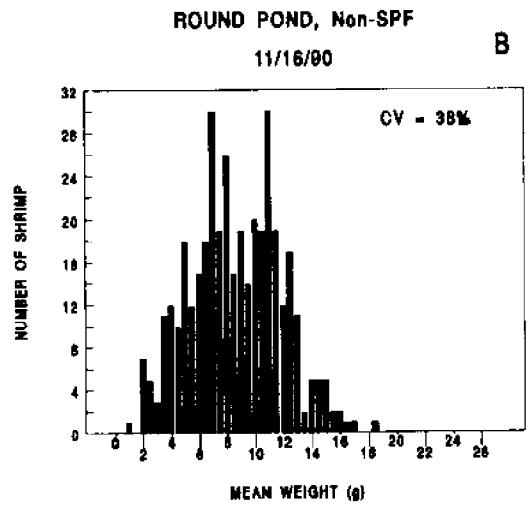


Figure 3. Size variation in Penaeus vannamei *in the nursery phase.*





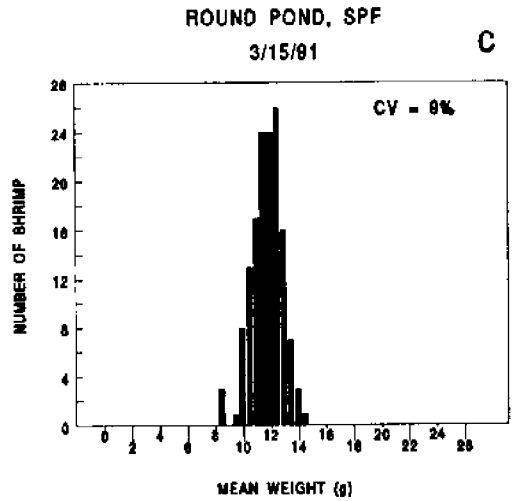


Figure 4. Size variation of Penaeus vannamei *in round pond growout trials.*

Improved production was noted immediately with the progeny of the SPF broodstock. Note, for example, the size distributions from two consecutive nursery harvests depicted in Figures 3b and 3c. Both ponds were stocked at a similar density and reared for the same number of days. Figure 3b represents non-SPF postlarvae; many of the harvested shrimp were 0.25 g or less. Figure 3c gives the data for the SPF animals; their average size at harvest was approximately 1 g.

We also observed a dramatic reduction of RDS in the earthen semi-intensive ponds and in the intensive round pond. Figure 2c is a size histogram for an earthen pond stocked with SPF *P. vannamei*. The CV for this pond was 19%. Figure 4c contains similar data for a population harvested from the round pond — the CV was 9%.

The production data for the growout ponds discussed above are in Tables 2 and 3. For the IHHNV-infected shrimp, there was a general decrease in growth rate, mean harvest size and lbs/acre/crop. However, once the SPF shrimp were stocked, there was less size variability and production improved.

As indicated by the data, one benefit of the SPF broodstock was a reduction in the level of RDS in growout and nursery. This was extremely important from a marketing point of view. Using three successive round pond harvests as an example (Table 3), the SPF crop yielded a 62.5% higher return than the non-SPF harvests. In the non-SPF harvest on Nov. 16, 1990, 8% of the shrimp were below marketable size and 53% were under 8.5 g; these animals commanded a low price. In comparison, 100% of the SPF crop was sellable, and only 3% weighed less than 9.5 g. Similar results were obtained for the earthen ponds.

IHHN virus was detected histologically in samples collected from ponds on the Amorient farm in 1989 (Table 1). In 1989, the average prevalence of IHHNV-infected *P. vannamei* was 61%, but increased to 89% in 1990. Since SPF shrimp have been stocked at Amorient, IHHN virus infection has not been detected histologically in *P. vannamei* sampled (N = 270) from ponds on the site. However, more study is required before the status of IHHN virus in the earthen ponds of the Amorient farm will be understood.

Furthermore, the prevalence of BP infection declined from 20% to 4% in pond-reared shrimp between 1990 and 1991. In laboratory experiments, LeBlanc and Overstreet (1991) demonstrated that BP is inactivated by desiccation. Perhaps the two-week drying period between crops partially inactivated infectious BP in the pond sediments. Further study is required to clarify this issue.

In summary, stocking the progeny of SPF broodstock on an IHHNV-contaminated farm where RDS was a serious problem resulted in the virtual elimination of RDS and improved production and profitability.

Table 2. Production data from non-SPF and SPF growout crop at Amorient in 1989-91.

Parameter	Semi-intensive growout			Intensive growout		
	Non-SPF	Non-SPF	SPF	Non-SPF	Non-SPF	SPF
Density (No./m^2)	15.0	14	13.7	110	98.5	91.5
Duration (days)	83	77	106	91	101	104
Growth rate (g/wk)	0.78	0.54	0.76	0.87	0.54	0.74
FCR	2.2	2.6	1.7	1.86	3.37	2.1
Harvest CV (%)	17	46	19	34	38	9
Harvest size (g)	9.2	6.9	11.8	11.9	8.5	11.8
Survival (%)	66	73	74	66	86	91
Total crop (lbs.)	826	623	1,055	3,838	3,140	4,271

Table 3. Marketing impact of using non-SPF versus SPF shrimp.

Count	Size (g)	# Shrimp	Percent	Pounds	Price	Value ($)
IHHNV-positive round pond crop — July 27, 1990						
Unsellable	< 4	6	2	75	0.00	0
71-110	4 - 6	23	6	227	3.75	851
51-70	6.5 - 8.5	47	13	496	4.00	1,984
46-50	9.0 - 9.5	20	5	189	4.25	803
41-45	10.0 - 11.0	45	12	458	4.50	2,061
36-40	11.5 - 12.5	63	17	649	4.75	3,083
31-35	13.0 - 14.5	83	22	841	5.25	4,415
26-30	15.0 - 17.5	55	15	561	5.75	3,226
21-25	18.0 - 21.5+	32	9	342	6.25	2,137
Total		374		3,838		18,560
IHHN-positive round pond crop — Nov. 16, 1990						
Unsellable	< 4	32	8	248	0.00	0
71-110	4 - 6	67	17	531	3.75	1,991
51-70	6.5 - 8.5	108	28	876	4.00	3,504
46-50	9.0 - 9.5	33	8	248	4.25	1,054
41-45	10.0 - 11.0	69	18	562	4.50	2,529
36-40	11.5 - 12.5	48	12	374	4.75	1,776
31-35	13.0 - 14.5	23	6	184	5.25	966
26-30	15.0 - 17.5	11	3	90	5.75	517
21-25	18.0 - 21.5+	1	1	27	6.25	169
Total		392		3,140		12,506
SPF round pond crop — Mar. 15, 1991						
51-70	6.5 - 8.5	3	2	77	4.00	308
46-50	9.0 - 9.5	1	1	35	4.25	149
41-45	10.0 - 11.0	38	27	1,145	4.50	5,152
36-40	11.5 - 12.5	74	52	2,212	4.75	10,507
31-35	13.0 - 14.5	27	19	802	5.25	4,210
Total		143		4,271		20,326

References

Brock, J.A., Unpublished.

Humason, G.L. 1979. Animal Tissue Techniques. 4th ed., W.H. Freeman and Company, San Francisco.

LeBlanc, B.D. and R.M. Overstreet. 1991. Efficacy of calcium hyperchlorite as a disinfectant against the shrimp virus *Baculovirus penaei*. J. Aquatic Animal Health. 3: 141-145.

Lightner, D.V., R.M. Redman, R.R. Williams, L.L. Mohney, J.P.M. Clerx, T.A. Bell and J.A. Brock. 1985. Recent advances in penaeid virus disease investigations. J. World Maric. Soc. 16: 267-274.

Luna, L.G. (Ed.). 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed., McGraw-Hill, New York, N.Y.

Shrimp Production in Texas Using Specific Pathogen-Free Stocks

Fritz Jaenike, Kieth Gregg and Louis Hamper
Harlingen Shrimp Farms, Ltd.
P.O. Box 4120
Los Fresnos, Texas 78566, U.S.A.

Abstract

Harlingen Shrimp Farms has obtained yields averaging 2.5 - 3 MT/ha in recent years, but some ponds have produced more than 4.5 MT/ha/crop. To achieve more consistent yields, a cooperative research agreement was entered into with the Gulf Coast Research Laboratory Consortium (GCRLC) in September 1990. The GCRLC supplied Harlingen Shrimp Farms with specific pathogen-free (SPF) broodstock to produce postlarvae for commercial-scale comparisons with selected farm stocks, termed Texas broodstock source (TBS), which were IHHNV positive. The SPF broodstock were maintained in isolation from the farm stocks housed in the same facility. Regular inspection of the postlarvae indicated that the offspring were also SPF. The ponds stocked with postlarvae produced from the SPF broodstock outperformed the TBS postlarvae in terms of survival, overall yield and decreased size variation.

Introduction

Harlingen Shrimp Farms, located in South Texas and originally founded in 1980 as Laguna Madre Shrimp Farms, was formed in October 1990. The farm produces *Penaeus vannamei* in 180 ha of semi-intensively managed ponds. Postlarvae are supplied by a hatchery on site. Ponds range in size from 2 - 15 ha, and are direct-stocked at densities up to 50 postlarvae/m^2.

The climate in South Texas is considered subtropical, and the growing season for a tropical shrimp such as *P. vannamei* is restricted to 245 days at the farm site. The need for higher returns per ha, per unit time has pushed most farms toward intensification. To maximize yields, most U.S. farms use high-quality feeds and aerate at 5 - 9 hp/ha. With high aeration and efficient application of high-quality feeds, stocking densities have been increasing. Some farms stock more than 70 postlarvae/m^2 and achieve yields over 6 MT/ha in a single crop. A consistent supply of high-quality postlarvae is required to make such intensive systems successful.

At Harlingen Shrimp Farms, yields have averaged 2.5 - 3 MT/ha in recent years, but some ponds have produced

more than 4.5 MT/ha/crop. These inconsistent yields have resulted from low survival and growth rates. The low growth rates have been impacted by runt-deformity syndrome (RDS), which has been causally linked with IHHNV (Kalagayan et al., 1991), but the low survival rates remain unexplained. In an attempt to achieve more consistent yields, a cooperative research agreement to utilize high-health stocks of shrimp was entered into with the Gulf Coast Research Laboratory Consortium (GCRLC) in September 1990.

The GCRLC supplied Harlingen Shrimp Farms with enough specific pathogen-free (SPF) broodstock to produce postlarvae for commercial-scale comparisons with selected farm stocks ("Texas broodstock source," or "TBS"), that were IHHNV positive. SPF broodstock, by definition, are free of IHHNV, hepatopancreatic parvo-like virus, *Baculovirus penaei*, microsporidians, gregarines, nematodes and cestodes. Ponds stocked with postlarvae produced from the SPF broodstock (hereafter referred to as "high-health" animals) outperformed the offspring of the TBS in terms of survival, overall yield and decreased size variation.

Methods and Materials

The hatchery at Harlingen Shrimp Farm is housed within a 3,600-m^2 concrete building and includes a water treatment facility, two maturation rooms, broodstock holding and acclimation areas and over 500 tons of larval rearing capacity. The maturation area consists of two light- and temperature-controlled rooms, each containing 12 8-ton circular fiberglass tanks. The tanks are plumbed in groups of four; each group has a biofilter. In preparation for receiving SPF broodstock, one of these four tank systems was physically isolated from the rest of the tanks with a plastic curtain. A new biofilter was installed, and the whole area was carefully sterilized. A protocol for receiving and isolating the SPF broodstock and their progeny was developed in conjunction with Dr. Paul Frelier and the Gulf Coast Research Laboratory Consortium (Frelier, pers. comm). The protocol was strictly followed. Standard maturation methodologies utilizing unilateral eyestalk ablation were used with both groups of broodstock. Nauplii produced from the SPF broodstock were isolated from the TBS nauplii, and initially were reared in an isolated part of the larval rearing area.

The larval rearing area consisted of rows of fiberglass tanks that drain into trenches for harvesting. One of these rows of tanks was physically isolated from the others with a plastic curtain. Identical, standard hatchery methods were used for both groups, except that the high-health postlarvae were initially stocked at lower densities because fewer broodstock were sourced for nauplii. The high-health nauplii were initially isolated from the TBS nauplii. After several million postlarvae were produced in isolation, the larval rearing area was no longer physically segregated. The nauplii produced from the two broodstock groups were segre-

Table 1. Comparison of the average growth rate, feed conversion ratio (FCR), survival and percentage of population with rostral and/or tail deformities at harvest.

Pond	Animal type	Stocking density (No./m^2)	Ave. growth per week after 1g (g/wk)	FCR	Survival (%)	% deformed at harvest
N-1	TBS	50	0.80	3.1	43	30
G-4B	TBS	50	0.84	2.6	46	11
A-4	TBS	75	0.85	3.6	40	16
G-2	TBS	18	0.87	3.2	30	29
N-2	SPF	37	0.74	2.7	43	4
G-3	SPF	37	0.77	2.5	72	2
N-3	SPF	50	1.06	2.3	25	*
N-4	SPF	50	0.89	2.3	32	3
N-6	SPF	50	0.90	2.3	60	5
N-7	SPF	37	1.03	2.3	64	*
A-2	SPF	75	0.67	2.9	60	*

*Data not available.

gated into separate tanks whenever possible; however, a number of mixed tanks were stocked to maximize tank space and optimize production. After six weeks of completely isolated production, the two broodstock groups were mixed in mass spawning tanks for several days.

Eleven ponds of varying sizes, totaling 50 ha, were stocked with either high-health postlarvae or TBS postlarvae (Table 1). Although the hauling tank and transfer baskets were sanitized with chlorine prior to stocking with high-health postlarvae, no attempt was made to use segregated equipment or supplies in the management of the growout ponds. Management strategies were applied according to stocking density. For example, aeration rates ranged from no aeration at the stocking density of 18 postlarvae/m^2 to nearly 15 hp/ha for the smaller ponds stocked at 75 postlarvae/m^2. The average daily water exchange rates ranged from ten to 45% per pond, depending on the biomass estimate. All ponds were fed two to three times per day using 45% protein crumbles until shrimp reached 1 g; thereafter, 30% protein prawn pellets were used. Feeding rates were calculated using a standard feed curve based on percent biomass, and were adjusted according to observed consumption rates monitored with feed trays. Ponds were evaluated on a weekly basis. Each week, 100 to 200 shrimp from each pond were sampled using cast nets. The average weight was determined, and shrimp were inspected for state of health and vigor, signs of stress, feeding activity, deformities, size variation and shell lesions. Samples of stocked postlarvae, 30 day-old juveniles and adults nearing harvest were collected and fixed in Davidson's solution for disease testing. All samples were examined at Texas A&M University, where diagnoses for

Table 2. Average number of nauplii produced per sourced female and percentage of total females mated and sourced per day.

	March		April		May		June
	TBS	SPF	TBS	SPF	TBS	SPF	TBS
Nauplii x 10^3	88.5	144.9	87.4	139	87.1	148.7	70.1
Percent females mated and sourced per day	6.1	5.2	5.5	5.6	5.0	6.4	4.6

IHHNV and other disease agents were made by direct histology. The size distribution in each pond was determined by individually weighing samples of shrimp and by examining final processing packout reports.

Results

Over 85 million nauplii were produced by 140 female, SPF broodstock from March through June, 1991. The percentage of females mated per day and the average number of nauplii per spawn for both broodstock groups are listed in Table 2. The average number of nauplii per spawn takes into account all females that were mated and placed into spawning tanks (sourced).

The SPF and TBS broodstock performed similarly in terms of percent females mated per day; however, the SPF broodstock averaged more nauplii per spawn.

Not all of the nauplii produced from the SPF females were segregated in larval rearing. Overall, 36 million postlarvae were produced from segregated SPF nauplii. Survival from nauplii to postlarvae was better in the high-health postlarvae (Table 3). The survival rates listed in Table 2 represent only the results from 9,000-L tanks. The size, vigor and appearance of the high-health and TBS postlarvae were similar.

Approximately 11.5 million high-health postlarvae were segregated in growout ponds. The results from eleven ponds stocked with either high-health or TBS postlarvae will be discussed here (Table 1). Growth rates did not differ greatly between the high-health and TBS ponds. Average time from PL5 to 1 g average weight was 32 d for high-health animals and 38 d in the TBS ponds. Furthermore, growth from 1 g to harvest weight averaged .87 g/wk and .84 g/wk in high-health and TBS ponds, respectively (Table 1).

A dramatic difference, however, was observed in the degree of size variation observed in the high-health and TBS groups. A typical TBS pond population averaging 1 g in size contained some shrimp that were less than 0.1 g and others that weighed more than 3 g. A typical high-health population, by contrast, had a size distribution ranging only from 0.5 g to 1.5 g. This difference became more pronounced as the growout period progressed — the size distribution in TBS ponds increased

Table 3. Total postlarvae produced per month and survival from nauplii to harvested postlarvae.

	April		May		June		July	
	TBS	SPF	TBS[1]	SPF	TBS[1]	SPF	TBS[1]	SPF
No. postlarvae produced (x 10^6)	29.5	8.6	30.1	7.4	17.8	12.9	18.2	7.4
Survival (%)	59	72	62	61	48	54	42	52

[1]TBS or mixed.

weekly, whereas the high-health populations maintained a tight size distribution throughout the culture period. As a result, a much more uniform product was harvested from the high-health ponds (Fig. 1a, b).

The level of rostral or tail deformities detected during weekly samples and at harvest typically ranged from 15 to 25% in TBS ponds, but was less than 3% in the high-health animals (Table 1).

Survival rates were higher in high-health ponds as compared to the TBS ponds. Average survival from PL5 to harvest for high-health animals was 51%; the best pond had 72% survival at harvest. Average survival for the TBS animals was only 40%; the best pond had a 46% survival rate. Feed conversion rates (FCRs) were also much better in SPF ponds (Table 1).

Discussion

The SPF broodstock and their high-health offspring outperformed the TBS broodstock and their progeny in the hatchery; however, the differences between the two groups were even more pronounced during growout.

The SPF broodstock produced more nauplii per spawning female than the TBS broodstock; however, the SPF broodstock were much larger, and there is a positive correlation between broodstock size and spawn size. The percentage of females mating and spawning per day was good for both groups and did not appear to be different. The sourcing pressure on the SPF broodstock was slightly more intensive due to fewer females producing.

Overall survival of the high-health nauplii was higher than that of the TBS nauplii. The greatest difference was in April, when survival of the high-health and TBS nauplii was 72% and 59%, respectively (Table 3). At that time, however, the high-health tanks were stocked at lower densities, and in our hatchery, lower densities in larval rearing often translate into increased survival. In May, the two groups were similar in terms of survival from nauplius to postlarva, but in June and July, when densities were similar, the high-health tanks yielded better survivals

than TBS tanks. Finally, the appearance of postlarvae was similar between the two groups.

Additional comparisons between the high-health and TBS nauplii and postlarvae were conducted at two universities. Individual families of nauplii were sent to the University of Houston Clearlake for comparison of resistance to *Baculovirus penaei* (BP). The experiment, which was designed to compare differences between broodstock groups and between families within broodstock groups, entailed feeding BP-laden *Artemia* to mysis larvae and monitoring mortality. No differences in resistance to BP were detected (Lester, pers. comm.).

Four groups of postlarvae from each broodstock source were sent to Texas A&M University for a replicated experiment conducted in a controlled envi-

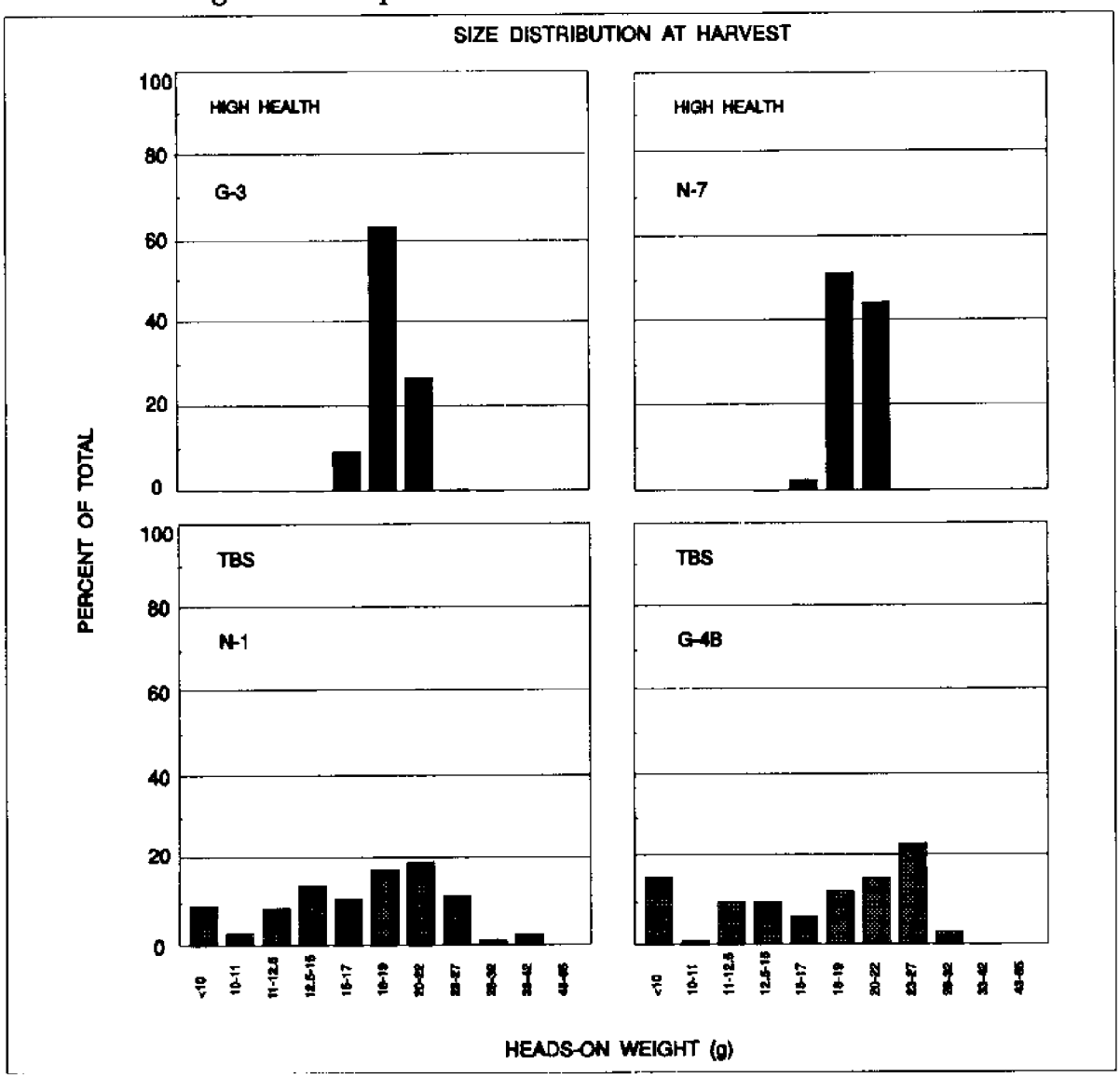


Figure 1a. Comparison of size distribution at harvest for four ponds.

ronment tank system (Castille et al., 1992). This comparison evaluated postlarval performance for 30 and 60 days, beginning with PL6. Survivals were similar; however, high-health postlarvae posted significantly better growth rates and also had a significantly smaller size variation than the TBS postlarvae.

The differences between high-health and TBS animals were more evident in growout. Initial sampling revealed a uniform size distribution in high-health ponds and a wide size variation in the TBS ponds. These differences increased as growout progressed (Fig. 1a, b). The uniform size distribution of the high-health animals allowed for more accurate average weight and total biomass estimates and resulted in more efficient feed management, as revealed by the FCRs (Table 1). Feed is the largest operational cost in produc-

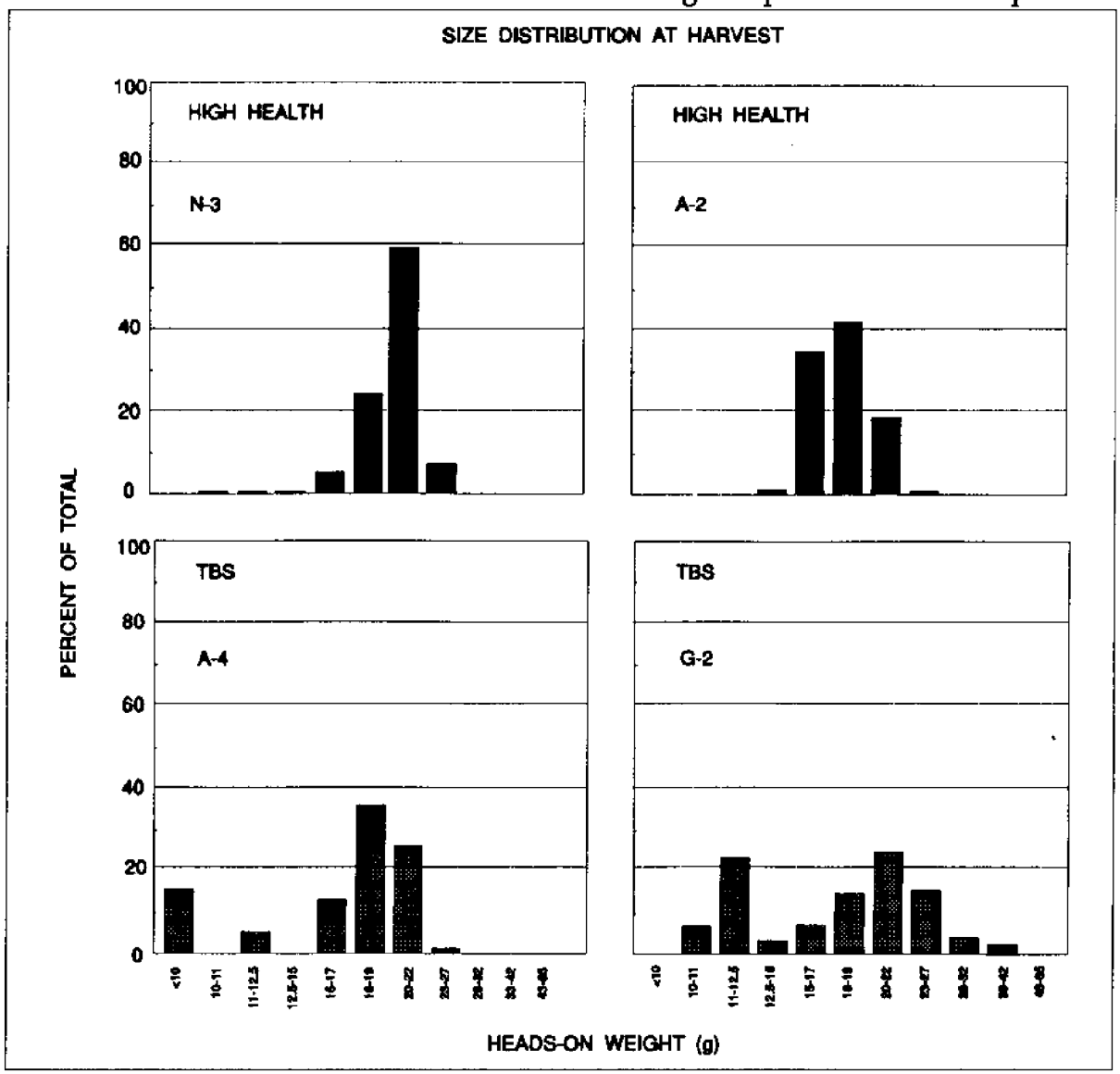


Figure 1b. Comparison of size distribution at harvest for four ponds.

ing shrimp — the lower the FCR, the better the profit margin.

The more uniform size distribution was also advantageous at harvest. Shrimp buyers prefer a uniform product; runts are regarded as liabilities. The uniform distribution of the high-health animals benefited fresh or heads-on marketing where grading is more labor intensive. Batches that could be sold either as whole shrimp at pondside, or as "bulk-ungraded" at market, had lower handling and processing costs.

The average survival rate of high-health animals was better than that of the TBS shrimp (Table 1). Historically, our farm site has experienced wide ranges in pond survival rates, which are still largely unexplained. The high-health shrimp ponds also exhibited a wide range of survival rates (25% to 72%). There is increasing evidence that the low survival rates at our farm are due to unknown factors related to the growout ponds and are not the direct result of postlarval quality. The higher average survivals of the high-health animals simply indicates that that they have a greater capacity to tolerate these unknown factors. In addition, the growth rates of high-health shrimp were slightly higher than the TBS animals.

The average growth rate in the SPF ponds, .87 g/wk after 1 g, was lower than anticipated. This may have been due to underfeeding resulting from higher-than-expected survivals.

In summary, with better survival, more uniform size, easier management resulting in lower FCRs and more marketing options, the high-health animals performed better than the TBS progeny.

Acknowledgments

Harlingen Shrimp Farms is grateful to The Oceanic Institute and the Gulf Coast Research Laboratory Consortium (GCRLC) for providing the SPF shrimp stocks. It is important for research groups such as the GCRLC to be at the forefront of production-oriented projects. Dr. Paul Frelier and his staff at Texas A&M University spent hours analyzing the samples and helping to develop the protocol for maintaining the SPF stock as disease-free. Finally, the entire staff at Harlingen Shrimp Farms was instrumental in producing the shrimp and being conscientious about the SPF project.

Literature Cited

Castille, F.L., T.M. Samocha, A.L. Lawrence, H. He, P. Frelier and F. Jaenike. 1992. Variability in growth and survival of early postlarval *Penaeus vannamei* Boone, 1931. Aquaculture. In Press.

Kalagayan, H., D. Godin, R. Kanna, G. Hagino, J. Sweeny, J. Wyban and J. Brock. 1991. IHHN Virus as an etiological factor in runt-deformity syndrome (RDS) of juvenile *Penaeus vannamei* cultured in Hawaii. J. World Aquacult. Soc. 22: 235-243.

Part II:

Contributed Papers -

Research, Regulations, and Health Management

Shrimp Culture Technologies Inc.: Research to Improve Shrimp Genetics and Health

Rolland Laramore
Shrimp Culture Technologies Inc.
Harbor Branch Oceanographic Institution
5600 Old Dixie Hwy., Ft. Pierce, Florida 34964, U.S.A.

Abstract

This paper presents the results of three postlarvae vaccine studies and a broodstock vaccination study. Vaccines were administered to postlarvae and evaluated in nursery and growout ponds. Survival in the nurseries and direct-stocked growout ponds improved by an average of 19% and 33%, respectively. Vaccinated animals that were harvested from the nursery ponds and placed in growout did not have a higher survival rate than the control group; however, survival was more than 80% in both the vaccinated pond and in the nonvaccinated control pond. The results of the vaccination of broodstock were disappointing with respect to survival; however, improved production in the first two months suggests that research should be continued. The results of a survey for infectious hypodermal and hematopoietic necrosis virus (IHHNV) on six shrimp farms in Honduras are also presented.

Introduction

Shrimp Culture Technologies Inc. (SCTI) is a company newly formed by Shrimp Culture Inc. to improve the genetics of cultured shrimp through selective breeding. Selection will be imposed upon a pool of shrimp that has been certified as specific pathogen-free (SPF).

Other areas of research are:

- Efficacy testing and licensing of a water-administered *Vibrio* vaccine for larvae, and the testing of an injectable vaccine for broodstock;
- Stability testing of a concentrated, live, packaged algae for use in hatcheries;
- The use of probiotics to control bacterial infections in larval culture tanks; and
- Experiments toward the development of polyploid shrimp.

Broodstock

A selection program, under the direction of Dr. James Lester, University of Houston, Clear Lake, Texas, was instituted at Agromarina de Panama in

Table 1. *Penaeus vannamei* vaccination studies.

	Vaccine	Control	Increase
Nursery evaluation[1], 36 days			
Stocking density (No./acre)	600,000	600,000	—
Survival (%)	77.4	64.8	19%
Yield (lbs/acre)	699	567	23%
Growout evaluation[2], Agromarina 116 days			
Stocking density (No./acre)	41,000	41,000	—
Survival (%)	83	85	-2%
Yield (lbs/acre)	1,021	869	17%
Growout evaluation[1, 3], MIDA[4] Study, 114 days			
Stocking density (No./acre)	34,000	35,500	—
Survival (%)	67.6	50.8	33%
Yield (lbs/acre)	519	372	40%

[1]Average of four replications.

[2]Average of two replications. Difference in survival is not significantly different. Increase in yield due to larger size shrimp at harvest.

[3]Studies conducted in the dry season under adverse growing conditions.

[4]Conducted in the Ministry of Agricultural Development of Panama's research ponds.

1988. Progeny from the selected animals produced an average increase in weight of 7.4% at harvest, when compared to the progeny of the nonselected maturation animals. In addition, the uniformity of size was enhanced. Unfortunately, poachers drained and harvested the pond containing the second generation animals reserved for broodstock. The infectious hypodermal and hematopoietic necrosis virus (IHHNV) status of the animals in this selection program is unknown.

The Oceanic Institute, located in Hawaii, reported improved growth rates and uniformity of size in selected SPF animals (see Wyban, this volume). Also, postlarvae purchased from Laguna Madre's hatchery in Texas and shipped to Honduras outproduced nonselected animals during the dry season of 1990 (Unpublished report, Dr. Bill MacGrath, Shrimp Culture Inc.).

There is little doubt in my mind that within the next decade the larger, more progressive shrimp farms will be stocking ponds with postlarvae produced from selected SPF broodstock.

Vaccine — Postlarvae

Efficacy testing of a killed *Vibrio* bacterin, grown on agar plates, was conducted in Panama in 1989/90 (Table 1). Survival increased in nursery pond evaluations, conducted over a 36-day period, from 64.8% to 77.4% (average of four replications). Yields increased from 567 lbs/acre to 699 lbs/acre. Stocking density was 600,000/acre.

Table 2. Maturation vaccine trials, Panama, 1987.

Group	Naupliar Production x 10^6								
	RED			BLUE			WHITE		
	1	2	3	1	2	3	1	2	3
1	1,910	3,182	267	2,785	2,631	144	1,368	3,078	0
2	1,054	2,058	903	1,710	2,776	1,221	288	1,263	822
3	237	1,871	1,256	240	2,260	1,626	708	3,183	2,314
4	1,910	3,182	165	2,845	2,739	258	1,368	3,521	66
5	1,710	2,866	1,284	1,054	2,158	1,101	288	1,563	1,038
6	237	1,943	1,220	240	2,260	1,746	792	3,617	2,608
Total	7,058	15,102	5,095	8,874	14,824	6,096	4,812	16,225	6,848
Females	50	41	34	50	42	36	50	49	42

Growout evaluations were conducted on four 10-acre ponds (two vaccinated and two controls), stocked with juveniles from the nursery trial. Survival was excellent in both cases; 83% for the vaccinated ponds, 85% for the controls. However, the yields from the vaccinated ponds were higher than the control yields; 1021 lbs/acre vs. 869 lbs/acre. The higher yields were due to a 17% increase in the size of the harvested shrimp.

The reason for the size difference is not readily apparent. Some have suggested the possibility of less stress from microbial assault or, perhaps, nonspecific protection against one or more viruses (neither group was tested). While the difference was significant, there were only two replications.

A third study was conducted in ponds at the experimental station of the Ministry for Agricultural Development (MIDA) in Aquadulce, Panama. The ponds were direct stocked at 35,000/acre. Survival increased from 50.8% to 67.6%, and yields increased from 372 lbs/acre to 519 lbs/acre (average of four replications). Harvest was after 114 days.

Tests are currently underway in Ecuador and Honduras evaluating a vaccine produced from a broth culture using three strains of *Vibrio*. Antigens produced in broth culture are less expensive than those produced on solid media.

Vaccine — Maturation

Two prototypes of an injectable *Vibrio* vaccine emulsified in an oil-based adjuvant were tested against unvaccinated controls. Six tanks were tested for each treatment in this three-month study. Evaluation was for survival and naupliar production. Only females were vaccinated (see Table 2). Survivals for the "RED" vaccine, "BLUE" vaccine, and "WHITE" control groups were 68%, 72% and 84%, respectively (Fig. 1).

Naupliar production per treatment group was 27.2 million, 29.8 million and 27.8 million, respectively. These differences are not significant.

It was interesting to note that both vaccines outproduced the nonvaccinated control the first month: Red, 7.1 million; Blue, 8.9 million; and the Control, 4.8 million. Production was essentially the same the second month; 15.1 million, 14.8 million, and 16.2 million, respectively, but dropped slightly below the control the third month: 5.1 million, 6.1 million, and 6.8 million, respectively; although, the difference, again, was not significant (Fig. 2).

If naupliar production is corrected for survival, that is, number of females remaining in the tanks after removing mortalities, the vaccinated groups outproduced the nonvaccinated group slightly (Fig. 3). The average production of nauplii per female over the three-month period was: Red, 679,000; Blue, 725,000; and Control, 556,000.

Higher mortality in the treated groups may have resulted from the trauma of vaccination (no sham injection was made on the controls). It is also possible that the greater number of females handled during the first month could have increased mortality in the more productive tanks. In any case, the early increases in production dictate that additional research be conducted.

IHHNV Survey

At the request of the US/AID shrimp program in Honduras, six Honduran shrimp farms were surveyed for IHHN virus. All farms tested positive for IHHNV. The rate of infection ranged from 20% to near 100% (Fig. 4). The shrimp selected for the survey were juveniles weighing approximately 3 g. A severity index (scores from one to four based on the prevalence of Cowdry A inclusion bodies found in susceptible tissue) was positively correlated with the percentage of animals infected.

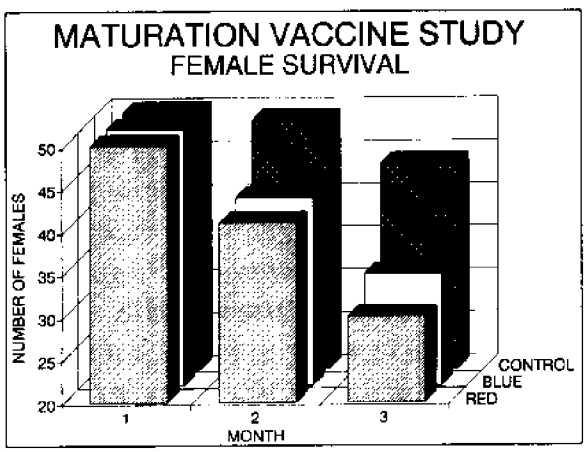


Figure 1. Number of vaccinated female broodstock surviving after one, two and three months in production.

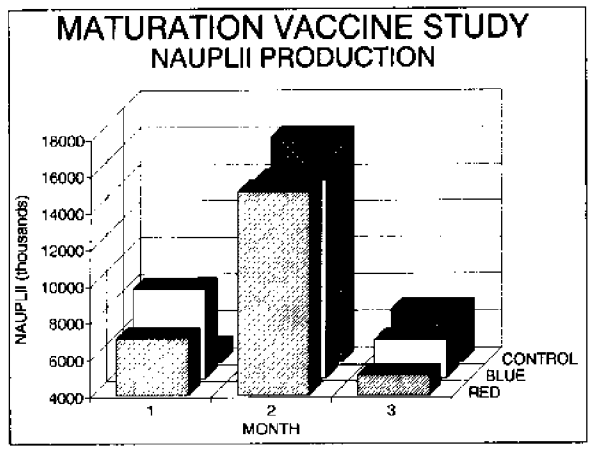


Figure 2. Number of nauplii produced per treatment at the end of the first, second, and third months. "Blue" and "Red" indicate two different vaccine formulations.

This survey was conducted in the dry season (April 92). Pond harvest data was not available at the time this report was prepared. During the dry season, growth rates are normally depressed, declining rapidly after about 7 or 8 g, and virtually stopping at 12 - 13 g. Deformities were less than 5% in all the ponds sampled. Attempts will be made to obtain data on stunting and deformities following harvest.

It is interesting to note that wild-caught postlarvae were stocked into virgin ponds (ponds not previously stocked) on farm No. 128. Yet, this farm had an 88% incidence rate and a severity score of one, indicating that IHHNV came in with the wild postlarvae.

An additional, more extensive survey, was made on farm No. 132, testing animals closer to harvest. A comparison was made between survival at harvest, severity of infection, and the percentage of wild postlarvae in the population. There was no correlation between the percentage of wild animals in the population or severity of infection, to survival. Pond 19 was free of detectable IHHNV but had a lower survival rate (39%) than pond 21 (68%). The latter had the highest severity score of the six ponds studied, but was also among the highest in survival (Fig. 5). No records were available on runt-deformity syndrome (RDS).

In practice, I have been unable to correlate RDS with IHHNV on extensive and semi-intensive farms in Latin America. I also believe there is evidence that RDS is not the result of a single agent or stress, at least in Latin American ponds. This is not to say that we should not strive to produce SPF broodstock. Common sense dictates that virus-free animals are desirable. However, very little is understood about the mode of infection and susceptibility of *P. vannamei* to IHHNV. One farmer asked, "If SPF postlarvae were available, and I went to the expense of stocking them, what would prevent wild stock from entering my

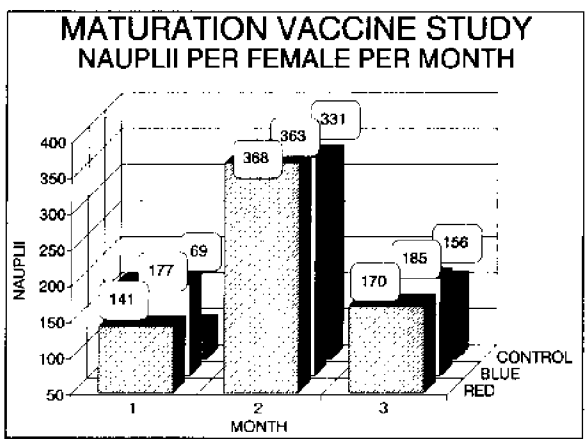


Figure 3. Average number of nauplii produced per female after adjusting for survival.

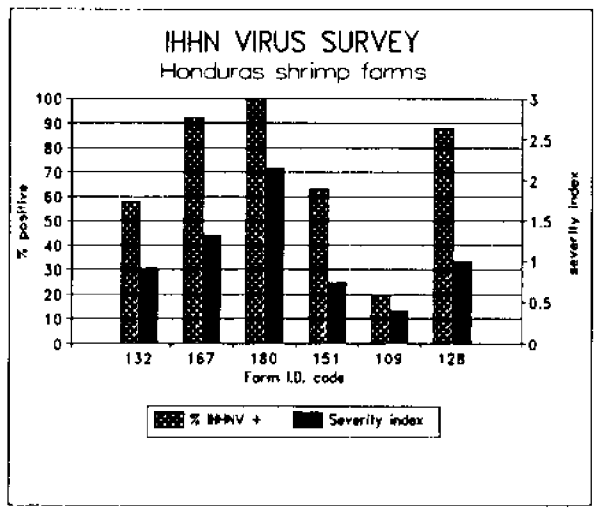


Figure 4. The percentage of IHHNV-positive juvenile shrimp and the relative severity of infection based on the number of inclusions observed for six farms in Honduras.

pond and infecting the SPF animals?" This is a good question.

At this point, I believe that any attempts to require the stocking of certified IHHNV-free postlarvae into ponds where *P. vannamei* is **indigenous** would be counterproductive at the least, and, at the worst, could destroy much of the shrimp industry in Latin America.

It remains to be seen, however, whether SPF progeny will also outperform wild-caught postlarvae or first generation maturation postlarvae in Honduras.

With regard to selective breeding, it is possible that virulence and/or the level of viral infection will increase in closed populations, resulting in a higher incidence of RDS. If this is the case, a successful domestication program will be impossible without SPF stock.

Note: Dr. Wyban and coworkers from The Oceanic Institute and Mr. Jaenike of Harlingen Shrimp Farms presented impressive data at this meeting showing significantly faster growth rates and a more uniform size distribution in SPF animals compared to IHHNV-infected shrimp. The infected shrimp used in the comparisons were from 5th or 6th generation broodstock.

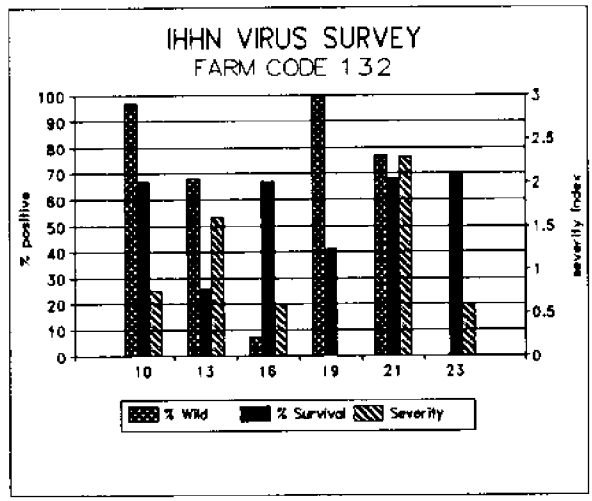


Figure 5. Incidence of IHHNV on a single farm for which six ponds were sampled. "Wild" refers to the percentage of postlarvae captured from the estuaries. "Survival" indicates the number surviving to harvest.

Acknowledgments

Thanks to Jorge Pang and his staff at Agromarina de Panama and Dr. Richard Pritto, Director of the Ministry of Agricultural Development and his staff at the MIDA Research Station at Aquadulce, Panama, for their assistance in running the vaccine trials. Also, to Ana Carolina Eisenman and the staff at Agromarina de Panama's maturation facility, Veracruz, Panama for their assistance in evaluating the vaccine for the broodstock. Finally, I thank Mr. Roberto Chamorro, Project Manager, USAID/FPX Honduras Shrimp Project, for funding the IHHNV survey.

Drugs and Chemotherapeutants for Shrimp Diseases: Their Present Status in the United States, with an Overview of Research and Approval Processes

Thomas A. Bell
University of Arizona
Department of Veterinary Sciences
Tucson, Arizona 85721, U.S.A.

Abstract

Diseases are playing an increasingly important role in the culture of shrimp. Controlling diseases has been, and will continue to be, essential to the expansion of the industry. At present, only one compound has been approved by either the Environmental Protection Agency (EPA) or the Food and Drug Administration (FDA) for use in shrimp culture within the United States. This paper summarizes the present status of drug research and the basic requirements for drug approval in the United States.

Introduction

As most researchers in the field of shrimp pathology are quite aware, international shrimp culture is in a strong growth phase. The 1991 world production of shrimp was approximately 2.4 million MT, with 28% coming from aquaculture. By comparison, the total world production in 1983 was approximately 1.8 million MT, but only 7% was contributed by aquaculture (Rosenberry, 1991, 1992; Food and Agriculture Organization, 1989). With increased production, the prevalence of infectious and noninfectious diseases in cultured shrimp has also increased substantially. Although data is lacking to quantify this increase, a rise in the volume of shrimp disease-related literature, along with more conferences, workshops and higher diagnostician caseloads are all indicators of the increased importance of shrimp diseases.

Many shrimp diseases fall into the potentially treatable category, primarily those with bacterial, protozoan and fungal etiologies. Further discussion in this paper will focus on treatable diseases. The actual mechanics of drug therapy will not be discussed here; readers should refer to Bell (1992) for that information.

Drugs Approved in the United States

In the United States, the process for approving food animal drugs and chemotherapeutants is extremely complicated, time-consuming and expensive. Approval process details will be discussed later; the process is so complex that only five drugs/chemotherapeutants are approved for use in aquaculture in general, only one of which can be used in shrimp culture.

The one chemotherapeutant for which the approval process has been completed for shrimp use in the United States is Aquatrine® (more commonly referred to as Cutrine®-Plus, Applied Biochemists Inc., Milwaukee, Wisconsin). Aquatrine®, a chelated copper sulfate compound, is registered and approved by the U.S. Environmental Protection Agency (EPA) for use in fish and shrimp ponds, tanks and raceways. It is registered for use as an algaecide against common algal species such as *Chara, Spirogyra, Cladophora, Microcystis, Spirulina, Enteromorpha, Oscillatoria* and other algae. Its label also permits use against the filamentous bacteria *Leucothrix*. Fish or shrimp in water treated with Aquatrine® can be harvested immediately after treatment. Depending upon the specific circumstances, Aquatrine® can be applied at levels up to 1.0 ppm for up to approximately 24 h. Aquatrine® is nontoxic at the recommended dose rates; therefore, shrimp may be left in the water during treatment and will coincidentally be cleared of any attached algae or filamentous bacteria. There is at least one generic copy of Aquatrine®; however, its use in shrimp ponds, tanks or raceways within the United States would be considered illegal.

In May 1991, public notification was officially made, via the *Federal Register,* that the data package submitted for the use of Formalin on shrimp had been accepted by the U.S. Food and Drug Administration (FDA). Such notification permits any commercial company to submit a New Animal Drug Application (NADA) for approved use of Formalin in shrimp culture. It is our understanding that Argent Chemical Laboratories Inc. (Redman, Washington) submitted a NADA in January 1992; an FDA decision is expected within six months from the submission date. At least one other company may be preparing a Formalin NADA package for the FDA.

Besides Aquatrine® and Formalin, no other compounds have formal FDA or EPA approval for use on cultured shrimp within the United States. There are, however, several compounds presently considered by FDA as "low regulatory priority chemicals." This means that FDA has reviewed all relevant literature and data on the use of these compounds and has decided that their use poses minimal risk if the following conditions are met (Guest, 1992):

- The compounds are used only for indicated conditions;
- The compounds are used only at prescribed rates;
- The compounds are used according to good management practices;
- The compound is of appropriate grade; and
- There is not likely to be an adverse effect on the environment.

Unfortunately, these compounds are seldom effective in combating shrimp diseases. This list includes (Geyer, 1992):

- Sodium sulfite (improves hatchability of fish eggs — not used with shrimp);
- Sodium chloride (osmoregulatory aide and parasiticide — not used with shrimp);
- Sodium bicarbonate (anesthetic in fish — not used with shrimp);
- Acetic acid (parasiticide — rarely used in shrimp culture); and
- Carbon dioxide gas (fish anesthetic — rarely used with shrimp).

The FDA is reviewing a long list of compounds that may eventually be placed in this category. They have also reserved the right to remove compounds from this list as new information becomes available indicating the compound's lack of safety and effectiveness (Geyer, 1992).

Groups Involved In Research and Approval Process

The entire drug approval process has traditionally been monetarily supported by the pharmaceutical firm manufacturing the drug for which approval is being sought. Unfortunately, even an investment in drug research to assist a large industry (relative to shrimp) like farm-raised catfish seldom holds the potential for required profit margins.

Therein lies our dilemma; an industry in a significant growth phase and with the genuine need for chemotherapeutants, but still too small to provide sufficient incentive for pharmaceutical firms to invest in aquaculture drug research. Fortunately, a few drug companies are either investing in aquaculture drug research based on the perceived potential of aquaculture in the United States, or they are investing in drug research in the United States with a worldwide market in mind.

The majority of existing shrimp drug work is being supported by public funds. In particular, support is being provided by the U.S. Department of Agriculture (USDA) through two separate agencies. The Regional Aquaculture Centers, and more specifically, the Center

for Tropical and Subtropical Aquaculture (CTSA), has provided funding for the past five years. CTSA normally funds projects within Hawaii and the Pacific Basin protectorates.

The USDA also supports minor animal drug work (including aquaculture) through the Interregional Project No. 4 (IR-4) program. The IR-4, through its four regional offices and via USDA funds, helps groups other than pharmaceutical firms obtain minor use approvals for new animal drugs. Minor use is defined as that intended for use on minor animal species (anything other than the major species: cattle, swine, chickens, turkeys, horses, dogs and cats) or that intended for use on minor diseases of major species.

There are several groups involved in drug research for aquaculture species. Only three groups are involved in formal shrimp drug research in the United States: the Gulf Coast Research Laboratory (GCRL) in Mississippi, Texas A&M University's College of Veterinary Medicine (TAMU), and the University of Arizona's Department of Veterinary Science (UA).

The GCRL group has concentrated on research not necessarily intended to support applications for drug approval, such as chronic toxicity studies and preliminary drug screening. They have also been involved in the initial screening of previously untested compounds. Much of their work is most applicable as supplemental data to NADAs.

The UA group, on the other hand, has concentrated its efforts on research aimed specifically at fulfilling NADA requirements. FDA-required work conducted by the UA group includes: *in vitro* efficacy trials (minimum inhibitory concentration, or MIC testing), *in vivo* safety trials, *in vivo* efficacy trials, efficacy field trials, medicated feed manufacturing stability trials, and human safety trials (shrimp tissue residue trials). In addition, the UA group has conducted studies that were prerequisite to the FDA-required studies or that would be considered supplemental studies. The prerequisite and supplemental studies include: palatability, pharmacokinetic and feed manufacturing studies. The UA group played a major role in the data package generation for both Aquatrine® and Formalin. The UA group involved in drug studies comprises two aquaculture pathobiologists, one aquatic toxicologist, one microbiologist and several technicians and graduate students.

The TAMU group has only recently become involved in formal drug testing studies. They have been assisting and/or coordinating the UA efficacy field trials for the past two years and will be doing the same for at least the next two years. They have also been involved in protocol development for field trials. The TAMU group comprises primarily a certified veterinary pathologist (specializing in aquaculture species), several advanced degree veterinarians and technicians.

Present Drug Research to Support Ultimate Approval

Several shrimp-use compounds are at various stages of investigation. The majority of the research is aimed specifically at FDA or EPA approval for use in shrimp culture. A few of the compounds listed have been dropped from further consideration. The list of promising compounds includes:

- Oxytetracycline;
- Romet-30®;
- Sarafin® (sarafloxacin);
- Baytril® (enrofloxacin);
- Florfenicol;
- Unnamed fluoroquinolones from Parke-Davis; and
- Treflan® (trifluralin).

Oxytetracycline approval studies have been underway for approximately 15 years. The original funding was provided the National Marine Fisheries Service (Corliss et al., 1977; Corliss, 1979) and later by Pfizer (the manufacturer) and Marine Culture Enterprises (a business partnership between the Coca-Cola Co. and the F.H. Prince Co.). The initial funding and work conducted by the UA ceased in approximately 1986. Work began again in approximately 1988, with funding from the CTSA; this work continues (Bell et al., submitted; Mohney et al., In press; Williams et al., In press). This latter period of work has focused on efficacy field trials and has been conducted by TAMU and UA. Pfizer officially sponsored the studies, but provided only minimal support. Pfizer's support has been primarily in the areas of medicated feed assays, protocol review and joint meetings with FDA. Future work on efficacy field trials will also be partially funded by another USDA-funded agency, the U.S. Marine Shrimp Farming Program (operating under the Gulf Coast Research Laboratory Consortium).

The potentiated sulfonamide Romet-30® has been the subject of required FDA studies since approximately 1988. CTSA began funding preliminary MIC and larval toxicity studies in 1988 (Mohney et al., In press; Williams et al., In press) and continues to fund other Romet-30® studies. Since approximately 1990, Hoffmann-LaRoche has also provided financial support, specifically in the areas of larval toxicity, juvenile pharmacokinetics, field bioavailability and field feed manufacturing studies. Hoffmann-LaRoche has indicated an interest in continuing penaeid shrimp studies in the areas of juvenile/adult palatability, toxicity and residue. The work on Romet-30® has almost exclusively been conducted by the UA. A portion of upcoming work, clinical efficacy trials, may be supported by the Marine Shrimp Farming Program, which will provide funds to TAMU.

Sarafin®, generically referred to as sarafloxacin, is a fluoroquinolone compound. CTSA began funding preliminary MIC and larval toxicity studies in 1988 (Mohney et al., In press; Williams et al., In press) and continues to finance other Sarafin® studies. Since approximately 1990, Abbott Labs has also provided financial support, specifically in the areas of larval and juvenile toxicity trials, juvenile/adult palatability and exploratory residue studies. Abbott initiated FDA Investigational New Animal Drug Application (INADA) submission for Sarafin® and has agreed to fund continuing penaeid shrimp studies in the areas of infectivity model development and testing. The work on Sarafin® has been conducted solely by the UA. Abbott Labs has expressed interest in having the UA test a similar fluoroquinolone in a like fashion.

Another fluoroquinolone compound, Baytril®, generically referred to as enrofloxacin, was investigated preliminarily by the UA with funding by CTSA that was received in 1989-90. The work completed to date included preliminary MIC trials and *in vivo* larval toxicity trials (Mohney et al., In press; Williams et al., In press). The manufacturer of the compound, Mobay (Shawnee Mission, Kansas), was approached for sponsorship and possible monetary support of future work; they declined any major participation, but agreed to provide the UA with research quantities of the drug. The UA has approached the IR-4 (Western Region) for possible sponsorship and funding, and verbal approval has been granted. The IR-4 (Eastern Region) is presently sponsoring and funding Baytril® studies on trout.

Florfenicol (a chloramphenicol analog manufactured by Schering-Plough, Kenilworth, New Jersey) was tested preliminarily. All work on the compound was conducted in 1988-89 by the UA and funded by CTSA. Preliminary tests included *in vitro* MIC studies and *in vivo* larval toxicity studies. The results of these studies were presented to Mobay in a request for sponsorship and potential funding of future studies. Schering-Plough declined to provide sponsorship or funding for future work. Further florfenicol work was dropped from the list of CTSA-funded work; however, IR-4 may be approached for financial support and sponsorship. It is our understanding that Schering-Plough has been concentrating their efforts on acquiring Japanese approval for use of florfenicol in yellowtail (*Seriola quinqueradiata*) culture.

Several fluoroquinolones produced by Parke-Davis (Ann Arbor, Michigan) were tested by the UA. These compounds are not commercially available for any animal use and, thus, were only identified by a code number. Both MIC and larval toxicity studies were conducted (Mohney et al., In press; Williams et al., In press) and two of the compounds provided the highest estimated Margins of Safety of any drugs tested to date. This work was funded by the CTSA, but ceased early in 1991. Parke-Davis has been approached for sponsorship and funding, but has declined

in both regards. One of the compounds has since been licensed to Upjohn, Inc. (Kalamazoo, Michigan); a similar request for sponsorship and assistance has been presented to Upjohn and a response is pending.

Treflan® is a commercially available product manufactured by Elanco (Indianapolis, Indiana). It is Elanco's formulation of trifluralin that is traditionally used in agriculture as a pre-emergent herbicide. Over the years, it has been found by the aquaculture industry to be an effective and safe fungistatic /fungicidal compound used against *Lagenidium callinectes* and *Sirolpidium* sp. Both of these organisms cause the ubiquitous disease known as larval mycosis. Elanco has been approached to sponsor and fund research to verify empirical findings, but has declined the offer. CTSA has begun (1992) to fund UA research in this area and the IR-4 (Western Region) has agreed to sponsor and provide additional funds for the research. A formal argument for the classification of trifluralin as a pesticide (and, thus, under the jurisdiction of EPA) as opposed to a drug (and, thus, under the jurisdiction of FDA) has been submitted to FDA. FDA has verbally informed the UA and IR-4 that the use of trifluralin in shrimp culture is, in their opinion, consistent with that of a pesticide and not a drug. Preliminary work is now underway to seek EPA approval for Treflan® use in shrimp culture.

As was noted earlier, total drug development in the United States is extremely complex and expensive. The costs can be substantially reduced if application processes and requisite testing can be abbreviated. One of the easiest means to achieve this is to seek a "label extension" for a product already approved for other species, in particular, other aquatic species. This procedure allows for the referencing, in the application for shrimp use, of information generated for the original species. Such data would most often include environmental and animal safety data. Because nearly all, if not all, new animal drug development costs are either clearly outside the limits of public funding and/or are unattractive to the pharmaceutical firms, the UA group has decided to pursue compounds for which only a label extension is required. Unfortunately, the drugs in this category may not be the safest and/or most efficacious drugs available. However, several available approved drugs of moderate efficacy/safety are of more use to a farmer than no drugs, even if they have excellent efficacy/safety.

Need For Approved Therapeutants

The need for approved chemotherapeutants in shrimp culture seems obvious. The prevalence of diseases and concomitant mortalities appears to be increasing exponentially relative to the growth of the industry.

The use of drugs and chemotherapeutants within shrimp culture falls under the jurisdiction of the local, state, provincial and/or federal government in which it is being conducted. Unfortunately, there is no uniform set of international codes governing the use of such compounds. Some governments cannot control their use, and this is where shrimp culture has flourished. In countries like the United States, where shrimp culture is inherently marginal due to basic environmental and climatic constraints, strict procedures for the approval of drugs has hindered the growth of the shrimp culture industry.

Within the United States, shrimp produced with only FDA-approved drugs and chemicals are perceived by the consumer to be of higher quality. Providing shrimp culturists with a useful set of tools with which to combat diseases will discourage the illegal use of chemotherapeutants.

Approval Requirements

There are two requirements for FDA or EPA approval for the use of drugs or chemotherapeutants in aquaculture, scientific research and administrative tasks.

Research Studies

The studies required by the FDA are outlined below. These basic studies are dictated by common sense — the use of chemotherapeutants in any situation, be it in aquaculture or with humans, can be allowed only when there is requisite information in four basic areas.

- **Efficacy.** Does the compound achieve the desired results?
- **Animal Safety.** Can the results be attained without further jeopardizing the health of the patient, whether it be a shrimp, a cow or a human?
- Human Safety. Does its use pose potential dangers to humans (other than the patient in the case of human medicine)? In the treatment of food animals, will there be unsafe drug residues in the edible portion of the animal when a person consumes the product? In any situation, does the use of the compound pose a danger to the person administering it to the patient?
- **Environmental Safety.** Does the therapeutant harm the environment? In the case of aquaculture, when a drug is administered to the cultured species, does it eventually make its way into the environment at harmful levels?

For terrestrial animals, there are relatively well-defined protocols for generating information in each of the four areas noted above. Extrapolation from terrestrial animals to shrimp has not been simple, however. The following is a brief summary review of such procedures as they apply to shrimp.

Efficacy or Effectiveness

One of the first steps in establishing the effectiveness of a prospective compound is to test it against potential pathogens to demonstrate that they are sensitive to the drug. This is accomplished by determining, via in vitro testing, the Minimum Inhibitory Concentrations, or MICs, for the drug/pathogen combinations.

To accomplish this, a "standardized test battery" of shrimp disease-associated isolates was assembled (Mohney et al., 1992). The test battery comprises 18 gram-negative isolates representative of the numerous geographic regions, shrimp genera, bacterial genera and antibiotic profiles. It also includes two reference bacteria, *Escherichia coli* and *Pseudomonas aeuroginosa*, from the American Type-Culture Collection. In our studies, we use the standardized test battery to determine an MIC profile for each prospective compound as the first screening test. Acceptable MICs are normally less than 2.0 ppm.

Assuming the drug is determined to be safe (see Animal Safety Section below), the same drug must be established as being effective at approximately the same dose levels in *in vivo* trials. These latter trials entail the use of dose-titration studies where the disease is intentionally induced (with the pathogen), followed by administration of the drug at varying levels. If the drug is effective, there should be a positive relationship between the level of drug administered and the level of disease reduction. These latter procedures have yet to be developed for shrimp, primarily due to the lack of truly obligate bacterial pathogens.

Safety When Used on Test Animal

The lowest dose toxic to the test animal must be established. Toxicity is not just the lowest level causing mortality; rather, toxicity is the level that causes death and any other deleterious effect (e.g., lethargy, poor growth, aesthetic considerations, etc.). These values are normally established by means of one or more of the following standardizedprocedures: Lethal Concentration (LC), Lethal Dose (LD), Effective Concentration (EC) or Effective Dose (ED).

As one might expect, the toxicity testing procedures established for cattle are not directly applicable to shrimp. Therefore, a standardized protocol for the initial testing of any prospective drug against shrimp has been developed (Williams et al., 1992). The procedure calls for an initial 48-h EC trial to be run on protozoea III - mysis I (Z_3/M_1) larvae. This trial is actually begun 24 h before the protozoea stage metamorphoses into the mysis stage and continues for 24 h into the mysis stage. Assuming these EC values are acceptable (a Therapeutic Index greater than 4.0), additional larval toxicity studies are conducted. These include a 24-h EC trial during the first 24 h of the nauplius stage, followed by a 48-h EC trial for the first 48 h of each of the remaining larval stages (protozoea, mysis and post-

larvae). All larval toxicity trials are conducted at a commercial shrimp hatchery or a commercial-sized research hatchery. A complete set of standardized test containers and supportive equipment and supplies are brought to the hatchery; the host hatchery need only provide larvae, algae, electricity and a work area of approximately 2 - 3 m^2.

A combination of the toxic level and the efficacious level yields the Margin of Safety (difference between values) or Therapeutic Index (ratio between values). This index is the relationship between the highest level of drug required to inhibit the growth of the pathogen and the lowest level toxic to the shrimp. A five-fold Margin of Safety is generally required before our group investigates a prospective compound further.

Human Safety

If it is assumed, from the previous tests, that the drug is nontoxic to shrimp at levels that are effective against the target pathogens, it must then be further assumed that effective levels will be achieved in the shrimp muscle. Of greatest concern to the consumer is the question, "How long are these effective levels retained within the muscle of the shrimp?" It is expected that no compound considered for use in shrimp culture would be retained within the shrimp tissues indefinitely. The drug should be degraded and excreted via the shrimp's metabolism and the compound's inherent half-life. Typically, a culturist must halt treatments of a given drug for a specified period of time before marketing the product for human consumption. That period of time, known as the "withdrawal period," is the amount of time a given drug persists in the edible flesh of treated shrimp at detectable levels.

The studies used to establish the withdrawal period are referred to as residue or depletion studies. They are time-consuming and expensive, requiring a significant amount of detailed laboratory analyses. An extremely rigid set of analytical guidelines, referred to as Good Laboratory Practices (or GLP) must be followed for the analytical data to be acceptable. A laboratory must be certified by the FDA as being able to conduct GLP; hence, there are very few GLP labs in the United States. Typically, the majority of these data are generated by the pharmaceutical company making the compound.

In the past, our activities in this area included feeding medicated feeds (uptake) and holding shrimp after medication (depletion). Samples of shrimp collected during uptake and depletion were typically frozen and shipped on dry ice to the drug manufacturer for analyses of drug levels in the edible portion.

The most important aspect of any product is its inherent safety to the end user or consumer. In response to the U.S. consumer, the FDA appears to have become more sensitive to the issues of contamination in food products. The recent formation of the FDA's Office of Seafood, which is responsible for en-

suring the final purity of domestic and imported products, is indicative of this concern for consumer safety. Hence, this aspect of the drug approval process may be most important, and surely is the most expensive step.

Environmental Safety

The FDA is primarily concerned with reviewing information to support the premise that the prospective drug does not harm the environment. A data package for an aquatic animal should include information that demonstrates rapid degradation within the cultured animal, short half-life within the culture system, a low effluent volume, a highly diluted effluent, a further dilution of the effluent once it enters natural water systems, etc.

Although, to our knowledge, the FDA is only concerned with the prospective drug harming the environment as a direct toxicant, there are other factors that should be of equal concern to shrimp culturists. Probably highest on this list is the direct or indirect effects on the microbial flora inside and outside of the shrimp facility. The use of antimicrobials, especially at suboptimal levels, can unnaturally shift the bacterial composition toward a resistant species. Theoretically, each successive use of the compound could increase the proportion of drug-resistant microbes. Such changes would be considered a direct aquaculture-specific impact.

The environment can also be indirectly affected. As the number of drug-resistant microbes increases, the chances for this characteristic to be transferred to previously drug-sensitive bacteria (related or unrelated to the original) increases. Drug resistance may be transferred via genome or extra-chromosomal plasmid exchange. The effectiveness of that particular drug can thus be further reduced.

Administrative Procedures

Unfortunately, the scientific studies involved in the new animal drug clearance are not the only requirements. Administrative tasks can be more difficult than the science. The following is a brief summary of various types of FDA applications and procedures that need to be followed to acquire drug approval in the United States.

Protocol Review

Prior to undertaking any experiments required for ultimate drug approval, FDA strongly recommends (but does not require) that you submit protocols for their review. Their rationale is that it will be much easier for you to change a protocol on paper than to repeat an experiment because of inadequate or inappropriate procedures. It is often prudent for investigators to follow protocol review submissions with a visit to FDA to discuss the protocols.

By law, the FDA must respond to nearly every type of submission within a set period of time; unfortunately,

from the investigators point of view, these time frames are quite liberal (up to 180 days) and the response does not have to be definitive. For example, 180 days after you have submitted an INADA, FDA may respond by telling you that a particular required item was not included with your application. Again, frequent contacts with FDA following a submission, either by phone or in person, may hasten the process.

Investigational New Animal Drug Application (INADA)

An approved INADA allows for the use of an unapproved drug; however, INADAs are intended to be used for a specific purpose and with several restrictions:

- **Meaningful Data.** Use of the prospective drug under the authority of an INADA will only be permitted when such use will generate data applicable to the submission of a New Animal Drug Application (NADA). Therefore, the drug can only be used under conditions specified in the INADA protocols. For the INADA to be approved, these protocols must be reviewed either prior to INADA submission or during the INADA review.

- **Human Safety.** Use of the compound should present virtually no hazard to consumers. Reasonable information must be available to indicate that rapid depletion of the drug does or will occur in treated animals.

- **Environmental Safety.** The use of the compound should cause minimum impact on the environment. Information must be presented that supports the rapid natural or induced (artificial) degradation, innocuous nature and/or minimal effluent of the prospective drug.

- **Investigators.** INADAs may be submitted by any qualified researcher or entity; however, FDA is now encouraging producer groups, state agencies, universities, regional aquaculture centers and other such organizations to submit the INADA instead of every individual desiring to use the drug. Such entities can supervise the use of the drug and allow only those individuals agreeing to follow protocol to use the compound, thus maximizing the chances of generating meaningful data.

New Animal Drug Application (NADA)

NADAs provide for the submission of required data in support of a request to gain the approval of a new drug for use with animals. As noted before, the submission, excluding the actual cost of scientific investigations, can be extremely high and time-consuming. Typically, NADAs are submitted by the pharmaceutical firm manufacturing the drug; often a specialized division within the firm handles this task. As noted previously, the NADA must contain supportive data in four specific areas:

- Efficacy;
- Animal safety;
- Human safety; and
- Environmental safety.

Abbreviated New Animal Drug Application (ANADA).
An ANADA is a NADA containing less information due to special circumstances, such as:

- A new use or dose rate on the same species, i.e., a supplement to an approved application or label extension;
- A new use on a different species, i.e., a supplement to an approved application or label extension; or
- A generic copy of an existing approved drug.

As in the case of a NADA, an ANADA must contain all the appropriate safety and efficacy data. However, data generated for the original approval that is applicable to the newly proposed usage may be referenced in the ANADA. In some instances, consideration may be given to the similarity of a new species to the original species.

In January 1991, the FDA began accepting generic drug ANADAs. In a generic ANADA, a sponsor is permitted to reference nearly all data generated for the original or "pioneer" product. Within a generic ANADA, however, the sponsor must provide data to support bioequivalency between the generic and the pioneer drug, or data that supports the generic being chemically identical to the pioneer. It is our understanding that no generic drugs are yet approved for food animals.

Summary

Shrimp cultured within the United States represents approximately 0.1% of the total world production of cultured shrimp (Rosenberry, 1992); however, the United States consumes in excess of 11% of the total world production (captured and cultured) of shrimp (Food and Agriculture Organization, 1989). The ability to legally and safely combat potentially treatable diseases should improve domestic production of cultured shrimp and reduce our reliance on imported products.

Literature Cited

Bell, T.A. 1992. Principles of shrimp culture chemotherapy. *In*: J.A. Wyban (Ed.). Proceedings of the Special Session on Shrimp Farming. World Aquaculture Society, Baton Rouge, LA., U.S.A. pp. 227-237.

Bell, T.A., D.V. Lightner, P.F. Frelier, R.A. Creelman and Y.S. Juan. Submitted. Treatment of Texas necrotizing hepatopancreatitis with oxytetracycline-medicated feeds: 1990 field trial and epizootiological studies. J. Aquatic Animal Health.

Corliss, J.P. 1979. Accumulation and depletion of oxytetracycline in juvenile white shrimp, *Penaeus setiferus*. Aquaculture. 16: 1-6.

Corliss, J.P., D.V. Lightner and Z.P. Zein-Eldin. 1977. Some effects of oral doses of oxytetracycline on growth, survival and disease in *Penaeus aztecus*. Aquaculture. 11: 355-362.

Food and Agriculture Organization. 1989. Fishery Statistics; Catches and Landing, Vol. 68. Food and Agriculture Organization of the United Nations.

Geyer, R.E. 1992. FDA Regulation of Drugs for Use in Aquaculture: Law and Policy. Presented at the National Aquaculture Extension Workshop, Ferndale, Arkansas. 3 March 1992.

Guest, G.B. 1992. Regulating Drug Use in Aquaculture. Presented at the Catfish Farmers of America National Convention, Orange Beach, Alabama. 28 February 1992.

Mohney, L.L., T.A. Bell and D.V. Lightner. Submitted. Shrimp antimicrobial testing. I. *In vitro* susceptibility of thirteen Gram-negative bacteria to twelve antimicrobials. J. Aquatic Animal Health.

Roedel, P.M. 1973. Shrimp '73 - A billion dollar business. Mar. Fish. Rev. 35(3-4): 1-6.

Rosenberry, B. 1991. World Shrimp Farming 1990. Aquaculture Digest, San Diego, California.

Rosenberry, B. 1992. World Shrimp Farming 1991. Aquaculture Digest, San Diego, California.

Williams, R.R., T.A. Bell and D.V. Lightner. Submitted. Shrimp antimicrobial testing. II. Toxicity testing and safety determination for twelve antimicrobials with penaeid shrimp larvae. J. Aquatic Animal Health.

Precautions for Importing and Culturing Non-native Shrimp

Carl J. Sindermann
National Marine Fisheries Service, NOAA
Oxford, Maryland 21654, U.S.A.

Abstract

Disease risks from importing non-native shrimp for aquaculture have received increased attention as a consequence of significant production losses that were traced to introduced viruses. A number of precautions should be taken during importation as well as later in culture development. A proposed code of practice oriented toward disease control emphasizes careful examination of the initial introduction, dissemination of progeny but not the original import, and the establishment of specific pathogen-free seed sources. Effective international communication and acceptance of the principles of uniform practices governing movements of shrimp stocks are critical to limiting the spread of pathogens.

Introduction

A proposal for deliberate introduction of a non-native marine species results in a potential crisis situation for decision-makers (and often, unknowingly, for the recipient ecosystem as well). Questions must be asked and answered about possible genetic, environmental and disease implications of the introduction. Since this workshop focuses on diseases of penaeid shrimp, and since disease risks are usually near the top of any perceived or actual problem list, it is logical to emphasize the kinds of precautions that should be taken to reduce disease risks associated with the importation and culture of non-native species.

Unlike the situation in terrestrial animal husbandry, risks of disease from introduced pathogens in marine animals concern possible effects on commercially important wild stocks, as well as on cultured populations. We must, therefore, be sensitive to threats to native species, as well as to the danger of spreading introduced pathogens among cultured populations of other shrimp species.

This discussion is divided into three segments: (1) precautions to be taken during the initial phases of an introduction; (2) precautions to be taken during later phases, when production is expanding; and (3) a codification of uniform practices that should reduce the likelihood of catastrophic losses in

cultured and wild stocks due to accidental introduction of nonindigenous pathogens.

Precautions During the Initial Phases of an Introduction

Precautions for introducing non-native species should begin long before any actual movement of animals. The species proposed for introduction should be subjected to a detailed risk assessment based on these areas of concern:

- A review of genetic information about the species, especially from viewpoints of selective forces in the new environment and potential hybridization with native stocks if escapes occur.

- A review of ecological characteristics of the species especially from viewpoints of competition/predation or colonization potential in recipient waters if escapes occur.

- An examination made of disease problems in natural populations of the species proposed for importation, and the history of disease outbreaks or unexplained mortalities encountered during any previous culture attempts should be reviewed.

- Examinations for the presence and effects of pathogens of shrimp, which concentrates on viruses — but not to the exclusion of other pathogen groups. If viral pathogens are found in natural populations, pathogen-free stocks will be sought for introduction. Additionally, the potential infectivity of each pathogen to other shrimp species should be determined clearly, as should carrier state relationships and possible viral synergisms. The search for viral pathogens should include carrier state bioassays and stress enhancement tests (Lightner et al., 1985).

If a decision based on adequate scientific information is made to introduce a non-native species of shrimp, then an adequate facility must exist in the recipient country — a fail-safe quarantine system immune to accidents that could lead to escapes, into which the imported animals can be received and maintained throughout one entire life cycle and preferably longer. This, ideally, should be a facility created expressly for the purpose of quarantine, and not merely a converted commercial hatchery, or (even worse) a collection of assorted tanks in the basement or on the grounds of a university marine laboratory.

Precautions During Culture of Non-native Shrimp

Once the quarantine period has been completed and close continuous examination of at least one entire life cycle has not disclosed the presence of pathogens, the F_1 generation of the introduced species can become a possible base for aquaculture production in

the recipient country. Seven additional precautions apply to shrimp culture generally, but to the introduced species in particular:

- If feasible, the facility rearing the introduced species should be spatially isolated from facilities rearing other species, preferably for several generations of the stocked species, and any unusual mortalities should be examined carefully.

- If other species are being produced in the same facility, they should be kept completely isolated from the introduced species, which may be susceptible to pathogens carried by but not lethal to the other species. This isolation means no exchange of species, staff, equipment or water.

- A routine disease monitoring program should be developed, based principally on gross observations, histopathology, stress enhancement tests and carrier bioassays. This activity should be augmented by a stress monitoring program based on the kinds of gross signs shown in Figure 1. A competent virologist should be available on retainer, to confront perceived emergencies.

- Introducing a non-native species must be considered as a single circumscribed event, designed to create a broodstock population. Successful completion of the proposed steps does not imply that subsequent importations of that species can be made without controls or restrictions, particularly insofar as disease risks are concerned.

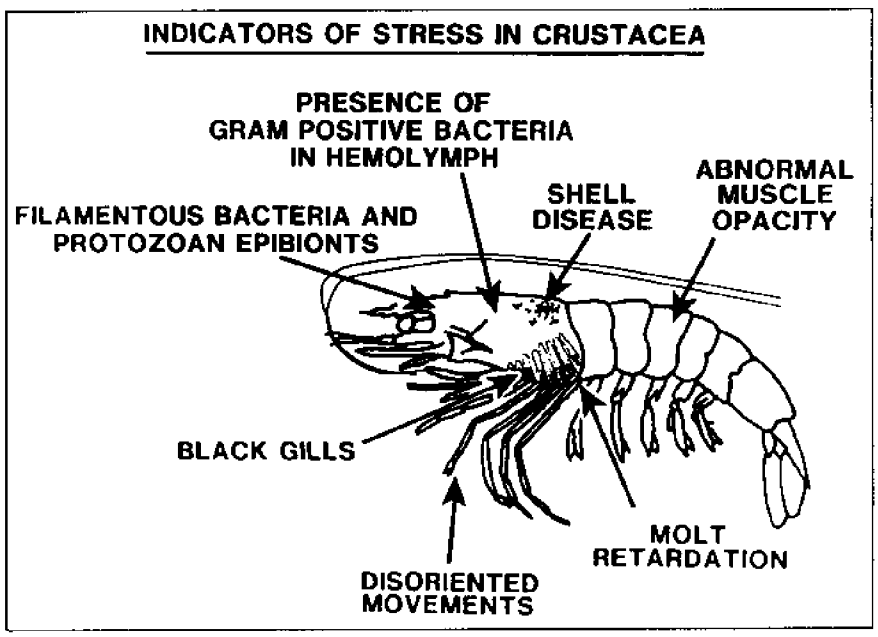


Figure 1. Some gross indications of stress in shrimp.

- Batches of shrimp of unknown origin or uncertain disease history should never, at any life stage, be brought into the culture facility.

- Detailed records should be maintained on the source, performance and disposition of every batch of shrimp that enters or is hatched in the culture facility.

- Every effort should be made to achieve and maintain specific pathogen-free status for the culture operation. A classification system for shrimp hatcheries should be developed that is comparable to the one used to classify and certify salmonid hatcheries in the United States.

A Proposed Code of Practice for Introducing Non-native Shrimp

How does all this come together? It does so in a **code of uniform practices for introduced species**, modified and expanded for shrimp from the ICES (International Council for the Exploration of the Sea) Code of Practice Regarding Introductions of Non-indigenous Marine Organisms (Fig. 2). I offer this adaptation for your examination and comments. I know that some will say (as others have already said) that the code is too extreme or too idealistic — that producers can't live with it. Such a code may mean the difference between viability and failure of shrimp culture enterprises and, if ignored, it

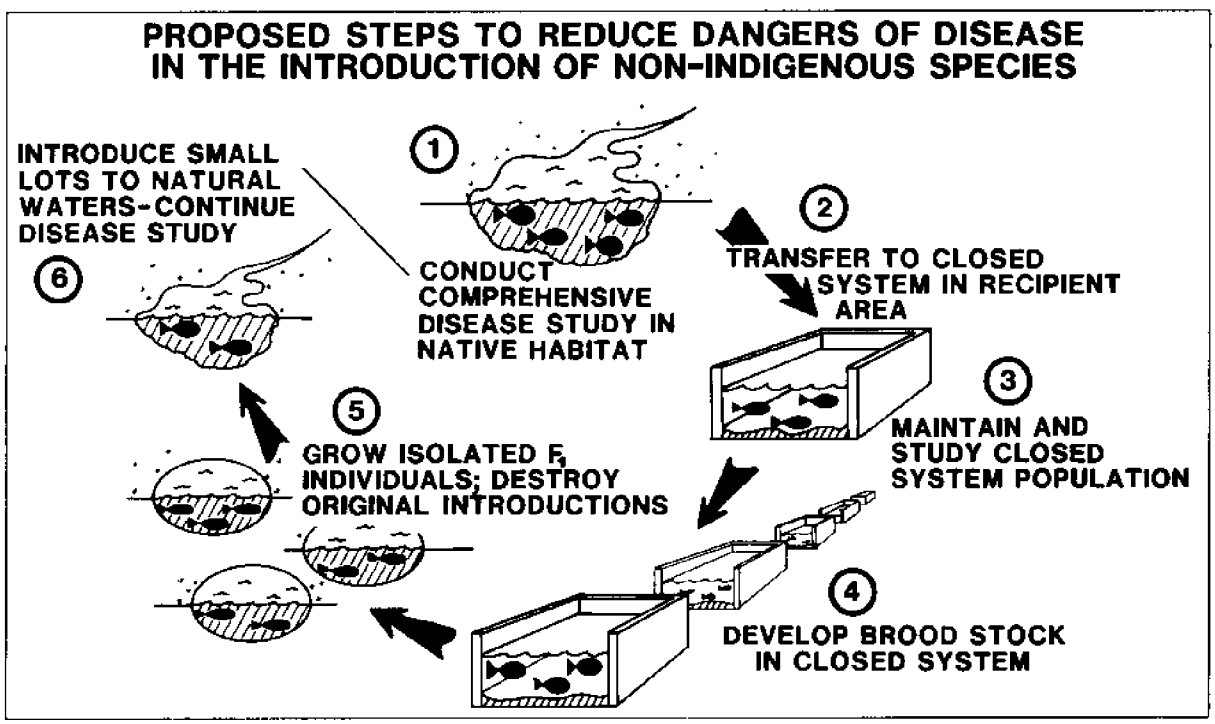


Figure 2. An illustration of some major features of the ICES Code of Practice Concerning Introductions of Non-indigenous Species (from Sindermann, 1986).

may also result in actions that can affect productivity of native wild stocks.

Several clarifications need to be stated early:

- The proposed code applies to introductions planned for either intensive, semi-intensive, or extensive culture, since experience has shown that escapes from any coastal culture facility are inevitable.

- Some of the guidelines may seem overly restrictive, but introducing a new species is a pre-emptive and often irreversible ecological step; therefore, the decision-making process governing introductions is critical. Some details of the proposed code follow.

Recommended procedure for all species of shrimp before reaching a decision regarding new introductions.

(a) Individuals, companies, governmental entities and research groups contemplating any new introduction should assemble and present to appropriate state and federal agencies, at an early planning stage, information on the species, stage in the life cycle to be introduced, area of origin, proposed place of introduction, and objectives of the introduction, with detailed information on its environmental requirements, epifauna, diseases, associated organisms and potential competition with species in the recipient environment.

(b) Appropriate governmental authorities, state and federal (including fishery management agencies), should examine the information on the "candidate for admission" to assess: the rationale and justification for the introduction, its relationship with other members of the ecosystem, and the role played by parasites and diseases. Inadequate information should be grounds for rejection of the application.

(c) The probable effects (genetic, disease and ecological) of the introduced species in the new area should be assessed carefully, including examination of the effects of any previous introductions of this or similar species in other areas.

(d) The appropriate governmental entities should consider all data and the possible outcome of the introduction, and reach a decision to approve or disapprove the proposed introduction.

If the introduction is approved, the following actions should be taken:

(a) A broodstock population should be established in an approved fail-safe quarantine facility. The progeny of the

introduced species may be transplanted to the natural environment if no diseases or parasites become evident during the quarantine period, but not **the original import.** The quarantine period will provide opportunity for observation for diseases and parasites. Duration of the quarantine period should be at least one complete life cycle, regardless of the stage at which the shrimp were introduced.

(b) Any unusual mortalities at any life cycle stage in native stocks of the country of origin or in quarantine, or low average hatchery survival rates (5 - 10%), should be considered as possibly induced by hitherto unidentified viral infections, and moribund specimens should be examined closely for occlusion bodies or ultramicroscopic viral arrays.

(c) Diagnostic procedures for suspected viral diseases should be extended to include carrier state bioassays with susceptible species and stress enhancement (overcrowding) tests (Lightner et al., 1985).

(d) All effluents from quarantine facilities must be sterilized in an approved manner, which means killing all living organisms in the effluents.

(e) If evidence of disease is obtained during the quarantine period, the introduced animals and their offspring should be immediately destroyed, the facility sterilized, and the approval for introduction withdrawn.

(f) Every effort should be made, in the United States and elsewhere, to develop and use certified specific pathogen-free broodstocks and certified hatcheries, modeled on the highly successful Conwy (Wales) program for molluscan shellfish.

After an introduction has been effected, a continuing study should be made of the introduced species in its new environment, and progress reports should be submitted to the authorizing governmental agencies annually.

Discussion and Conclusions

A code of uniform practices such as this one is a necessary first step, but it must be followed quickly by **protocol** development — by detailed procedural instructions. We need detailed protocols for everything related to new introductions — inspection, certification and quarantine. Additionally, we need to develop protocols for repeated introductions of species that have become part of established commercial practice — a reduced program of continuous disease monitoring and inspection. We also need to develop protocols for species that have been introduced previously and then reintroduced after passing through culture facilities in a third country — where new pathogens

may have been acquired. These and other protocols give the necessary form and substance to any proposed program to reduce risks from introduced diseases.

A code of uniform practices and the development of detailed protocols are not enough, however. We need a **regulatory framework** to ensure compliance with the codes and protocols—and this is not easy, since the last thing that any aquaculturist wants is more regulations.

All of this activity takes place on a **national** level, but to be really effective, such a code must achieve **international** acceptance, since shrimp culture is truly global, with a pathogen transfer network that almost defies description (although Lightner has made an excellent attempt in his recent [1990] paper). The best vehicle to facilitate communication is not clear either, although it could be proposed as an early initiative of the newly formed North Pacific Marine Science Organization (a Pacific counterpart of the Atlantic-oriented ICES) (Stewart, 1991). Alternatively, FAO, through its various regional fisheries commissions, could form a nucleus for the network that would be required— as could the OIE (International Office of Epizootics) through its four regional groups.

This final step of international acceptance is a difficult one. In the North Atlantic, where the celebrated ICES Code has existed for almost 20 years, some movement toward acceptance of the Code has been seen in many member countries. I know of no comparable activity in the Pacific, except for a workshop on exotic aquatic organisms held in Australia in 1988.

There is some reason for optimism, however. Even in the absence of a strong legal structure to control introductions, the risks from exotic pathogens can still be reduced significantly by the ready availability and utilization of specific pathogen-free (SPF) stocks. The desirability and utility of SPF seed sources seem clear, and we have several examples (salmonid culture in the United States and bivalve mollusc culture in Britain) of the effectiveness of the approach. Every effort should be made to develop and use SPF technology, including provision of certified broodstock as well as postlarvae. Panaceas are rare, however, and SPF stocks are not always as advertised. Probably the best recent example of this melancholy conclusion is the spread of crayfish plague in Britain since 1981, principally because of shipments of an infected introduced crayfish species from a supposedly "disease-free" hatchery (Thompson, 1990). The shipments were carrying the plague fungus, *Aphanomyces astaci*.

In addition to possible deficiencies in SPF technology, other disease problems may develop from unknown or unrecognized pathogens in the imported populations — pathogens that have not been described in the technical literature, but that may have the potential for outbreaks in stressed populations or in susceptible native species of the recipi-

ent country. An excellent recent example is the introduction and dissemination of the protozoan pathogen, *Perkinsus karlssoni*, together with its host, the bay scallop, *Argopecten irradians*, in waters off eastern Canada — after **three generations** in quarantine, during which time the pathogen was not recognized, except as an "idiopathic granuloma" (McGladdery et al., 1991). So the **risks from unknown pathogens are never zero**—and this may be especially true for shrimp, in which new disease agents are being discovered with distressing frequency.

In conclusion, it is obvious from the present status and stature of disease problems in shrimp culture that precautions must be taken to reduce the spread of pathogens. Regulatory measures constitute one approach to risk reduction, but development and use of SPF stocks offer a complementary approach. In my opinion, to make a significant impact on disease risks from introductions of shrimp, we need a combination of readily available SPF stocks and acceptance of national and international uniform codes of practice when we move those stocks, or any non-native stocks.

The forces encouraging the chaos of indiscriminate introductions can be overcome, and a rational conservative system can be assembled to ensure that shrimp introductions will be made according to agreed-upon uniform protocols, preferably from certified SPF sources. With such a system in place, risks from introduced pathogens can eventually be reduced to manageable levels.

Literature Cited

Lightner, D.V. 1990. Viruses section: introductory remarks. *In*: Perkins, F.O. and T.C. Cheng (Eds.). Pathology in Marine Science. Academic Press, New York. pp. 3-6.

Lightner, D.V., R.M. Redman, R.R. Williams, L.L. Mohney, J.P. M. Clerx, T.A. Bell and J.A. Brock. 1985. Recent advances in penaeid virus disease investigations. J. World Maric. Soc. 16: 267-274.

McGladdery, S.E., R.J. Cawthorn and B.C. Bradford. 1991. *Perkinsus karlssoni* n. sp. (Apicomplexa) in bay scallops, *Argopecten irradians*. Dis. Aquat. Org. 127-137.

Sindermann, C.J. 1986. Strategies for reducing risks from introductions of aquatic organisms: a marine perspective. Fisheries. 11(2): 10-15.

Stewart, J.E. 1991. A brief review of the International Council for the Exploration of the Sea (ICES) on the occasion of the formation of the North Pacific Marine Science Organization. Can. J. Fish. Aquat. Sci. 48: 2543-2550.

Thompson, A.G. 1990. The danger of exotic species. World Aquaculture. 21(3): 25-32.

Issues Related to Regulation of Penaeid Shrimp Diseases in Texas, U.S.A.

Sterling K. Johnson
Extension Fish Disease Specialist
Department of Wildlife and Fisheries Sciences
Texas A & M University, College Station, Texas 77843, U.S.A.

Abstract

Regulation of exotic shellfish has been stated in Texas regulatory code for almost two decades. Requirements and implementation of the regulation was rather orthodox. Those involved in shrimp aquaculture and other interests, however, had to address perceptions and issues arising from the influence of a number of factors. An abbreviated analysis of influences and impacts is offered, as well as suggestions for those who must address the general issue of disease regulation for penaeid shrimps.

Introduction

Regulation by government has become more prevalent in the world and especially in the United States. Texas has had a part in this and to some degree provides an example of how people have approached regulations related to penaeid shrimp diseases.

Because this is a discussion of regulation, it will be helpful to briefly explain the form of government that is present in the United States. The United States is a federal union composed of national, state, and local governments. Its constitution specifies the authoritative power that each segment of government possesses. The people control that authority through their elected representatives in the different places of government. Regulation, therefore, comes from either national, state or local government.

Protection for the common good has always been a function of government. The continual problems are: who defines and determines the common good? How will protection be achieved? As governments enlarge and societies become more complex, authority to formulate and enforce regulation is normally delegated to more remote places in bureaucracy. As regulators approach a "setting in stone" of regulation, they often find that the political process is not inclusive of enough suitable viewpoints. They must make extra effort to obtain people's input in order to avoid unbalanced results.

Issues are what people believe to be important at a particular time. The importance of a particular issue varies as time takes its course and according to a variety of influences. Shrimp disease regulation could be described as a single issue from the perspective of validity or current response. I have chosen instead to accept the issue's existence as fact and consider the issues that relate to its present state of development in the state of Texas. The viewpoint is that of an extension specialist whose role is education and service in the area of aquatic animal health. That role includes no exercise of regulatory authority.

This paper will give a brief background on important aspects of development and status of shrimp disease regulation in Texas, speak to the key influences and impacts, and mention some general and particular helps. In regard to the particular helps, they are addressed primarily to the principal component of this conference's participants — the research scientist.

Development and Status

Shrimp Aquaculture in Texas

Interest in marine aquaculture increased in Texas in the early 1970s. By the 1980s, several marine facilities were established. The emphasis on shrimp aquaculture in Texas was partly due to the existing and well-developed shrimp fishery, with its established processing and marketing channels. During the developmental phase, a number of native and exotic (nonindigenous) species were cultured. Of the species used, one emerged as the commercial species of choice: *Penaeus vannamei.*

In 1991, Texans cultured shrimp intensively in more than 350 ha of ponds distributed among less than ten farms. These were clustered mid-way up the coast near Palacios, Texas and in the southern-most county of the state near Brownsville. There were two small inland efforts. Two hatcheries existed, with a production capacity of 60 to 80 million postlarvae per month. The estimate for imported seed last year was 40% of total stock. One or two more hatcheries are planned for 1992-93.

Regulatory Authority

For regulations to exist, there must be some seat of authority. Regulations that relate directly to **shrimp disease** in Texas are found within the statutes of the natural resource agency, the Texas Parks and Wildlife Department (TPWD). They relate to the use of nonindigenous species (now called harmful or potentially harmful species). The Texas Department of Agriculture and a commission on animal health do not presently regulate shrimp disease. The former does issue a fish farming license, which is necessary for general operation.

On a federal or national level, regulation is still rather indirect. The U.S. Fish and Wildlife Service (USFWS), a resource agency of the Department of the

Interior could, in certain instances, assure compliance to state regulations based on a legislated act (Lacey Act). That agency also requires an import license for wildlife, including shrimp introduction. A simple health certificate or statement of health for port-of-entry check is a requirement of the U.S. Department of Agriculture.

Environmental Concerns

Disease is only one of several environmental concerns. This is important for disease specialists to recognize because regulation of disease, especially if introductions are at issue, is often interwoven with regulation of other concerns. The development and status of other concerns influence the existence of disease regulation. Regulation in Texas reflects more the concern for the natural environment than for aquaculture.

Regulation Relating to the Control of Shrimp Disease

Regulation of exotic shellfish imports was added to the Texas Regulatory Code in 1973. The licensed producer was to fulfill certain requirements in accordance with the permit issued. Later, when it was recognized that examination of nauplii for viruses was not practical, new rules were adopted.

The crawfish industry convinced the resource agency to exclude crawfish from this regulation. They did this by showing the need to import animals on a regular basis from neighboring states and noting that the movements would occur within the natural range of the species. Subsequently, introductions and distributions of Australian crawfish without disease inspections have been a common occurrence.

Shrimp farmers took the opportunity early in the development of regulation to influence how regulations were to be implemented. The decision for implementation was to examine subsamples of each batch of imported stock for diseases. The examination was to follow a set of general guidelines and the inspector would be someone approved by the agency.

Current rules include the stipulations that imported nauplii must be quarantined and examined monthly as they reach postlarval sizes, and that hatcheries from which the nauplii come must be certified as free of disease. Regular shipments of postlarvae and larger animals are inspected on a shipment-by-shipment basis and held in quarantine until they are declared clean by the regulatory agency.

The focus of the regulation relating to introduction of exotic shrimps and their diseases is the protection of the natural resource, in particular, the shrimp fishery, which is valued at $500 million. The regulations emphasize prevention of escape of exotic species and inspection of exotics for disease.

Key items of regulation intended to control escapes are: an acceptable design capable of stopping effluent, a

screen barrier between stocks and discharge capable of containing any life stage, dikes around farms within a 100 year flood plain, and adequate security to prevent removal by people.

Impacts

What are some important influences to the development of shrimp disease regulation in Texas? I will discuss first the nature of the influence and then its impact.

Societal, Agricultural and Fisheries Development

Will development of shrimp culture regulations proceed too fast? Will unnecessary regulations emerge under pressure from strange places, as regulators say, "better safe than sorry." Or, because of stalemates, will there be too little too late? The general state and pace of development of our society, agriculture, and fisheries, impacts everything, including regulation.

Impact: It is difficult to obtain a clear reading on regulatory expectations when change is rapid and structures are slow to develop. Regulatory concepts and stipulations may be in writing but implementation is vague.

Early disease regulations for exotics in Texas were drafted primarily with the intent to control oyster diseases and to have a mechanism (i.e., permit) to keep track of where animals were coming and going (Terry Leary, GCSMC, personal communication). Shrimp were included, but expectations were stated on the permit and on a case-by-case basis. Expected beneficiaries and hazards were not clearly defined.

Governmental Mandates

The basic mission of an agency is reflected in the regulation it directs. For example, agriculture agencies tend to support regulatory disease programs that would enhance or safeguard the production and marketing of an agricultural commodity. Natural resource agencies focus more on protection or conservation of natural systems.

Most government agencies that control aquaculture were not instituted to serve aquaculture. Yet, government agencies will, under pressure from without and within, tend toward an expansion of their roles to include things such as aquaculture if financial resources are available and when efforts can be justified. When conflicts arise, however, they generally retreat to the mandates that originally established their role and the will of the clientele they were originally intended to serve. This is no small problem for aquaculture.

A call for action often follows a perceived need for regulation or a concern about how to implement existing regulation. But an agency's effort toward aquaculture (including regulatory) often suffers in terms of its priority and the conviction and clarity of delivery. This is because societal leadership relies on agencies or agency groups whose fundamental mandates make them

more or less inappropriate for particular regulatory applications.

This is one of the reasons that private production interests and their representatives do not give hearty support to government initiatives concerning regulation, even when they are told that they will be beneficiaries. Private production interests tend to favor a suppression of regulation by government. In the United States, there are fewer regulations in states with large aquaculture industries. Consumer and environmental groups, in contrast, tend to favor adoption of regulation.

Impact: There is essentially no regulation of disease in Texas by the national government. This is not to imply that there needs to be. National government does little to reinforce state regulatory laws concerning aquatic animal disease. The U.S. Fish and Wildlife Service is active in control of movements of endangered species, but shrimps are not considered endangered. National guidelines and policies are undeveloped in regard to aquatic animal introductions. Only recently has legislative mandate developed to deal with harmful nonindigenous species. The legislation is so targeted toward a single species that its regulatory mandate may be too weak to form the basis for development of meaningful regulation from the national level.

The state government of Texas has focused on regulation of aquatic animal disease through its natural resource agency (TPWD). Due to an emerging environmental awareness, an environmentally conscious Texan perceives the introduction by release of nonindigenous animals or their diseases as very detrimental. Concern is also expressed by those who are dependent on exploitation of natural shrimp stocks. The agency's mandate is to protect the natural resource, so the focus is on protection against organisms perceived as biological pollutants.

Research

The desire for reliable information in an expanding industry is great. Knowledge of disease is popular. Mystique is often king in shrimp culture where biologists vie for attention and disease is a subject by which sweeping statements have the ability to gain attention. Disease also is often an easy answer for failure — and failure is common in the rush to adapt new and unproven technologies.

Research brings us information based on accepted scientific methods. Accurately documented and presented, it will hopefully pass the scrutiny of peers. Scientific information is supposed to eclipse expert opinion as "the best information available" and be a notch above "rumor has it."

Impact: Research has had a considerable impact on the regulatory level of decision making in Texas and other places. During the past few years, government agency personnel wanted to know whether or not the time was right to take prudent action by regulatory

control. They also wanted to know which endpoints to establish for diseases considered to have significant harmful effect. For certain viral diseases of shrimp, research had stated that danger existed and caution was needed. Warnings had been made by respected scientists, at least from the theoretical and predictive point of view. Texas regulation of shrimp disease, at last reading, does show an inclusion of what research has said on the matter. It has an implementation design that is workable for aquaculturists even though the regulations are based primarily on protection of natural resources.

Perception

Perception = how things are seen or understood; **reality** = how they really are. Science as a method helps us to gain a better grasp of reality. And people who do science have their own perception. And, obviously, much of what I am saying is my own perception.

Regulation is based on the gifts (good and bad) of research, but only to a degree. Environmental problems in our society gain response more on the basis of public perception than scientific reality. In the area of shrimp disease, the perception of the public, managers and regulators of what scientific data says may be somewhat different from what it said. Yet it is that perception that has had more force in bringing issues to the regulatory table. Once there, defenders of reality usually have a chance to fend for themselves.

Impact: I have no opinion polls that can document the history of the influence of perception on Texas regulation. I have by experience, however, seen its importance time and again and have spent a considerable amount of time relating research results to decision-makers to help them do their best.

Political Process

People and groups have particular views concerning what is important for the common good. If they are active and yet open to compromise, their efforts will serve the progress of all.

Impact: Several groups have engaged in the political process of formulating Texas disease regulations and the way they are implemented. The Texas Aquaculture Association has been the primary advocate group for aquaculture. The Texas Shrimp Association (shrimp fishermen, or "shrimpers") has been the primary group advocating protection of the fishery resource. Environmental and conservation groups have had some influence, usually in support of the shrimpers' positions. Those opposed to the use of nonindigenous species have not failed to wave the banner of virus danger before a public already sensitized by media to the perception of harmful effects of human viruses.

The shrimpers have had an important influence on bringing regulation to the table because they represent the largest fishery, with a value many times that of shrimp aquaculture. Authority has

been rather considerate to aquaculture in Texas in this regard. It has respected and involved the aquaculture position even when aquaculture was comparatively weak in force and organization. Several state agencies made efforts to be responsive even when those agencies had limited staffing and resources devoted to aquaculture. TPWD facilitated common forums for shrimpers and aquaculturists and met with aquaculturists on multiple occasions for input.

Producer Actions

In respect to regulation, shrimp growers are expected to comply and make attempts to provide a good example. They are not expected to engage in illegal actions or to otherwise greatly offend the perception of what the public considers acceptable. If they do what is noncompliant or offensive, it can result from malice, accident, ignorance or somewhere in between.

Impact: On the whole, Texas aquaculturists have presented a good example. Most of what is good and workable in our regulations are a result of sensible and accurate inputs from this group, whose membership is highly regarded by public officials. Mistakes, however, have fueled the need for tighter controls, especially in regards to escapement. Escapes of small proportion are anticipated in time, but toward the end of the 1991 growing season, several hundred pounds of juvenile shrimp were released into natural waters in South Texas. Public awareness was roused throughout the state and new and more restrictive regulations were printed, as adopted in the Texas Register in March 1992. Environmental activists have targeted the responsible farms and are searching for some way to find fault in compliance so that someone can be brought to justice. Cameron County has filed charges against a number of people, partnerships and corporations with production units at the release site.

Helps

Regulation is praiseworthy in many respects, but it is greatly weakened if from the view of the complier or others it is vague, politically motivated, ineffective, or unpredictable. Time, effort and expense of producers and others are often wasted at adversarial forums where the focus is merely a defense of self interest. It would be much better for all if the mind-set and environment were conducive to conflict resolution. Much anxiety could be avoided on the subject of regulation if the need for good endpoints is seen and efforts structured so that endpoints could be properly established.

Endpoints

Endpoints are the descriptors used to determine the fulfillment of objectives. They are expressed by measures in the form of criteria (fixed standards) or guidelines (broad performance standards). Guidelines are more appropriate measures for shrimp disease regulation because they better reflect the reality of

industry, the dynamics of biology and the use of professional judgment. They have a flexibility that is concurrent with changing but definable circumstances. There has been a tendency in shrimp disease regulatory efforts to focus on the criterion of presence or absence of a virus agent. This is because avoidance is a favored management strategy for aquatic animal viruses, and because unique biological characteristics favor a technological approach based on host specificity.

Regulation is shaky and unclear to a great extent because of the lack of acceptable endpoints. Good and better endpoints are needed if shrimp disease is to be regulated. Also needed is agreement on the commonly derived objectives that state what people want. They form the basis for endpoint selection. Common objectives are reached by communication, political process, consideration of valid information, scientific input, and other means. Time and effort must be devoted to derivation of common objectives so that good endpoints can be selected.

Regulatory endpoints should be effects-based because if there is no effect, there is no problem. In practice, however, we often have regulations before we have data that determines harmful effects. Prediction is very important to the process of endpoint selection, but prediction is dangerous if accepted wholly and without question. This generally happens when science becomes regulation. Supporters of shrimp aquaculture, in my view, have spent much time (and money) considering well-founded but predictive approaches. The consideration of the distinctive realities of the diverse problems are ignored in most cases. It is worthwhile again to note that both scientific and unscientific factors influence the selection of endpoints and objectives.

Common Sense

In a technological society, the use of common sense is often bypassed. Disease regulation should recognize the often vast differences in species biology, regional settings, and types of culture systems. It should recognize the true complexity of problems associated with disease and that natural systems have, at any point in time, a unique dynamism.

A recognition of actions of scale is also helpful when considerations are given to protecting systems of nature or aquaculture. Can, for example, a small producer respond to requirements that present unbearable economic hardship?

Level of Implementation of Regulations

Certainly the global nature of shrimp disease introduction will necessitate international cooperation. From where will implementation of regulations be directed once there is basic agreement on common objectives and discernment of recognizable differences from the global to local level? I think, for effec-

tiveness sake, it should be directed from the lowest level possible.

Helps From Scientists in Reaching Regulatory Endpoints

Wise Use of Limited Resources for Research

There are few people researching shrimp disease. More people, along with more support, of course, are needed. As a specialist responsible for transmitting research-based knowledge, my opinion is that we need "more of this and more of that" instead of "a little of this and a little of that." Some research needs to be repeated; in some cases, several times.

It is fortunate that many researchers have a strong degree of solidarity with what would be called aquaculture research. Those that say they are in aquaculture because of the research money usually do not have enough acquaintance with aquaculture to make wise choices on research direction. This is not to say that there is less need for viewpoints and active involvements from as broad a base as possible.

Balancing Reductionist and Holistic Approaches

Reductionist or "bottom-up" approaches try to rely on the simplest laboratory data. They are helpful in prediction and diagnosis but give a view that is remote from real world situations. Holistic or "top-down" approaches give a closer view of reality by direct measurements of problem impacts (i.e., field data basis). They are not as predictive or diagnostic. Both approaches, however, are needed for proper determination of endpoints used in regulatory controls.

Honesty in Recommendation of Technology-based Standards

As specialists, we have a basic responsibility to explain to decision-makers the array of possible strategies available to reach solutions on regulatory issues of shrimp disease. This includes the use of proper endpoints. We must, to the best of our ability, communicate the actual strengths and weaknesses of each, and not be afraid to take stands against those that are ill-founded or unscientific. And if the field of choices is bleak and there are areas with little or no answers, then we must say so. This is one of the best things that we can do. To say that more research is required may not be appreciated, but it may be just the kind of help that is needed.

Appreciation of Impact of Perception

It has been surprising to me to find that researchers have been just about as clumsy in respecting the value of perceptions as anyone else. Perception, however, does steer the ship. It fuels the development of issues. Poor per-

ception does not do much for direction or choice of action. Who will speak for science and what will they say? Why do some respected aquaculture spokespersons give credence to what could be nothing more than rumor in conversations with managers, regulators and the public?

Careful attention by specialists to portray repeatedly and with conviction what is accurate will do much to aid the process toward good regulation. One only has to look to recent struggles in quelling divergent and confused perceptions of the U.S. public about infectious viruses (HIV) to see the need for accurate information.

I have seen perception along with "facts" aired in a vehement and adversarial manner in regulatory hearings on shrimp introductions in attempts to sway opinion. Social science gives little help in those sessions. This says something for the need of more social scientists to be involved in aquaculture, especially in social settings beset with factions.

Appreciation of Various Roles

We are often asked to make contributions from a scientific perspective. Most of us welcome the chance to help in new and different ways. The call for contribution often comes from unfamiliar people beyond our ranks as disease specialists. It comes because we were perceived to have certain competence — an attribute which we may confirm by self-perception. Someone else with similar competence may have a role that better fits the need. If this is the case, and we are aware of it, then we as professionals should redirect the request of our inquirer. Also, we usually have a perspective on problems that allows us to identify others in auxiliary roles who could be very supportive. We should identify them to our inquirer and get them involved. Appreciation of roles can help to reach appropriate endpoints for regulation.

Resource Person to Coalitions

We must not be afraid to engage as resource persons in coalitions. Factions are present and a few resource people. Coalitions are formed in order to resolve conflicts, often in time of crises and often because of the need for, or the reaction to, regulation. If we are involved, we can do much good, and personal risk is minimized if we stay on track in our role. It is important to remember to stay within one's role in conflict resolution. Our position is often flattering, and momentary abandonment of role can mean that credibility will be lost in an instant.

Summary

Shrimp aquaculture and knowledge of shrimp disease have developed during the past two decades. It is difficult for government to provide proper regulatory response for this new and expanding development because of the necessity to cope with a variety of impacts. These include societal development, insufficient government

mandates, research results, producer actions, and the influence of perception on the political process. General helps toward establishing good regulation include selection of suitable endpoints, use of common sense and implementation of regulation at the best level. Scientists can do much toward the realization of good regulatory endpoints. They can urge the adoption of research programs with balance and efficiency. They can make their weight felt in the decision-making process by giving factual information to inquirers and at public forums and elsewhere. Scientists can also provide help by directing decision-makers to persons in roles best able to facilitate the establishment of good endpoints.

Selected References

General

Geasler, M. and J. Poley, 1991. Science, social science, policy, and environment. *In*: D.T. Smith (Ed.). Agriculture and the Environment. The 1991 Yearbook of Agriculture, USDA, Washington, D.C. pp. 217-222.

Ravera, O. 1991. Terrestrial and Aquatic Ecosystems: Perturbation and Recovery. Ellis Horwood, Ltd. London. 613 p.

Turner, G.E. 1988. Codes of practice and manual of procedures for consideration of introductions and transfers of marine and freshwater organisms. EIFAC/CECPI Occasional paper no. 23, 44 p.

Development and Status

Chamberlain, G.W. 1990. Texas aquaculture: status of the industry. Review draft for 1990 Texas Aquaculture Conference, January 30 - February 1, 1990, Corpus Christi, Texas. Printed by the Texas Water Commission, 1700 N. Congress Ave., Austin, Texas, 133 p.

Courtenay, W.R. and C.K. Kohler. 1988. Exotic fishes in North American fisheries management. *In*: R.H. Stroud (Ed.). Fish Culture in Fisheries Management. American Fisheries Society, Bethesda, MD. pp. 401-413.

Culotta, E. 1991. News & Comment: Biological immigrants under fire. Science. 254: 1444-1447.

Johnson, S.K. 1990. Aquaculture of Texas. World Aquaculture. 21(4): 52-56.

Johnson, S.K. 1991. The need for regulation in aquaculture: The case of introductions and transfers of biological disease agents. Draft of a presentation made at the U.S./Latin American Aquaculture Congress, Houston, Texas. 7 p.

State of Texas, Secretary of State, 1992. Official Texas Administrative Code, Title 31, Chapter 57.

State of Texas, Secretary of State, 1992. Texas Register, Adopted Sections, March 17, 1992. pp. 2009-2017.

Societal, Agricultural and Fisheries Development

Avery, D.T. 1991. Global food progress 1991: a report from Hudson Institute's Center for Global Food Issues. Chapter 5. Farming and the Environment, multiple authors. Hudson Institute, Indianapolis, Indiana. 268 p.

FAO 1987. Thematic Evaluation of Aquaculture. 85 + 44 pages. FAO, Rome, Italy.

New, M.B. 1991. Turn of the millennium aquaculture: Navigating troubled waters or riding the crest of the wave? World Aquaculture. 22(3): 28-49.

U.S. National Marine Fisheries Service, NOAA. 1990. Fisheries of the United States.

Research

Lightner, D.V. and R.M. Redman. 1991. Hosts, geographic range and diagnostic procedures for the penaeid virus diseases of concern to shrimp culturists in the Americas. *In*: P. DeLoach, W.J. Dougherty and M.A. Davidson (Eds.). Frontiers in Shrimp Research. 9: 28-33.

Pantoja-Morales, C. and D.V. Lightner. 1991. Status of the presence of IHHN in wild penaeid shrimp from the coast of Sonora, Mexico. (abstract). Society for Invertebrate Pathology, XXIV Annual Meeting. Flagstaff, Arizona, August 4-9, 1991.

Producer Actions

Dailey, R. 1991. Tougher regulations sought after release of exotic shrimp. Valley Morning Star, Harlingen, Texas, December 20.

Garza-Trejo, H.F. 1991. Alien shrimp threaten Laguna Madre. The Brownsville Herald. Brownsville, Texas, November 15.

Waite, M. 1990. Shrimpers fear farms' exotic species will taint bay. Valley Morning Star. Harlingen Texas, November 27.

Waite, M. 1991. Experts, shrimpers debate quarantine system. Valley Morning Star. Harlingen, Texas, August 29.

Waite, M. 1992. Charges filed against shrimp farms. Valley Morning Star. Harlingen, Texas, May 8.

General Helps

Chapman, P.M. Environmental quality criteria: What type should we be developing? Environ. Sci. Technol. 25(8):1353.

Elston, R. 1981. Discussion of shellfish certification issues aimed at formulating sensible solutions. Aquaculture Magazine. May-June Issue. pp. 28-33.

Glaze, N.H. 1991. Good science and the scientist. Environ. Sci. Technol. 25(8):1341.

Plumb, J. 1982. Influence of regulations on the spread of fish disease. *In*: Aquaculture: Public Health, Regulatory and Management Aspects. Sea Grant Publ. No. TAMU-SG-82-119. Texas A&M Univ. pp. 87-97.

Shrimp Health Management Procedures

Brad R. LeaMaster
The Oceanic Institute
Makapuu Point
Waimanalo, Hawaii, 96795, U.S.A.

Abstract

Increasing demand for marine shrimp will pressure shrimp producers to intensify their culture techniques to increase production. Intensive shrimp production systems will invariably encounter health and disease obstacles. This paper describes general preventive health recommendations and approaches that can be used to develop a preventive health program for marine shrimp. Specifically, the text demonstrates how preventive health measures can be applied to specific shrimp diseases.

Introduction

The modern era of shrimp culture began in the late 1970s and early 1980s. Technologies to enhance productivity combined with the lucrative Japanese and North American markets provided the necessary economic returns to establish a viable industry. Today, the world shrimp industry produces 28% of the shrimp placed on world markets. In 1991, the world's shrimp farmers produced an estimated 690,100 MT of shrimp from approximately one million ha of ponds (Rosenberry, 1991).

The profile of shrimp farming is shifting from extensive culture systems (50 - 500 kg/ha/yr) to semi-intensive (500 - 5,000 kg/ha/yr) and intensive (5,000 - 10,000 kg/ha/yr) farming (Brock, 1991a). Unfortunately, diseases have accompanied the expansion and intensification of shrimp farming. Sano and Fukuda (1987) reported that the annual losses related to disease in 1984 totaled 70.2 MT, 3.4% of the entire production from kuruma shrimp hatcheries and farms in Japan. Lightner (In press) reported that in most of the major shrimp farming regions of the world, hatchery and farm production losses due to diseases are increasing. In Taiwan, diseases contributed to the near collapse of the industry (Lin, 1989). Indeed, a discerning shrimp farmer must be knowledgeable in the area of diseases and preventive medicine. This paper summarizes preventive health measures and demonstrates how they can be applied on shrimp farms.

Etiologic Agents

A variety of biotic and abiotic agents cause shrimp diseases (Lightner, 1983, 1988; Brock, 1991b). The important viral

Table 1. Principal viral diseases of farmed penaeid shrimp.

Diseases	Etiologic Agents
Baculoviral midgut gland necrosis disease (BMN virus disease)	Nonoccluded baculovirus
Penaeus monodon baculovirus disease (MBV virus disease)	Occluded baculovirus
Baculovirus penaei virus disease (BP virus disease)	Occluded baculovirus
Infectious hypodermal and hematopoietic necrosis disease (IHHN virus disease)	Parvo-like virus
Hepatopancreatic parvo-like virus disease (HPV virus disease)	Parvo-like virus

diseases of penaeid shrimp are listed in Table 1, and the principal bacterial, fungal and parasitic diseases of shrimp in Table 2. An important consideration is the dynamic interplay between husbandry practices, stress, and the causal agents in many disease outbreaks. These interactions increase the difficulty of an accurate diagnosis and proper control. Infectious agents, environmental factors (soil and water, chemicals, biotoxins and pesticides), host characteristics, and husbandry practices all contribute to the causation-complex of shrimp diseases.

Preventive Health Principles

The production of healthy animals is the goal of any commercial enterprise. The goal of a preventive health program is to minimize loss due to clinical and subclinical disease and to maximize reproduction and quality of shrimp. Providing conditions to insure the good health of cultured shrimp includes the period from hatching to marketing. Additionally, as production methods are intensified, and the efficiency of conversion of feed to meat product is in-

Table 2. Principal bacterial, fungal and parasite diseases of farmed shrimp.

Disease	Etiologic Agent
Texas necrotizing hepatopancreatitis syndrome (TNHPS)	Unclassified rickettsia or bacterium
Hepatopancreatic rickettsiosis	Unclassified rickettsia
Vibriosis	*Vibrio* spp.
Filamentous bacterial fouling	*Leucothrix mucor* and other filamentous forms
Larval mycosis	*Lagenidium* sp., *Sirolpidium* sp.
Fusariosis	*Fusarium solani*
Microsporidosis	*Ameson* sp., *Agmasoma* sp. and *Pleistophora* sp.
Haplosporiosis	Unclassified protozoan
Intestinal gregarines	*Nematopsis* sp.
Epicommensal protozoa	*Zoothamnium* sp., *Epistylis* sp., etc.

creased, stresses are introduced that increase the potential for disease.

Preventing disease is much more economical than providing expensive treatments following a disease outbreak. There is not a single, ideal, universal preventive program that can be applied by every producer. Specific considerations must be taken into account for individual enterprises; however, there are some general preventive recommendations that can be made including (after Fraser, 1986):

1. Provide adequate and clean water.
2. Provide adequate space.
3. Provide adequate and properly balanced feed.
4. Prevent undue temperature changes.
5. Always use sanitary procedures.
6. Remove feces as often as practicable, remove dead fish, prevent the accumulation of other organic matter such as uneaten feed and the accumulation of a biofouling community; i.e., algae and slime.
7. Follow an "all-in, all-out" concept when feasible.
8. Thoroughly clean and disinfect between crops.
9. Separate age (size) groups as much as is practical. Avoid mixing species. Polyculture practices are an exception.
10. Avoid unnecessary handling.
11. Immunize against diseases common to the geographic area if feasible.
12. Control internal and external parasites.
13. Provide diligent surveillance to recognize early signs of disease.
14. All new incoming shrimp should be quarantined from resident stock. Movement of shrimp should be restricted from a suspected or unknown disease status area. Detention time should be at least as long as the incubation period of the suspected disease.
15. Isolate or destroy diseased shrimp.
16. Provide adequate nursing for diseased shrimp.
17. Begin treatment of diseased animals as soon as possible after disease is diagnosed.

Developing a preventive health program entails assessing the current needs and objectives of the producer, the current management and husbandry practices, the current health and disease problems, education of the producer if appropriate, and the future anticipated needs. The basic principles used to protect the producer, as well as customers, are 1) begin by establishing

Table 3. Shrimp disease prevention and control options for medical action.

Biotic Agents	Abiotic Agents
Barrier rearing and restriction of movement	Remove exposure
Disinfection	Feed supplementation
Disruption of pathogen/parasite life cycle	Stock management practices
Chemoprophylaxis	Resistant species/strain
Chemotherapy	
Resistant species/strain	
Enhanced host's defenses	
Stock management practices	

healthy, disease-free animals; 2) establish barriers to prevent disease from entering the farm; 3) surveillance and monitoring for the presence of disease; 4) have disease-fighting programs in place and initiate prompt action when disease does occur; and 5) continuous effort to upgrade the overall health status of the program.

Application of Preventive Health Principles to Shrimp Diseases

Options for prevention and control of shrimp diseases have evolved from traditional veterinary and animal husbandry methodology. For diseases of biotic origin, quarantine and restriction of movement, disinfection and sterilization, enhanced species resistance, disruption of the parasite/pathogen life cycle, chemoprophylaxis and chemotherapy, enhancement of host's defenses, and stock management practices have been applied on a commercial or experimental basis. For diseases of abiotic origin, removal of the opportunity for exposure, feed supplementation, altering or improving stock management practices, and utilization of resistant species have been used (Brock, 1991a). Table 3 summarizes these various methods.

Biotic Agents

Barrier Rearing and Restricting Movement

An example of preventing disease by utilizing the principles of restricting movement (quarantine) and barrier rearing would apply for the infectious hypodermal and hematopoietic necrosis virus (IHHNV). IHHNV is a parvo-like virus that is highly contagious to all species of penaeid shrimp tested (Bonami et al., 1990; Lightner, 1988). IHHNV is widely distributed in cultured penaeids, but its range in wild shrimp has not been fully determined (Brock, 1991a).

For *P. stylirostris* and *P. vannamei*, IHHNV is extremely threatening. In juvenile through adult *P. stylirostris*, IHHNV occurs as a rapidly disseminating disease characterized by high morbidity and mortality (Bell and Lightner, 1987a; Brock, 1991a). In *P. vannamei*, infection by IHHNV during early devel-

opmental stages results in a disease characterized by poor growth, various cuticle and appendage deformities, and reduced survival during later stages of culture (Kalagayan et al., 1991). This phenomenon has been termed "runt-deformity syndrome." Larval stages of both *P. stylirostris* and *P. vannamei* are commonly infected; however, IHHNV is not associated with clinical disease in these younger life stages. IHHNV infections are persistent in shrimp, so once a group of shrimp becomes infected, the virus remains in the population and is transmitted between generations (Brock, 1991a). Several reports have indicated that IHHNV has probably been widely disseminated as a result of the movement of live shrimp associated with aquaculture practices (Lightner, 1983; Brock et al., 1983; Colorni et al., 1987).

Improved diagnostics have contributed to the prevention and control of IHHNV. Specific pathogen-free (SPF) stock are showing promise as a means to prevent IHHNV. The Gulf Coast Research Laboratory Marine Shrimp Consortium at The Oceanic Institute has SPF *P. vannamei* derived from a founder population of postlarvae from a commercial hatchery in Northern Mexico and introduced into Hawaii in 1989 (Brock, 1991a). Strict enforcement of rigorous quarantine protocols and routine surveillance (viz., barriers and restriction of movement) have kept these shrimp free of IHHNV to date.

Disinfection

Bacterial infections result in severe economic losses in shrimp hatcheries (Nash, 1988; Brock, 1991a). Bacterial disease in hatcheries can be associated with sudden, high mortality and almost complete loss of production. The types of bacteria associated with these infections are facultative pathogens that reservoir in the water, larval feeds or the hatchery environment (Lightner, 1983, 1985; Brock, 1991a). Disease occurs when bacterial populations increase, and high densities of potentially pathogenic species dominate the hatchery tank microflora. Unfortunately, antibiotics have been indiscriminately used in shrimp hatcheries as a quick control measure instead of changing poor husbandry practices. Besides being costly, the routine use of antibiotics has led to the emergence of antibiotic-resistant bacteria as well as posing health risks to hatchery workers (Brown, 1989).

Successful prevention of bacterial diseases can be achieved by a properly balanced culture medium coupled with an "all-in, all-out" practice that allows for routine disinfection and sanitation of equipment, pipes, and tanks between uses (Brock, 1991a).

Disruption of Pathogen/Parasite Life Cycle

The Microsporida are protozoa that have been associated with the disease "cotton shrimp." Microsporida are common parasites of wild shrimp (Lightner, 1988) and have been identified from penaeid shrimp in the Eastern and Western

hemispheres (Anderson et al., 1989; Lighter, 1988). Wild-caught adults stocked into maturation systems can be infected. Moderate to heavy Microsporida infections are diagnosed by the gross appearance of involved organs which appear fuzzy or "cotton-like." Even though Microsporida infection of cultured shrimp is not common, infections of growout shrimp populations result in reduced survival and economic loss related to rejection of the product at the packing plant.

The life cycle of these protozoa is complex; shrimp serve as an intermediate host and a predatory fish is the final host. Vertical or horizontal transmission does not occur (Lightner, 1988). Prevention of microsporidosis involves disruption of the parasite's life cycle. This can be achieved by screening to exclude infected shrimp from the farm, or by removing the predatory fish that serves as the final host from ponds (Brock, 1991a). Shrimp with microsporidosis should not be transported; infected shrimp should be destroyed.

Chemoprophylaxis

The strategic and responsible use of chemical products can be important in a preventive health program. In shrimp hatcheries, larval mycosis is a ubiquitous problem that can lead to devastating losses if not properly controlled. Motile zoospores are responsible for disseminating larval mycosis infections. These fungi are saprophytes and are thought to reservoir in the hatchery water system and possibly in the broodstock shrimp. *Lagenidium* sp. is normally associated with disease outbreaks in the nauplii and protozoea stages, while *Sirolpidium* sp. infection is common in the late protozoea to mysis stages. Older shrimp are rarely infected because their thicker cuticle inhibits penetration of the zoospore germ-tube (Brock, 1991a).

The use of trifluralin (Treflan®) is very efficacious in the prevention of larval mycosis. Trifluralin is a herbicide that effectively destroys the motility of the fungal zoospores and prevents widespread infection (Bell and Lightner, 1987b; Brock, 1991a, 1991b). In addition, proper disinfection of hatchery equipment helps reduce the presence of the fungal reservoir in the environment (Baticados, 1988).

Chemotherapy

Chemotherapy has been used to successfully control rickettsial infections in shrimp. A pleomorphic, intracellular infectious agent has been associated with a serious disease syndrome of pond-cultured *P. vannamei* in localized geographic areas of Texas. This disease has been termed "Texas necrotizing hepatopancreatitis syndrome" (TNHPS, formerly known as "Texas pond mortality syndrome") (Bell and Frelier, 1991). The presence of the infecting organism can be verified by histologic demonstration of the characteristic microcolonies in the hepatopancreas of *P. vannamei* using special stains or electron microscopy. The use of oxytetracycline-medicated feed has been reported to be

effective in controlling TNHPS (Bell, 1991; Bell and Frelier, 1991).

Resistant Species/Strain

Fusarium disease is a fungal disease that can be a serious affliction of subadult to adult cultured penaeid shrimp. The causative agents are *Fusarium solani* and possibly other *Fusarium* spp. *F. solani* is a ubiquitous organism with a worldwide distribution. It is a saprophytic fungus and can be abundant in culture systems where excess decaying organic matter is present or where culture practices are conducive to cuticular wounding. *F. solani* invades dead or damaged tissue such as cuticular wounds. Any site is susceptible, but gills, appendages and uropods are most often involved (Brock, 1991b). There is a strong correlation between species and age and susceptibility to infection (Lightner, 1988). Among Asian penaeids, *P. japonicus* is highly susceptible (Lightner, 1988; Brock, 1991b). Practical prevention measures recommended in culture systems where Fusarium disease is a problem include using a resistant species of shrimp such as *P. monodon*, reducing organic matter, and reducing wound-creating husbandry practices (Brock, 1991a).

Enhanced Host's Defenses

Increased understanding of the immune response and mechanisms of resistance to disease by marine shrimp has led to the development of immunoprophylaxis regimens for certain bacterial diseases. In juvenile and adult shrimp, the bacteria that invade shrimp tissues are predominately gram-negative, oxidase-positive rods belonging to the genus *Vibrio* (Brock, 1991a). Septicemic vibriosis and localized internal *Vibrio* sp. infections are associated with different clinical signs. Gross signs include opaque abdominal musculature, anorexia, and darker pigmentation. In larval and early postlarval shrimp, signs of vibriosis include melanization and necrosis of appendage tips (Lightner, 1988).

Studies have shown that immunization protected shrimp from experimental challenge to bacterial pathogens (Itami et al., 1989). Further, Lewis and Lawrence (1985) reported that vaccinating penaeids with a killed *Vibrio* sp. bacterin resulted in improved protection against vibriosis in pond-raised shrimp. Vaccinating to enhance the shrimp's defenses against bacterial diseases may become a practical preventive health technique for shrimp farmers in the future.

Stock Management Practices

It is generally understood that conscientious and responsible husbandry practices are essential for successful production. Proper stock management practices have a major impact on lowering stressor pressure. Several diseases associated with biotic agents can be prevented by adhering to sound stock management practices. For example, bacterial fouling is a frequent problem in penaeid hatcheries. It is a disease condition that results from the heavy colonization of cuticular surfaces by

noninvasive bacteria. All age classes of shrimp can be affected, but the disease is commonly associated with larval and postlarval mortality. At low densities, bacterial colonization causes no apparent harm to the shrimp. When nutrients are plentiful in the culture water, multiplication of bacteria is stimulated and the microbial numbers increase to levels where physiological function is impaired. When fouling organisms heavily colonize the gill lamellae, respiration is compromised (Lightner, 1988; Brock, 1991a).

Therapeutic antibiotic or chemical treatment may be indicated in certain cases of bacterial fouling to lower bacterial numbers; however, successful prevention is closely related to the proper management of the predisposing conditions. These include improved water quality, increased water exchange, lower stocking density, and close surveillance to minimize overfeeding (Brock, 1991a).

Abiotic Agents

Remove From Exposure

Formulated feeds subjected to storage under humid tropical conditions are commonly infested with the fungus *Aspergillus flavus*. This mold species produces several classes of aflatoxins. Of these, AF B_1 is one of the most potent naturally occurring hepatocarcinogens. Aflatoxicosis is not considered a significant disease of cultured penaeids. However, mortalities were induced in a population of juvenile *P. vannamei* after feeding diets containing 50- to 300-ppm aflatoxin B for 28 days (Wiseman et al., 1982). The point is that the mechanism for aflatoxicosis to become an important disease in shrimp is in place, because penaeids reared in intensive and semi-intensive systems are fed diets that are typically formulated with ingredients that occasionally contain aflatoxins. Aflatoxin could also be produced in feeds improperly stored under the warm and humid conditions typical of the regions where penaeids are cultured (Lightner, 1988).

Practical preventive measures would include removing the opportunity for exposure by proper handling and storage of feeds, and the raw ingredients. Feeds and feed ingredients that appear moldy should be avoided.

Feed Supplementation

Supplementing feed for potential deficiencies is a common technique. Vitamin C deficiency, or "black death disease" can affect all penaeid species and is known to affect juvenile to subadult shrimp cultured intensively in tanks. Black death disease results from a low level of vitamin C in the diet (Lightner, 1988). Shrimp with clinical vitamin C deficiency may experience a 1 - 5% daily mortality. More typically, exposure to other stressors triggers massive losses (Lightner, 1988; Brock, 1991b). The extent of the disease in cultured crustacean populations is unknown. Therefore, subclinical vitamin C deficiency may be quite common in intensive culture establishments.

Control and prevention of black death disease is achieved by proper diet formulation to supplement sufficient vitamin C required for metabolic needs. Shrimp tissue content of 0.03 mg ascorbic acid/g is recommended (Lightner, 1988; Brock, 1991b).

Stock Management Practices

As previously mentioned under the biotic agents of disease, stock management strategies that promote an overall decrease in culture-related stressors will greatly increase production efficiency. Again, some of these strategies include adequate and unpolluted water supply, proper stocking densities, adequate and a properly formulated feed, undue temperature changes, and diligent sanitation at all times.

Resistant Species/Strains

In temperate regions, seasonally cold temperatures are often a limiting abiotic factor for farming shrimp. The commercial aquaculture community has expressed an interest in identifying a shrimp that could be cultured in the United States during periods when *P. vannamei* culture is temperature limited. Data suggest that *P. chinensis* is a cold-resistant species. Besides its good growth at low temperatures, *P. chinensis* is easy to culture, has broad salinity tolerance and could be readily marketed in the United States, Europe and Japan (Main and Fulks, 1990). Future research in the area of penaeid genetics may yield varieties of shrimp that are resistant to a broader range of disease-causing agents.

Summary

Preventive health is not just the routine application of disease prevention procedures; it encompasses a way of thinking, philosophy, and goals. Preventive health programs must be customized for each farm. Successful preventive health systems require input from many areas, including nutrition, environment, health and physiology, disease (microbiology and pathology), epidemiology, genetics, management, and economics.

Meticulous planning and constant surveillance are required to decrease the danger of disease. The routine day-to-day data that is generated by system monitoring should be stored in an orderly fashion to allow for easy and frequent analysis. Such analysis of data can provide advanced warning of disease problems in many instances. The application of computer software programs to aid in preventive health is in its infancy. Without question, computer programs will become increasingly useful to help monitor and prevent disease, thereby increasing the efficiency and production of shrimp farms in the future.

The aquatic environment, as well as the physiology of marine shrimp, offer distinctive challenges for the producer and health professional. However, these challenges can be met with innovative application of traditional veterinary preventive health principles.

Literature Cited

Anderson, I.G., M. Shariff, M. Nash and G. Nash. 1989. A hepatopancreatic microsporidian in pond-reared tiger shrimp, *Penaeus monodon*, from Malaysia. J. Invertebr. Pathol. 53: 278-280.

Baticados, M.C.L. 1988. Diseases of prawns in the Philippines. SEAFDEC Asian Aquaculture. 10: 1-8.

Bell, T.A. and D.V. Lightner. 1987a. IHHN disease of *Penaeus stylirostris*: effects of shrimp size on disease expression. J. Fish Dis. 10: 165-170.

Bell, T.A. and D.V. Lightner. 1987b. An outline of penaeid shrimp culture methods including infectious disease problems and priority drug treatments. Vet. Hum. Toxicol. (Suppl. 1). 29: 37-43.

Bell, T.A. and P.F. Frelier. 1991. The treatment of Texas Pond Mortality Syndrome (TPMS) with oxytetracycline medicated feeds: 1990 field trial results. World Aquaculture '91. San Juan, Puerto Rico (Abstract). p. 18.

Bell, T.A. 1991. Overview of diseases and drug needs for major aquaculture species: shrimp. Vet. Hum. Toxicol. (Suppl. 1). 33: 19-23.

Bonami, J.R., T. Bronwen, J. Mari, M. Brehelin and D.V. Lightner. 1990. Purification and characterization of the infectious hypodermal and hematopoietic necrosis virus of penaeid shrimps. J. Gen. Virol. 71: 2657-2664.

Brock, J.A., D.V. Lightner and T.A. Bell. 1983. A review of four virus (BP, MBV, BMN and IHHNV) diseases of penaeid shrimp with particular reference to clinical significance, diagnosis, and control in shrimp aquaculture. Int. Council for the Exploration of the Sea, C.M. 1983/Gen: 10/Mini-Symposium.

Brock, J.A. 1991a. An overview of infectious diseases of cultured penaeid shrimp with an emphasis on those caused by obligate pathogens. U.S. Marine Shrimp Farming Program Shrimp Breeding Workshop. July 16-18, 1992. The Oceanic Institute, Waimanalo, HI.

Brock, J.A. 1991b. An overview of diseases of cultured crustaceans in the Asia Pacific region. Report on a Regional Study and Workshop on Fish Disease and Fish Health Management. Asian Development Bank Agriculture Department Report. Series No. 1. June 1991. pp. 347-395.

Brown, J.H. 1989. Antibiotics: Their use and abuse in aquaculture. World Aquaculture. 20: 34-43.

Colorni, A., T. Samocha and B. Colorni. 1987. Pathogenic viruses introduced into Israeli mariculture systems by imported penaeid shrimp. Bamidgeh. 39: 21-228.

Fraser, C.M. 1986. Management, husbandry, nutrition, introduction. *In*: C.M. Fraser (Ed.). The Merck Veterinary Manual. Merck & Co., Inc., Rahway, N.J., U.S.A. pp. 1022-1025.

Itami, T., J. Takahashi and Y. Nakamura. 1989. Efficacy of vaccination against vibriosis in cultured kuruma prawns, *Penaeus japonicus*. J. Aquatic Animal Health. 1: 238-242.

Kalagayan, H., D. Godin, R. Kanna, G. Hagino, J. Sweeney, J. Wyban and J. Brock, 1991. IHHN virus as an etiological factor in runt-deformity syndrome (RDS) of juvenile *Penaeus vannamei* cultured in Hawaii. J. World Aquacult. Soc. 22: 235-243.

Lewis, D.H. and A.L. Lawrence. 1985. Immunoprophylaxis to *Vibrio* sp. in pond reared shrimp. *In*: G.L. Rogers, R. Day and A. Lim, (Eds.). Proceedings of the First International Conference on Warm Water Aquaculture—Crustacea. Brigham Young University, Laie, HI. pp. 304-307.

Lightner, D.V. 1983. Diseases of cultured penaeid shrimp. *In*: J.P. McVey (Ed.). CRC Handbook of Mariculture. Vol. I, Crustacean Aquaculture. CRC Press, Boca Raton, FL. pp. 289-320.

Lightner, D.V. 1985. A review of the diseases of cultured penaeid shrimps and prawns with emphasis on recent discoveries and developments. *In*: Y. Taki, J.H. Primavera and J.A. Llobrera (Eds.). Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps. Aquaculture Dept., SEAFDEC, Iloilo, Philippines. pp. 79-103.

Lightner, D.V. 1988. Diseases of cultured penaeid shrimps and prawns. *In*: C.J. Sindermann and D.V. Lightner (Eds.). Disease Diagnosis and Control in North American Marine Aquaculture, 2nd ed. Elsevier, New York. pp. 8-127.

Lightner, D.V. In Press. Diseases of cultured penaeid shrimp. *In*: J.P. McVey (Ed.). CRC Handbook of Mariculture. Crustacean Aquaculture. CRC Press, Boca Raton, Florida.

Lin, C.K. 1989. Prawn culture in Taiwan. What went wrong? World Aquaculture. 20: 19.

Main, K.L. and W. Fulks. 1990. The Culture of Cold-tolerant Shrimp: Proceedings of an Asia-U.S. Workshop on Shrimp Culture. The Oceanic Institute, Waimanalo, Hawaii, U.S.A. 215 p.

Nash, G. 1988. Diseases of shrimp and prawns. Fish Farming International. 15: 30-31.

Rosenberry, B. 1991. Five billion shrimp cocktails to go! World Shrimp Farming. November 1, 1991. pp. 1-19.

Sano, T. and H. Fukuda. 1987. Principal microbial diseases of mariculture in Japan. Aquaculture. 67: 59.

Wiseman, M.O., R.L. Price, D.V. Lightner and R.R. Williams. 1982. Toxicity of aflatoxin B to penaeid shrimp. Appl. Environ. Microbiol. 44: 1479-1481.

Part III:

Discussion Group Summaries

Discussion Group Summaries

Introduction

Conference participants were given the opportunity to discuss a variety of shrimp disease-related topics in six 90-minute discussion groups. These sessions were designed to generate ideas, raise issues, solve problems and provide information to fellow experts. Relatively narrow topics were assigned to most sessions, and moderators were charged with facilitating the discussions.

In the context of the following pages, the term **disease** is defined as "any departure from normal structure or function" (after Sindermann, 1990). Furthermore, a **pathogen** is considered to be any biotic agent that may cause disease (as defined above). No attempt has been made to distinguish between obligate and facultative pathogens.

Discussion Group A was titled: "Country-by-Country Concerns." Each participant listed his or her most important shrimp disease-related concerns. Common themes included the effects of shrimp culture on the natural environment, the indiscriminate use of drugs, and the need for more diagnostic techniques, better shrimp stocks, and improved husbandry techniques.

Subsequently, **Discussion Group B** focused on the "Prevention and Treatment of Diseases in Growout." Biotic diseases were highlighted, and much of the time was devoted to preventing viral diseases. In addition, one of the concerns aired during this session was the quality of both wild-caught and hatchery-reared postlarvae. There was much discussion over how postlarval quality has changed over the past 10 - 15 years and what factors might be responsible for lowered performance.

"Prevention and Treatment of Diseases in Hatcheries" was the topic for **Discussion Group C**. Attention was given to the methods used to prevent and/or treat diseases of viral, bacterial, protozoan and fungal etiology. Most of the discussion centered around procedures used in *P. monodon* and *P. vannamei* hatcheries.

Issues relevant to the topic, "Disease Diagnosis" were dealt with in **Discussion Group D**. In particular, the problem of standardizing diagnostic methods and certifying diagnosticians, shrimp stocks, and culture facilities was discussed at length. The group issued a set of recommendations to address this issue.

Discussion Group E focused on an important issue raised in the previous discussion group, the certification and

possible quarantining of transported stocks and the certification of stocks and hatcheries as SPF.

In **Discussion Group F**, participants prioritized both the overall concerns and the research concerns that were raised during Discussion Group A. The need for SPF stocks and more diagnostic techniques were identified as two of the most important issues for researchers and farmers alike.

Discussion Group A: Country-by-Country Concerns

To begin the discussion group sessions, participants were asked, simply, what their primary concerns were with regard to diseases of cultured shrimp. As one might imagine, responses were varied, reflecting each individual's home country and experience, in addition to their role as scientists, farmers or extension agents.

Central and South America

Because the majority of his experience has been with the shrimp culture industry in Central and South America, Rolland Laramore focused on the situation there. Topping his list was the problem of poor growth during dry seasons. Noting that several factors probably contribute to the phenomenon, Dr. Laramore was optimistic that genetic selection of robust and disease-resistant strains of *Penaeus vannamei* could alleviate some of the problems. Other key avenues of research mentioned were developing bacterial vaccines for nauplii, postlarvae and broodstock, and "probiotics", beneficial bacteria that displace or offset pathogens.

Japan

In Japan, *P. japonicus* is the dominant species of cultured shrimp. Kazuo Momoyama noted that the most economically important diseases of *P. japonicus* in Japan are caused by *Vibrio* spp. and *Fusarium* sp. Vibrio diseases have become increasingly important in recent years; they are estimated to cause 20% mortality during growout. In particular, there is a new, highly pathogenic Vibrio strain called "*Vibrio* sp. PJ" that is the subject of much concern in Japan. While there are two antibiotics sold in Japan to combat Vibrio diseases, their beneficial effects are only temporary.

Among penaeids, *P. japonicus* is known to be particularly susceptible to Fusarium disease, especially under intensive culture conditions. Reducing shrimp densities is the only effective means of controlling this fungus. Baculoviral midgut gland necrosis virus (BMNV) used to be a major problem, but, as a result of effective preventive measures, it is now relatively rare.

In more general terms, Tokuo Sano discussed the need for a clean culture environment to discourage facultative pathogens and the problems associated with developing bacterial vaccines, while Kazuo Momoyama aired con-

cerns about the international transport of shrimp stocks and feeds.

Malaysia

Speaking on behalf of both Malaysia and Asia in general, Mohd. Shariff called for more ecological studies of shrimp culture situations — studies that would enable researchers to monitor changes in potential pathogens. He further noted that chemotherapeutants are not necessarily the best means of combating shrimp diseases — not only can they harm the environment, but they are not always effective. Therefore, immunological studies should receive a high priority. Finally, Dr. Shariff called for the standardization of shrimp research methodologies. Perhaps a manual similar to the American Fisheries Society "Blue Book" could be issued for shrimp disease workers. Such a project would benefit from cooperation between the Asian Fisheries Society and the American Fisheries Society.

People's Republic of China

In what was to become a common theme, Dou Chen joined Mohd. Shariff in cautioning against the use of chemotherapeutants in shrimp culture. Stating that the most serious diseases of cultured shrimp in China are those caused by *Vibrio* spp., Prof. Chen listed several disadvantages to using antibiotics, including the possibility of promoting fungal diseases and fostering resistant strains of bacteria. For example, as a result of overuse/abuse, oxytetracycline is now completely useless. Since not all bacteria are harmful, Prof. Chen believes that the best way to control the potentially pathogenic species is by ecological or biological control.

Although there is concern about viruses in China, viral diseases are not as economically significant as those caused by bacteria. Anxiety is increasing, however, over a number of diseases of unknown etiology, including black-white spot disease (see D. Chen, this volume). Finally, epicommensal diseases also significantly impact shrimp culture in China.

Philippines

José Natividad was another who spoke out against the indiscriminate use of antibiotics in shrimp culture. He also shared his concerns about the current widespread use of probiotics and other drugs in the Philippines. Because the impact of probiotics is largely unknown, and because great quantities of probiotics, as well as other drugs, are present on the farms, Dr. Natividad called for some sort of government control or clearance process for probiotics and other compounds that are now unregulated. Secondly, Dr. Natividad said that he needed field diagnostic techniques to help him quickly assess production problems. Finally, some diseases that affect the marketability of shrimp, most notably "black meat disease" and "tail rot disease", have resulted in rejection of shipments, alarming shrimp farmers.

South Korea

Most shrimp culture in South Korea is extensive; as a result, relatively few disease problems have been encountered. Myoung Ae Park did, however, list some disease agents that had been encountered in farms and hatcheries in Korea: BMNV (in larval *P. japonicus*), hepatopancreatic parvo-like virus (HPV; in *P. chinensis*) and *Vibrio* spp. Ms. Park pointed out that better water quality management can alleviate some disease-related problems.

Taiwan

When it comes to shrimp diseases, Taiwanese farmers and researchers have, unfortunately, gained a great deal of knowledge through experience. According to S.N. Chen, the most important problem is environmental impact. How does shrimp culture impact the surrounding environment, and, in turn, how do environmental changes affect cultured shrimp? Vibriosis is also a key concern, and while progress is being made in the area of vaccines, delivery technology has been problematic. Shrimp viruses also need to receive more attention, as does the problem of drug residues in harvested shrimp in some Asian countries.

As noted by Cheng-Fang Chang, intensification is responsible for many of the problems encountered on shrimp farms. In intensive systems, epicommensal diseases are important; water quality management is key to controlling fouling organisms. Interestingly, Mr. Chang also listed gregarines as a major concern. While their affect on shrimp performance is unknown, a recent survey in Taiwan found that 80% of cultured *P. monodon* were infected with these intestinal parasites (see Liao et al., this volume). Finally, Mr. Chang reminded the group that the relationship between the culture environment and diseases should not be ignored.

Thailand

Timothy Flegel called for rapid diagnostic techniques that can be used in the field. Noting that such techniques are needed for abiotic as well as biotic diseases, Dr. Flegel recounted some of his experiences with pesticides that were toxic to shrimp at levels in the range of pg/L. A second concern is the need for better preventives, including vaccines, probiotics and management techniques. Finally, Dr. Flegel advocated using specific pathogen-free (SPF) broodstock as a way to simultaneously improve the disease situation and breed better strains of shrimp.

United States

Recognizing that many diseases can be avoided by improved husbandry techniques, a number of the experts from the United States called for studies to address issues such as sustainability, the ramifications of intensification, site selection/environmental planning, feeds, preventive health maintenance, and the impact of certain culture practices on the environment. Carl Sindermann challenged researchers to help

farmers "manage around pathogens," while James Wyban said the shrimp culture industry had a great deal to learn from other meat production industries such as the cattle, poultry and swine industries.

The two commercial representatives, Nick Carpenter and Fritz Jaenike, were among several participants who shared concerns about the spread of viral diseases. The movement of stocks threatens efforts to establish disease-free facilities and may also impact populations of wild shrimp. In a related topic, SPF technology was mentioned by several members of the U.S. contingent as a means of improving the shrimp industry's image, increasing production, and beginning the process of domestication.

Bacterial diseases, however, were also listed as concerns by three U.S. participants. For example, Donald Lightner pointed out that farmers need to have effective, government-approved chemotherapeutants, and Nick Carpenter wondered whether vaccines could be a realistic solution to the problem of bacterial diseases. Finally, Carl Sindermann strongly encouraged researchers studying shrimp diseases to quantify the economic impact of disease using standardized measures. This is the only way researchers are going to receive needed support, said Dr. Sindermann; scientists need to convince the shrimp industry, governments and funding agencies that their work is economically significant. He encouraged consultation with economists and experts in population management and epidemiology, and the uniform statistical treatment of data.

Discussion Group B: Prevention and Treatment of Diseases in Growout

Although the varied means by which viral, bacterial and protozoan diseases are prevented and treated was the topic of this session, most of the discussion centered around two issues: preventing viral diseases, and poor performance of animals in growout in many areas, possibly as a result of 10 - 15 years of culture activities.

Virus Prevention

As evidenced in Discussion Group A, viruses are not the only important pathogens of penaeid shrimp. The relative importance of viruses to cultured shrimp depends on a number of factors, including the type of shrimp cultured, the type of viral and nonviral pathogens present, the stressors present in the culture environment, and how heavily infected a given shrimp population is. There are environmental and regulatory issues as well. If a certain viral pathogen is absent from a given area, it is important to exclude that virus from nearby culture facilities.

In many cases, the best way to prevent viral diseases is to use certified SPF broodstock. An SPF program for *Penaeus vannamei* has already been implemented in the United States (see

Wyban, this volume). Maintaining SPF *P. monodon* in Southeast Asia may prove much more difficult, however. Steve Psinakis, a shrimp farmer from the Philippines that participated in the workshop, asked about the possibility of breeding disease-resistant strains, thereby eliminating the need to keep animals isolated from some pathogens.

What about the problem of introducing the progeny of SPF broodstock (hereafter referred to as high-health animals) into ponds that once held virus-infected shrimp? How do these animals perform? Do they become infected with viruses during the growout cycle? Preliminary results from several shrimp farms in the United States indicate that, in these situations, the incidence of viral disease is greatly reduced, significantly improving production. Factors to consider are the treatment of the pond bottom prior to stocking, and the nature of the virus(es) of interest. For example, it is likely that occluded viruses such as MBV will be more difficult to eliminate from ponds than nonoccluded viruses.

In Hawaii, growout ponds were dried for 10 to 14 days and treated with 800 lbs $CaCO_3$/acre prior to being stocked with high-health postlarval *P. vannamei*. *Baculovirus penaei* (BP) and infectious hypodermal and hematopoietic necrosis virus (IHHNV) were the viruses of concern. Shrimp yields were very good, and the incidence of runt-deformity syndrome was greatly reduced. Some of the shrimp, however, did test positive for BP (see Carpenter and Brock, this volume).

In Taiwan, growout ponds are routinely dried between harvests and then treated with chlorine (10 - 20 ppm for 48 h). These levels of chlorine are effective against some viruses and other pathogens; however, they probably also harm the natural environment. The results of a number of studies were discussed with regard to eliminating pathogenic viruses. For example, a treatment of 0.1 ppm iodine for 6 - 7 s eliminated 99.9% of the trout virus, IHN, from water (Batts et al., 1991). Studies on BP were performed at the Gulf Coast Research Laboratory (Le Blanc and Overstreet, 1991a, b). BP-infected hepatopancreases were subjected to a variety of treatments: desiccation, calcium hypochlorite, heating, pH extremes, etc. Though the results are not directly transferable to treating pond bottoms, the researchers found that BP could be inactivated rather easily using several methods. Finally, it was mentioned that the microbial activity in the pond bottom could be a significant factor in destroying infective viruses in the sediment.
In a related question, participants discussed the best means to test sediments for the presence of viruses. Bioassay studies are presently being used in Mississippi for BP. Gene probe and

PCR (polymerase chain reaction) technology may also be applied to this problem in the future.

The Impact of Shrimp Farming on Postlarval Quality

During the course of the discussion, Steve Psinakis, a shrimp farmer from the Philippines, submitted the following hypothesis for discussion: "In many places around the world over the past 10 - 15 years, shrimp farming activities have done something to reduce the quality of both wild-caught and hatchery-reared postlarvae. Farms are experiencing higher feed conversion ratios, lower growth rates and lower survivals, and the problem is getting worse. The animals seem much more sensitive to perturbations than they used to be." As evidence to support his hypothesis, Mr. Psinakis noted that despite improved culture techniques and better trained staff, production declines year by year. Furthermore, the phenomenon has been observed in ponds of varying ages using both wild seed and hatchery-raised seed from wild-caught spawners. Other participants related experiences that seemed to support the above hypothesis. For example, *P. vannamei* was once considered asymptomatic for the IHHN virus; now it is clearly not asymptomatic. Is this a result of deteriorating stocks? Also, while postlarval quality problems have not been observed in Panama over the past 15 years, farms in Guayaquil, Ecuador, have experienced lower production in recent years, and increased incidence of disease. Problems are worse in areas with a high density of farms.

What specific activities might be responsible for harming wild stocks of penaeids? Two possibilities were discussed, the use of chemotherapeutants, and the release of hatchery-reared postlarvae into the natural environment. Chemotherapy in the hatchery allows animals that are naturally weak and susceptible to disease to survive, thereby selecting for shrimp that are less fit. These animals typically perform poorly in growout. Most hatchery managers, moreover, have no incentive to produce animals that will perform well in growout, they profit by producing large numbers of healthy-appearing postlarvae. This makes it difficult to obtain animals that will remain healthy during growout.

In a related problem, it is a common perception in many parts of Asia that countless numbers of diseased, hatchery-reared postlarvae are regularly released into coastal areas. If some of these shrimp survive and reproduce, these artificially selected animals may alter the composition of the gene pool of the wild population. Secondly, because of the widespread practice of transporting stocks without regard to their disease status, viruses have been introduced to previously "clean" culture facilities. Furthermore, we are learning that a number of wild shrimp populations now carry pathogenic viruses (see Lotz, this volume), possibly

as a consequence of nearby shrimp culture activities. The impact of these viruses on natural stocks is unknown.

The above hypothesis has not been proven — it has not even been tested. Because there are so many interrelated factors, finding answers will be difficult. Clearly, though, more emphasis should be placed on determining the capacity of a given culture area to support shrimp. Also, precautions should be taken to avoid new viral introductions, and chemotherapeutants should be used more judiciously. Finally, the practice of releasing cultured animals into the natural environment should involve consideration of animal health and genetic diversity.

Discussion Group C: Prevention and Treatment of Diseases in Hatcheries

A key conclusion from Discussion Group B was that the performance of shrimp in growout depends greatly on the treatment the animals receive in the hatchery. There are a number of different "hatchery philosophies" whereby the water and the animals are managed to encourage growth and prevent disease. In general, hatchery diseases are prevented and treated by

- Managing the culture water;
- Adding chemicals to the culture water (or, more rarely, to the feed);
- Monitoring the culture environment; or
- Managing the animals.

Prevention

Viruses. No one in the group reported using specific water management techniques to prevent viral diseases. Many culturists, however, pretreat culture water, either with chemicals such as chlorine or iodine, or by other means such as ozonation or ultraviolet radiation (Table 1). Often culture water is filtered beforehand to increase the effectiveness of these other sterilization methods.

In some places, cultured shrimp are routinely monitored for the presence of viruses. Such measures, depending on the sensitivity of the assay used, could help prevent the spread of a virus from infected tanks to uninfected tanks. Animal management, however, is the best way to prevent viral diseases. Simply put, stocking with high-health postlarvae is the most effective means of keeping viruses out of the hatchery. Secondarily, eggs and/or nauplii can be rinsed or chemically treated to remove external virus particles. This has effectively prevented BMN outbreaks in Japan, and is also used to lower the incidence of MBV in *P. monodon* hatcheries and BP outbreaks in commercial shrimp hatcheries in South and Central America. Finally, many hatcheries these days are using batch methods; drying out their system in between cycles. While this is quite effective for

Table 1. Preventing diseases in hatcheries in the Americas and Asia[1].

Potential pathogens	Water management	Chemicals	Monitoring	Animal management	Other
Viruses	None.	Pretreatment of water with ozone, chlorine, iodine, UV (filtration will enhance effectiveness of above).	Periodic diagnostic screening, broodstock history	Begin with high health animals, rinse or treat shrimp eggs and/or nauplii[2]	Use batch techniques with dry-outs in between.
Bacteria	Increase exchange rate, use microalgae and other means to condition water, optimize temperature.	Disinfection with chemicals listed above, antibiotics[3], malachite green, vaccines, EDTA (5 ppm in Taiwan, 10 ppm in the Philippines).	Daily monitoring with agar plates, e.g., TCBS counts with water and larval homogenates.	Rinse shrimp nauplii and eggs, optimize stocking density.	Rinse *Artemia* nauplii, use batch techniques with dry-outs in between, optimize nutrition, add microalgae that have inhibitory effects.
Protozoa	Increase exchange rate, optimize temperature.	Formalin, malachite green, copper sulfate, EDTA.	Observe water with microscope.	Rinse shrimp nauplii and eggs.	Rinse *Artemia* nauplii, optimize nutrition.
Fungi		Treflan, malachite green, EDTA	Observation with microscope.		Optimize nutrition.

[1]Preventive methods appearing in this table are used either singly or in combination in hatcheries producing *P. vannamei, P. monodon, P. chinensis* or *P. japonicus*. Some may be effective only for specific agents or shrimp species, and some may not be effective at all.

[2]Sano and Momoyama, this volume; Liao et al., this volume.

[3]The prophylactic use of antibiotics was strongly discouraged by most of the workshop participants.

preventing bacterial problems, it may also destroy viruses.

Bacteria. Increasing the rate of water exchange can prevent the build-up of high levels of bacteria in a hatchery system. It is also important to maintain optimum temperatures (Table 1). Furthermore, many believe that healthy microalgae blooms can inhibit the growth of potentially pathogenic bacteria.

The chemical pretreatments used to prevent bacterial problems include those listed above for preventing viruses. Additionally, a number of different chemicals may be added to the culture water to inhibit bacterial growth, including malachite green, EDTA and antibiotics.

Most of the participants were strongly against the prophylactic use of antibiotics because of the dangers of selecting

for and disseminating antibiotic-resistant strains of bacteria, and because it may be detrimental to the shrimp in the long run. However, antibiotics are used regularly in *P. monodon* hatcheries in the Philippines, Taiwan and elsewhere to prevent bacterial diseases. S.N. Chen noted that much effort is being expended in Taiwan looking for alternatives to antibiotics. In general, hatcheries that do not use antibiotics produce animals that perform very well during growout, but production is reduced in these hatcheries. Timothy Flegel added that oxytetracycline (OTC) is used successfully to improve production in *P. monodon* hatcheries in Thailand, even though most of the bacterial strains isolated from diseased shrimp are resistant to OTC. He also noted that the addition of very small amounts of OTC improves the hatching rate of *P. monodon* eggs. He hypothesized that OTC was having some other effect; one that was unrelated to its antibiotic activity.

Penaeus vannamei, in contrast to *P. monodon*, appears to be much more tolerant of high levels of bacteria. Whereas antibiotics are sometimes used prophylactically in Central and South America, their use is not ubiquitous. Antibiotics cannot legally be used either to prevent or to treat bacterial diseases in the United States, and the commercial representatives present indicated that, under most circumstances, they would not consider using antibiotics prophylactically even if such use were legal.

In the People's Republic of China, where the use of antibiotics is not regulated, *P. chinensis* hatcheries do not routinely add antibiotics to the culture water. Finally, in Japan, the use of antibiotics is strictly regulated. Prefectural extension agents instruct farmers on the proper usage of antibiotics (only a few of which have government approval) and antibiotics are not used prophylactically.

Many hatchery managers have pinned their hopes on vaccines to prevent bacterial diseases. While bacterial vaccines for penaeid shrimp are not now widely available, several researchers at the workshop had developed and tested vaccines that could eventually be useful in hatcheries (e.g., see Laramore, this volume).

There are a number of ways to monitor hatcheries for bacteria, including doing total plate counts or TCBS counts of water and/or larval homogenates (Table 1). This is usually only practiced routinely in areas where there is a high density of farms and/or the water available is not of optimal quality. The widespread practice of rinsing or treating shrimp eggs and/or nauplii to remove bacteria before stocking falls under the category "animal management". Similarly, *Artemia* nauplii, a common feed in shrimp hatcheries, are routinely rinsed before use to minimize the spread of bacteria.

Employing batch techniques can also help minimize bacterial problems (Table 1). And, since bacteria are ordinarily considered facultative pathogens of shrimp, another good way to prevent

infection is to minimize stress. This can be accomplished, in part, by providing adequate quantities of high-quality feed and by optimizing stocking density. Finally, there is some evidence (see D. Chen, this volume) that certain species of microalgae inhibit the growth of *Vibrio* spp. and other potential pathogens — adding these microalgae to the culture water should, therefore, lower the density of these bacterial species in the tanks.

Protozoa. Many of the methods used to prevent bacterial diseases also apply to diseases caused by protozoa (Table 1). In addition, chemicals such as Formalin and copper sulfate are also used in hatcheries, and it is important to monitor the culture water regularly with the aid of a microscope to monitor protozoan populations.

Fungi. Larval mycosis is the most well-known fungal disease afflicting larval penaeids. This disease is usually prevented by adding Treflan® to the rearing water, although chemicals such as EDTA and malachite green have also been used (Table 1).

Treatment

Viruses. Once a shrimp population has been infected with a virus, there is no way to get rid of the virus. There are, however, techniques that can maximize the performance of virus-infected animals in culture. The most commonly cited method is simply to minimize the stress placed on the animals. For example, in the past, Nick Carpenter has effectively "managed around" BP at his *P. vannamei* hatchery by lowering shrimp densities (Table 2). In addition, monitoring the level of infection can help in making management decisions.

Bacteria. Increasing water exchange and optimizing temperature are water management techniques that can be used to treat diseases of bacterial etiology (Table 2). Antibiotics, of course, may also be effective, but, as Tokuo Sano noted, treatment should be preceded by sensitivity analyses and determination of minimum inhibitory concentrations. In Ecuador and elsewhere, hatchery managers are adding 1 - 10 ppm sucrose to culture tanks to encourage the growth of bacteria that might outcompete potentially pathogenic species and strains. Although the technique is still being refined, shifts in the types of bacteria revealed in TCBS counts have been documented. Sucrose is also being used in growout, but its effect in ponds is more difficult to document. Similarly, some culturists in Asia and in the Western Hemisphere are adding so-called "beneficial" bacteria, or "probiotics" to hatchery tanks, and even to growout ponds, in the hope that they will prosper and outcompete *Vibrio* spp. and other potential pathogens.

Protozoa. Flushing to lower the densities of microbes on which epicommensal protozoa feed is one management method for protozoan fouling disease (Table 2). Alternatively, chemicals such as copper sulfate, malachite green and EDTA can be added to tanks. In fact,

Table 2. Treating hatchery diseases in the Americas and Asia.

Potential pathogens	Water management	Chemicals	Monitoring	Animal management	Other
Viruses	None.	None.	Periodic diagnostic screening to make treatment decisions.	Avoid stress, lower or optimize densities.	
Bacteria	Increase water exchange, optimize temperature.	Antibiotics, 1 - 10 ppm sucrose (to encourage growth of "beneficial" bacteria.	Antibiotic sensitivity profiles to make treatment decisions.		Do not overfeed if animals are sick.
Protozoa	Increase rate of exchange.	Treflan®, copper sulfate, malachite green, EDTA.			
Fungi	Increase rate of exchange.	Copper sulfate, malachite green, EDTA.		Lower density.	

Cutrine®-Plus, a copper sulfate compound, is the only substance that has received formal approval in the United States to treat cultured shrimp (see Bell, this volume).

Fungi. Larval mycosis is variously treated by increasing the rate of water exchange, by using chemicals such as malachite green and EDTA, and by lowering larval densities (Table 2).

Discussion Group D: Disease Diagnosis

One of the key disease-related issues facing the global shrimp industry is certifying stock and hatcheries as SPF. For example, stock that is certified as "virus-free" is worthless without knowledge of the diagnostic procedures used to detect viruses. Furthermore, all economically important infectious agents should be targeted, not only viruses. Another topic discussed was the development of new diagnostic techniques and guidelines for investigating idiopathic observations and cases in which there are multiple pathogens.

Standardization of Diagnostic Procedures and Certification of Stocks, Pathologists and Facilities

With currently available technology, how do we standardize diagnostic procedures? Then, once we have standard procedures, how do we certify diagnosticians, and how do we certify shrimp stocks and culture facilities as SPF? Several of the researchers present were in favor of a committee approach; form-

Table 3. Recommendations.

1. Develop priority pathogens list containing pathogens that are economically or ecologically significant, that can be diagnosed with existing technology. – Should we apply certification? Where? – What will be the criteria for sensitivity (what level is acceptable or should it be zero tolerance)?
2. Select and organize committees comprised from members of the following: the World Aquaculture Society, the Asian Fisheries Society, the Office of Internationale Epizooties and the European Association of Fish Pathologists. Committees will review and standardize diagnostic procedures. – Committee must be representative. – Take advantage of computer networking to alleviate need for many formal meetings. – Handbooks need to be developed by committees and so do SPF procedures.
3. Reference labs. Specimen exchange will be very helpful in the diagnosis of certain diseases.

ing one or more representative committees of experts to:

- Develop or endorse handbooks that contain detailed diagnostic procedures; and

- Develop or endorse guidelines for certifying diagnosticians, shrimp stocks and culture facilities (Table 3).

Perhaps committees could be formed within each of the following organizations: the World Aquaculture Society, the Office Internationale des Epizooties, the European Association of Fish Pathologists and the Asian Fisheries Society.

Regional committees, it was decided, would probably be needed to develop lists of pathogens to be excluded from stocks. Different species and various regions are expected to have different lists. For example, if a certain pathogen is endemic to an area, that is, present in the wild shrimp population, it would be impractical to exclude that pathogen from that region.

Research and Development on New Diagnostic Techniques

Tissue Culture. There was general agreement that much more needs to be done in the area of shrimp tissue culture. The lack of progress in this area has hindered the development of new diagnostic techniques.

Idiopathic Syndromes. Some participants believed that the phrases "idiopathic lesions" and "idiopathic syndromes" are overused in the scientific literature. Furthermore, diagnosticians would benefit from a standard set of steps to be used to investigate idiopathic observations in cultured shrimp.

Multiple Pathogens. Similarly, diagnosticians often encounter shrimp that contain multiple pathogens. In this case, it is very difficult to determine if one pathogen is more important than

the other(s), and if so, which one(s). Perhaps a set of guidelines could be developed to assist researchers who encounter these cases.

Morphological Diagnosis vs. Etiological Diagnosis. Citing Texas as one example, Ken Johnson observed that hepatopancreatic granulomas are being reported with increasing frequency in many culture areas. Because shrimp have a nonspecific immune response, these granulomas are generally regarded to be caused by a number of unrelated factors. In many cases, however, it is impossible to determine the etiological agent. It is extremely important for pathologists to carefully report the structural changes they observe, that is, to make a morphological diagnosis, if the disease agent is unknown.

Discussion Group E: Quarantine/SPF

Quarantine

Country Descriptions. To begin, the group heard descriptions of the inspection/quarantine procedures used in Hawaii, South Korea, and the People's Republic of China.

In Hawaii, permit requirements for imported shrimp have drastically reduced introductions in the past seven years. Importers are required to quarantine imported stock, and, because the likelihood that the shrimp will carry obligate, exotic pathogens is so great, shrimp introductions to Hawaii are now rare. Most producers are not willing to risk the financial losses that would result from an infected shipment.

In South Korea, fish health inspections are conducted under the auspices of the National Fisheries Research and Development Agency. A serious hindrance, however, is the lack of a specialized quarantine facility. Right now, pathologists check imported fish for bacteria and parasites; they presently lack the means to test for viruses.

There are also standardized procedures in the People's Republic of China for importing shrimp stocks. Implementation, however, is sometimes difficult because the regulations are too broad.

Purposes. There are at least two reasons to implement quarantine procedures. First of all, many countries are interested in protecting native shrimp populations from diseases that may be carried by imported shrimp. Quarantine procedures may not be effective in attaining this goal, however, if there are nearby countries that import and culture shrimp without regard to their disease status. Secondly, quarantining can protect the shrimp culture industry from the potentially devastating effects of new pathogens.

Implementation. The heart of the problem lies in implementing quarantine procedures without crippling the shrimp culture industry within a given country or region. Many of those present at this session were in favor of

establishing reliable domestic supplies of SPF stocks prior to the adoption of regulations. Another issue raised was the need for international cooperation in establishing workable, reasonable quarantine procedures.

Furthermore, as was pointed out in the previous discussion, before one can begin to certify stocks and hatcheries on a large scale, standard diagnostic procedures must be in place, and there must also be qualified diagnosticians to use those procedures.

In a related problem, how should we develop priority pathogen lists? This issue was touched on in the previous discussion. Some researchers wondered whether enough is known about the geographic ranges of pathogens to develop pathogen lists. Furthermore, any pathogens on an exclusion list must be able to be diagnosed with certainty. Other participants were worried that priority pathogen lists would be misused by governments in some countries and become regulatory lists.

The SPF Issue

Motivations. There are a number of reasons countries or groups might want to develop SPF shrimp stocks. One is the shortage of broodstock in some areas. Domesticated stocks are needed because wild sources of high-quality broodstock are becoming scarce. Logically, if one is going to begin a domestic stock program, he or she should begin with SPF animals. One could also argue that domesticated stocks are needed so that genetically "superior" strains of animals can be developed. Alternatively, some companies simply want to have a reliable supply of high-health animals for growout. Finally, other companies may be motivated by the economic incentive of selling high-health seed to other farms.

Approaches to Certification. Finally, when it is time to certify animals and hatcheries with regard to their pathogen status, what approach should be taken? Some fish hatcheries are classified based on the number of pathogens present in their stocks. For example, a Class A hatchery may contain SPF animals, whereas animals in a Class B hatchery might carry one known pathogen, and so on.

It may also be desirable to categorize the pathogens themselves. For a given area, disease agents might be divided into groups based on 1) the presence or absence of the agent in the natural environment, and 2) the threat posed by the agent in question. Finding answers to the above mentioned questions for all of the known shrimp pathogens affecting all the various species and culture regions will certainly require a great deal of study. The Fish Disease Commission of the Office International des Epizooties has already begun to develop a list of excludable fish, shrimp and mollusc pathogens (see Sano and Momoyama, this volume).

Table 4. Overall disease-related concerns, in order of decreasing importance.

1.	The need for SPF stocks.
2.	The use/abuse of chemotherapeutants, including antibiotics.
3.	The need for rapid diagnostic techniques.
4.	The natural environment — the need for ecological studies.
5.	Problems associated with movement of shrimp.
6.	Better environmental planning.
7.	Bacterial diseases.
8.	Vaccines/preventives

Discussion Group F: Open Session and Wrap-Up

This session was devoted to prioritizing the concerns raised in Discussion Group A. Two summary lists were made that reflected 1) the participants' overall disease-related concerns, and 2) their priorities for future disease research. There was some overlap; for example, the need for SPF stocks was both a research priority and an overall concern. Everyone was asked to individually prioritize the issues on the two lists. The results are in Tables 4 and 5.

The need for SPF stocks was ranked highest among the overall shrimp disease-related concerns and was also second on the research priority list. This reflects, in large part, the perceived threat of viral diseases to the global shrimp culture industry, and the need for a reliable supply of seed. The use or abuse of chemotherapeutants was the second most important overall concern. In particular, the prophylactic use of antibiotics and the presence of drug residues in harvested shrimp were at issue. Third on the list was the need for rapid diagnostic techniques. Those present emphasized the need for two different types of techniques, those that could be used on farms and others that could be applied at "minimal clinical labs."

Table 5. Research priorities, in order of decreasing importance.

1.	Rapid diagnostic techniques.
2.	SPF stocks.
3.	Probiotics.
4.	Vaccines/preventives
5.	Effects of disease on wild shrimp populations

The need for rapid diagnostic techniques was also the highest research priority (Table 5). SPF stocks were the second priority, and the need for more studies on probiotics was third. Finally, the commercial representatives stated that they wanted more research efforts to be directed toward developing more and better chemical preventives, including vaccines (number eight and four on the overall concerns and research priority lists, respectively). Better disinfection methods are needed, in addition to antibiotics that are designed for aquaculture, and immunostimulants.

Literature Cited

Batts, W.N., M.L. Landolt and J.R. Winton. 1991. Inactivation of infectious hematopoietic necrosis virus by low levels of iodine. Appl. Environ. Microbiol. 57(5): 1379-1385.

LeBlanc, B. and R. Overstreet. 1991a. Effect of desiccation, pH, heat and ultraviolet irradiation on viability of *Baculovirus penaei*. J. Invertebr. Pathol. 57: 277-286.

LeBlanc, B. and R. Overstreet. 1991b. Efficacy of calcium hypochlorite as a disinfectant against the shrimp virus *Baculovirus penaei*. J. Aquatic Animal Health. 3: 141-145.

Sindermann, C. 1990. Principal Diseases of Marine Fish and Shellfish. Vol. 2. 2nd edition. Academic Press, San Diego. 516 p.

Appendices

Appendix I: Workshop Participants

Japan

Dr. Kazuo Momoyama
Yamaguchi Pref. Naikai Fisheries
Exp. Station
Yamaguchi 754
JAPAN

Dr. Tokuo Sano
Laboratory of Aquatic Pathology
Dept. of Aquatic Biosciences
Tokyo University of Fisheries
4-5-7 Konan, Minato-ku
Tokyo 108, JAPAN

Malaysia

Dr. Mohammed Shariff
Faculty of Fisheries and Marine Science
Universiti Pertanian Malaysia
43400 Serdang, Selangor, MALAYSIA

People's Republic of China

Professor Dou Chen
Institute of Oceanology, Academia Sinica
7 Nan-Hai Road, Qingdao
PEOPLE'S REPUBLIC OF CHINA

Philippines

Dr. José Natividad
BFAR-IDRC Fish Health Project
Bureau of Fisheries & Aquatic Resources
4th Ffloor Estuar Building,
880 Quezon Avenue
Quezon City, Metro Manila 3008
PHILIPPINES

South Korea

Ms. Myoung Ae Park
Pathology Division
National Fisheries Research &
Development Agency
Shirang-ri, Kijan-up, Yangsan-gun
Kyongsang nam-do 626-900
REPUBLIC OF KOREA

Taiwan

Mr. Cheng-Fang Chang
Tungkang Marine Laboratory
Taiwan Fisheries Research Institute
Tungkang, Pingtung
TAIWAN 92804

Dr. S.N. Chen
Department of Zoology
National University of Taiwan
Taipei, TAIWAN

Thailand

Dr. Timothy Flegel
BP Nutrition Aquaculture Research Centre
577 Thanadee Complex,
Koryor-Songkhla Road,
Amphur Muang Songkhla 9000
THAILAND

United States

Mr. Thomas Bell
Division of Therapeutic Drugs for Food Animals
New Animal Drug Evaluation
Food and Drug Administration
Center for Veterinary Medicine
7500 Standish Place
Rockville, Maryland 20855
U.S.A.

Dr. James Brock
Department of Land and Natural Resources
Aquaculture Development Program
335 Merchant Street, Rm. 348
Honolulu, Hawaii 96813
U.S.A.

Mr. Nick Carpenter
Amorient Aquafarm, Inc.,
P.O. Box 131
Kahuku, Hawaii
U.S.A.

Mr. Fritz Jaenike
Harlingen Shrimp Farms, Ltd.
Rt. 3, Box 300K Centerline Road
Los Fresnos, Texas 78566
U.S.A.

Dr. Sterling K. Johnson
Extension Fish Disease Specialist
Department of Wildlife and Fisheries Sciences
Texas A&M Univerisity
College Station, Texas 77843
U.S.A.

Dr. Rolland Laramore
Shrimp Culture Technologies Inc.
Harbor Branch Oceanographic Institution
5600 Old Dixie Highway
Fort Pierce, Florida 34964
U.S.A.

Dr. Brad R. LeaMaster
The Oceanic Institute
Makapuu Point
Waimanalo, Hawaii 96795
U.S.A.

Dr. Donald V. Lightner
Department of Veterinary Science
University of Arizona
Tucson, Arizona 85721
U.S.A.

Dr. Jeffrey Lotz
Gulf Coast Research Laboratory
P.O. Box 7000
703 E. Beach Boulevard
Ocean Springs, Mississippi
U.S.A.

Dr. Carl J. Sindermann
National Marine Fisheries Service, NOAA
Oxford, Maryland 21654
U.S.A.

Dr. James A. Wyban
The Oceanic Institute
Makapuu Point
Waimanalo, Hawaii 96795
U.S.A.

Appendix II: Agenda

April 27, 1992
East-West Center, Jefferson Hall, Asia Room

8:30 am	Dr. Paul Bienfang The Oceanic Institute	*Introduction and Welcome*
8:45 am	Dr. Mohammed Shariff Universiti Pertanian, Malaysia	*An Overview of the Shrimp Disease Situation in Asia*
9:15 am	Dr. Donald Lightner University of Arizona Arizona, USA	*New Developments in Penaeid Virology: Application of Biotechnology in Research and Disease Diagnosis for Shrimp Viruses of Concern in the Americas*
10:00 am	Dr. Jeffrey Lotz Gulf Coast Research Lab Mississippi, USA	*Developing Specific-Pathogen-Free (SPF) Animal Populations for Use in Aquaculture: A Case Study for IHHN Virus of Penaeid Shrimp*
10:45 am	Dr. James Brock Anuenue Fisheries Research Center Hawaii, USA	*Current Diagnostic Procedures for Diseases of Marine Shrimp*
11:30 am	Dr. José Natividad Bureau of Fisheries and Aquatic Res. Philippines	*Prevalence and Geographical Distribution of* Penaeus monodon baculovirus *(MBV) and Other Diseases in Hatchery-Reared and Pond-Cultured Giant Tiger Prawn (*Penaeus monodon *Fabricius) in the Philippines*
12:30 pm	LUNCH—Garden level, Jefferson Hall	
2:00 pm	Discussion Group A — Sarimanok Room (Third floor) *Country by Country Concerns*	
3:30 pm	Discussion Group B — Sarimanok Room *Prevention and Treatment of Diseases in Growout*	
5:00 pm	RETURN TO HOTEL	
6:30 pm	DINNER — The Oceanarium	2490 Kalakaua Ave. Pacific Beach Hotel

April 28, 1992
East-West Center, Jefferson Hall, Asia Room

8:30 am	Dr. S.N. Chen National University of Taiwan Republic of China	*Studies on the Epizootiology and Pathogenicity of Bacterial Infection in the Cultured Giant Tiger Prawn,* Penaeus monodon, *in Taiwan*
9:15 am	Dr. James Wyban The Oceanic Institute Hawaii, USA	*Selected Breeding of SPF Shrimp for High Health and Increased Growth*
10:00 am	Dr. Kazuo Momoyama Naikai Fisheries Exp. Station Japan	*Viral Diseases of Cultured Penaeid Shrimp in Japan*
10:45 am	Mr. Nick Carpenter Amorient Aquafarm Hawaii, USA	*A Comparison of Virus-Diseased vs. SPF* Penaeus vannamei *on a Commercial Scale in Hawaii*
11:30 am	Ms. Myoung Ae Park Natl. Fisheries Res. & Devel. Agency Republic of Korea	*An Overview of the Shrimp Disease Situation and Diagnostic Techniques and Methods of Treatment Used on Shrimp Farms in Korea*

12:30 pm LUNCH—Garden level, Jefferson Hall

1:30 pm GROUP PHOTO — Garden behind Jefferson Hall

2:00 pm Discussion Group C — Sarimanok Room
Prevention and Treatment of Diseases in Hatcheries

3:30 pm Discussion Group D — Sarimanok Room
Disease Diagnosis

5:00 pm RETURN TO HOTEL

6:30 pm DINNER — Camellia Restaurant — 2460 Koa Ave. Waikiki Resort Hotel

April 29, 1992
East-West Center, Jefferson Hall, Asia Room

8:30 am	Dr. Tokuo Sano Tokyo University of Fisheries Japan	*Baculoviral Infections of Cultured Shrimp in Japan*
9:15 am	Mr. Fritz Jaenike Harlingen Shrimp Farms, Ltd. Texas, USA	*Shrimp Production in Texas Using SPF Stocks*
10:00 am	Dr. Brad LeaMaster The Oceanic Institute Hawaii, USA	*Shrimp Health Management Procedures*
10:45 am	Professor Dou Chen Academia Sinica Inst. of Oceanology People's Republic of China	*An Overview of the Shrimp Disease Situation, Diagnostic Techniques and Methods of Treatment Used on Shrimp Farms in China*
11:30 am	Dr. S.Ken Johnson Texas A & M University Texas, USA	*A Review of the Regulatory Issues Related to Treatment of Penaeid Shrimp Diseases in Texas*
12:30 pm	LUNCH—Garden level, Jefferson Hall	
1:30 pm	RETURN TO HOTEL — Free time	

April 30, 1992
East-West Center, Jefferson Hall, Asia Room

8:30 am	Dr. Rolland Laramore Shrimp Culture Technologies Inc. Florida, USA	*Shrimp Culture Technologies Inc.: Implementing Research to Improve Shrimp Genetics and Health*
9:15 am	Dr. Timothy Flegel BP Nutrition Aquacult. Res. Center Thailand	*Occurrence, Diagnosis and Treatment of Shrimp Diseases in Thailand*
10:00 am	Dr. Carl Sindermann National Marine Fisheries Service Maryland, USA	*Precautions to be Taken in Importing and Culturing Non-Native Shrimp*
10:45 am	Mr. Thomas Bell University of Arizona Arizona, USA	*Drugs and Chemotherapeutants for Shrimp Diseases: Their Present Status in the USA, With an Overview of Research and Approval Processes*
11:30 am	Mr. Cheng-Feng Chang Tungkang Marine Laboratory Republic of China	*Diseases of Grass Prawn* (Penaeus monodon) *in Taiwan: A Review from 1977 to 1991*
12:30 pm	LUNCH—Garden level, Jefferson Hall	
2:00 pm	Discussion Group E — Sarimanok Room *Quarantine/SPF*	
3:30 pm	Discussion Group F — Sarimanok Room *Open Session and Wrap-up*	
5:00 pm	RETURN TO HOTEL	
6:30 pm	DINNER — Cannon Club	Diamond Head Road

May 1, 1992
Optional Tour of Aquaculture Facilities on Oahu

7:45 am	DEPART HOTEL
8:30 am	The Oceanic Institute
11:30 am	Mariculture Research and Training Center
1:00 pm	LUNCH — Pat's at Punaluu
2:30 pm	Amorient Aquafarm
4:00 pm	RETURN TO HOTEL

Index

A

Abbreviated New Animal Drug Application
See ANADA
Acetic acid, 313
Acineta sp., 5, 10, 43, 51, 79, 115 - 116, 220, 275
Aeromonas sp., 75, 77, 127, 132, 143, 196 - 197
Aflatoxicosis, 25, 224
Aflatoxin, 143, 352
Agmasoma penaei, 43, 80
Agmasoma sp., 220, 275, 346
Aldrin, 86
Alexandrium sp., 94
Alexandrium tamarense, 224
Altermonas sp., 79
Ameson sp., 43, 220, 275, 346
Ampicillin, 75
ANADA, 323
Aphanomyces astaci, 331
Aquatin, 222
Aquatrine, 312, 314
Artemia sp., 43, 49, 216, 225, 300, 367 - 368
ASCC(Asian Shrimp Culture Council), 61
Astaxanthin, 11, 44, 222

B

Bacillus sphaericus, 154
Bacteria induced hepatopancreatitis, 8, 19, 23
Bacterial gill disease, 19 - 20
Baculoviral midgut gland necrosis virus
See BMNV
Baculovirus, 6, 38 - 39, 61, 96, 139, 146, 153, 155, 169, 182, 189, 216, 235, 249, 296
Baculovirus penaei
See BP
Baytril, 315 - 316
Benthiocarb, 86, 93
Benzalkonium chloride,
See BKC
Bipenicillin, 8 - 9
BKC, 80, 118, 125, 127 - 128, 130, 132, 171, 190
Black gill disease, 5 - 6, 12, 17, 20, 42, 48, 52, 114, 122 - 123
Black gill syndrome, 224
Black spermatophore disease, 6
Black spot disease, 21, 23
Blue disease, 11 - 12, 44, 133, 222
BMN,
See BMNV
BMNV, 15, 18 - 19, 38, 151, 155, 163, 169 - 171, 173, 185 - 191, 214, 216, 233 - 235, 238, 346, 360, 362, 366
BMPC, 88
BP, 24, 26, 189, 212 - 215, 233 - 235,238, 249 - 251, 271, 274 - 277, 282, 285 - 287, 289, 300, 346, 364, 366
Bubble disease, 17
Buthylphenyl N-methyl-carbamate
See BMPC

C

Cadmium, 122, 143
Calcium hypochlorite, 10, 49, 54, 364
Cancer magister, 86, 89
Carbamate, 88
Carbaryl, 88
Carbon dioxide, 313
Carbophenothion, 86
Carchesium sp., 16 - 17, 43

Carcinus sp., 90
Cephalolobus sp., 51, 117, 121 - 122, 220
Chemotherapy, 8, 20, 28, 42, 80, 135, 348, 350, 365
Chitinolytic bacteria, 5
Chloramine T, 80
Chloramphenicol, 5, 8 - 9, 15, 28, 40, 48 - 49, 75, 77, 132 - 133, 140, 316
Chlorine, 171, 190 - 191, 277 - 279, 287, 297, 364, 367
Chloropyrifos, 86, 93
Chloroquine diphosphate, 10 - 11
Chronic soft-shell syndrome, 11 - 12, 43, 133
Chrysanthemum, 91
Cladocera, 85, 87 - 88, 91
Copper, 122
Cramped tails, 11 - 12, 44
Crangon septemspinosa, 86 - 88
Cutrine-Plus, 8 - 9, 28, 41, 312, 370
Cypermethrin, 57, 81 - 84, 88, 91 - 93

D

Daphnia magna, 85, 87 - 88
DDT, 85 - 86, 92, 223
Decapoda, 87 - 88
Diaoxathan, 86
Diazinon, 87
Dichlorvos, 86, 89
Dieldrin, 86, 223
DNA probe, 61, 171, 213, 215, 237, 245, 249

E

Ectocommensal ciliates, 23
EDTA, 5, 25, 132, 223, 367, 369 - 370
ELISA, 27, 214, 238, 242 - 246
Endrin, 86
Enteromorpha sp., 51, 120
Environmental Protection Agency
See EPA
Enzyme-linked immunosorbent assay
See ELISA
EPA, 311, 315, 318
Ephelota gemmipara, 10
Ephelota sp., 220
Epicommensal ciliates, 17, 19 - 20, 51, 114 - 115
Epicommensal species, 24
Epidemiology, 363
Epistylis sp., 5, 10, 16 - 17, 22, 43, 51, 79, 115, 117, 164, 220, 346
Erythromycin, 5, 75, 140
Erythromycin phosphate, 8 - 9
Escherichia coli, 319
Ethyl-parathion, 87
Euphelota sp., 43

F

FDA, 312 - 313, 316 - 318, 320 - 322
Federal Regulations (Code of), 25
Fenthion, 87
Fentitrothion, 87
Fenvalerate, 91 - 93
Filamentous bacteria, 24 - 25, 29, 40 - 41, 50, 77, 79, 123 - 124, 128, 139, 146, 150, 153, 155, 218, 346
Flavobacterium sp., 127, 132, 143, 196, 204
Floating head syndrome, 17, 52
Florfenicol, 315 - 316
Flucythrinate, 87, 93
Fluoroquinolone, 316
Food and Drug Administration
See FDA
Formalin, 9 - 11, 17, 19, 23 - 25, 28, 41, 49 - 51, 117 - 118, 124, 127 - 128, 164, 171, 182 - 183, 190, 312, 314, 367, 369
Furanace, 5, 8 - 10, 28, 127, 132 - 133
Furazolidone, 5, 19 - 20, 125, 128, 132
Fusarium disease, 5, 20 - 21, 42, 50, 212,

220
Fusarium moniliforme, 20
Fusarium solani, 5, 20, 42, 50, 212, 219 - 220, 274, 346, 351
Fusarium sp., 6, 10, 42, 49 - 50, 52, 79, 213, 219, 351

G

GABA, 92
Gama amino butyric acid
See GABA
Gas bubble disease, 6, 12, 20, 25, 29, 44, 133, 135, 143
Gill rot disease, 19 - 21
GNS, 19, 21, 212
Gregarines, 6, 10 - 11, 121 - 122, 220, 259, 296, 346
Gustathion A, 222
Gut-and-nerve syndrome
See GNS

H

Haliphthoros philippinensis, 42, 143, 155
Haliphthoros sp., 5, 9 - 10, 42, 219
Heavy metal poisoning, 12
Hemocytic enteritis, 12, 212
Hepatopancreatic parvo-like virus
See HPV
Heptachlor, 86
HP
See HPV
HPV, 7, 15 - 16, 21 - 25, 48, 52, 73 - 74, 141 - 142, 157, 163, 234 - 235, 238, 246 - 247, 259, 274, 346, 362
Hyalophysa sp., 275
Hypertrophy, 66 - 67, 70 - 72, 98, 108, 142, 144, 187 - 188, 217
Hyphomyces sp., 10

I

IHHN
See IHHNV
IHHNV, 7, 24, 27 - 28, 39, 73, 141, 157, 186, 212 - 216, 226, 233 - 248, 250, 259, 269 - 271, 274 - 276, 278 - 280, 282, 285 - 289, 291, 295 - 296, 298, 305 - 306, 308 - 310, 346, 348 - 349, 364 - 365
Immunostimulants, 76, 107
Infectious hypodermal/hematopoetic necrosis virus
See IHHNV
Isopods, 133, 221

L

Lagenidium callinectes, 5, 9 - 10, 42, 317
Lagenidium sp., 16, 42, 50, 79, 143, 219, 346, 350
Lagenophrys sp., 5, 52, 220
Larval black spot syndrome, 13, 96
Larval mycosis, 5, 9 - 10, 25, 42, 50, 139, 146, 150, 153, 155, 212, 220, 346, 370
Laspevresia pomonella, 154
Leptolegnia marina, 10
Leucothrix mucor, 5, 41, 50, 143, 151, 153, 155, 164, 219
Leucothrix sp., 9, 16, 19 - 20, 312, 346
Lindane, 86
LOPV, 28
Luminous bacterial disease, 8 - 9
Lymphoidal parvo-like virus
See LOPV

M

Malathion, 87
MB
See MBV
MBV, 3, 7, 9, 14, 21 - 23, 38 - 39, 41, 57, 60 - 64, 106, 128 - 131, 139 - 141, 144 -

151, 153 - 154, 156 - 157, 169, 178, 180 - 181, 183, 191, 212, 214, 233 - 235, 238, 249, 346, 364, 366
Metapenaeus ensis, 3, 169, 171, 185 - 186
Metazoa, 211, 221, 259
Methoxychlor, 85 - 86
Methyl-parathion, 81 - 82, 84 - 85, 87, 89 - 91, 93, 100
Mevinphos, 87
Microsporida, 5, 10, 17, 43, 50, 80, 259, 296, 346, 350
Microsporidosis, 5
Mirex, 215, 223
Moina macrocopa, 85
Muscle necrosis, 12, 17, 20, 52, 133, 200
Mysidacea, 87 - 88
Mysidopsis bahia, 86 - 88

N

NADA, 312, 314, 316, 322
Nalidixic acid, 75
Nematodes, 117 - 119, 259, 296
Nematopsis sp., 51, 212, 220, 273,346
New Animal Drug Application
See NADA
Noctiluca scintilans, 94
Noctiluca sp., 94
Nosema sp., 16 - 17, 50
Nucleocapsid, 62

O

One month mortality syndrome, 13, 81, 97, 100, 106
Organochlorides, 86, 89
Organophosphorous, 86, 89 - 91, 93, 100
Oscillatoria sp., 94, 123 - 124, 312
OTC, 74 - 76, 106, 127, 130, 132 - 133, 140, 164, 315, 350, 361, 368
Oxytetracycline
See OTC
Ozone, 367

P

Pagurus longicarpus, 86 - 87
Palaemon macrodactylus, 79
Palaemonetes vulgaris, 86 - 87
Paranophrys carcini, 16 - 17, 51
Paranophrys sp., 221
Parasitic ciliate disease, 17, 51
Paratya compressa improvisa, 85, 87
Parvoviridae, 39, 61, 65, 234 - 235
Penaeus aztecus, 88, 196, 236, 250
Penaeus brasiliensis, 236
Penaeus californiensis, 213, 226, 236, 250, 271
Penaeus chinensis, 3, 15, 17 - 18, 22, 39, 47 - 48, 50, 142, 161 - 163, 169, 171, 185 - 186, 212, 217, 236, 247, 353, 362, 367 - 368
See also *P. orientalis*
Penaeus duorarum, 87 - 88, 182, 215, 236
Penaeus esculentus, 3, 28, 38 - 39, 236
Penaeus indicus, 3, 9, 39, 236
Penaeus japonicus, 3, 5 - 6, 15, 18, 20 - 21, 23, 38 - 42, 155 - 156, 161 - 164, 166, 169, 182 - 183, 185 - 189, 191, 214, 217, 220, 226, 236, 351, 360, 362, 367
Penaeus kerathurus, 3, 38, 236
Penaeus latisulcatus, 185
Penaeus marginatus, 4, 236
Penaeus merguiensis, 3 - 4, 9, 21 - 22, 28, 38 - 40, 42 - 43, 47, 80, 142, 151, 236
Penaeus monodon, 3 - 4, 6 - 9, 11 - 15, 22, 28 - 29, 38 - 44, 57 - 58, 60 - 61, 66, 68, 70, 73 - 76, 79 - 84, 91 - 92, 94, 97, 100 - 101, 113 - 117, 119, 121 - 124, 129 - 131, 134, 139, 141, 143 - 151, 153 - 155, 169, 171, 178, 180, 183, 185, 191, 195 - 197, 199, 201 - 204, 212, 217 - 218, 223, 233, 235 - 236, 247, 258, 286 -

288, 346, 351, 359, 362, 364, 366 - 368
Penaeus monodon-type baculovirus
See MBV
Penaeus orientalis, 3
Penaeus paulensis, 236
Penaeus penicillatus, 3, 13, 18, 22 - 23, 38 - 39, 47, 236
Penaeus plebejus, 3, 151, 236
Penaeus plebejus baculovirus(PBV) 38
Penaeus schmitti, 236
Penaeus semisulcatus, 3, 13, 38 - 39, 142, 169, 171, 185 - 186, 236
Penaeus setiferus, 6, 143, 196, 236
Penaeus stylirostris, 4, 6, 142, 213, 215 - 217, 220, 226, 236 - 237, 239, 242, 244 - 245, 247 - 251, 269, 271, 276, 278, 287, 348 - 349
Penaeus subtilis, 236
Penaeus vannamei, 3 - 4, 6, 24 - 25, 39, 142, 189, 212 - 218, 223, 226, 236 - 238, 240, 244, 247, 249 - 250, 257 - 261, 266, 269 - 272, 274 - 276, 279 - 280, 285 - 291, 295, 306, 309 - 310, 334, 348 - 350, 352 - 353, 359 - 360, 363 - 365, 367 - 369
Penicillin, 140, 220
Perkinsus karlssoni, 332
Permethrin, 91, 93
Phloxine, 27
Phycomycete fungi, 212
Pleistophora sp., 16 - 17, 50, 212, 220, 275, 346
Polymycin, 75
Potassium permanganate, 10, 51, 122
Povidone iodine, 80
Probiotics, 107, 360 - 362, 369, 374
Protogonyaulax sp., 94
Protozoa, 4 - 5, 16, 19, 22 - 24, 37, 42 - 43, 211, 220, 259, 274, 346, 349, 367, 369 - 370
Pseudomonas aeuroginosa, 319
Pseudomonas sp., 8, 15 - 16, 41, 132, 143, 151, 204
Pyrethroid, 57, 85, 87, 91 - 92, 94

Q

Quarantine, 264, 273, 275 - 278, 280, 286, 326, 329 - 330, 335, 348, 372

R

Red discoloration, 13, 114, 125 - 126, 132
Red disease, 11 - 12, 41, 44, 146, 153, 156
Red leg disease, 18, 22 - 23, 49
REO, 7, 19, 24, 40, 142, 212, 217, 234 - 235, 238
Reo-like virus
See REO
Resiguard, 10
Rhizosolenia, 94
Rickettsia, 4, 6, 22, 41 - 42, 60, 211 - 212, 217 - 218, 274, 346
Romet-30, 315
Runt-deformity syndrome, 24, 270, 285, 291, 296, 309 - 310

S

Saponin, 10, 19, 23, 117, 120, 133, 222
Saprolegnia parasitica, 10
Sarafin, 315 - 316
Seriola quinqueradiata, 316
Sirolpidium sp., 5, 9 - 10, 16, 24 - 25, 42, 50, 143, 219, 317, 346, 350
Sodium bicarbonate, 313
Sodium chloride, 313
Sodium sulfite, 313
Soft shell syndrome, 18, 53, 222 - 223
Specific pathogen free
See SPF
SPF, 26 - 27, 257 - 263, 265 - 266, 269, 272, 279 - 281, 286 - 288, 291 - 292, 295, 297 - 299, 302, 305 - 306, 309 - 310, 325,

328, 330 - 332, 349, 360, 362 - 364, 370, 373 - 374
Spirillium sp., 127, 196
Spirocamallanus pereirai, 118
Spongy muscle syndrome, 13, 96
Streptomycin, 8 - 9, 75, 220
Sulfamethazin, 8 - 9
Sulfamethosazole, 75

T

Tail rot, 12, 126 - 128, 132
Tail rot disease, 361
TCBS, 369
Teaseed cake, 51, 54, 117, 120, 127,133
Terramycin, 5, 48 - 49, 132
Tetracycline chlorohydrate, 4, 8 - 9
Thelohania sp., 16 - 17, 22, 43, 50, 275
Thiothrix sp., 16, 50
Thymascar sp., 117 - 118
Thymascaris sp., 118 - 119
Treflan, 5, 9 - 10, 24 - 25, 28, 42, 79, 315, 317, 350, 367, 369-370
Trichodesium sp., 94
Trichodesmium erythraeum, 94
Trimethoprim, 75

U

U.S. Department of Agriculture
See USDA
USDA, 270, 282, 313, 315

V

Vibrio alginolyticus, 41, 48 - 49, 143, 204
Vibrio anguillarum, 49, 125, 132, 195-196, 198, 204
Vibrio campbellii, 49
Vibrio cholera, 273
Vibrio damsela, 125, 195 - 198, 204
Vibrio harveyi, 40, 76, 125, 140, 195 - 204
Vibrio nereis, 195, 197 - 198, 204
Vibrio parahaemolyticus, 41, 48 - 49, 74, 125, 132, 143, 197 - 198, 204
Vibrio sp., 4, 8, 15 - 16, 19 - 20, 26, 40 - 41, 47, 49 - 50, 52, 54, 74 - 78, 127, 132, 143, 156, 163 - 164, 166, 196 - 197, 204, 218, 225, 273, 305, 307, 346, 351, 360 - 362, 369
Vibrio splendidus, 40, 76, 142, 198
Vibrio tubiashii, 196
Vibrio vulnificus, 74, 76, 195, 198
Vibriosis, 5, 8, 15, 19 - 21, 40, 47 - 48, 53, 74, 76, 163 - 164, 212, 218 - 219, 225, 346, 351, 362
Viral occlusion
See VO
Virbrio harveyi, 142
Virions, 62 - 65, 69, 73, 96, 103 - 105, 182, 239, 246 - 247, 249
VO, 61
Vorticella sp., 5, 10, 16 - 17, 43, 51, 79, 139, 146, 150, 153, 155, 220

W

White spot disease, 18, 53
White-black spot disease, 18, 52

Z

Zoothamnium sp., 5, 10, 16 - 17, 22, 43, 51 - 52, 79, 115 - 117, 139, 146, 150 - 151, 153, 155, 164, 220, 274, 346