

Diseases in Farmed Mud Crabs *Scylla* spp.: Diagnosis, Prevention, and Control



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Aquaculture Department
Southeast Asian Fisheries Development Center
Government of Japan Trust Fund

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Aquaculture Department
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Philippines
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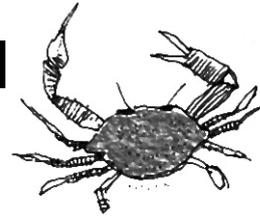
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Foreword



Aquaculture production has suffered many set-backs due to the occurrence of diseases. Many of the diseases are caused by infectious organisms that are difficult to detect and need sophisticated instruments for diagnosis, but most disease occurrence and mortality in farmed aquatic animals are related to poor rearing water quality. It is, therefore, important for technicians and farmers to recognize the relationship between the animals they culture and the aquatic environment. The Government of Japan, through the Regional Fish Disease Project, funded research on diseases affecting mud crabs in order to come up with sound prevention and control methods.

This book is a collection of observations gathered from various research and commercial culture activities, and gives emphasis on disease recognition using simple techniques and gross observations of affected crabs. However, since many of the diseases are caused by microorganisms, microscopy is an important technique for their diagnosis. The authors of the book encourage active cooperation between farmers and diagnostic laboratories for disease identification, prevention, and control in order to build up more information to increase production. The Regional Fish Disease Project supports sustainable mud crab production and hopes that farmers and other users of this book will attain their production goals.

A handwritten signature in black ink, appearing to read 'K. Nagasawa', with a long, sweeping horizontal line extending to the right.

Kazuya Nagasawa, Ph.D.

Fish Disease Expert and Leader
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December 2004

Message



The SEAFDEC Aquaculture Department is mandated to develop and promote aquaculture technologies that enhance productivity and provide benefits to aquaculture communities in Member Countries. The high demand for mud crabs in the local and export markets generated more interest among farmers, who found in mud crabs an alternative to shrimp. From traditional mud crab fattening activities, farming of the mud crabs *Scylla* spp. has recently diversified into their culture in ponds or pens in mangroves. Anticipating intensified collection of wild seeds for grow-out culture, SEAFDEC Aquaculture Department initiated crab seed production in hatcheries and nurseries to ensure the sustainability of mud crab farming. Complementary research activities in crab nutrition and health management were conducted hand-in-hand with farming activities to guarantee fast development of technology.

The contents of this book is a product of research and support activities of the Mud Crab Team done at the SEAFDEC Aquaculture Department since 1998 with funding from various sources, including those from the Australian Centre for International Agricultural Research (ACIAR), the European Union's International Cooperation Project on Culture and Management of the Crab *Scylla* (INCO-CAMS), and the Trust Fund on Fish Diseases from the Government of Japan. This book serves as a guide to technicians who are involved in crab hatchery operations, and aims to complement the contents of the manual "Biology and Hatchery of Mud Crabs *Scylla* spp." that was published in 2003. This book also serves as a source of information for extension workers, as well as of students of fisheries and aquaculture.

A handwritten signature in black ink, appearing to read 'R. Platon'.

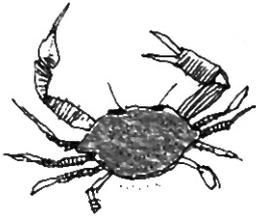
Rolando R. Platon, Ph.D.

Chief

SEAFDEC Aquaculture Department

Tigbauan, Iloilo, Philippines

December 2004



Acknowledgments

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For the duration of the studies, valuable technical assistance was provided by Demy D. Catedral, Sharon Ann G. Pedrajas-Mendoza, and Helen Marcial. We also acknowledge colleagues in the Mud Crab Team composed of Emilia T. Qunitio, Fe Dolores Parado-Estepa, Jurgenne H. Primavera, and Eduard M. Rodriguez. Remia Traviña and Milagros G. Paner of Fish Health Section's Diagnostic Services Laboratory, as well as Susan Torrento and Fely Torreta of the Microtechnique Laboratory, extended valuable help in processing specimens. Several specimens that were studied came from farmers who submitted their samples for analysis at the Diagnostic Services Laboratory – our sincerest thanks to the farmers.

We thank Dr. Emilia T. Qunitio for providing photos of larval stages of mud crab that are in Appendix 7 and for giving us valuable comments to improve the book. Dr. Teodora U. Bagarinao and Dr. Kazuya Nagasawa thoroughly reviewed the draft and gave important suggestions that improved the lay-out, format, and presentation of contents. We recognize the artistic talent Raph Nacepo, who patiently did the lay-out.

Our heartfelt thanks to Mr. Hermès Alègrè, one of the Philippines' best selling visual artists, for his rendition of "Crab Circle of Life". Notwithstanding the fact that we have not met, he created and entrusted to us his original artwork to cover this manual. Portions of this artwork are also used to highlight section separators.

Celia R. Lavilla-Pitogo and Leobert D. de la Peña
December 2004

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Introduction



Mud crabs, also known as mangrove crabs, belong to Family Portunidae under genus *Scylla* with four species, *S. serrata*, *S. tranquebarica*, *S. olivacea* and *S. paramamosain*. These large marine and estuarine crustaceans live in soft muddy bottom in sheltered estuaries, tidal flats and rivers lined with mangroves. However, females carrying eggs are present in deeper waters up to 50 km offshore in tropical to warm temperate waters. Mud crabs vary in colour from dark olive-brown to greenish and blue-black, and patterns of lighter colored dots cover the walking legs.

Mud crab aquaculture has been practiced for many years in Southeast Asia and is an important source of income among fisherfolks. Traditionally, these activities were mainly based on stocking wild-caught juveniles and adults for grow-out culture and fattening. The demand for mud crabs in the export market has multiplied farming activities leading to intensified collection of wild seed. Thus, SEAFDEC Aquaculture Department developed technology for large-scale production of juvenile mud crabs, *S. serrata*, *S. olivacea* and *S. tranquebarica* to ensure the sustainability of mud crab farming and reduce the fishing pressure on wild stocks.

With the state-of-the-art of mud crab larval rearing in hatcheries, not all mortality is caused by disease and much remains to be learned to improve the survival of hatchery-reared zoeae to megalopae, which is currently at 4%. Although hatchery production of megalopae is already feasible, the initial source of spawners and broodstock is mostly wild stock. Thus, broodstock development is also an important component of SEAFDEC Aquaculture Department's project on mud crabs to avoid undue environmental impact due to over-harvesting and continuous collection of gravid females from nature. This activity is aimed at learning more about domestication of crabs for sustainable aquaculture development and independence from natural populations of spawners.

Because crab culture operations have not taken off to the level of other crustacean species like shrimps and prawn and stocking densities in culture systems have been comparatively low, numerous diseases with potential economic impact may not have been observed and caused problems. For example, the septicemic "orange crab disease that was responsible for losses of crabs (*Scylla serrata*) cultured in floating cages in Singapore is not included in this manual since not a case was observed in the course of our study. *Scylla* baculovirus (SBV) and the parasite *Hematodinium* sp. that have been reported infecting the digestive organs of juveniles, sub-adult and adult *S. serrata* in Australia, were not observed or probably have been missed out in routine diagnosis. *Hematodinium* infection can be difficult to diagnose and is believed to cause substantial mortality in the field because infections are generally considered terminal. Another reported disease of unknown prevalence is *Sacculina granifera*, a parasite that causes sterility among infected crabs and alteration of taste of infected meat. If such diseases remain unreported or unsolved during culture, they will

hamper production or affect the marketability of products. Moreover, infected crabs may potentially transmit pathogens to various rearing facilities, adjacent farms, and, worse, into the natural environment. At present, most diseases are observed in crab grow-out only when the quality of the water or the soil is poor.

We recognize that many of the diseases and associated fouling organisms on crabs presented here are classified under non-specific taxonomic groupings. This is because not much in-depth study has been conducted on the pathogens so far and our aim is to provide a practical guidance rather than an academic review. Our endeavor to bring academic significance to our work is continuing by collaborating with various scientists and specialists. For now, this book intends to inform crab industry stake-holders on diseases commonly observed during culture of various stages of crabs, and methods and practices to minimize losses from diseases. The diagnostic techniques described for each disease or abnormality gives emphasis on gross observation, and simple microscopy and microbiology. Specifically, the goals of the book are to:

- present the disease problems commonly observed in larvae, juvenile and adults
- provide simple diagnostic background for hatchery and farm technicians
- introduce techniques for disease prevention
- increase awareness about hazards of diseases to the operation and the environment

Background information on the development of disease and the relationships between the hosts, pathogens and the environment that result to outbreaks, as well as sources of more information can be obtained by starting with the references and links below. It has been observed that diseases of crustaceans have many similarities. Thus, references on shrimps and other species of crabs are useful materials in studying diseases of mudcrabs. Interestingly, the internet offers a vast array of information, though not necessarily on mud crab that will surely widen everyone's perspective on crab culture. The few links to web-based information that are listed below, as well as after every disease problem described, are intended to lead readers to the wealth of web-based information.

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Web-based Resources



<http://aquanic.org/> = This is the website of The Aquaculture Network Information Center which provides a gateway to the world's electronic aquaculture resources.

<http://www.dpi.qld.gov.au/extra/pdf/fishweb/mudcrab.pdf> = This is the link to a leaflet on mud crab available for download and shows a simple life cycle illustration.

www.pac.dfo-mpo.gc.ca = The website has an array of diseases of aquatic animals. Find the AQUACULTURE pages and search for crab diseases.

<http://www.dpi.qld.gov.au/fishweb/2698.html>

www.blue-crab.org

<http://www.blue-crab.org/lifecycle1.htm>

[http://www.aciar.gov.au/web.nsf/doc/ACIA-5VL3HK/\\$file/WP54%20Web.pdf](http://www.aciar.gov.au/web.nsf/doc/ACIA-5VL3HK/$file/WP54%20Web.pdf)

http://www.enaca.org/AquacultureAsia/Articles/July-Sept-2002/Seed_production_crucifix.pdf

<http://www.dec.ctu.edu.vn/sardi/AacrabCWare/Publication/309CRA.htm>

http://www.spc.org.nc/aquaculture/site/commodities/PDF/MudCrab_page.pdf

Discussion Groups



Aquataalk at <http://www.data-case.com/index.htm>

AquaFarmers' Corner at <http://www.seafdec.org.ph/ubbthreads/ubbthreads.php>

Training



AquaHealth Online at: <http://www.seafdec.org.ph/training/aquahealthonline.html>

Health Maintenance and Monitoring



For aquaculture species with mature technologies, the major constraints in production are usually related to health maintenance, disease occurrence, and product quality. These issues are inter-related, especially if disease prevention or control implements have long-term effects on the environment or produce residues that make products unacceptable for consumption. Although Fish Health is a relatively young discipline, numerous publications are already available for reference. One of the best references that discuss about disease, health, monitoring and surveillance is the Survey Toolbox for Aquatic Animal Diseases. Most of the information in this section is derived from various sections of that book.

Health and Disease

Disease is usually defined as any abnormality of structure or function. This means that whenever there is something abnormal about the animal, we can consider it to be a disease.

Health is simply the normal state of an animal, or the absence of disease. Determining if an animal is healthy or not does not just mean that we have to identify some physical abnormality or a disease agent. The level of production can be an indicator of whether an animal is healthy or diseased. Measures of production to indicate health status can be very useful.

Ensuring good farm production and animal health starts at the planning stage of every aquaculture venture. At each step of planning and production, questions and answers should be anticipated on how to decrease the possibility of pathogen entry and environmental contamination. These considerations include the following:

- Selection of a production site
- Selection of water source
- Water quality management and control
- Maintenance of various life stages
- Feed quality and feeding practices
- Fish health management
- Proper use of chemicals and veterinary drugs
- Proper sanitation
- Harvesting, holding and transport
- Detailed record keeping

Sources of Infection

Different measures are needed to maintain cleanliness and hygiene within culture premises. Prevention and control measures for specific diseases are discussed in the sections on Diseases in Eggs and Larvae, and in Diseases in Juveniles and Adults. The Appendices section also contains specific methods for disinfection and identification of sources of infection.

Hatchery Facilities. Maintenance of hygiene in the hatchery can be done simply by disinfecting with chlorine all facilities (reservoir, larval rearing tanks, algal tanks, rotifer tanks, *Artemia* tanks, etc.) and materials (nets, hoses, pails and other paraphernalia). A well-designed hatchery should have a disposal system for contaminated effluents to prevent contact with natural bodies of water. If a broodstock facility is incorporated in the design, this should be separated from larval rearing facilities since it is well-known that broodstock harbor and transmit various infectious diseases. Precautionary measures such as provision of footbaths at entrance and exits of production buildings are effective measures to prevent diseases.

Animals. Any stage of live animals for culture that is brought into new sites or aquaculture facilities may carry with it associated microorganisms, including those that cause diseases. Therefore, disinfection and quarantine are carried out to avoid introduction of new and exotic pathogens. Guidelines and protocols regarding introductions are discussed in various manuals and agreed-upon Codes of Practices for prevention and spread of diseases.

Natural food. Hatchery technicians should ensure that their phytoplankton and zooplankton cultures are free of contaminants such as saprophytic protozoans that may become nuisance during culture. The bacterial populations associated with *Brachionus* and *Artemia* can be checked for harmful and opportunistic pathogens through microbial culture. Microbial load of natural food can also be reduced by rinsing them in clean seawater before feeding.

Artificial feeds should be stored properly under refrigeration or in well-ventilated rooms to avoid rancidity or fungal growth resulting in toxic by-products.

Regular Record Keeping

Individual experiences in hatchery and farm operation build up information on acceptable survival and growth rates. However, careful monitoring and record keeping provide data on most profitable operational protocols that result in highest production possible. Records of water quality, stocking rate and date, daily feeding quantities, water management schemes, harvesting dates and quantities, etc. provide a picture of how each culture unit performs under a certain management regime. The accumulated information, if properly analyzed, can be linked with production rates and become the basis for optimized and profitable operations in the hatchery and farm.

The survival and growth rate of each population depends on factors like stocking density, predation, feed, temperature and other site-specific and farmer-specific aspects. One way to increase the predictability of production outcomes is by monitoring the health status of animals. Carefully analyzed records of the presence of morphological deformities or of indicator microorganisms can generally prevent sudden mass kills. The records also enable farmers to investigate the causes of low level mortality over a period of time.

Disease Monitoring and Sampling

Data obtained from frequent and regular monitoring of farmed animals has predictive value if the examinations are based on a good number of samples. Diseases or characteristics to evaluate during a microscopic examination of larvae may include the following:

- Larval stage
- Presence and severity of microbial fouling and infection
- Presence of shell necrosis
- Presence of missing appendages or body parts, and abdominal or appendage deformities
- Gut fullness
- Discoloration of larvae

The above observations can be recorded in the sample form (Fig. 1). Diseases and abnormalities that affect cultured crabs are presented in Section III.

EGG/LARVAL MONITORING FORM

Tank No.: _____ Sampling Time: _____ Date: _____
 Source: _____ Larval Stage: _____ Species: _____

Findings	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Filamentous bacteria																				
Fungi																				
Protozoa: Vorticella																				
Zoothamnium																				
Others																				
Nematodes																				
Necrotic tissues																				
Shell disease																				
Incomplete molting																				
Empty gut																				
Weak																				
Dead																				
Others																				

Comments: _____

Fig. 1. Sample form to record observations during daily monitoring of eggs and larvae of crabs

In aquatic culture systems, it is difficult to determine the exact number of animals in the population because of their small size at the larval stages and because they are swimming in the water column. Obtaining the representative number and kind of samples for monitoring purposes is difficult. Sampling is the process of selecting this group from the population. Each member of the sample will be examined and the results are used to generate a picture of the status of the entire population from which the sample was drawn.

For routine examination to determine the condition of hatchery-reared crab larvae, the recommended sample size for daily monitoring follows:

Larval Population	Sample Size (Number of Larvae)
1,000 to 199,000	20
200,000 to 399,000	40
400,000 to 599,000	60
600,000 to 799,000	80
800,000 up	100

However, when disease is suspected in the population, a different sampling guideline and sample number should be used. Below are the sample sizes based on assumed pathogen prevalence in the population.

Population size	Sample Size		
	at 2% prevalence	at 5% prevalence	at 10% prevalence
50	50	35	20
100	75	45	23
250	110	50	25
500	130	55	26
1,000	140	55	27
1,500	140	55	27
2,000	145	60	27
4,000	145	60	27
10,000	145	60	27
10,000 or more	150	60	30

Therefore, in a population of 10,000 crab larvae in one rearing tank where a serious disease caused by an infectious organism is assumed to occur at 5% prevalence, the sample to be obtained should be 60 larvae. In cases where the assumed disease prevalence is unknown, it is best to obtain and examine the maximum number of sample recommended.

Levels of Diagnosis

The Asia Diagnostic Guide provides guide for the pathogens and diseases listed in the NACA/FAO/OIE Quarterly Aquatic Animal Disease Reporting System. It was developed from a large amount of technical contribution from aquatic animal health scientists in the Asia-Pacific region who supported the regional programme. The Asia Diagnostic Guide could be effectively used for both farm and laboratory level diagnosis in the region. It complements the Manual of Procedures for the Implementation of the Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals. It also assists countries in expanding national and regional aquatic animal health diagnostic capabilities to meet the requirements in the International Aquatic Animal Code and the Diagnostic Manual for Aquatic Animal Diseases of the Office International des Epizooties (OIE).

Definition of levels of diagnosis

- Level I:** Diagnostic activity includes observation of animal and the environment and clinical examination (Diagnosis site: Field);
- Level II:** Diagnostic activity includes parasitology, bacteriology, mycology, and histopathology (Diagnosis site: Laboratory);
- Level III:** Diagnostic activity includes virology, electron microscopy, molecular biology and immunology (Diagnosis site: Laboratory).

Sending Samples for Diagnosis

Farm site diagnosis is very important in order to gather information about diseases, especially for emerging problems caused by infectious microorganisms. Strong disease recognition capability at Level I, coupled with more understanding about the course of disease after Level II and III diagnoses, will fast-track our understanding of disease problems affecting aquatic animals. Recognizing the limited facilities for laboratory diagnosis, it is very important for farmers and technicians to know where samples can be sent. Based on the recommended number of representative samples, good quality specimens can be submitted. Farmers and technicians should know where to contact fishery officers and laboratories near their culture sites so that disease outbreaks can readily be reported and investigated.

Sample preparation, fixation, packing and submission are in Appendix 1. Formulae of fixatives and fixation procedures are in Appendix 2.

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Web-based Resources



<http://aquanic.org/publicat/state/il-in/as-503.htm> = Fish Farmer's Guide to Understanding Water Quality can be downloaded from this site

http://aquanic.org/publicat/usda_rac/tr/ctsa/mangro95.pdf = This site contains a document by the Center for Tropical and Subtropical Aquaculture (CTSA) entitled "Mangrove Crab as a Model for Development of a Quarantine System to Screen Species for Aquaculture in Guam"

www.oie.int, specifically at http://www.oie.int/eng/normes/fmanual/A_00046.htm

www.fao.org/docrep/003/w7499e/w7499e23.htm = link to a document on developments and issues in fish health, application of chemicals in aquaculture and quarantine

<http://www.fao.org/DOCREP/005/X8485E/x8485e06.htm> = this site provides a link to the "Beijing Consensus and Implementation Strategy"

<http://www.seafdec.org.ph/information/publication.html> = link to the website of SEAFDEC Aquaculture Department that provides free downloads of information materials and proceedings of meetings

<http://www.dpi.qld.gov.au/thematiclists/14515.html> and <http://www.dpi.qld.gov.au/thematiclists/14527.html> = these links to the Department of Primary Industries and Fisheries of the Queensland Government, Australia with information about submitting samples for laboratory examination and crab culture

<http://www.enaca.org/modules/mydownloads/singlefile.php?cid=23&lid=52> = This is the download site of the Asia Diagnostic Guide to Aquatic Animal Diseases or 'Asia Diagnostic Guide'

Diseases in Eggs and Larvae

Luminescent Vibriosis

Shell Disease

Fungal Infection

Fouling by Filamentous Bacteria

Fouling by Filamentous Diatoms

Fouling by Sessile Protozoans

Fouling by Saprophytic Protozoans and Nematodes

Fouling by Suspended Debris

Incomplete Molting



Luminescent Vibriosis

Pathogen or Cause:

Luminescent vibriosis or luminous bacterial infection is caused by *Vibrio harveyi*, a rod-shaped bacterium with single polar flagellum (Photo 1; SEM \times 5,000). *Vibrio harveyi* reproduces by simple cell division (Photo 2; SEM \times 5,000).

Background Information on Marine Luminous Bacteria:

Originally reported in cultured shrimp, luminescent vibriosis is also a devastating disease in crab larvae. An analysis of the origin of luminescent bacterial disease outbreaks in the shrimp hatchery showed that the shift in husbandry and feeding practices led to ecological imbalance in the culture system. *Vibrio harveyi* is commonly found in nearshore sea water and infection among cultured crustaceans may be expected when the health of hosts is compromised.

Effect on Crabs:

- Affects eggs and larvae where infections result in mortality reaching up to nearly 100% of the population

Diagnostic Techniques:

Gross Observations (Level I):

- Heavily infected larvae exhibit a continuous greenish luminescence when observed in total darkness
- The condition is best observed by monitoring the tanks at night and watching out for luminous larvae. This occurs as a result of bacterial multiplication in infected larvae resulting to mortalities
- Affected larvae become weak and opaque-white, and settle to the bottom forming a dense mat after mass mortality

Microscopic Examination (Level II):

- Fresh mounts of weak and dying larvae show internal tissues densely packed with highly motile bacteria
- Occasionally, the region near the hepatopancreas appears dark

Microbiological Techniques (Level II):

- Media and method for isolation of bacteria are in Appendix 4a. Infected larval tissues streaked on nutrient agar medium produce luminescent colonies after 18 - 24 hours incubation (Photo 3)
- Colonies on nutrient agar are cream to off-white in color, and round with entire edges (Photo 4)
- Green colonies dominate on thiosulfate citrate bile sucrose (TCBS) agar, a selective culture medium for vibrios (Photo 5)
- Regular monitoring of spawned eggs show *Vibrio* counts ranging from 10^3 to 10^8 cfu/gram
- The presence of luminous bacteria in larvae may be a reflection of luminous bacteria in the water

Mode of Transmission:

- The number of luminous bacteria gradually builds up in the rearing water
- When the critical number of luminous bacteria reaches 10^2 per/ml, infection through oral route occurs within a few days
- The egg mass gradually builds up luminous bacteria if spawning occurs in contaminated water

Methods of Prevention and Control:**a). Spawning**

- Use only previously chlorinated water (Appendix 5) for spawning and rearing to ensure a clean environment for newly hatched and developing larvae
- Remove the mothers from the tanks immediately after spawning and rinse the zoeae with chlorinated water to prevent its colonization with luminous bacteria

b). Larval Rearing

- Prevent the entry of luminous bacteria into the hatchery system by using chlorinated water, or ultraviolet-irradiated water, or by employing a series of filtration equipment (sand filters, filter bags, cartridge filters, 0.45 micrometer filter, etc.). Higher levels of chlorine may be used if necessary, but care must be taken to ensure complete dechlorination prior to use. Take note that biofilm formation has been found to promote the survival of the bacteria against chlorination so that care should be taken to ensure that tank wall and other rearing paraphernalia have not developed protective biofilms.
- Rinse *Artemia* nauplii and other zooplankton before introducing them as food into larval rearing tanks
- Siphon out sediments and debris from the tank bottom since these could serve as substrates for bacterial growth
- Since the onset of mortality is preceded by the dominance of luminous bacteria in the rearing water, monitor bacterial profile regularly using microbial culture media
- Use reservoirs where settling of sediments, disinfection, conditioning and effective monitoring of bacterial load of the rearing water can be done

c). Termination

- Disinfect infected stock with 200 ppm active chlorine for at least one hour before finally discarding them. Complete clean-up and disinfection of hatchery paraphernalia should be done after every larval rearing period

Note

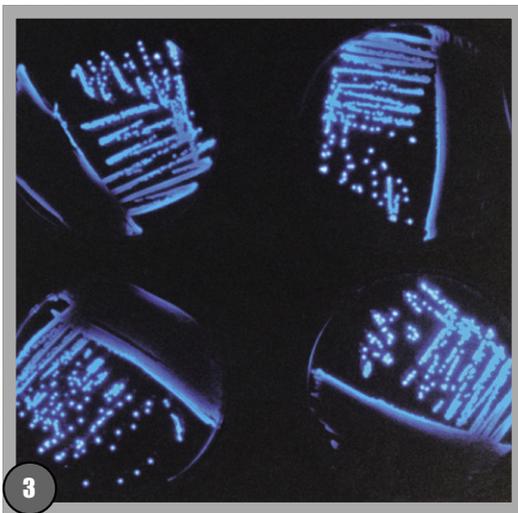
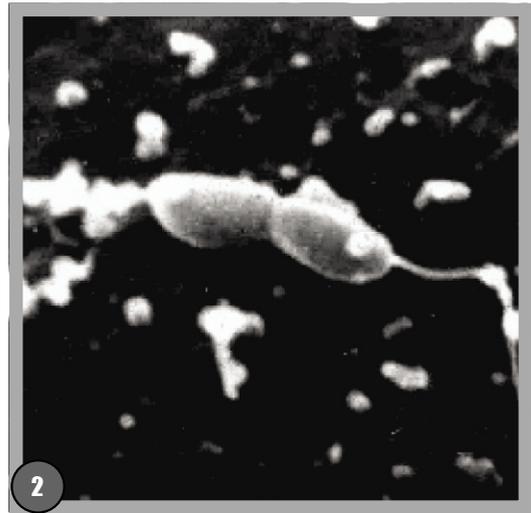
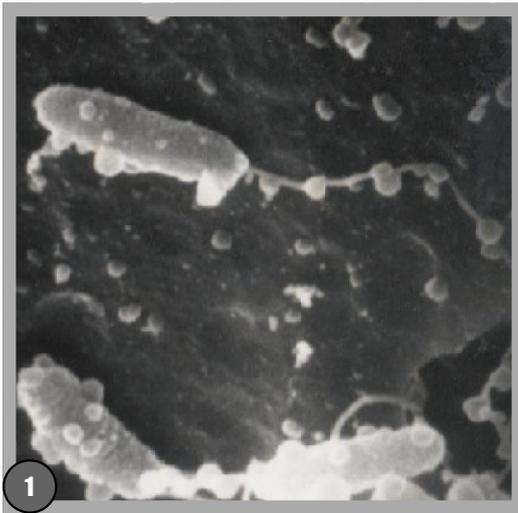
Chemical control of the disease based on efficacy of available drugs appears limited because of the restricted tolerance of crab larvae to drugs and the possible development of resistant strains of bacteria.

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Web-based Resources

- [http://www.biology.pl/bakterie sw/index.en.html](http://www.biology.pl/bakterie/sw/index.en.html) = the site provides information about the habitat, physiology, isolation and maintenance of luminous bacteria from various sources
- <http://141.150.117:8080/prokPUB/index.htm> = this is the download site of The Prokaryotes, an evolving electronic resource for the microbiological community



Shell Disease

Pathogen or Cause:

Various shell-degrading or chitinolytic bacteria belonging to the genera *Pseudomonas*, *Aeromonas* and *Vibrio*

Description:

Shell disease is characterised by progressive erosion of the exoskeleton due to microbial action. The disease may play an important role in the development of systemic disease in various stages of cultured crabs, and such has also been reported in juveniles and adults in the wild. It may be fatal when large areas of the exoskeleton become eroded.

Effect on Crabs:

- Discoloration of affected parts because of deposition of the brown pigment melanin
- Originally intact spine (Photo 1, arrow) become shortened because of tissue erosion (Photos 2 and 3; arrows)
- Severe cases may lead to loss of affected appendages (Photo 4)
- Usually not a cause of mortality in larval stages that molt regularly since affected parts go off with the old exoskeleton during molting
- A more serious form of shell disease may progress to systemic infection and mortality may occur

Diagnostic Techniques:

Gross Observations (Level I):

- Microscopy is needed to see affected parts and degree of erosion in larval stages

Microscopic Examination (Level II):

- Prepare fresh mounts of live larvae for light microscope examination (Appendix 3)
- Look for blackened or brownish eroded areas on the shell, especially at the tips of spines, appendages and tail (Photos 2-4)

Mode of Transmission:

- Chitinolytic bacteria are ubiquitous in the marine environment and they have close affinity with chitin, the material that makes up the exoskeleton of crustaceans. Shell disease is an outcome of bacterial build up on the exoskeleton and is a function of intermolt duration
- More shell disease occurs when larvae grow slowly and molt infrequently
- Shell disease may also be caused by chitinolytic bacteria attacking sites of mechanical injury on crabs

Methods of Prevention and Control:

Since the appearance of fouling protozoans indicates high organic matter load in the rearing system, the following are effective preventive methods:

- Siphon regularly excess feeds, dead larvae and natural food, and debris that settle to the bottom to keep organic load low
- Siphon out molted exoskeleton which harbor high numbers of bacteria on parts affected with shell disease
- Minimize handling and overcrowding to avoid mechanical injuries that may lead to shell disease

References

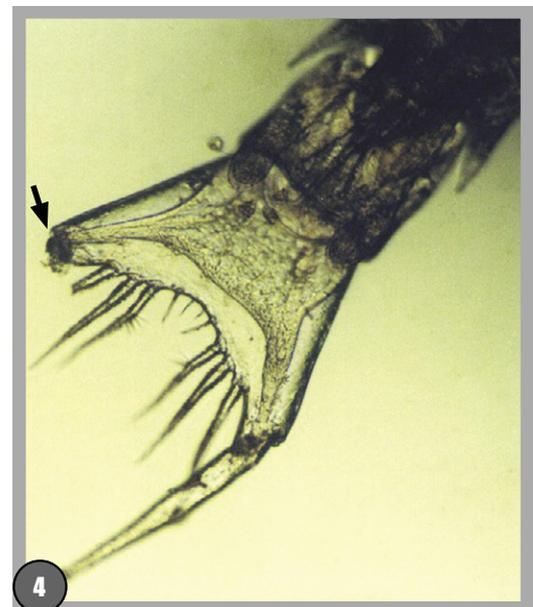
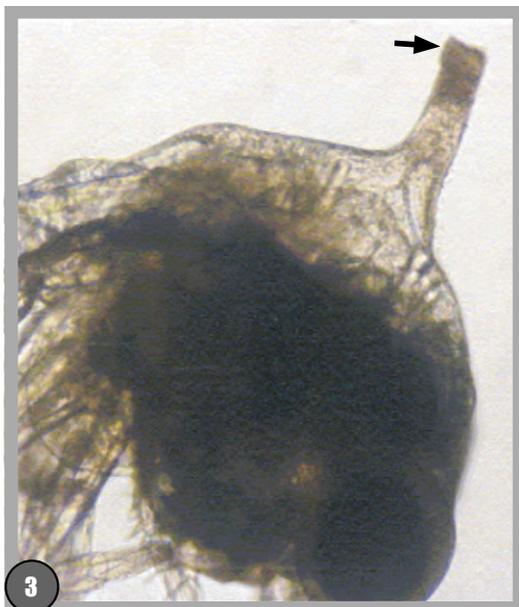
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Web-based Resources

<http://www.seafdec.org.ph/downloads/hilites2k3.pdf>

<http://crabstreetjournal.com/articles/shelldisease/message26526.html>

<http://crabstreetjournal.com/articles/shelldisease/index.html>



Fungal Infection

Pathogen or Cause:

Fungi such as *Lagenidium*, *Sirolopidium*-, *Halocrusticida* and *Haliphthoros*

Background Information of Infections Due to Marine Fungi:

Aquatic fungi are widespread in the environment and they can tolerate wide ranges in salinity. Because of the exposed nature of the egg mass (Photo 1) and length of time between spawning and hatching (9-14 days), eggs become vulnerable to infections and diseases derived from the environment. Fungal infection is one of the more serious diseases affecting incubating crab eggs and can destroy the whole egg mass, in some cases.

Effect on Crabs:

Eggs

- Infected eggs may not hatch
- Infected egg masses can introduce significant numbers of infective zoospores into the rearing system upon hatching

Larvae

- Infected larvae mostly die
- Newly hatched and younger larvae are more susceptible because of their relatively thin exoskeleton
- Response to treatment is unsatisfactory in advanced cases of fungal infections
- Surviving animals will be of low quality. Disinfect and dump affected tanks with terminal infections

Diagnostic Techniques:

Gross Observations (Level I):

- Abnormally long incubation period of eggs
- Affected larvae settle to the bottom forming a whitish mass

Microscopic Examination (Level II):

- Prepare fresh mounts of live larvae for light microscope examination (Appendix 3)
- Branching non-septate filaments can be readily seen within infected eggs (Photos 2-4) and larvae (Photos 5-7)
- Details about fungal isolation are in Appendix 4b

Mode of Transmission:

- Motile zoospores released from globose vesicles (Photos 3 and 7; arrows) or discharge tubes (Photos 2, 4 and 6; arrows) swim in the water and implant themselves into susceptible crabs (Fig. 2)
- Spore release in *Lagenidium* takes less than an hour after formation of vesicles, and spread of infection is rapid after uncontrolled onset

Methods of Prevention and Control:

- Monitor larval stocks by daily microscopic examination for early detection of fungus in infected larvae. Refer to the table in Section II for the recommended number of samples to monitor with the corresponding population being reared. Record observations using the form in Fig. 1, Section II

- Monitor incubating eggs; zoospores that are released from infected eggs cause fungal infection in newly hatched larvae
- Inhibit the transmission of the fungus from eggs to hatched larvae by placing ovigerous females in 25 ppm formalin in the hatching tank
- Short-term baths for disinfection are in Appendix 6
- Bath treatment with 25 ppm formalin inactivates the zoospores released from infected eggs without harming newly hatched zoeae
- Motile zoospores of the fungus *Lagenidium* sp. transferred to salinities ranging from 7–15 ppt (see box below) became immobile after 10-15 minutes showing that short dips to lower salinity has a potential to control the invasiveness of motile zoospores. This may be particularly important for berried females harboring eggs infected with fungi

Note**How to obtain salinities ranging from 7–15 ppt?**

Assuming that full strength seawater derived from the seawater with no freshwater intrusion has a salinity of 30 – 32 ppt, merely adding an equal volume of freshwater will produce 15 – 16 ppt seawater. Further addition of an equal volume of freshwater will produce 7.5 – 8 ppt salinities.

References

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Web-based Resources



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<http://www.dec.ctu.edu.vn/sardi/AacrabCWare/Publication/310CRA.htm> = this site links to an article “Review of mud crab culture research in Indonesia”

http://www.pac.dfo-mpo.gc.ca/sci/shelldis/pages/lagfdcb_e.htm

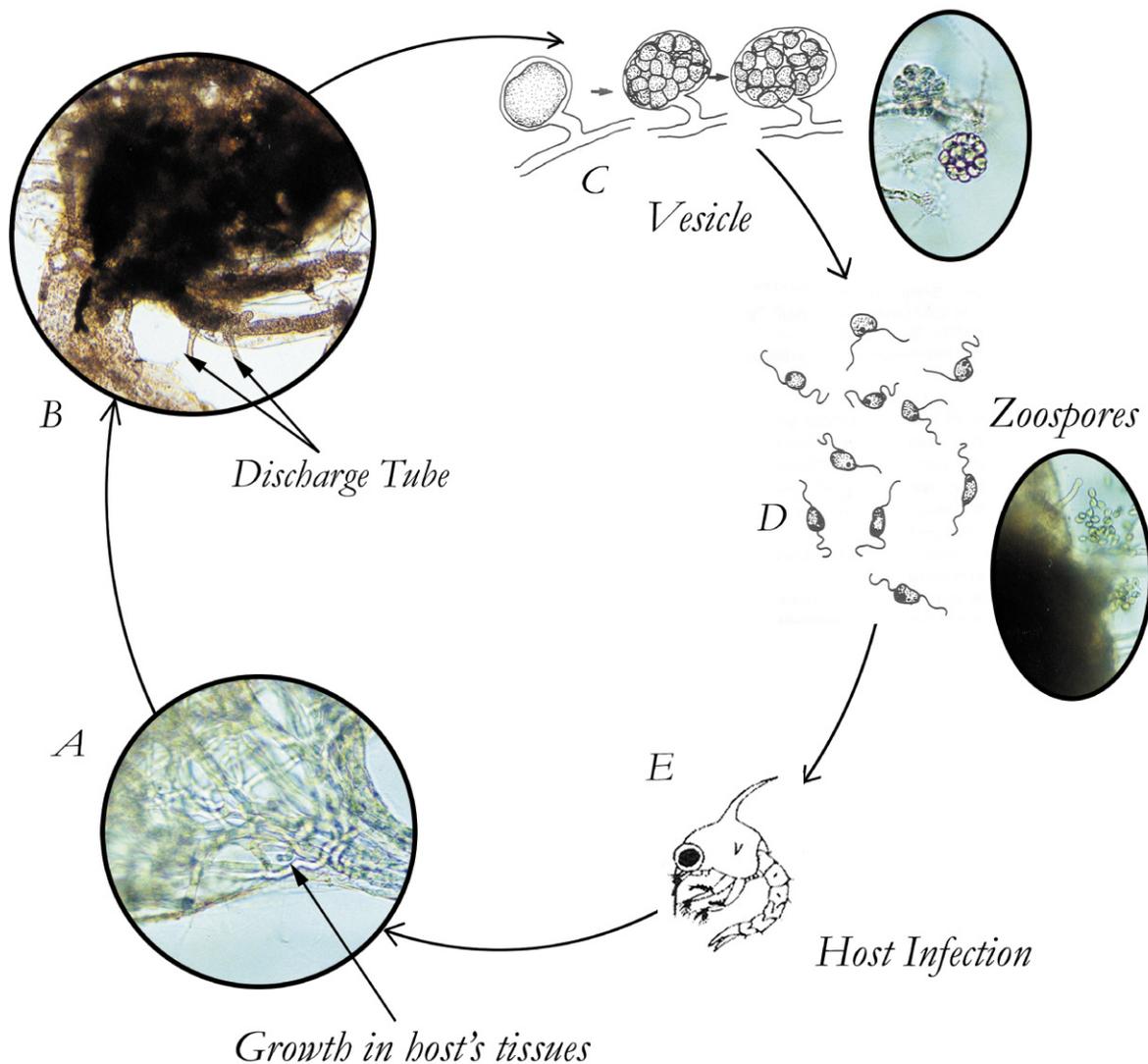
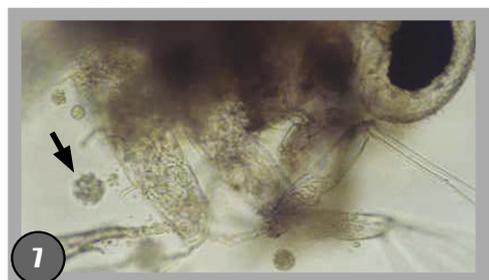
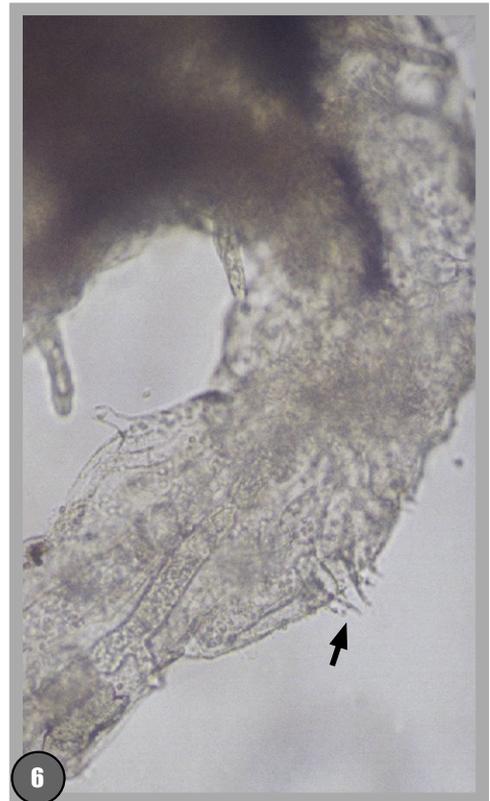
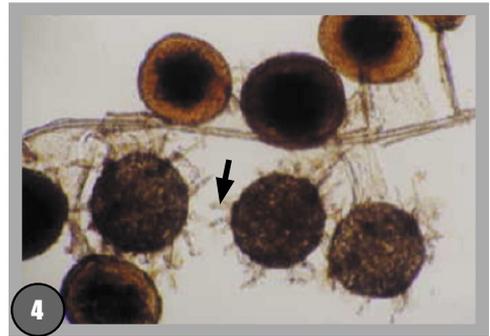


Fig. 1. Infection cycle of marine fungi in hatchery-reared crab larvae. (A) Fungus growing inside infected larva produce highly-branched filaments. Mature filaments produce zoospores and release them either through discharge tubes (B), or externally-developing vesicles (C). Released zoospores (D) find their hosts and start new infection (E).

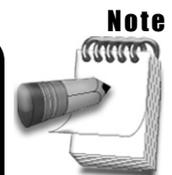


Fouling by Filamentous Bacteria

Organisms that become colonizers of various surfaces are widespread in nature and most of them benefit from aquaculture habitats because of the rich supply of organic matter. Many bacteria, fungi, sessile protozoans, and microscopic algae use fish and crustacean larvae as substrates, benefiting from the association by feeding on abundant microorganisms. Regularity of molting in crustaceans prevents a massive build-up of fouling organisms on the exoskeleton such that fouling problems may merely be manifestations of underlying problems related to water or feeding management.

The Molting Process:

Crabs must molt in order to grow. When it molts, the old shell is shed together with everything attached on it. A newly-molted crab has relatively soft shell and is susceptible to cannibalism.



Pathogen or Cause:

Leucothrix-like filamentous bacteria and other surface-living bacteria that are widely distributed in aquatic environments colonize any submerged surfaces including shells of crab eggs and various stages of larval crustaceans. The presence of filamentous bacteria is an indication of deterioration of water quality or animal health. The number of days per stage (compare with larval stage in Appendix 7) partly determines the load of fouling organisms on the larvae — the longer it is, the more build up of fouling organisms

Effect on Crabs:

- Heavy fouling on eggs may affect hatching
- Filamentous bacteria can obstruct respiration and cause asphyxiation when they colonize a significant portion of the gills and other surfaces
- Leads to accumulation of debris and increase in weight resulting to swimming difficulty
- The filaments trap other microbial disease agents and saprophytes
- Heavy fouling may contribute to difficulty or failure in molting

Diagnostic Techniques:

Gross Observations (Level I):

Filamentous bacterial infestation is difficult to observe without the microscope, but some gross signs that may indicate its presence are:

- Prolonged hatching of eggs
- Irregular molting
- Weak swimming movements with the tendency to stay near the bottom of tanks

Microscopic Examination (Level II):

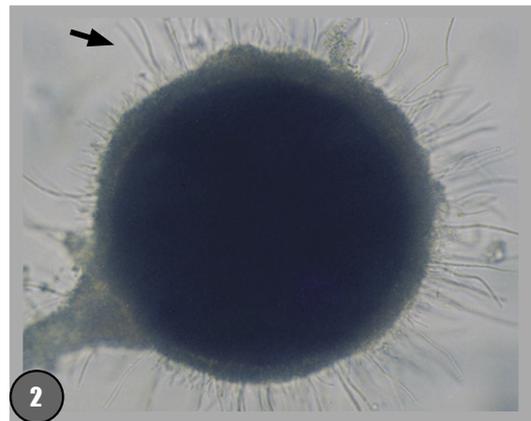
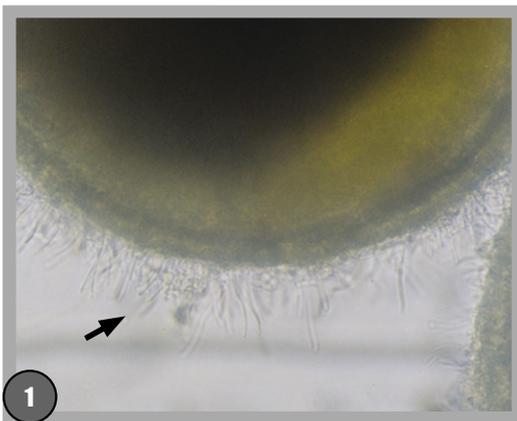
- Prepare fresh mounts of live larvae for light microscope examination (Appendix 3)
- Examine for the presence of fine, colorless and thread-like growths on the eggs (Photos 1 and 2; arrows) and larval surfaces (Photos 3 and 4; arrows)
- A scheme for numerical grading of the extent of filamentous bacteria or epibiotic fouling organism infestation can be done using the guide in Appendix 8

Methods of Prevention and Control:

- Effective prevention can only be done by regular monitoring using representative numbers of larvae or eggs (see Section II). At high density rearing, monitoring of larvae should be done at least once a day
- Maintain low organic load in the rearing system by regularly siphoning out excess and uneaten feeds
- Siphon out molted exoskeletons that harbor large numbers of bacteria to prevent recontamination
- Use only previously chlorinated water for rearing

References

Fisher WS. 1977. Epibiotic microbial infestations of cultured crustaceans. Proc. World Maricult. Soc. 8: 673-684



Fouling by Filamentous Diatoms

Pathogen or Cause:

Filamentous diatoms were observed in berried females with incubating eggs as off-white masses that covered portions of the egg mass

Effect on Crabs:

- Partial or total failure of egg hatching may occur depending on the area covered by the filamentous growth

Diagnostic Techniques:

Gross Observations (Level I):

- The egg sponge shows whitish to greenish discoloration

Microscopic Examination (Level II):

- Prepare fresh mounts of live larvae for light microscope examination (Appendix 3)
- Observe for presence of thick filamentous and greenish outgrowths on the eggs (Photo 1)
- Higher magnification show the filaments are actually chains of diatoms (Photos 2 and 3; arrows)

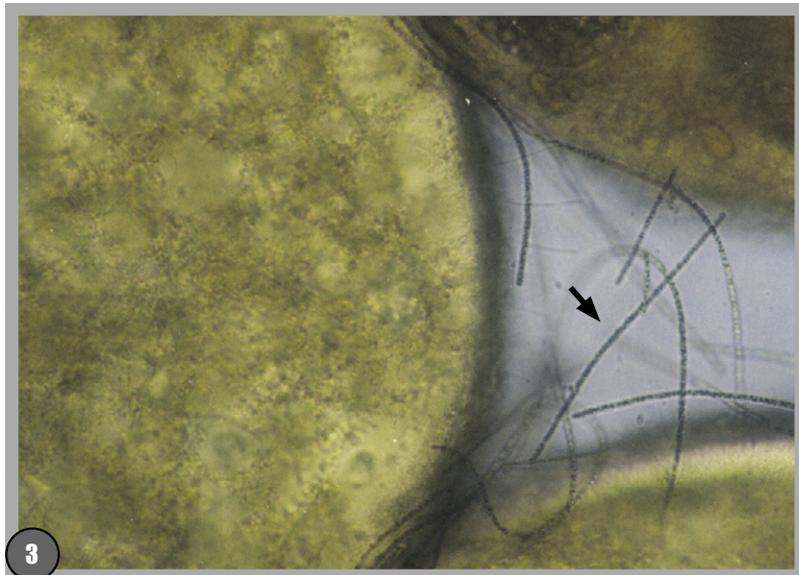
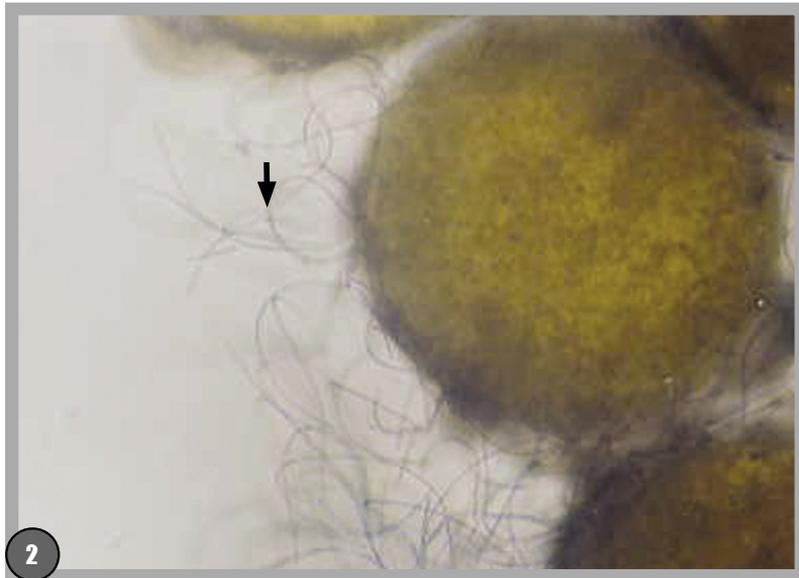
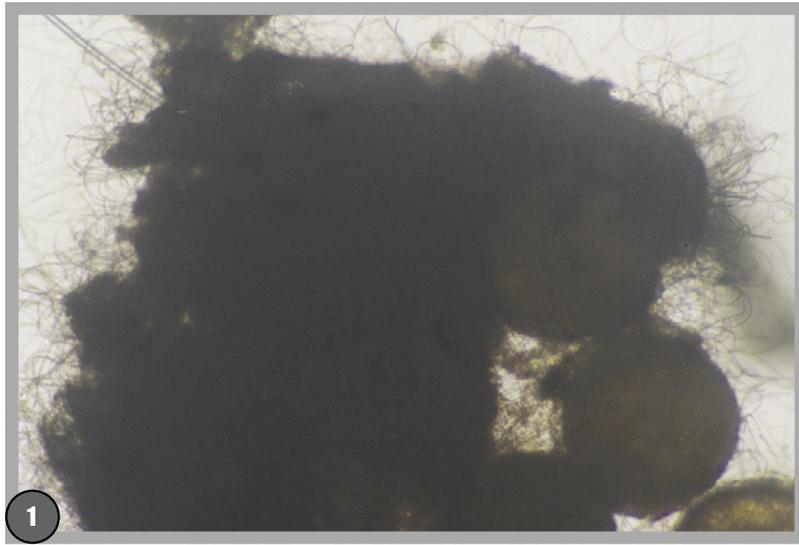
Methods of Prevention and Control:

- Disinfect berried females (spawners) in 150 ppm formalin for 30 minutes
- Maintain the spawners in clean water while eggs are undergoing development
- Avoid exposure to sunlight to prevent growth of diatoms

References

Quinitio ET, Parado-Estapa FD. 2003. Biology and Hatchery of the Mud Crabs *Scylla* spp. Aquaculture Extension Manual No. 34, SEAFDEC Aquaculture Department, Iloilo, Philippines. 42 p





Fouling by Sessile Protozoans

Pathogen or Cause:

Protozoans that possess stalks for attachment belonging to the genera *Vorticella*, *Epistylis*, *Zoothamnium*, *Acineta* and several others. These are widely distributed in the marine and brackishwater environments and colonize any submerged surface.

Effect on Crabs:

- Sessile protozoans interfere with gas exchange by blocking respiratory surfaces of the eggs and larvae, especially if found in high numbers (Photos 1-4)
- The longer the hatching, the more diverse the attached protozoan fouling organisms on egg surfaces (Photos 5-8)
- Although these organisms do not invade the underlying tissues, they make it difficult for the affected larvae to move and to feed
- If found heavily on appendages, it may cause swimming difficulty
- Molting eventually interrupts the colonization process. Delayed molting usually leads to build up of organisms on the skeleton (Photos 9-11)
- Attached protozoans come off with the old shell (Photo 12) upon complete molting

Diagnostic Techniques:

Gross Observations (Level I):

- As in other cases of fouling, slow swimming behavior among larvae and a tendency for them to stay near the bottom should be a suspected case of heavy fouling of surfaces

Microscopic Examination (Level II):

- Prepare fresh mounts of eggs or live larvae for light microscope examination (Appendix 3)
- Observe for the presence of protozoans of diverse form and numbers attached to various surfaces (Photos 1-11)

Mode of Transmission:

Molting give the crabs new shells completely devoid of sessile protozoans, but re-infestation occurs when the infested old shells remain in the tank too long.

Methods of Prevention and Control:

- Incidence of fouling organisms in hatcheries can be reduced by good management, especially chlorination or other forms of treatment of incoming water, and the proper cleaning of tank bottoms
- Since these organisms proliferate in water with high organic load, efficient and timely water change to prevent build up should discourage their growth
- Check underlying causes of molting or hatching delay to prevent serious implications to incubating or reared larvae
- Avoid overfeeding to keep water clean
- Calibrate aeration supply to constantly achieve a dissolved oxygen level of 5 ppm or higher. Optimum oxygen levels and other environmental parameters for rearing of crabs are in Appendix 9

References

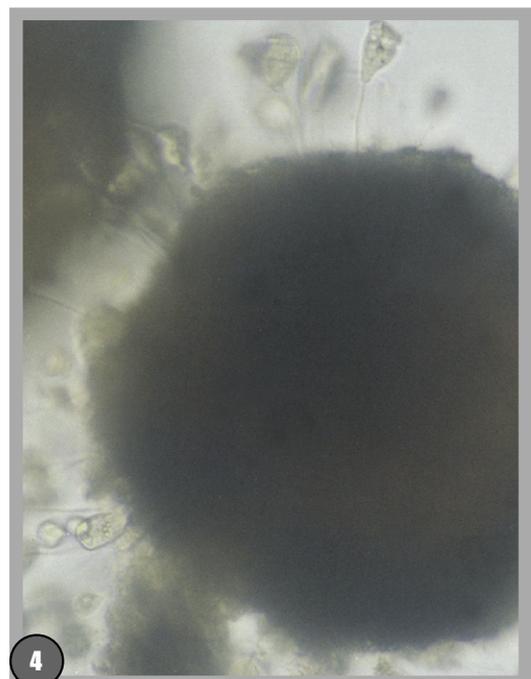
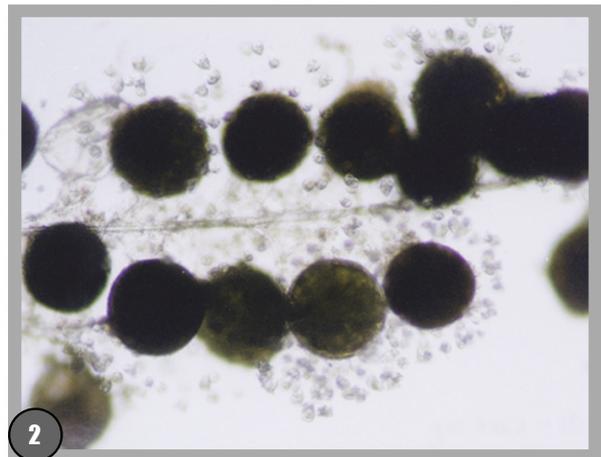
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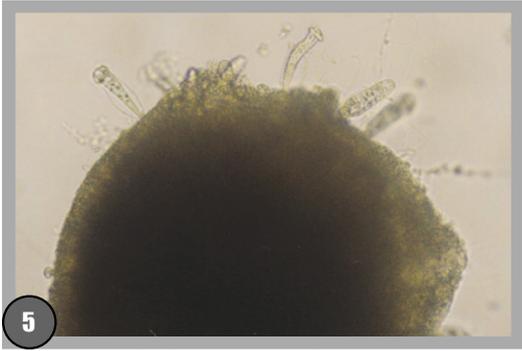
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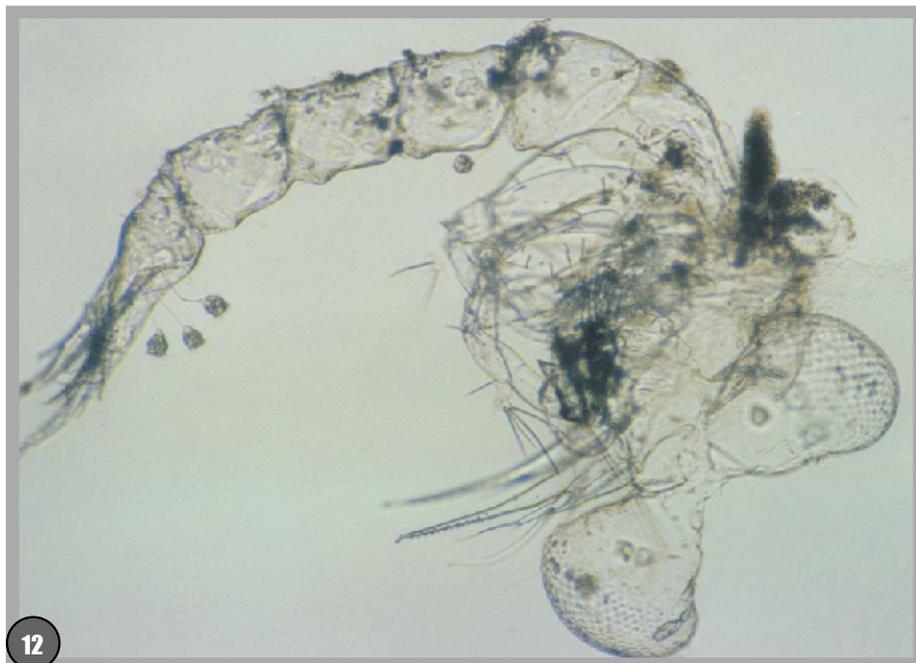
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Web-based Resources

<http://www.seafdec.org.ph/downloads/hilites2k3.pdf>







Fouling by Saprophytic Protozoans and Nematodes

Pathogen or Cause:

Free-swimming protozoans such as *Euphlotes* sp. and saprophytic nematodes commonly inhabit the egg mass of crabs.

Effect on Crabs:

- The movement of protozoans and nematodes within the egg mass may cause damage to incubating eggs
- Even if nematodes are considered egg predators and occur in relatively high prevalence in crab sponges, their overall effect may be limited by the high fecundity of their host
- Presence of protozoans and nematodes on eggs and larvae indicate advanced state of water deterioration due to accumulation of organic materials. Rather than the fouling organisms themselves, poor water quality can harm the larvae

Diagnostic Techniques:

Gross Observations (Level I):

- No gross signs are shown and microscopic examination is necessary to see the protozoans and nematodes

Microscopic Examination (Level II):

- Obtain egg samples by scraping gently the external part of the sponge and observe fresh preparations under a microscope (Appendix 3)
- Observe for the presence of motile organisms such as protozoans (Photo 1) and nematodes (Photo 2)

Mode of Transmission:

Saprophytes are ubiquitous in the environment and the presence of excess organic matter attracts them and encourages proliferation. Thus, the egg mass become easily colonized

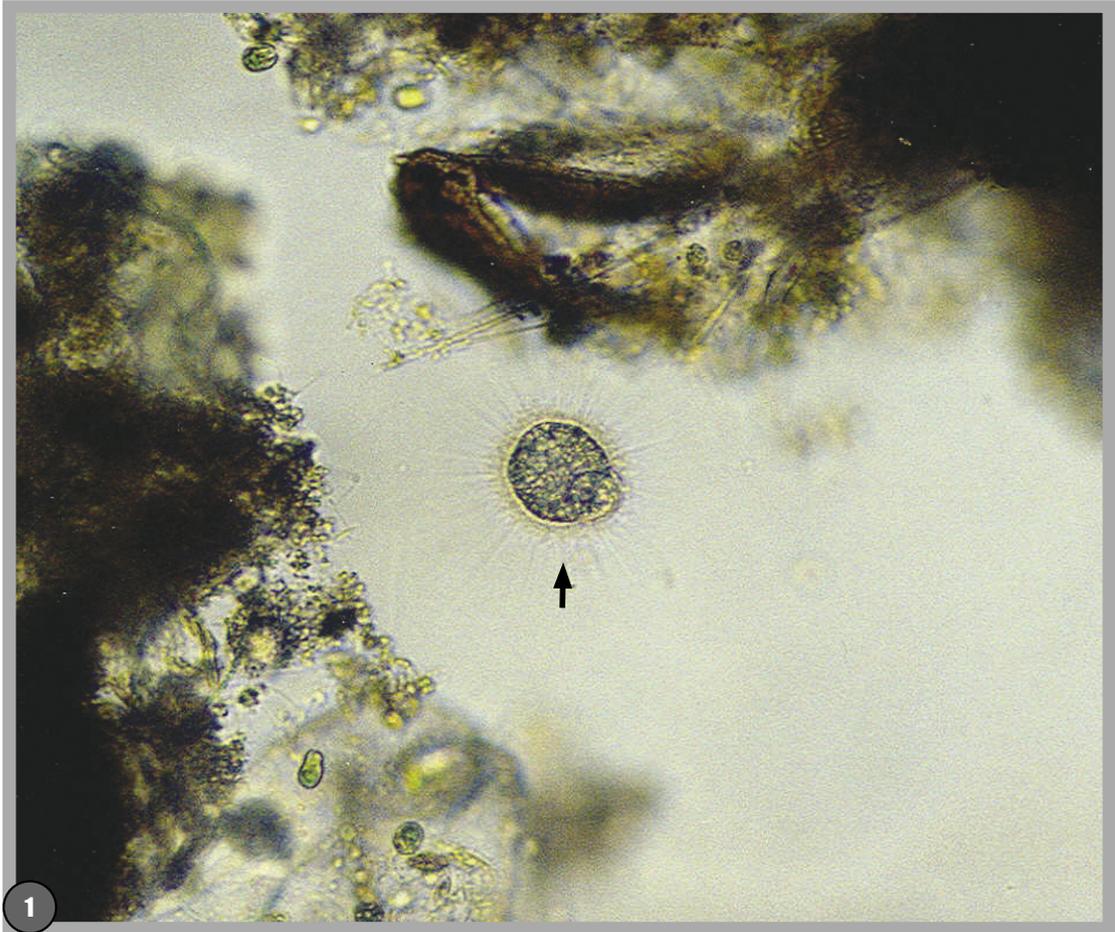
Methods of Prevention and Control:

- Disinfect newly-procured spawners in 150 ppm formalin to reduce associated saprophytic organisms with the egg sponge
- Maintain spawners in de-chlorinated sea water
- Maintain good water quality by regularly siphoning off accumulated sediments, uneaten feeds, dead larvae, etc. from the tank bottom

References

Couch JA. 1983. Diseases caused by protozoa, pp. 79-111. In: Provenzano Jr. AJ (ed), The Biology of the Crustacea, Vol. 6, Pathobiology, Academic Press, New York





Fouling by Suspended Debris

Debris is composed of dead microalgae and other dirt suspended in the rearing water, or settled at the bottom. Suspended debris settle on crab eggs and larvae.

Effect on Crabs:

- Heavy accumulation of debris on eggs (Photo 1; arrows) may cause hatching failure
- Presence of debris can cause entrapment of potential pathogens like fungus and saprophytic protozoans
- Debris accumulation on gills affects respiration and heavily loaded animals may die due to asphyxiation
- Debris accumulation on the body surface (Photo 2; arrows) may weigh down larvae and cause molting delay or failure

Diagnostic Techniques:

Gross Observations (Level I):

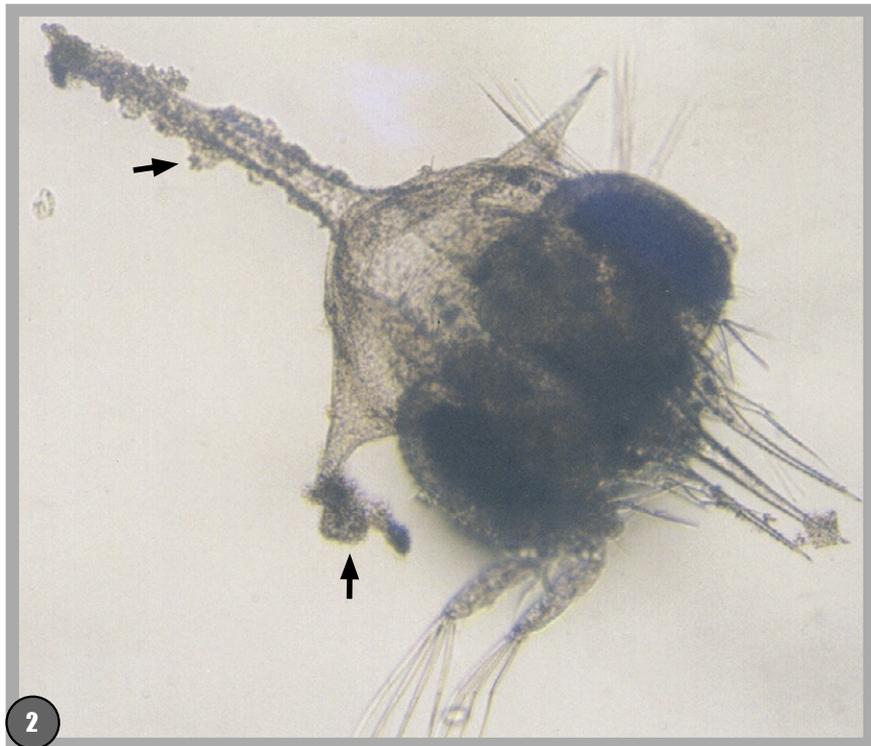
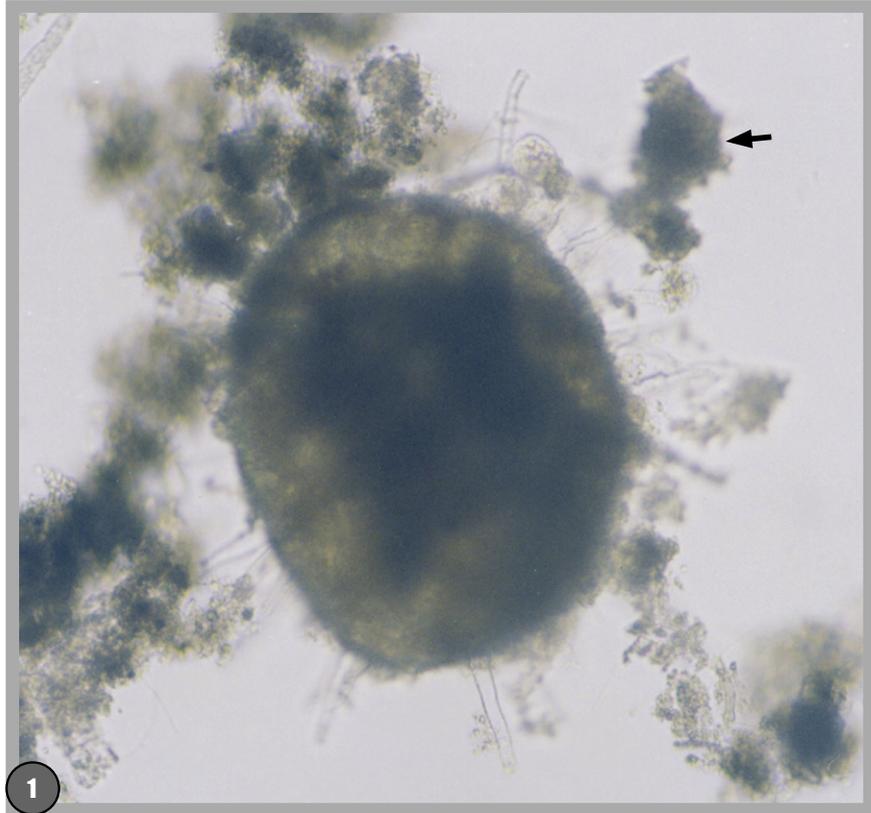
- Discoloration of egg mass different from its normal color change due to larval development
- Slow swimming behavior among larvae and a tendency for them to stay near the bottom

Microscopic Examination (Level II):

- The condition can only be observed on eggs and larvae under a microscope
- Presence of amorphous, transparent or opaque materials on eggs and larvae

Methods of Prevention and Control:

- Filter seawater with net bag having a mesh size of 5 micrometers or less. Alternatively, use fine silky cloth as filters
- Prevent phytoplankton collapse during rearing
- Control aeration to prevent re-suspension of accumulated sediments from the bottom



Incomplete Molting

Cause:

Incomplete molting is a non-infectious disease observed among hatchery-reared crab larvae that may be caused by exposure to low temperature levels. Poor nutritional condition of larvae may also contribute to its occurrence due to lack of energy to complete ecdysis

Effect on Crabs:

- Incomplete shedding of the old exoskeleton causes abnormal swimming and makes affected larvae susceptible to cannibalism
- Death

Diagnostic Techniques:

Microscopic Examination (Level II):

- Presence of old exoskeleton attached to newly molted larvae, especially in the area of the appendages (Photo 1; arrows)

Methods of Prevention and Control:

- Because the condition has been observed during rearing at sub-optimum temperatures, use heaters or provide tank enclosures that will maintain optimum temperature even at night (Appendix 9). Install canvass covers that will keep the heat inside the tanks (Photo 2)
- Adjust aeration supply so that no unnecessarily cold air is pumped into the system causing lowering of temperature
- Provide larvae with nutritious food containing enough energy for molting
- Avoid use of unnecessary chemicals that may cause delay and failure of molting

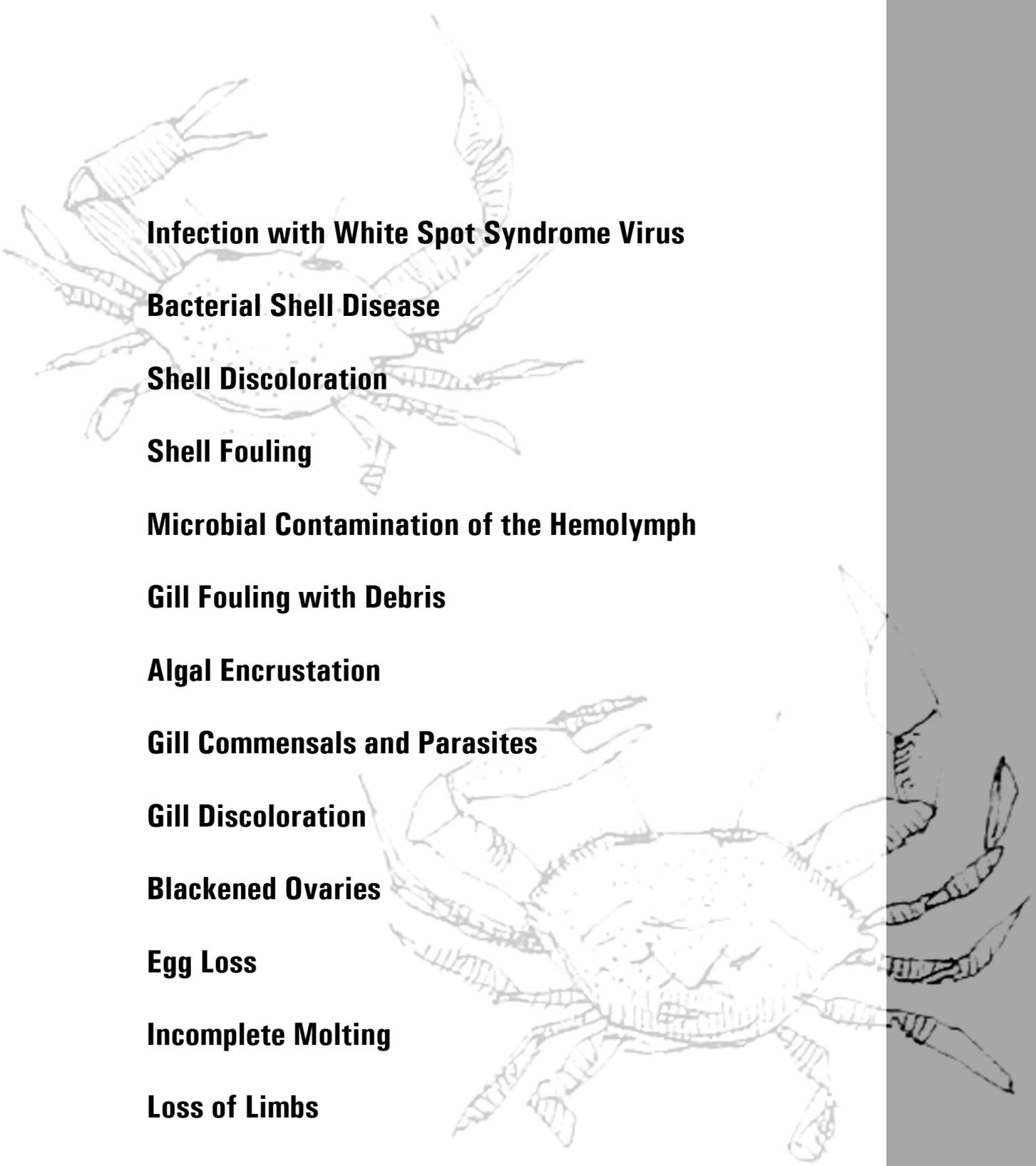
Web-based Resources

<http://www.seafdec.org.ph/downloads/hilites2k3.pdf>





Diseases in Juveniles and Adults



Infection with White Spot Syndrome Virus

Bacterial Shell Disease

Shell Discoloration

Shell Fouling

Microbial Contamination of the Hemolymph

Gill Fouling with Debris

Algal Encrustation

Gill Commensals and Parasites

Gill Discoloration

Blackened Ovaries

Egg Loss

Incomplete Molting

Loss of Limbs

White Spot Syndrome Virus (WSSV)

Pathogen or Cause:

WSSV is caused by a rod-shaped DNA virus belonging to genus Whispovirus. This virus is now widely distributed in crustacean culture systems and the wild. Crabs are known to be less susceptible compared with shrimps and all stages of crabs may carry WSSV without showing any disease sign.

Effect on Crabs:

- There are conflicting reports about mortalities of crabs due to WSSV
- Infected crabs become carriers of the virus and may introduce it to other rearing systems
- Given the carrier status of crabs, management decisions regarding polyculture with species highly susceptible to WSSV should be reconsidered

Diagnostic Techniques:

Gross Observations (Level I):

- Carrier crabs do not show any external signs

Molecular Techniques (Level III):

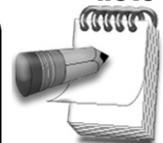
- Analyze tissue samples by Polymerase Chain Reaction (PCR) method
- Prepare appropriate samples as described in Appendix 1
- Obtain samples that will give a significant level of confidence when detecting the virus carrier status of specific samples. Use the table shown on Appendix 4 to determine sample sizes

WSSV is a disease reportable to the Office International des Epizooties (OIE) or World Organization for Animal Health. OIE is an intergovernmental organization with 166 members as of March 2004. Its mission is to guarantee the transparency of animal disease status world-wide. Each Member Country undertakes to report the animal diseases that it detects on its territory. The OIE then disseminates the information to other countries, which can take the necessary preventive action. The OIE collects and analyses the latest scientific information on animal disease control. This information is then made available to the Member Countries to help them to improve the methods used to control and eradicate these diseases.

Crustaceans, including crabs, are adversely affected by a number of diseases. The crustacean diseases and their etiological agents that are included in OIE's Aquatic Animal Health Code (the Aquatic Code) have restricted geographical range, have no therapeutic remedies or treatments, are potentially excludable, and are of significant social and economic importance. There are currently eight diseases of crustaceans listed by the OIE.

For more information about the OIE, log-in to www.oie.int

Note



Methods of Prevention and Control:

- Disinfect spawners to reduce microbial load and other substances that can entrap pathogens or their carriers
- Use de-chlorinated water (Appendix 5)
- Observe biosecurity in the rearing system, including natural food and feeds
- Quarantine and restrict movement and transfer of crustacean hosts and carriers
- Install screens and fences to ensure biosecurity of culture premises

References



Chen LL, Lo CF, Chiu YL, Chang CF, Kou GH. 2000. Natural and experimental infection of white spot syndrome virus (WSSV) in benthic larvae of mud crab *Scylla serrata*. Dis. Aquat. Org. 40: 157-161

Supamattaya K, Hoffmann RW, Boonyaratpalin S, Kanchanaphum P. 1998. Experimental transmission of white spot syndrome virus (WSSV) from black tiger shrimp *Penaeus monodon* to the sand crab *Portunus pelagicus*, mud crab *Scylla serrata* and krill *Acetes* sp. Dis. Aquat. Org. 32: 79-85

Web-based Resources



http://www.oie.int/eng/normes/fmanual/A_00046.htm

<http://www.fao.org/DOCREP/005/X8485E/x8485e06.htm> = this site provides a link to the “Beijing Consensus and Implementation Strategy”

Bacterial Shell Disease

Abnormalities of the shell usually start with the appearance of fuzzy mats composed of a community of filamentous blue-green algae, bacteria, sessile, ciliated protozoans; saprophytic ciliated protozoans and some flagellates. Unaffected crabs possess shiny exoskeletons. Shell disease commonly starts on the dorsal areas, especially the carapace (Appendix 10) since frequent brushing of the ventral region with the sand substrate during burrowing may have a cleaning effect. Conditions during maintenance of broodstock in tanks may result to severe fouling leading to either shell perforation or parasite settlement. These are brought about by inappropriate holding conditions, overcrowding, and absence of environmental factors that would have prevented the dominance of fouling organisms. Once the integrity of the shell is damaged, portals of entry for secondary and opportunistic pathogens may be created.

Pathogen or Cause:

Chitinolytic or chitin-digesting bacteria which are either sucrose-fermenting or non-sucrose-fermenting vibrios identified as *Vibrio vulnificus*, *V. parahaemolyticus*, *V. splendidus*, and *V. orientalis*. Majority of the strains associated with shell disease are within the Vibrionaceae, a common family of marine bacteria that is ubiquitous in the marine environment. The association of these bacteria both with lesions and with non-diseased crustaceans suggests that the natural flora is responsible for the shell disease lesions seen in these animals. Shell disease may also be induced by mechanical injury.

Effect on Crabs:

- Shell disease affects 100% of captive crabs after being held in tanks for three months, but is seldom found in newly-recruited crabs from ponds or the wild
- The condition seldom leads to mortality, but extensive shell erosion and perforation may create portals of entry for secondary bacterial or parasitic infections
- Around perforations of the shells are found small populations of saprophytic protozoans and nematodes, but not in the hemolymph

Diagnostic Techniques:

Gross Observations (Level I):

- Shell disease initiated by punctures and mechanical injuries inflicted during fighting shows blackening at the site of injury (Photo 1)
- Shell disease appears initially as discolored patches on the carapace (Photo 2), which later spread over the appendages (Photos 3 and 4)
- May cover more than 75% of the dorsal region of the shells (Photo 5). In severe cases, shell disease spreads to the ventral side of crabs causing extensive brownish to black discoloration (Photos 6-8)
- Many parts of the exoskeleton become soft and black as they lose the calcified tissue underneath
- These areas easily became perforated, exposing underlying tissues

Microbiological Techniques (Level II):

- Bacteria associated with shell disease can be easily cultured by streaking samples of affected tissues on Nutrient agar and TCBS (Appendix 4a). The bacterial population obtained in patches with shell disease can reach up to 10^7 total bacteria per 0.1 g of sample, 50 - 75% of these are chitinolytic. Aside from chitinase, these vibrios also

possess the enzymes gelatinase and lipase, which are considered compounding factors that enhance shell degradation

Histology (Level II):

- Tissues just beneath shell disease show melanization typical of those undergoing inflammatory reaction (Photos 9-10; H&E × 100)

Mode of Development:

- It is quite probable that the microbiological aggregate formed on the shell provided a good environment for chitinolytic vibrios to settle causing gradual damage resulting in perforation
- Injury inflicted during handling and crowding, and exposure to pollutants are some of the predisposing factors implicated

Methods of Prevention and Control:

- Provide a sandy substratum of appropriate thickness under which the crabs could burrow. Provision of an optimum amount of substrate may not only reduce stress, but also reduce the build-up of fouling organisms on the crabs
- Regularly brush and wipe the dorsal region of the exoskeleton of crabs with cotton dipped in iodine solution to prevent fouling during captivity and to minimize the buildup of organisms, which provide a favorable substrate for the establishment of chitinolytic bacteria
- Where feasible, induced molting since the condition is shed with the old shell except when underlying tissues are severely damaged

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- Rosen B. 1970. Shell disease of aquatic crustacea. In: SF Sniesko (ed). *A Symposium on Diseases of Fishes and Shellfishes*. American Fisheries Society Special Publication 5: 409-415
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Web-based Resources

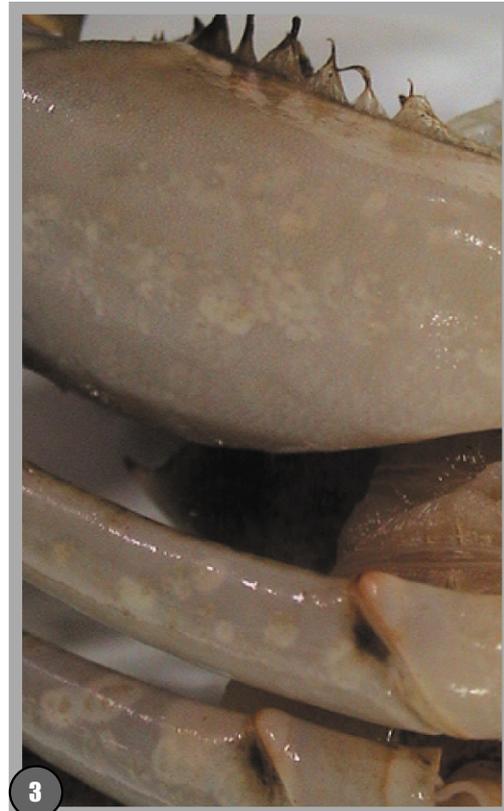
<http://www.frdc.com.au/about/index.htm> = this is the download site of “Port Curtis Mud Crab Shell Disease – nature, distribution and management”, specifically PDF1998-210

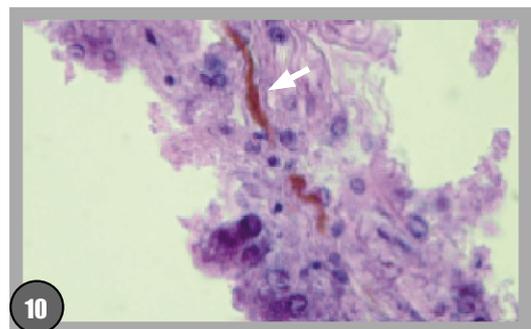
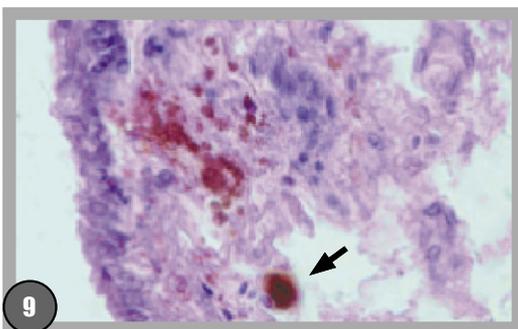


<http://www.seafdec.org.ph/downloads/hilites2k3.pdf>

<http://crabstreetjournal.com/articles/shelldisease/message26526.html>

<http://crabstreetjournal.com/articles/shelldisease/index.html>





Shell Discoloration

Description:

Crab shells are covered by yellowish-brown (Photo 1) to reddish-brown (Photo 2) deposits. The deposits are more obvious on the whitish ventral side but easily comes off after scraping (Photo 2).

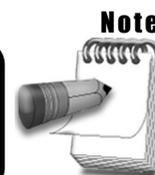
Cause:

Low soil pH and water pH. Acidic soil and water causes formation of iron precipitates on crab shells.

Effect on Crabs:

- Although shell discoloration *per se* has no effect on the calcareous shell, the factor leading to discoloration may adversely affect other organs like the gills and the eyes
- Exposure of crabs to acidic soil and water causes impairment of normal metabolism, leading to growth retardation and death

Very high pH levels in ponds can also cause mortalities, both because of the direct effect of the pH itself and because of the greater solubility of waste ammonia at high pH. High pH may also be due to dense phytoplankton blooms.



Diagnostic Techniques:

Gross Observations (Level I):

- Presence of orange to brown deposits on the crab shell that easily comes off after light scraping (Photo 2; arrow)

Methods of Prevention and Control:

- Prepare ponds properly to avoid acid sulfate problems and use lime to correct soil pH. Refer to additional information about pond preparation and crab culture in the references below
- Submit soil samples for analysis (Appendix 11) to determine acidity and corrective action

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Triño AT, Rodriguez EM, Coniza EB, Juanga BP. 1999. Mudcrab. Aquaculture Extension Manual No. 27, SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines. 32 p





Shell Fouling

Cause:

The general body surface of the crab can serve as a substrate for many types of fouling organisms like filamentous bacteria and algae, and single or colonial protozoa. The presence of crabs in the culture system that are covered with algae or showing signs of not having molted may indicate either that culture conditions are poor or that the animals are not healthy. Shells of juveniles are usually shiny with regular bumpy irregularities on the surface (Photo 1). Healthy crabs preen to keep their shells clean. Crabs may fail to preen due to limitation of movement especially if they are kept in individual enclosures to avoid cannibalism (Photos 2 and 3). This leads to accumulation of fouling organisms composed of a mixture of microorganisms, algae and debris (Photos 4 and 5). Molting provides an opportunity to get rid of the old shell with its associated fouling organisms and repair whatever damage it has gone through during the intermolt period. Barnacles may also be found attached to the carapace and chelipeds of crabs (Photo 6). These organisms, though not causing mortality, may affect the mobility of the crabs in severe infestations due to the extra weight of the barnacles leading to longer intermolt period.

Effect on Crabs:

- In light infestation, none, especially if molting is regular
- Heavily infested crabs may be burdened by the additional load of fouling algae with the possibility of slowed-down movement or longer intermolt duration
- Heavy fouling on the shell surface can reduce the market value of crabs

Diagnostic Techniques:

Gross Observations (Level I):

- Crab shell has attached velvety greenish to brown algae or barnacles and other encrusting organisms (Photos 4-6)

Methods of Prevention and Control:

- Provide rearing conditions that allow normal behavioral patterns like burying in the sediment, hiding in rock crevices, night-time activity, and exposure to air. These behaviors impede fouling through mechanical abrasion, lack of access, and desiccation
- Provide adequate space for preening and movement
- Increase water movement to inhibit attachment of fouling organisms
- Induce molting to temporarily get rid of these fouling micro-organisms
- Infestation by filamentous algae has been observed to occur in grow-out ponds with high transparency (above 40 cm). This problem can be reduced by encouraging phytoplankton growth to lower water transparency

References

Bauer RT. 2002. The ineffectiveness of grooming in prevention of body fouling in the red swamp crayfish, *Procambarus clarkii*. *Aquaculture* 208: 39-49

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Microbial Contamination of the Hemolymph

Pathogen or Cause:

Crabs from the wild as well as those that have been kept in tanks for several months harbor mixed populations of bacteria in the hemolymph, mainly dominated by sucrose-fermenting vibrios. The associated *Vibrio* bacteria are ubiquitous in the marine environment.

Effect on Crabs:

- Unknown and poorly understood
- The presence of bacteria in the hemolymph of new recruits, where shell disease was not seen, shows that shell perforation is not necessarily a precursor to internal contamination
- The presence of bacteria in the hemolymph has also been reported in other crustaceans
- The bacteria could invade the hemolymph through abrasions in the cuticle of the crab, and multiplies in the blood or some unknown route
- The true prevalence of crabs with vibrios and other bacteria in the hemolymph is unknown

Diagnostic Techniques:

Microbiological Techniques (Level II):

- Draw hemolymph from the soft tissues at the joints of shell plates or chelipeds (Appendix 10) using a tuberculin syringe, and deliver at least 0.1 ml on prepared microbiological culture media by spread plate method (Appendix 4a)
- Observe for bacterial colonies after 24 – 48 h incubation at room temperature. The presence of off-white bacterial colonies on nutrient agar (Photo 1), and green and yellow bacterial colonies on *Vibrio* selective agar (Photo 2) indicate hemolymph contamination

Methods of Prevention and Control:

- Unknown

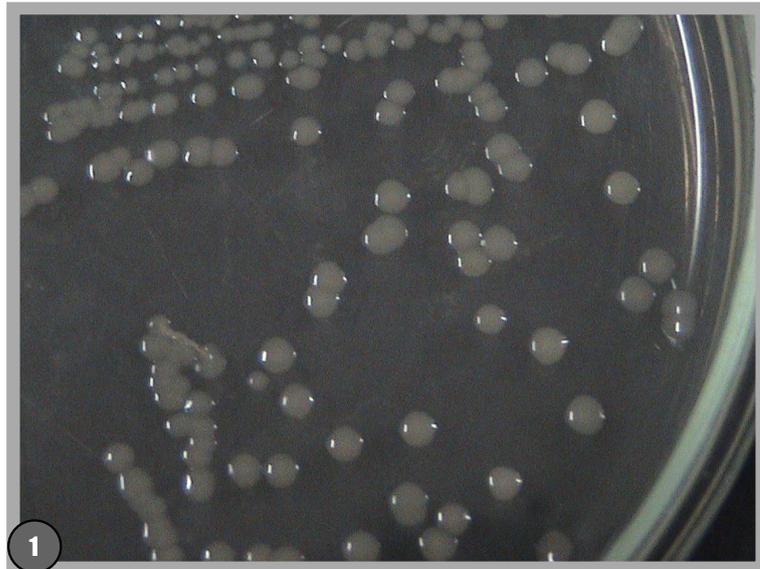
References

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Web-based Resources

<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=240053> = this links to an article about bacteria associated with crabs in cold waters



Gill Fouling with Debris

Cause:

The gills are very important for respiration and its function and location make it vulnerable to fouling with debris and suspended matter in the water. The prevalence of gill fouling varies with the preening activity and health status of the crab.

Effect on Crabs:

- Gill fouling is not a problem when water quality is good and dissolved oxygen content is optimum (> 5 ppm)
- Severe accumulation and build up of fouling organisms impairs water movement across the gills and can lead to suffocation

Diagnostic Techniques:

Gross Observations (Level I):

- Due to the opaque nature of the carapace (Appendix 10), gills can be observed only after lifting it off from the animal, thus the need to sacrifice them for examination
- In healthy animals, the sweeping movement of gill rakers or flabella (Photo 1 and 2; arrows) keep the gills clean

Microscopic Examination (Level II):

- Prepare specimens for microscopy (Appendix 3)
- Examine for presence of amorphous debris over the lamellae (Photo 3)

Methods of Prevention and Control:

- Keep rearing water clean and free from suspended particles that may clog the gills
- Avoid phytoplankton die-off since this contributes to clogging materials on the gills
- The normal motion of gill rakers or flabella keeps the gills clean in healthy crabs. In crabs where cleaning activity is ineffective, check underlying causes rather than providing temporary relief by curing the signs
- In cases where gill-cleaning structures are not effective against bacterial or ciliate fouling, molting should be induced to rid the crabs of heavy fouling organism build up

References

Bauer RT. 1999. Gill cleaning mechanisms of a dendrobranchiate shrimp, *Rimapenaeus similis* (Decapoda: Penaeidae): description and experimental testing of function. J. Morphol. 242: 125-139

Lavilla-Pitogo CR, Lio-Po GD, Cruz-Lacierda ER, Alapide-Tendencia EV, de la Peña LD. 2000. Diseases of Penaeid Shrimps in the Philippines. Second Edition, Aquaculture Extension Manual No. 16. SEAFDEC Aquaculture Department, Iloilo, Philippines. 83 p

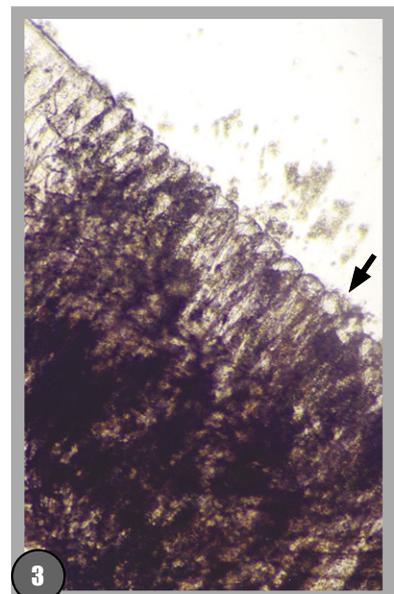
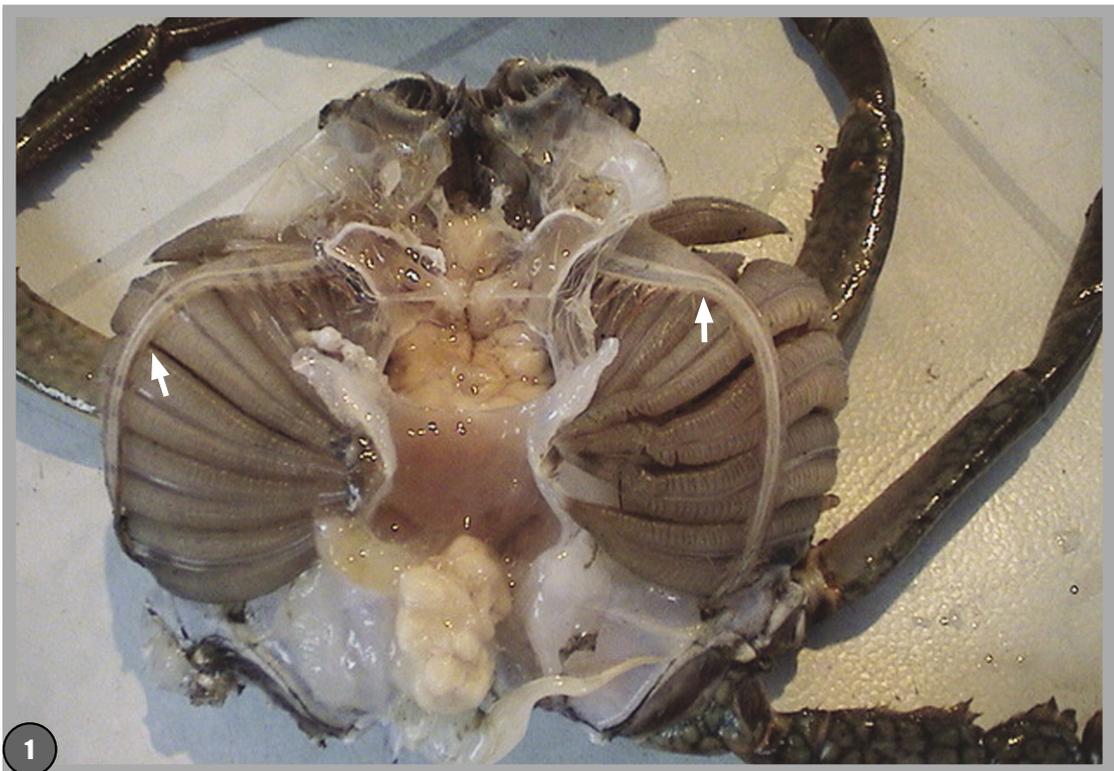


Web-based Resources

<http://www.usc.louisiana.edu/~rtb6933/shrimp/clean.html> = shows interesting photos on cleaning behavior of shrimp.

<http://www.vims.edu/adv/ed/crab/guts3.html> = shows an illustrated internal and external anatomy of a crab with an anatomical glossary

<http://www.lander.edu/rsfox/310callinectesLab.html> = a resource for the illustrated external and internal anatomy of the blue crab *Callinectes sapidus*, and notes on their functions. These should not be used as reference in the identification of mud crabs, *Scylla* spp.



Algal Encrustation

Cause:

Encrusting green alga that is widely distributed in marine and brackish water environments

Effect on Crabs:

- Unknown

Diagnostic Techniques:

Gross Observations (Level I):

- Brown to greenish discoloration of the gills, especially at the distal portion (Photos 1-3)

Microscopic Examination (Level II):

- Prepare fresh mounts of affected gills for light microscope examination (Appendix 1)
- Green algal cells form sparse to dense greenish multicellular mats over the gill lamellae (Photos 4 and 5)

Histology (Level II):

- Fix gill tissues in Davidson's fixative or other formalin-based fixatives (Appendix 12)
- Observe for entrapped debris in between gill lamellae (Photo 6; H&E \times 100) and dividing encrusted algae (Photo 7; arrows; H&E \times 200)

The importance of histology:

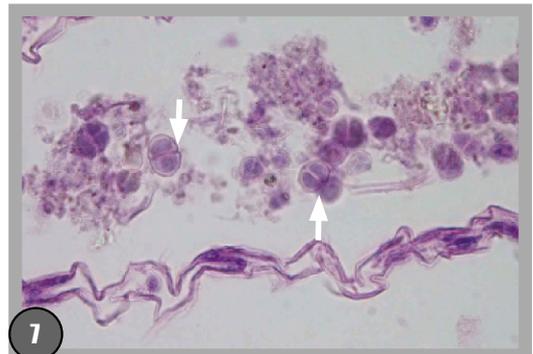
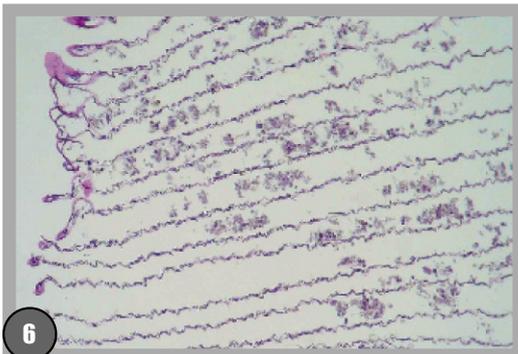
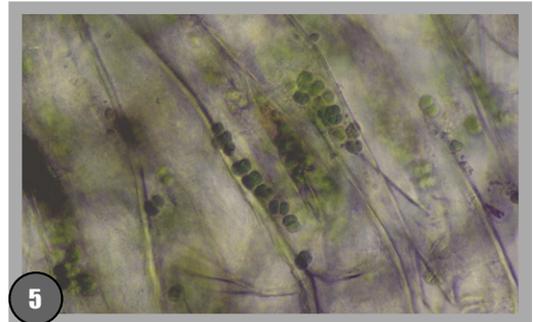
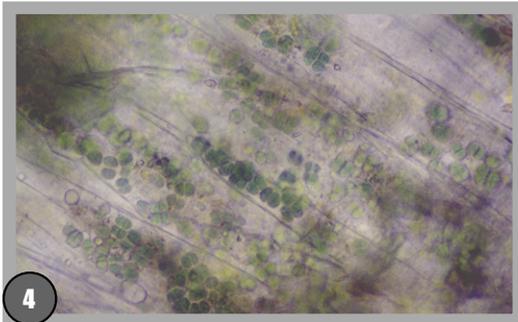
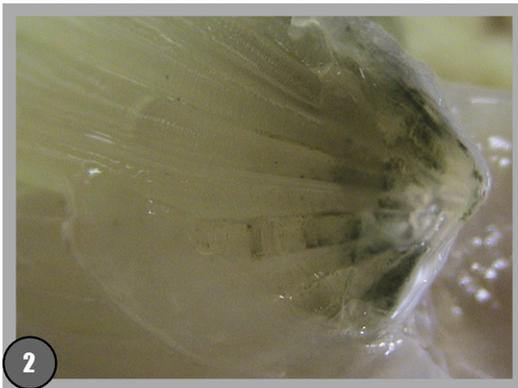
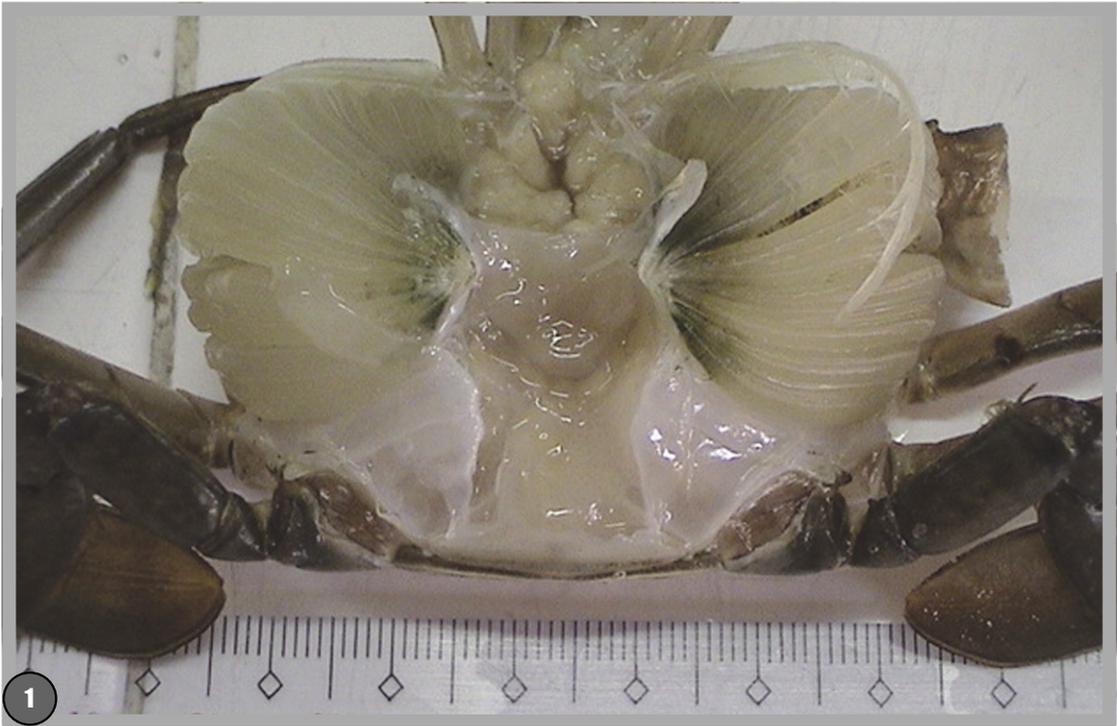
In some cases, examination of well prepared histological slides from adequately fixed specimens gives insight on the status of affected tissues and the extent of damage.

For more information about histology, log-in to: <http://histology-world.com>



Methods of Prevention and Control:

- Gill fouling is an outcome of poor water quality, thus improvement of water circulation and replenishment of clean rearing water may alleviate the condition
- Green algal encrustation is also a result of rearing crabs in waters with dense algal population, thus water change to thin out the algal load will remedy the condition
- Crabs should not be exposed to too much sunlight



Gill Commensals and Parasites

Stalked barnacles, which are morphologically similar to *Octolasmis* spp. were observed around the carapace at the edge of the inhalent aperture, at the base of the cheliped, and on the second and third maxillipeds (Photo 1; arrows). They are also lodge on the gills (Photos 2-4).

Pathogen or Cause:

Various types of barnacles such as *Octolasmis cor*, a stalked barnacle. Barnacles are widely distributed in marine and brackish water environments and colonize many types of submerged surfaces.

Effect on Crabs:

- Larval stages of the barnacle *Octolasmis* may colonize the gill chambers in the debilitating numbers
- The magnitude of infestation on individual crabs increases with their size
- The prevalence of *Octolasmis cor* is significantly higher in female crabs than in males.
- The stalked barnacles that inhabit the respiratory chambers occupy the space on the gills normally available for oxygen and carbon dioxide exchange
- Some of the negative effects of *Octolasmis* infestation are competition for oxygen or blockage of the gills due to accumulation of debris on colonized respiratory surfaces

Diagnostic Techniques:

Gross Observations (Level I):

- Presence of stalked organisms on the gills, around the carapace at the edge of the inhalent aperture, at the base of the cheliped, and on the second and third maxillipeds (Photos 1-4)

Methods of Prevention and Control:

- Unknown

References

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Overstreet RM. 1988. Aquatic pollution problems, Southeastern U.S. coast: histopathological indicators. *Aquatic Toxicol.* 11: 213-239

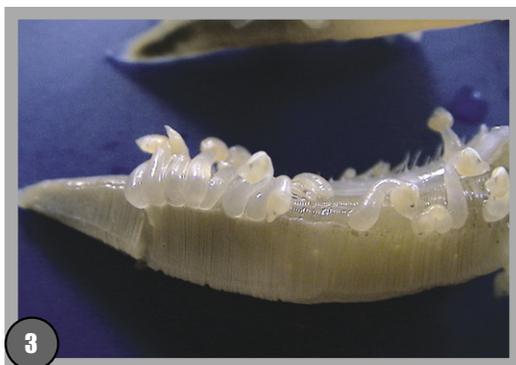
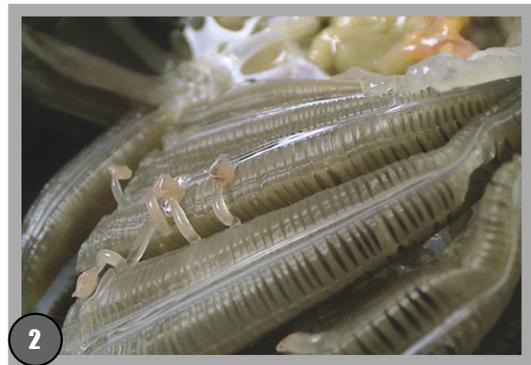
Voris HK, Jeffries WB, Poovachiranon S. 1994. Patterns of distribution of two barnacle species on the mangrove crab, *Scylla serrata*. *Biol. Bull.* 187: 346-354

Web-based Resources



www.crustacea.net/index.htm = this site offers leads regarding crustacean taxonomy

<http://www.fathom.com/feature/121900/> = this site provides information about types of barnacles, including parasitic ones



Gill Discoloration

Gray, brown to black discoloration of gill arches can occur in pond-cultured crabs (Photo 1).

Cause:

- Environmental failure due to improper pond preparation and accumulation of organic load due to uneaten and excess feed
- Poor environmental condition due to inadequate water exchange
- Heavy siltation

Effect on Crabs:

- Respiratory stress or failure due to clogging of gill surfaces
- Prolonged exposure to primary cause may lead to general weakness and onset of secondary infection by bacteria, fungus, and protozoans
- Loss of appetite
- Mortality

Diagnostic Techniques:

Gross Observations (Level I):

- Discoloration usually starts at the tips
- Gray, brown to black discoloration of gill arches (Photo 1)
- Complete blackening of the entire gill system (Photo 2)
- Observation can only be done by sacrificing suspected animals

Methods of Prevention and Control:

- Remedy siltation and other causes of debris and sediment suspension in the water
- Correct soil pH prior to start of culture
- Submit soil samples for analysis (Appendix 11)
- Avoid overfeeding
- Provide adequate means for water change

References

Lavilla-Pitogo CR, Lio-Po GD, Cruz-Lacierda ER, Alapide-Tendencia EV, de la Peña LD. 2000. Diseases of Penaeid Shrimps in the Philippines. Second Edition, Aquaculture Extension Manual No. 16. SEAFDEC Aquaculture Department, Iloilo, Philippines. 83 p

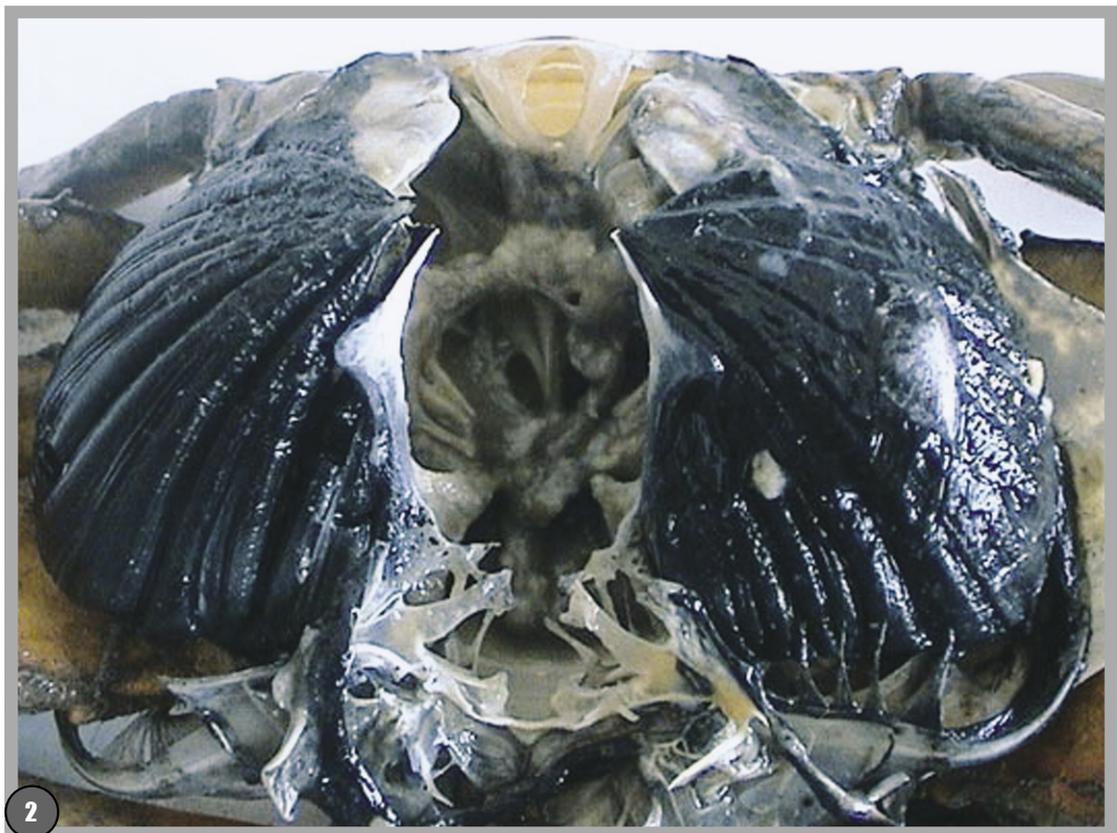


Web-based Resources

<http://www.lander.edu/rsfox/310callinectesLab.html> = this site shows the internal and external anatomy of the blue crab *Callinectes sapidus*



http://aquanic.org/publicat/usda_rac/efs/ctsa/126.pdf = this site provides information on the prevention of gill blackening in cultured shrimps



Blackened Ovaries

Broodstock in tanks were fed various artificial diets for at least 3 months. After the experiment, crabs showed various ranges of ovarian discoloration ranging from orange with black spots to black with remaining orange spots (Photo 1).

Cause:

Blackened tissues or the appearance of black spots on crustaceans have mostly been associated with deposition of the pigment melanin as a result of mechanical or microbial injury. Blackened tissues have been reported in shrimp fed artificial diets with little or no Vitamin C.

Effect on Crabs:

- May affect fecundity among females
- Causes spawning failure or incomplete spawning
- If the cause of blackening is Vitamin C deficiency, it may also be a manifestation of poor disease resistance or weakening

Diagnostic Techniques:

Gross Observations (Level I):

- Blackening or discoloration of ovaries (Photos 1a,b,c). Normal ovary with developing eggs are orange in color (Photo 2)

Histology (Level II):

- Presence of blackened and blood-infiltrated tissues of the ovary (Photos 3-5; arrows; H&E × 200)

Methods of Prevention and Control:

- Give crabs adequate and properly stored diets
- Ensure that Vitamin C sources are available in the diet or from natural food

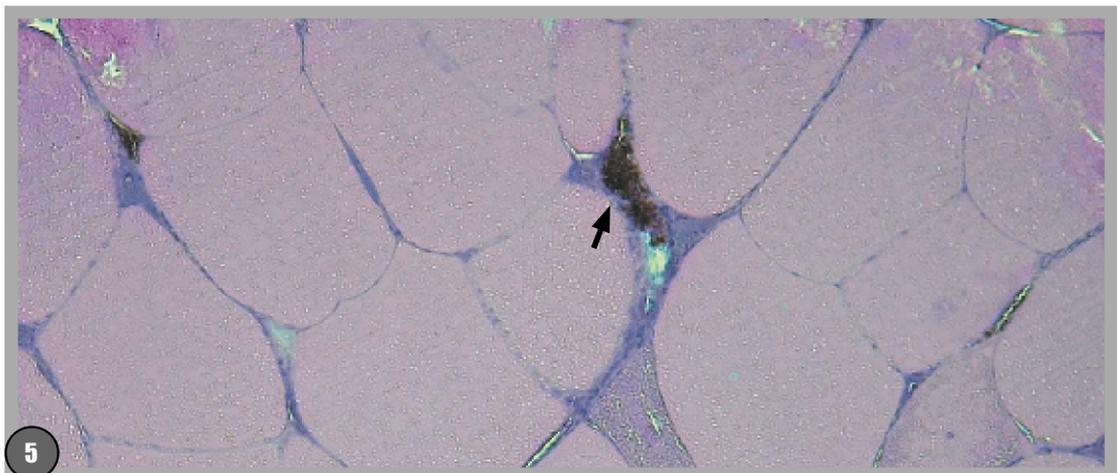
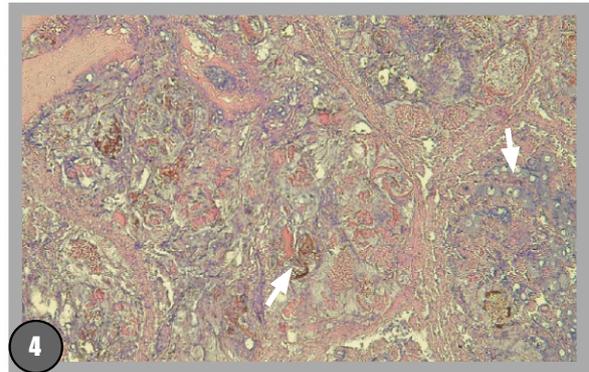
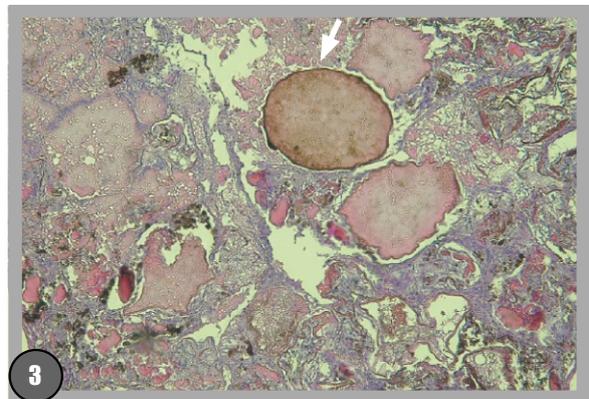
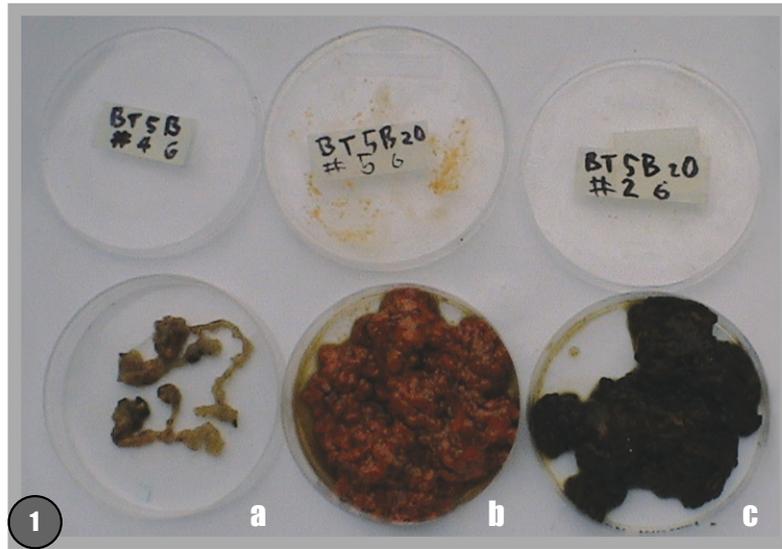
References

Lightner DV, Colbin LB, Brandt C, Danald DA. 1977. Black death, a disease syndrome related to a dietary deficiency of ascorbic acid. Proc. World Maricult. Soc. 8: 611-623

Djunaidah IS, Wille M, Kontara EK, Sorgeloos P. 2003. Reproductive performance and offspring quality in mud crab (*Scylla paramamosain*) broodstock fed different diets. Aquacult. Internat. 11: 3-15

Millamena OM, Quintio ET. 2000. The effects of diets on reproductive performance of eyestalk ablated and intact mud crab *Scylla serrata*. Aquaculture 181: 81-90





Egg Loss

Cause:

During incubation of eggs at optimum temperature range of 27 – 29.5°C, hatching normally occurs 9 – 14 days after spawning. However, due to failed fertilization, nutritional deficiency, microbial infection, heavy infestation with fouling organisms, and environmental stress, eggs fall off from the egg mass prior to hatching.

Effect on Crabs:

- Lowers fecundity and decreases number of larvae for rearing
- Causes spawning failure or incomplete spawning

Diagnostic Techniques:

Gross Observations (Level I):

- Egg mass changes from bright orange (Photo 1) to gray (Photo 2) without hatching a few days after spawning
- Eggs fail to develop into the next stage
- Eggs fall off gradually from the egg mass

Microscopic Examination (Level II):

- Fresh mounts of eggs show no “eyed” stage among incubating eggs in the sponge

Methods of Prevention and Control:

- Ensure that females are mated upon procurement or purchase
- Berried females should be kept in clean water during egg incubation to prevent microbial infection and fouling
- Spawners may be disinfected in 150 ppm formalin for 30 minutes to get rid of fouling organisms and fungi. This is especially for females who already have spawned upon procurement
- Broodstock that have not spawned should be separated from berried females to prevent cross-infection
- During egg incubation, 0.1 ppm Treflan can be applied to the water every 2 days

References

Djunaidah IS, Wille M, Kontara EK, Sorgeloos P. 2003. Reproductive performance and offspring quality in mud crab (*Scylla paramamosain*) broodstock fed different diets. *Aquacult. Internat.* 11: 3-15

Millamena OM, Quintio ET. 2000. The effects of diets on reproductive performance of eyestalk ablated and intact mud crab *Scylla serrata*. *Aquaculture* 181: 81-90

Quintio ET, Parado-Estepa FD. 2003. Biology and Hatchery of the Mud Crabs *Scylla* spp. *Aquaculture Extension Manual No. 34*, SEAFDEC Aquaculture Department, Iloilo, Philippines. 42 p





Incomplete Molting

Crustaceans shed their exoskeleton through a process called molting or ecdysis. This procedure is necessary for growth and requires a lot of energy to accomplish. Where conditions for complete molting are not met, affected animals fail to shed off their old shells completely resulting to parts of the old shell to remain attached to the new shell. Molting in crustaceans should not be prolonged so as not to reach a point-of-no-return, after which molting will never occur.

Cause:

- The factor mostly implicated with the occurrence of incomplete molting is low temperature and nutritional causes
- In one experiment, 37% of crab juveniles reared at ambient temperature (27-30°C) successfully molted during the 30-day holding period, but only 17% of crabs at 20-22°C molted in the same period

Effect on Crabs:

- Attached old shells cause abnormal movement or swimming behavior and affected crabs become easy prey to healthy individuals

Diagnostic Techniques:

Gross Observations (Level 1):

- Presence of old shell attached to newly-molted exoskeleton (Photo 1; arrow) in contrast to complete shedding of exoskeleton during successful molting (Photo 2; arrow)
- Note the size increment after molting

Methods of Prevention and Control:

- Give crabs adequate diet to provide energy for successful molting
- Grow crabs at optimum water temperature (Appendix 9)

Web-based Resources

<http://ourworld.compuserve.com/homepages/BMLSS/Ecdysis.htm>





Loss of Limbs

Description:

Crabs sacrifice their limbs through the process of autotomy in order to escape from predators. Other factors that have been ascribed to appendage loss in various species of crabs include too much exposure to dry environment and physical stress due to traumatic molt. Crabs are very aggressive animals and often suffer injury and loss of limbs (Photo 1). Severe muscular emaciation of some captive broodstock may result in the loss of limbs (Photo 2; arrow). Regeneration of the lost part, if it occurs, does not restore its original function because of the relatively small size of the new appendage (Photo 3; arrow). In cases where only one appendage is lost, the crab will have chelipeds with different sizes (Photo 4; arrow).

Effect on Crabs:

- The loss of a major appendage like the cheliped may lead to impaired feeding, mating, and capability for defense. A study done on spanner crabs showed that limb damage significantly affected survival, but not their ability to bury themselves and hide from predators.

Diagnostic Techniques:

Gross Observations (Level I):

- Absence of appendages (Photos 1 and 2)

Methods of Prevention and Control:

- Provide shelters to prevent aggressive behavior that might lead to fights and loss of appendages
- Maintain good conditions in the rearing tank to keep crab healthy in captivity
- Provide proper nutrition to prevent emaciation
- Avoid too much exposure to dry environment

References

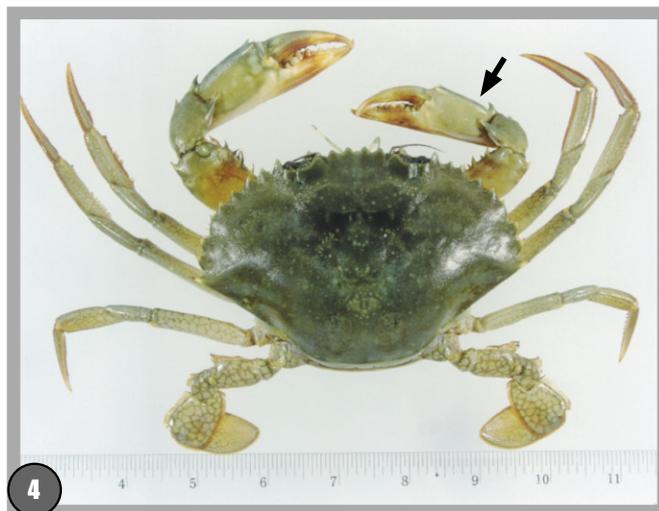
Kirkwood JM, Brown IW. 1998. Effect of limb damage on the survival and burial time of discarded spanner crabs *Ranina ranina* (Linnaeus). Mar. Freshwat. Res. 49: 41-45



Web-based Resources

<http://www.blue-crab.org/autotomy.htm>





Appendices

Appendix 1. Preparation of samples for diagnosis

Appendix 2. Fixatives and fixation procedures

Appendix 3. Preparation of fresh mounts for microscopic examination

Appendix 4. Techniques for isolation of microorganisms from various samples

a. Media for isolation of bacteria

b. Isolation of fungi from crab eggs and larvae

Appendix 5. Chlorination of water and rearing paraphernalia

Appendix 6. Disinfection and caring for berried crabs

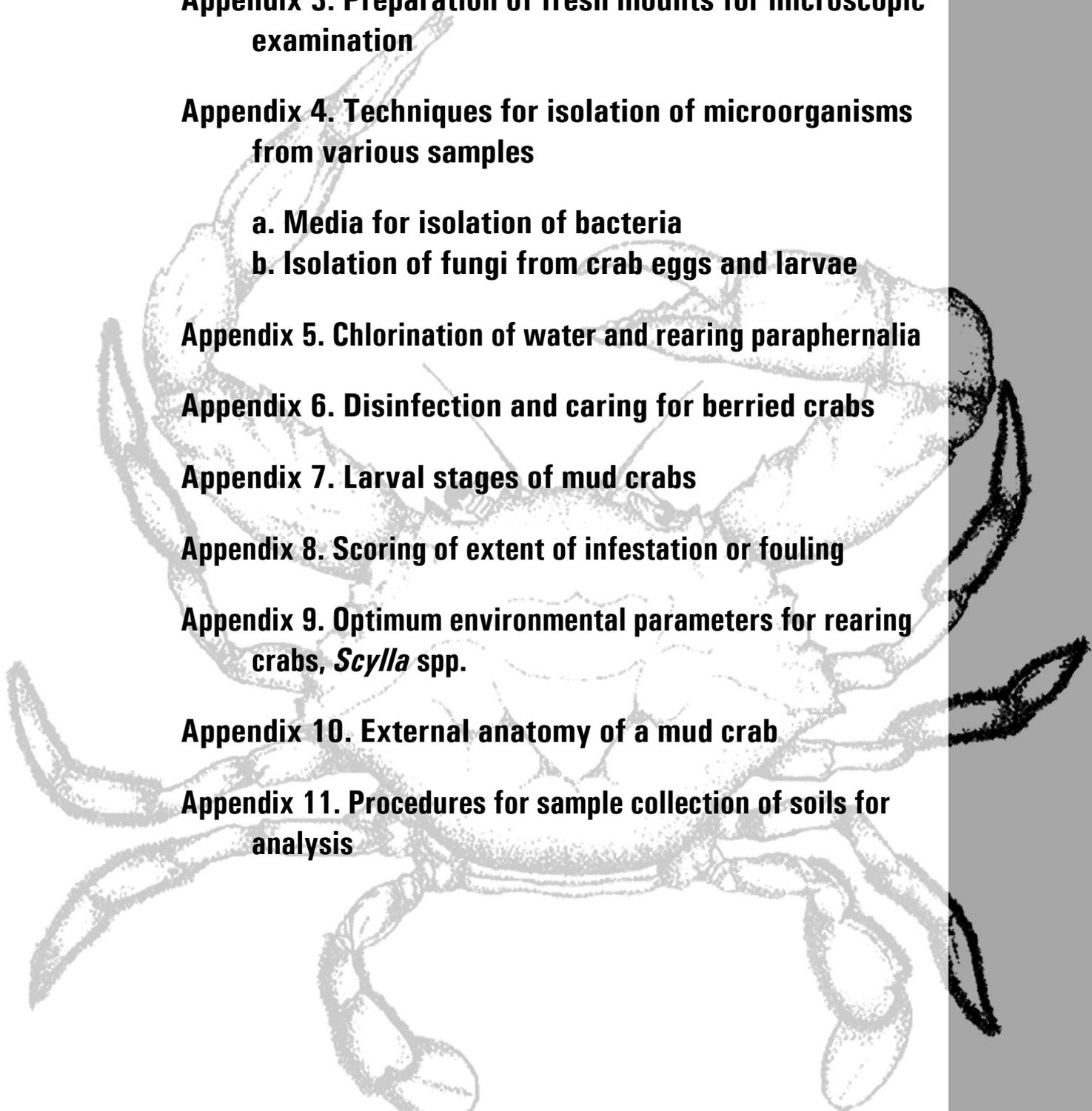
Appendix 7. Larval stages of mud crabs

Appendix 8. Scoring of extent of infestation or fouling

Appendix 9. Optimum environmental parameters for rearing crabs, *Scylla* spp.

Appendix 10. External anatomy of a mud crab

Appendix 11. Procedures for sample collection of soils for analysis



Appendix 1. Preparation of samples for diagnosis

A. Live samples

Obtain 3-5 or more diseased and moribund individuals, and an equal number of normal crabs from the pond and tank (Photo 1). Separately place crabs in boxes with moistened tissue paper for shelter during transport. Crabs can be transported even without water, but it is advisable to bring them to the laboratory early in the morning or late in the afternoon to avoid too much exposure to heat and prevent drying up.

B. Iced samples

Materials needed: ice packs, plastic bags, styrofoam box or other insulated containers

If delivery of live samples is not possible, samples can be sent iced or frozen (Photo 2). Obtain 3-5 or more diseased and moribund individuals, and an equal number of normal crabs from the pond and tank. Wrap individually in plastic bags to prevent water from damaging the tissues. Separate normal from diseased specimen. Place packed samples between layers of ice in a styrofoam box or other insulated containers. Specimens should reach the laboratory for analysis within 24 hours.

C. Fixed samples

Materials needed: Bottles with water-tight cap, fixatives (see Appendix 2), dissecting scissors, forceps, scalpels

If samples cannot be delivered to a laboratory within 24 hours, specimens should be fixed before submission. These specimens can be processed for histopathology only.

Put eggs and larvae in screw-cap containers. The number of specimens to be submitted should be based on the tabulated guides in page 8. For juveniles and adults, open the body (Photo 3) to allow the fixative to penetrate inner tissues. Place crab in a bottle or container. Then, add fixatives (Davidson's fixative or 10% buffered formalin) at the ratio of 10 parts fixative to 1 part tissues (Photo 4). Containers should be sealed tightly to prevent spillage and evaporation of fixative.

D. Samples for diagnosis of associated viruses by PCR

Samples for diagnosis of associated viruses by means of polymerase chain reaction (PCR) test should be fixed in 95% ethanol. For large specimens, dissect the gills (Photo 5) and fix tissues in 95% ethanol. These samples should be submitted to a laboratory as soon as possible.



Appendix 2. Fixatives and fixation procedures

Formulae of Fixatives

A. Davidson's Fixative

For 1 Liter:

95% Ethanol	330 ml
Formalin	220 ml
Glacial Acetic Acid	115 ml
Distilled water	335 ml

B. 10% Buffered Formalin

For 1 Liter:

Formalin	100 ml
Distilled water	900 ml
Sodium phosphate, monobasic	4 g
Sodium phosphate, dibasic, anhydrous	6 g

C. 95% Ethanol

For 1 liter:

Absolute ethanol	950 ml
Distilled water	50 ml

Caution:



- Store all fixatives in tightly capped containers and place unused fixatives in the refrigerator to prevent evaporation of water.
- Exercise caution when handling fixatives as they can be harmful when inhaled and toxic when ingested.
- Protect hands with gloves and use aspirators when dispensing them.
- Work in well ventilated rooms or under a fume hood to prevent lingering toxic fumes.

Web-based Resources

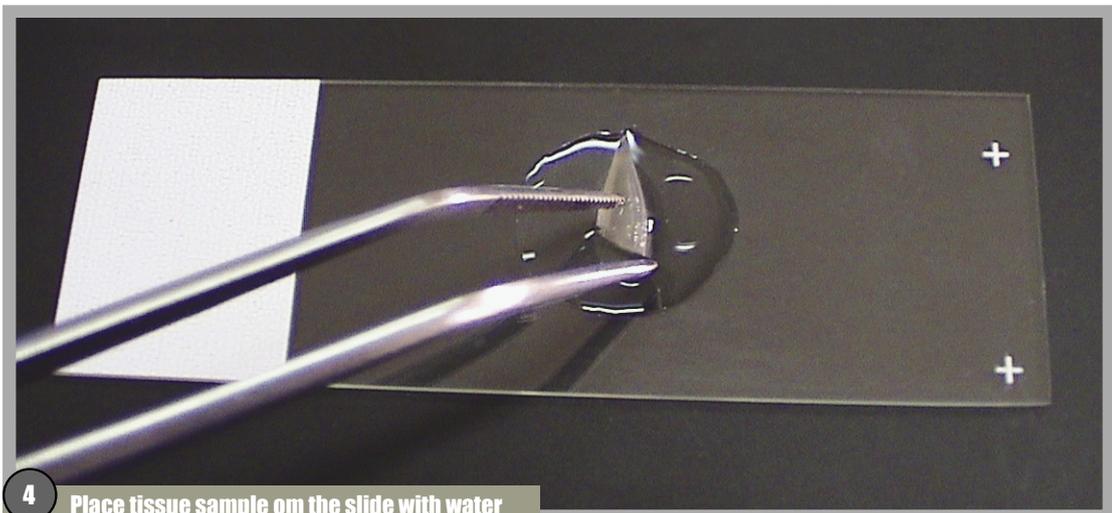
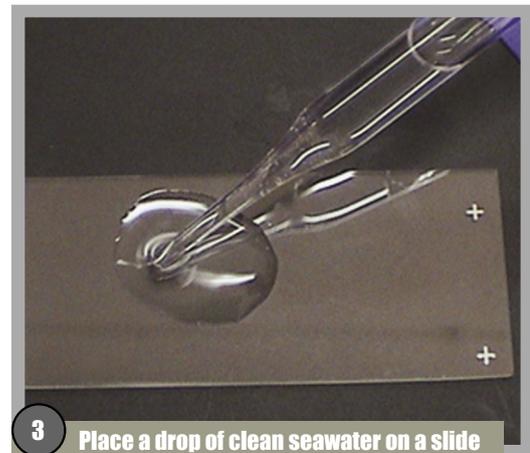
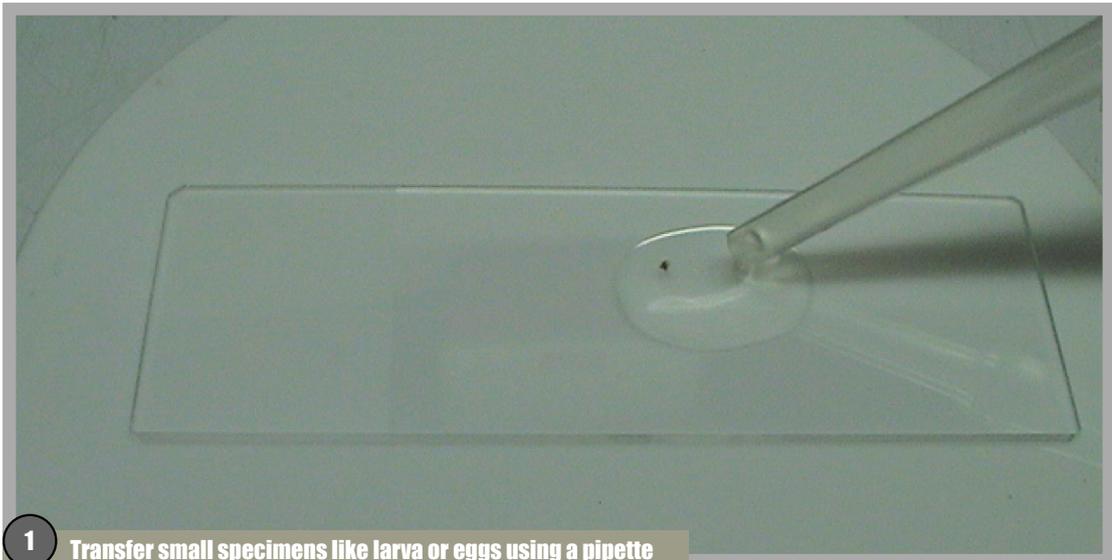
<http://www.dpi.qld.gov.au/thematiclists/14515.html> and <http://www.dpi.qld.gov.au/thematiclists/14527.html> = these links to the Department of Primary Industries and Fisheries of the Queensland Government, Australia with information about submitting samples for laboratory examination and crab culture

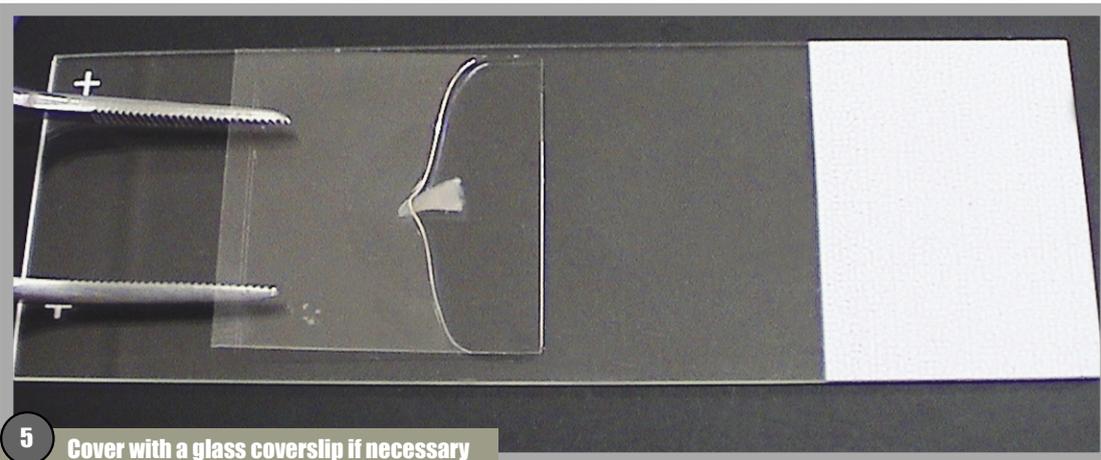


Appendix 3. Preparation of fresh mounts for microscopic examination

The simple techniques shown in Photos 1-6 allow microscopic examination of small live specimens like crab larvae and eggs, or small sections of gills, hepatopancreas, and other tissues.

Procedure:



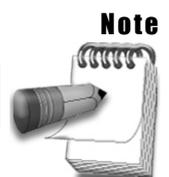


5 Cover with a glass coverslip if necessary



6 Place the slide on the microscope stage and adjust focus starting with the lowest power objectives before switching to higher magnifications

Do not flood glass slides with too much water to avoid wetting the microscope stage and objectives. Seawater is corrosive.



Note

Appendix 4. Techniques for isolation of microorganisms from various samples

A. Media for isolation of bacteria

Bacteria occurring at the sites of infection can be isolated using several types of culture media in plates (Photo 1). Dehydrated forms of media in powder or granular forms are commercially available from various suppliers.

Nutrient Agar with 1.5% salt

Preparation (for 1 Liter):

Nutrient Broth	8 grams
Agar	15 grams
Sodium chloride	15 grams
Distilled water	1000 ml

Weigh dry ingredients and put them in a heat resistant flask or bottle. Add distilled water. Cap lightly then sterilize by autoclaving at 121°C and at a pressure of 15 pounds per square inch (psi) for 15 minutes. After sterilization, allow medium to cool. Swirl bottle for thorough mixing before dispensing medium into sterile plates. Sea water may be used instead of distilled water. Do not add sodium chloride to the medium.

Thiosulfate citrate bile sucrose agar (TCBS)

Preparation (for 1 Liter):

TCBS medium	88 grams
Distilled water	1000 ml

Weigh dry powder and put in a heat resistant flask or bottle. Add distilled water. Cap bottle lightly, then sterilize by boiling in a water bath until TCBS agar powder is totally dissolved. After sterilization, allow medium to cool enough for easy dispensing into sterile plates.

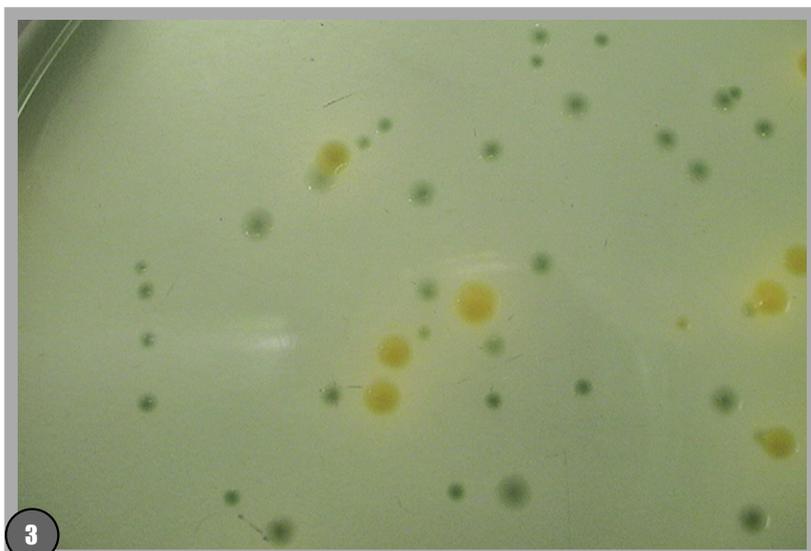
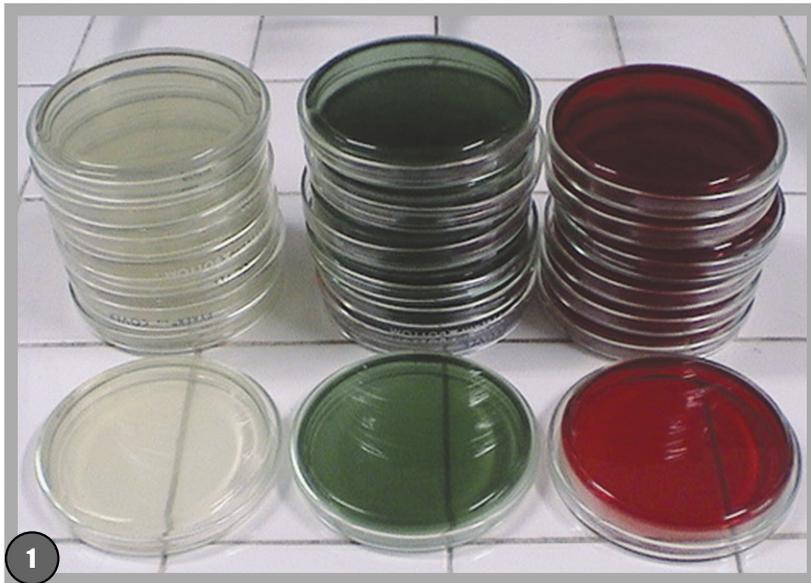
Note



Since TCBS medium already contains sodium chloride, do not prepare this using seawater.

These media can be inoculated by spread plate or streak methods. The table below gives the interpretation of colonies on TCBS after 18-24 hour incubation. Bacterial colonies growing on this medium are in Photos 2 and 3.

Appearance of Colonies	Microorganisms
Flat, 2-3 mm in diameter, yellow	<i>Vibrio cholerae</i> , <i>Vibrio cholerae</i> EI Tor
Small, blue-green center	<i>Vibrio parahaemolyticus</i> , <i>V. harveyi</i>
Large, yellow	<i>Vibrio alginolyticus</i>
Blue	<i>Pseudomonas</i> , <i>Aeromonas</i> , and others
Very small, translucent	Enterobacteriaceae and others



Appendix 4 (continuation)**B. Isolation of fungi from crab eggs and larvae****Media:****Peptone Yeast-extra Glucose (PYG) Broth**

- 1.25 g Bacto-Peptone
- 1.25 g Yeast Extract
- 3.0 g Glucose
- 1.0 liter sea water

Weigh dry ingredients and put them in a heat resistant flask or bottle. Add seawater. Cap lightly then sterilize by autoclaving at 121°C and at a pressure of 15 psi for 15 minutes. After sterilization, allow medium to cool. Add 500 ug/ml (final concentration) of Penicillin and Streptomycin to inhibit bacterial growth. Swirl bottle for thorough mixing before dispensing medium into sterile plates.

Note

To prepare PYG agar, add 15.0 g of agar/liter of PYG broth.

Isolation of fungi from eggs/larvae

1. Examine freshly sampled eggs or larvae
2. Under a dissecting microscope, separate fungi-infected eggs or larvae from normal ones
3. Isolate one egg or one larva and place in a test tube with 10 ml PYG broth with antibiotic
4. Observe for mycelial growth in the next 3 days
5. Remove hypha from the tube and rinse with sterile sea water
6. Place hypha in a petri plate with ~ 10 ml sterile sea water
7. Incubate at room temperature
8. After 18-24 hours, examine sporulation of fungi
9. If sporulation occurs, pipette out 0.1 ml aliquot and place 0.5 ml of sample on PYG agar plate with antibiotic
10. Spread the drops on the agar surface by shaking the plate
11. Incubate plate for more than 2-3 days to evidently see isolated hyphal growth of germinated spores

Propagate fungus on solid medium by cutting agar blocks that contain hyphae and placing them on new PYG agar plates.

References

Brock JA, Main KL. 1994. A Guide to the Common Problems and Diseases of Cultured *Penaeus vannamei*. The Oceanic Institute, Hawaii, USA. 241 p

Tonguthai K, Chinabut S, Somsiri T, Chanratchakool P, Kanchanakhan S. 1999. Diagnostic Procedures for Finfish Diseases. Aquatic Animal Health Research Institute, Bangkok, Thailand

Appendix 5. Chlorination of water and rearing paraphernalia

Chlorine is a widely used disinfectant to kill most bacteria, viruses and other microorganisms. It is added to water as hypochlorite powder (70% activity) or solutions (such as Chlorox, Purex, with 5% available chlorine).

Disinfection of rearing water using calcium hypochlorite (70% activity)

1. For chlorination with calcium hypochlorite powder, use Table 1 to determine the required amount of bleach powder for the volume of rearing water. For example, if the water volume is 0.5 ton or 500 liters and the desired chlorine concentration is 15 ppm, the amount of calcium hypochlorite needed is 10.7 g. Dissolve this amount first in a small volume of water (500 ml)
2. For chlorination with ordinary household bleach (Purex, Chlorox, etc. with 5% available chlorine), use Table 2 to determine the amount of bleach to be used for the volume of water.
3. Fill the tank with the desired volume of water then add the calcium hypochlorite solution
4. Allow chlorinated water to stand for at least 12 hours and up to 24 hours. Check the residual chlorine level by means of portable kits available in the market. Neutralize remaining chlorine with equal amount of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$), before using the water

TABLE 1. Guide for determining the amount of powder calcium hypochlorite to be added to get desired chlorine concentration for water disinfection

Volume of Water		Amount (grams) of Calcium Hypochlorite			
Tons	Liters	5ppm	10 ppm	15 ppm	20 ppm
0.25	250	1.4	3.6	5.4	7.2
0.50	500	3.6	7.1	10.7	14.3
1	1,000	7.1	14.3	21.4	28.6
2	2,000	14.3	28.6	42.9	57.1
3	3,000	21.4	42.9	64.3	85.7
5	5,000	35.7	71.4	107.1	142.9
10	10,000	71.4	142.9	214.3	285.7

TABLE 2. Guide for determining the amount of bleach solution (in milliliters) for water disinfection

Volume of Water		Amount (ml) of Bleach Solution			
Tons	Liters	5ppm	10 ppm	15 ppm	20 ppm
0.25	250	25	50	75	100
0.50	500	50	100	150	200
1	1,000	100	200	300	400
2	2,000	200	400	600	800
3	3,000	300	600	900	1,200
5	5,000	500	1,000	1,500	2,400
10	10,000	1,000	2,000	3,000	4,000

Equipment and Materials

- Provide properly labeled materials like beakers, scoop nets, pails, etc. for exclusive use in individual tanks
- Materials like brushes, pails, scoop nets, water hoses, and glasswares that are used in different tanks may be disinfected between use in different tanks by dipping in 400 ppm chlorine and thoroughly rinsing with clean freshwater
- The same amount of chlorine can be used to disinfect contaminated rearing water and infected animals for disposal
- Disinfect tanks between rearing periods

References



- Lio-Po GD, Fernandez RD, Cruz ER, Baticados MCL, Llobrera AT. 1989. Recommended Practices for Disease Prevention in Prawn and Shrimp Hatcheries. Aquaculture Extension Pamphlet No. 3, SEAFDEC Aquaculture Department, Iloilo, Philippines. 14 p

Appendix 6. Disinfection and caring for berried crabs

1. Disinfect newly procured berried crabs in 150 ppm formalin bath for 30 minutes. See formalin solutions preparation guide below
2. Place each berried crab in a 500-liter tank with aerated sea water
3. Feed crabs with mussel meat, fish, marine worms, or squid at 10-15% of crab biomass daily. Remove uneaten feeds after 4 hours. Discontinue feeding on the day eggs become brown
4. Siphon out detached eggs and excess food every day before water change. Change about 80% of the total water volume in the tank daily. Retain 20% of water in the tank to prevent egg desiccation
5. Obtain a few eggs from the egg mass 2-3 times during the incubation period (9-14 days) to examine embryonic development and biofouling
6. Apply 0.1 ppm Treflan (44% trifluralin) to water every other day to prevent fungal infection

TABLE 1. *Guide in the preparation of the formalin solutions*

Formalin concentration (ppm)	Volume of formalin solution (ml)	
	per 10 liters	per 100 liters
10	0.1	1.0
100	1.0	10.0
150	1.5	15.0
400	4.0	40.0
500	5.0	50.0

A 37-40% formalin solution should be considered as 100% stock. If a white precipitate forms, filter the formalin stock before use. Dilute the solution in sea water.

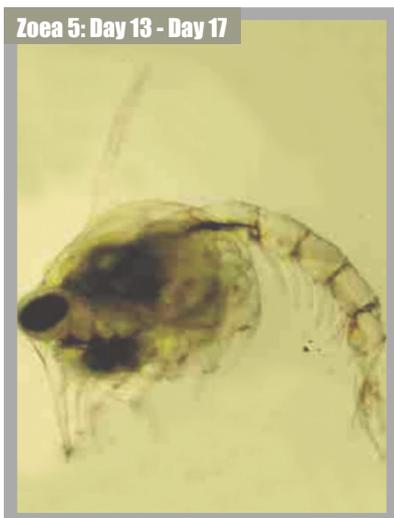
References

Lio-Po GD, Fernandez RD, Cruz ER, Baticados MCL, Llobrera AT. 1989. Recommended Practices for Disease Prevention in Prawn and Shrimp Hatcheries. Aquaculture Extension Pamphlet No. 3, SEAFDEC Aquaculture Department, Iloilo, Philippines. 14 p

Quinitio ET, Parado-Esteva FD. 2003. Biology and Hatchery of the Mud Crabs *Scylla* spp. Aquaculture Extension Manual No. 34, SEAFDEC Aquaculture Department, Iloilo, Philippines. 42 p



Appendix 7. Larval stages of mud crabs



Appendix 8. Scoring of extent of infestation or fouling

Numerical Score	Microscopic findings*
0	No fouling organisms
0.5	A few scattered fouling organisms
1	Fouling organisms present, but not abundant (i.e., not covering or affecting more than 10 to 25% of the total area examined)
2	Fouling organisms common and affecting or covering at least 25% of the total area examined.
3	At least 50% of the affected part covered.
4	All or nearly all (75 to 100%) of the affected surfaces covered

* To be determined by microscopic examination (at 50-100×) of the lamellae of a gill or appendages in unstained wet mounts or in histological section

References

Brock JA, Main KL. 1994. A Guide to the Common Problems and Diseases of Cultured *Penaeus vannamei*. The Oceanic Institute, Hawaii, USA. 241 p



Appendix 9. Optimum environmental parameters for rearing crabs, *Scylla* spp.

	Salinity (ppt)	Temperature (°C)	Dissolved Oxygen (ppm)	pH
Eggs	30-35	27-30	> 5.0	7.0-8.5
Zoea	24 - 32	26-30	> 5.0	7.5-8.5
Megalopae	20-25	26-30	> 5.0	6.5-9.0
Grow-out	16-32*	18-30	> 5.0	6.5-9.0
Broodstock	30-35	27-29.5	> 5.0	6.5-9.0

* 16-32 ppt = *S. serrata*; 16-24 ppt = *S. olivacea* and *S. tranquebarica*

References



Fortes RD, Baylon J, Marasigan E, Failaman A, Genodepa J, Garibay S, Mamon GA. 2002. A Guide to Hatchery and Nursery Production of Mud Crab (*Scylla serrata*) Juveniles. College of Fisheries and Ocean Sciences. University of the Philippines in the Visayas. Iloilo, Philippines. 46 p

Hoang DD. 1999. Description of mud crab (*Scylla* spp.) culture methods in Vietnam, pp. 67-71. In: Keenan CP, Blackshaw A. (eds), Mud Crab Aquaculture and Biology. Proceedings of an international scientific forum held in Darwin, Australia, 21-22 April 1997. ACIAR Proceedings No. 78, ACIAR, Canberra, Australia

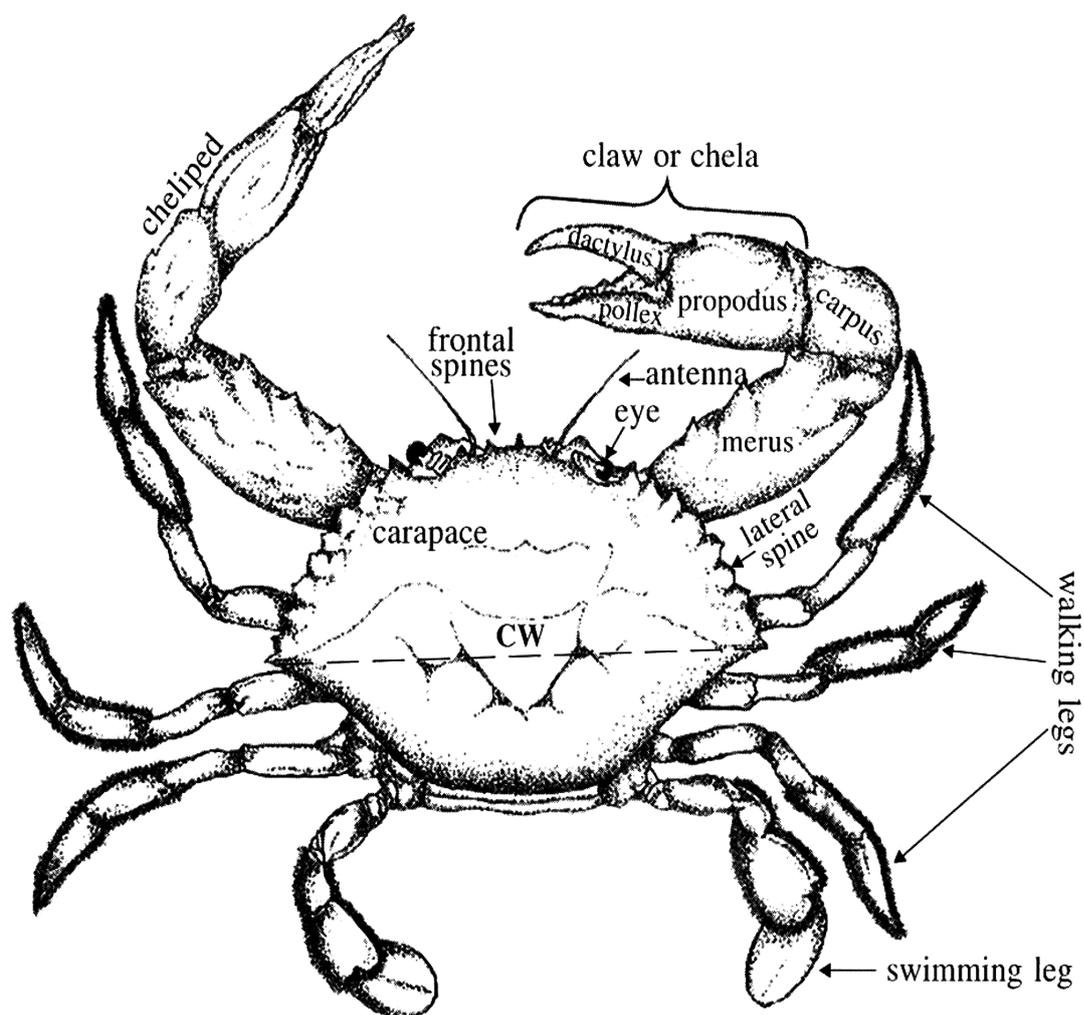
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Li S, Zeng C, Tanh H, Wang, Lin Q. 1999. Investigations into the reproductive and larval culture of the mud crab (*Scylla paramamosain*): A research overview, pp. 121-124. In: Keenan CP, Blackshaw A. (eds), Mud Crab Aquaculture and Biology. Proceedings of an international scientific forum held in Darwin, Australia, 21-22 April 1997. ACIAR Proceedings No. 78, ACIAR, Canberra, Australia

Luo Y, Wei S. 1986. A study on the experimental ecology of the mud crab *Scylla serrata* (Forsk.) . Donghai Mar. Sci. 4: 91-95

Quinitio ET, Parado-Esteva FD. 2003. Biology and Hatchery of the Mud Crabs *Scylla* spp. Aquaculture Extension Manual No. 34, SEAFDEC Aquaculture Department, Iloilo, Philippines. 42 p

Appendix 10. External anatomy of a mud crab



References

Quinitio ET, Parado-Esteba FD. 2003. Biology and Hatchery of the Mud Crabs *Scylla* spp. Aquaculture Extension Manual No. 34, SEAFDEC Aquaculture Department, Iloilo, Philippines. 42 p



Appendix 11. Procedures for sample collection of soils for analysis*

Soils are analyzed to determine its physical and chemical properties which are important in the culture and production of various species. It may be done before and during pond operation.

Following are the procedures for soil sampling to determine pH, lime requirement, organic matter, available phosphorous, available sulfur (sulfate -sulfur) and iron.

Soil Collection From Fish Ponds

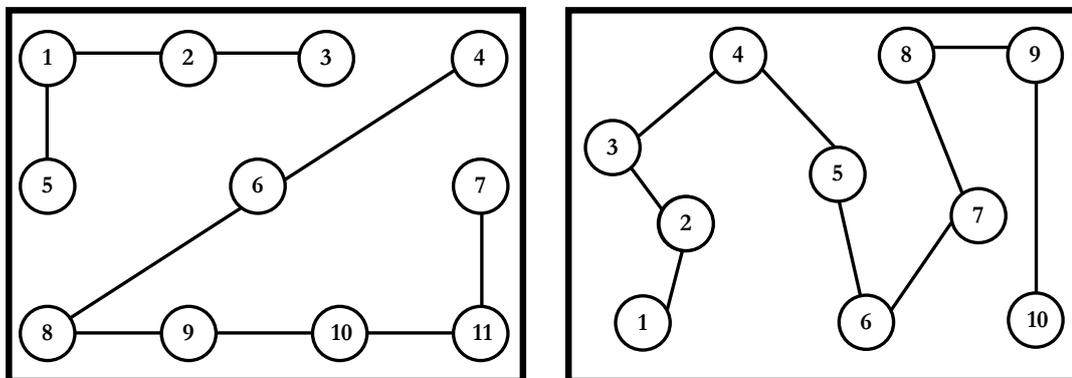
Proper collection and preparation of soil samples intended for analysis are extremely important. Correct interpretation of the tests can be made only when the samples are truly representative of the soil conditions in the field. Sampling is easy when the soil is moist. However, samples may also be taken when soil is dry or is naturally wet as in paddy fields.

Materials

Soil sampler = Core sampler or sampler made of bamboo or PVC pipes
Pail and plastic bags for collection and mixing

Procedure

1. Divide the fishpond into lots, as shown below:



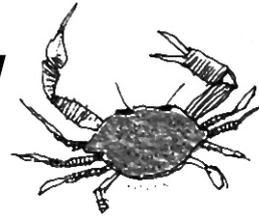
2. Collect core samples up to 1.5 feet deep in representative areas with uniform slope, texture and depth
3. Brush away any stone, rubbish, decayed wood or trash before taking soil sample. In collecting a composite sample, each of the lots in the pond should be represented. Take similar samples in 9 or more points of each lot. Avoid taking directly from fertilized band sector or portion
4. Mix all ten samples to obtain about 10 kg of composite sample
5. Place in containers and label properly to include information like location, area, surface or subsoil, etc.

* Based on guidelines provided by the Centralized Analytical Laboratory, SEAFDEC Aquaculture Department

Preparation of Soil Sample

1. Spread out the soil in thin layer on a labeled strong board or polyethylene film in a room protected from sunlight, dust and wind
2. Break the soil occasionally to hasten the drying process. It takes 4-7 days to dry samples
3. Pulverize the soil using a ball mill or wooden mallet
4. Sieve the ground soil sample through a 2 mm sieve. Crush the clods that do not pass and re-sieve
5. Store the soil sample in properly labeled plastic bags or glass jars prior to analysis
6. Submit samples to an analytical laboratory nearest your farm site

Glossary



- Amorphous** - having no fixed form or shape
- Antennae** - sense organ used to touch objects
- Antennules** - sense organs used to smell and find food
- Asphyxiation** - inability to breathe or exchange gases (carbon dioxide and oxygen), usually resulting in death
- Autotomy** - spontaneous removal or casting off of a body part (such as the tail of a lizard or claw of a crab) especially when the organism is injured or under attack
- Bacterium** - a single-celled microorganism which lack well-defined nucleus
- Biosecurity** - the sum of the management practices in place to reduce risk by ensuring the absolute health of the cultured animals, therefore protecting the financial investment and increasing profitability for the producer
- Brackish** - a mixture of salt and fresh water; moderately salty
- Broodstock** - adult crabs kept for purposes of reproduction
- Calcareous** - containing calcium carbonate
- Chitin** - the main component of crustacean exoskeletons
- Chitinolytic** - able to digest chitin
- Cirriped** - a type of barnacle
- Crustacean** - a class of organisms that have a hard outer skeleton called exoskeleton (includes crabs, shrimps, prawns and lobsters)
- Decapod** - crustaceans with five pairs of appendages for movement each joined to a segment of the thorax
- Debris** - the remains of something that has been destroyed or broken up
- Ecdysis** - molting
- Estuary** - a semi-closed body of water where freshwater from rivers meet sea water from the ocean
- Eukaryotic** - the type of cell containing a true nucleus bounded by a nuclear membrane
- Exoskeleton** - the hard outer covering of crustacean and other invertebrates
- Flabellum** - a hairy fan-like structure that sweeps over the gill lamellae to keep them clean
- Fungi, Fungi** (pl.) - any of numerous plants lacking chlorophyll, ranging in form from a single cell to a body of branched filaments. Includes the yeasts, molds, smuts, and mushrooms
- Habitat** - place where an animal or plant lives

- Hemolymph** - blood of crustaceans and other invertebrates that have open circulatory system
- Hepatopancreas** - the digestive organ of crustaceans that also functions in absorption and storage of food
- Histology** - the study of tissues
- Intermolt** - time or state of crab growth between molts
- Larva** - an early developmental stage of an animal
- Megalopa** - the second larval stage of crabs coming after the zoeal stages of crabs
- Molting** (ecdysis) - the process in which crustaceans including crabs shed their shells to allow them to grow
- Mycelium, Mycelia** (pl.) - a mass of interwoven filamentous 'threads' that make up the vegetative part of a fungus
- Nematode** - an elongated, cylindrical worm parasitic in animals, insects, or plants, or free-living in soil or water
- Parasite** - an organism that lives in or on another organism (the host) during some portion of its life cycle
- Pathogen** - a disease-causing organism
- Pedunculate** - has a stalk
- pH** - a measure of the concentration of hydrogen and hydroxide ions. pH values range from 0 to 14. A neutral solution has a pH of 7. A pH less than 7 indicates an acidic solution while a pH greater than 7 indicates an alkaline solution
- Population** - a group of individuals of the same species within a given space and time
- Predator** - an animal that attacks and feeds on other animals, normally killing several individuals during its life cycle
- Preening** - the act of cleaning, grooming and maintaining parts of the body
- Prokaryotic** - the type of cell whose nuclear substance is not enclosed within a membrane
- Protozoan** - a microscopic, single-celled organism that is largely aquatic and includes many parasitic forms
- Quarantine** - restrictions imposed on animals entering or leaving premises on account of suspected disease agents. It is the act of keeping animals separated for a period of time before, for instance, allowing them to enter another country. By doing this, it is possible to limit the risk of spreading disease
- Salinity** - the total amount of salt present in water
- Saprophytes** - organisms growing on decayed animal or plant matter, as most bacteria and fungi
- Septicemia** - blood poisoning caused by pathogenic organisms
- Soft-shell** - crabs that have shed their hard outer shells in preparation for growth
- Spawner** - a mature female crustacean that produces eggs in large numbers
- Spore** - a reproductive structure developing in certain bacteria and fungi which is strongly resistant to environmental influences but which will become active under suitable conditions
- Transmission** - the passing of a disease from an infected individual or group to a previously

Virus - any of various submicroscopic pathogens which can only replicate inside a living cell

Zoea - the free-swimming first larval form of some decapod crustaceans

Zoospore - a motile, asexually produced spore

Web-based Resources

www.aquatext.com = a free online aquaculture dictionary with over 2,500 aquaculture definitions including 280 tables, 250 pictures and 120 calculations



<http://www.fcps.k12.va.us/StratfordLandingES/Ecology/mpages/glossary.htm>

<http://www.mesa.edu.au/friends/seashores/glossary.html>

<http://www.seasky.org/aquarium/sea3a.html> = the site provides definitions for some of the most common terms used in the marine aquarium hobby.

<http://encyclopedia.thefreedictionary.com/Biology>

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he Southeast Asian Fisheries Development Center (SEAFDEC), a regional treaty organization based in Bangkok, Thailand, was established in December 1967 to promote fisheries development in the region. Its member countries are Japan, Malaysia, the Philippines, Singapore, Thailand, Brunei Darussalam, the Socialist Republic of Vietnam, Union of Myanmar, Indonesia, Cambodia and Lao Peoples Democratic Republic. The Council of Directors, who represents SEAFDEC Member Countries, is the policy-making body of the organization.

SEAFDEC does research on appropriate fisheries technologies, trains fisheries and aquaculture technicians, and disseminates fisheries and aquaculture technologies. Four departments were established to pursue these objectives:

- The Training Department (TD) in Samut Prakan, Thailand (1967) for marine capture fisheries training
- The Marine Fisheries Research Department (MFRD) in Singapore (1967) for fishery post-harvest technology
- The Aquaculture Department (AQD) in Tigbauan, Iloilo, Philippines (1973) for aquaculture research and development
- The Marine Fishery Resources Development and Management Department (MFRDMD) in Kuala Terengganu, Malaysia (1992) for the development and management of marine fishery resources in the exclusive economic zones (EEZs) of SEAFDEC Member Countries

SEAFDEC/AQD is mandated to

- Promote and undertake aquaculture research that is relevant and appropriate for the region
- Develop human resources for the region
- Disseminate and exchange information on aquaculture

The Aquaculture Department in the Philippines maintains four stations: in Iloilo Province, the Tigbauan Main Station and the Dumangas Brackishwater Substation; in Guimaras, the Igang Marine Substation; and in Rizal, the Binangonan Freshwater Station.



Tigbauan Main Station



Dumangas Brackishwater Station



Igang Marine Station



Binangonan Freshwater Station



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