

Diseases in Eggs and Larvae

Luminescent Vibriosis

Shell Disease

Fungal Infection

Fouling by Filamentous Bacteria

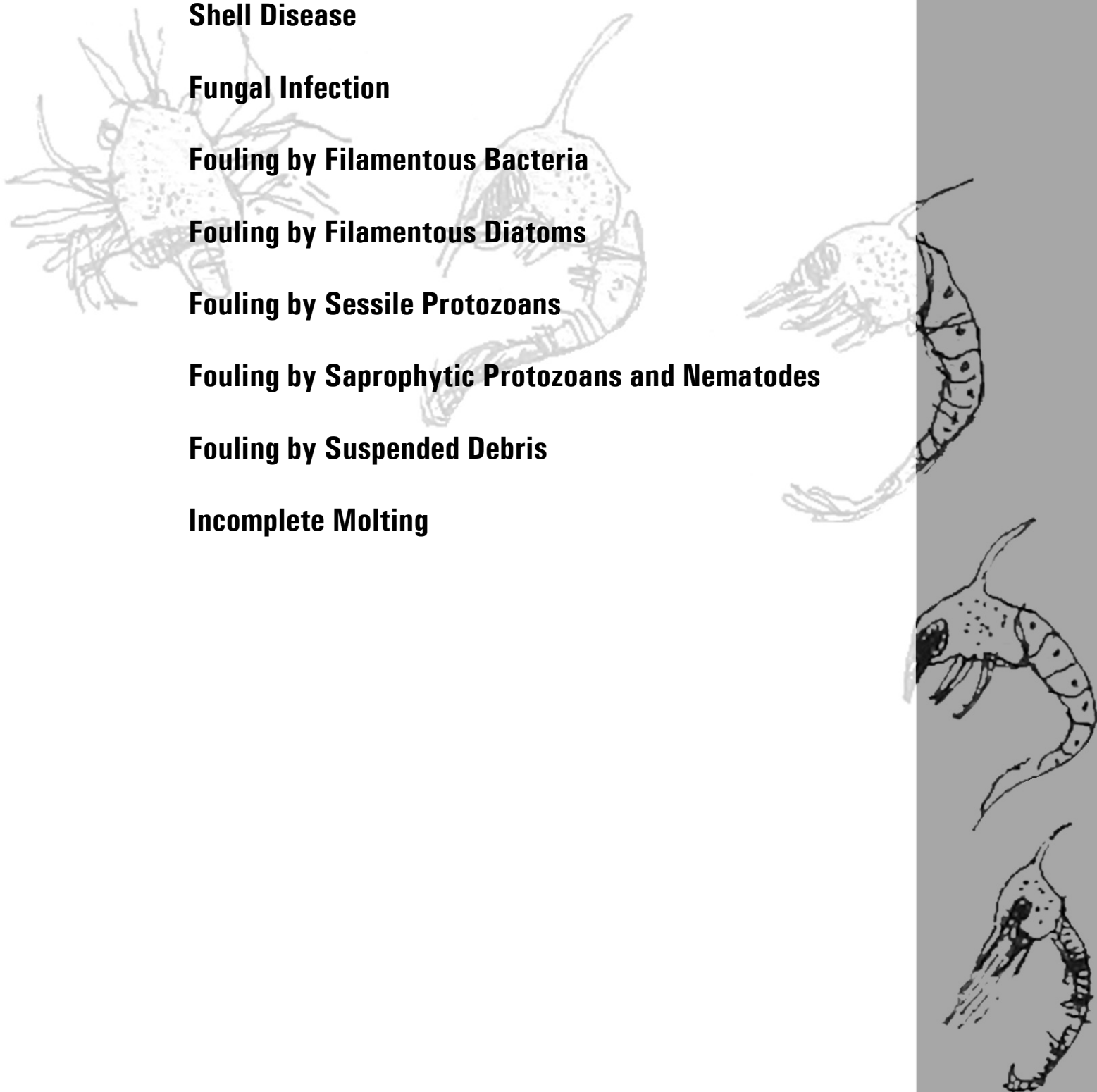
Fouling by Filamentous Diatoms

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**

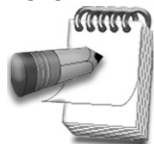
- Use only previously chlorinated water (Appendix 5) for spawning and rearing to ensure a clean environment for newly hatched and developing larvae
- Remove the mothers from the tanks immediately after spawning and rinse the zoeae with chlorinated water to prevent its colonization with luminous bacteria

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

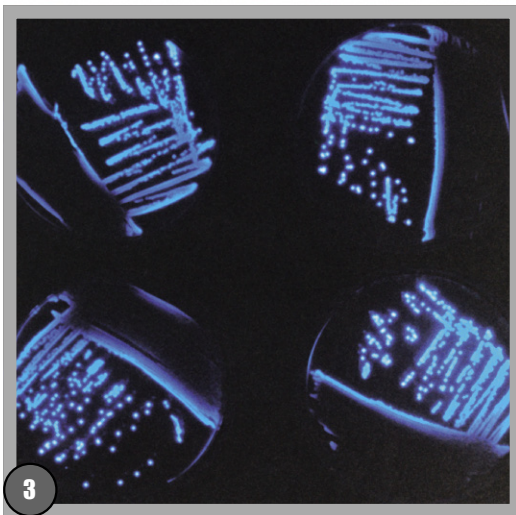
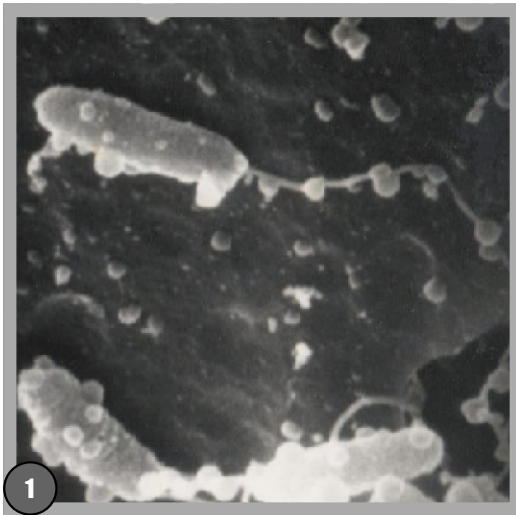
Chemical control of the disease based on efficacy of available drugs appears limited because of the restricted tolerance of crab larvae to drugs and the possible development of resistant strains of bacteria.

References

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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

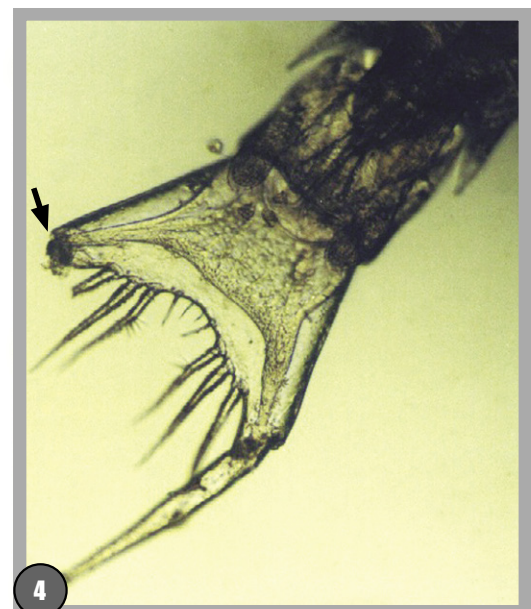
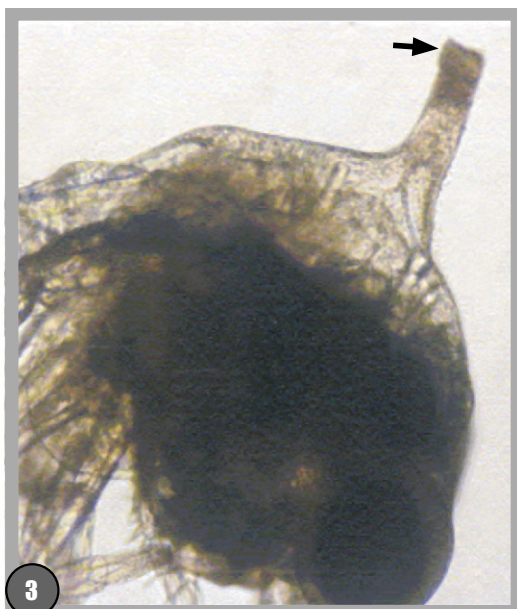
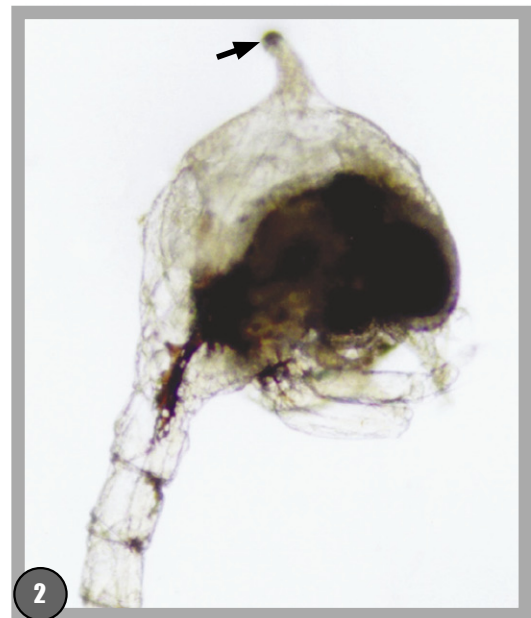
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Web-based Resources

<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

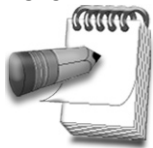
Mode of Transmission:

- 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



<http://www.dec.ctu.edu.vn/sardi/AacrabCWare/Publication/338CRA.htm> = this site links to an article “Improvement of mud crab larviculture (*Scylla paramamosain*) in Vietnam by using a recirculation system”

<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

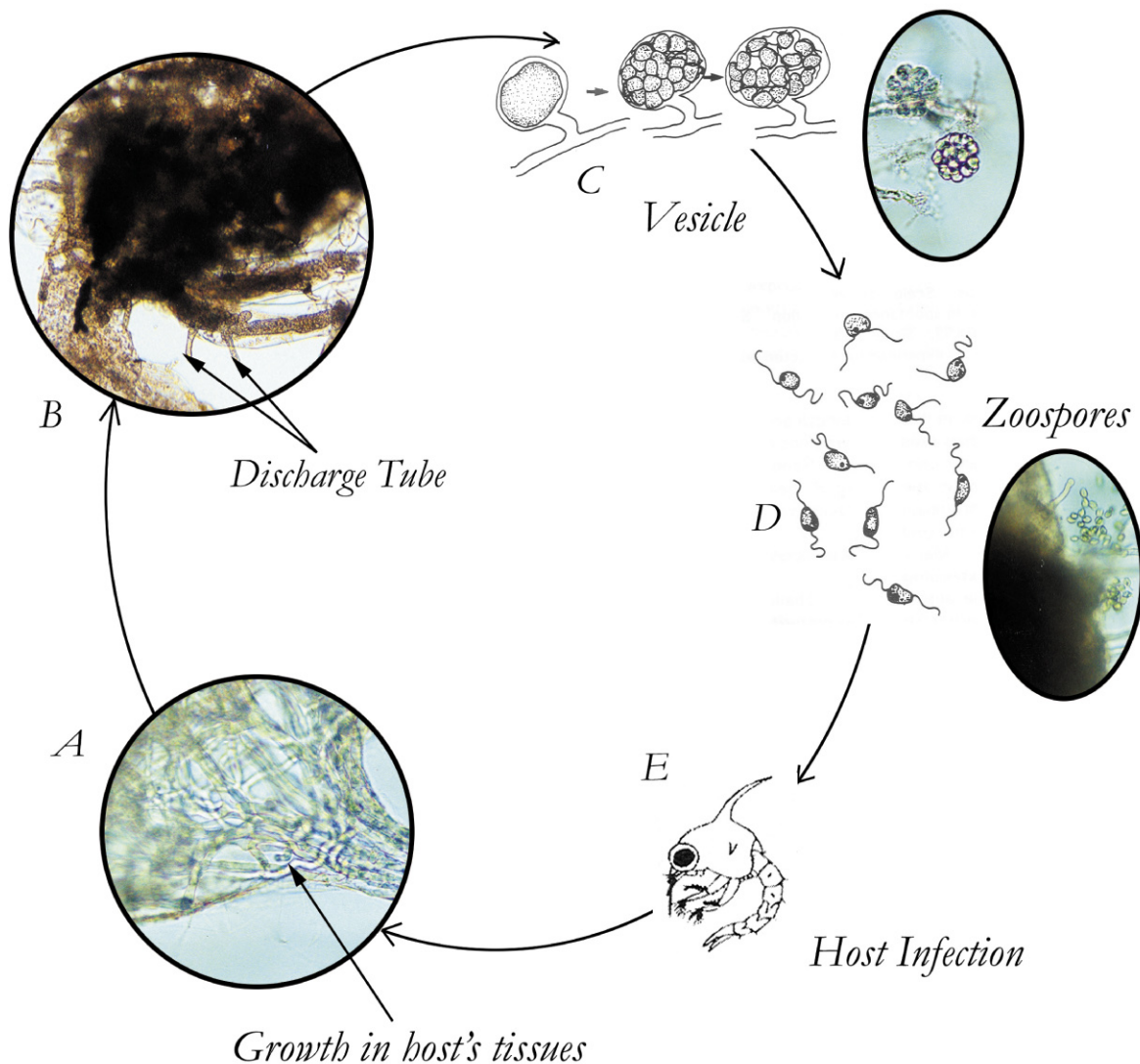
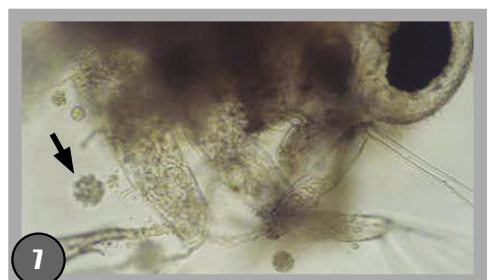
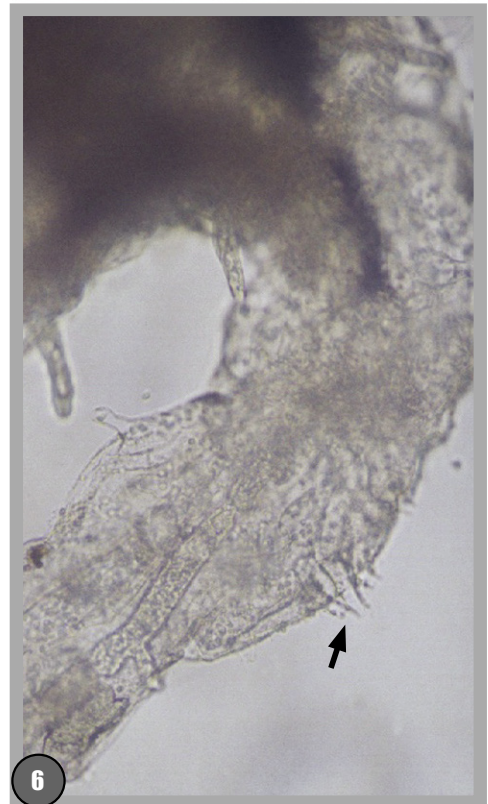
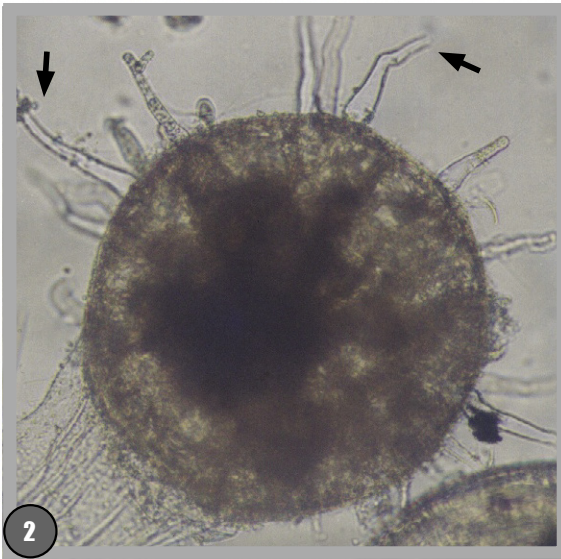
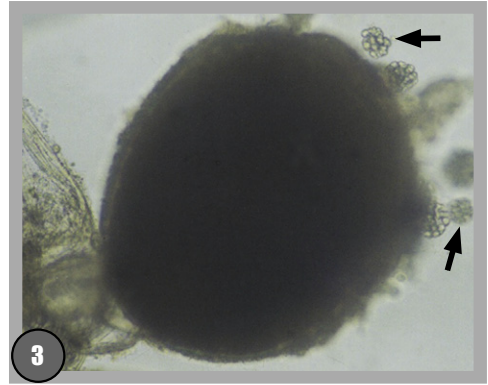


Fig. 1. Infection cycle of marine fungi in hatchery-reared crab larvae. (A) Fungus growing inside infected larva produce highly-branched filaments. Mature filaments produce zoospores and release them either through discharge tubes (B), or externally-developing vesicles (C). Released zoospores (D) find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

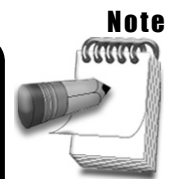


Fouling by Filamentous Bacteria

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):

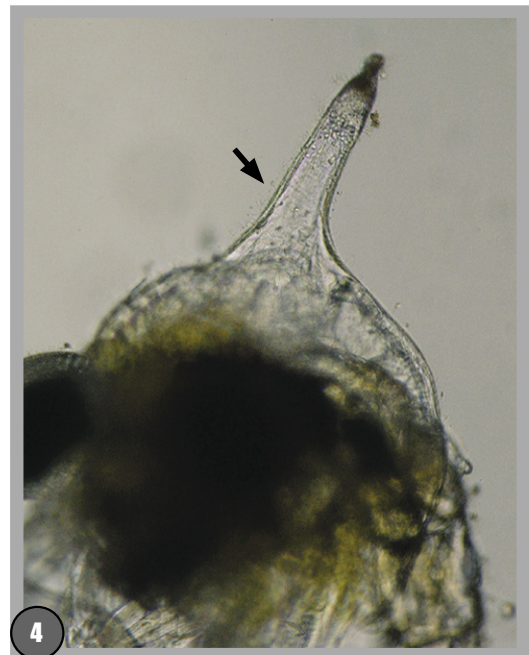
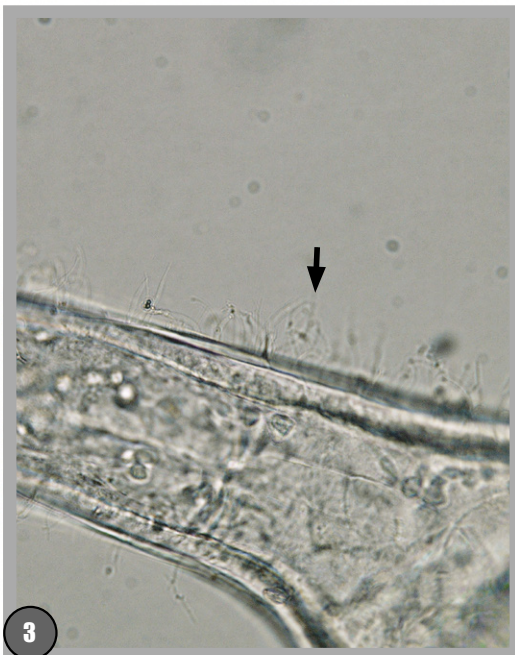
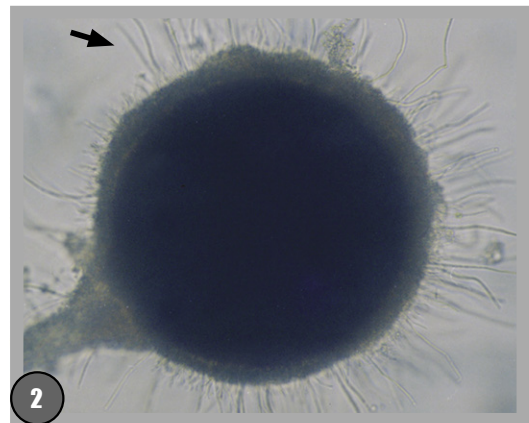
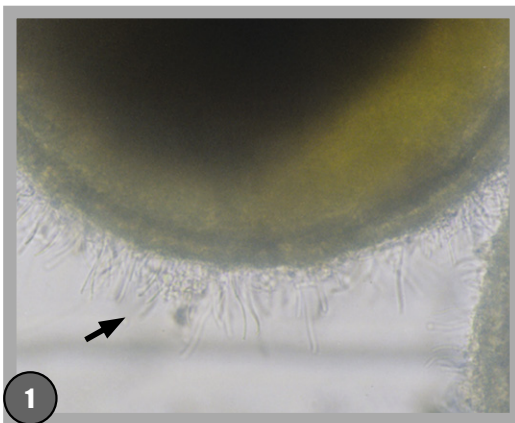
- Prepare fresh mounts of live larvae for light microscope examination (Appendix 3)
- Examine for the presence of fine, colorless and thread-like growths on the eggs (Photos 1 and 2; arrows) and larval surfaces (Photos 3 and 4; arrows)
- A scheme for numerical grading of the extent of filamentous bacteria 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)

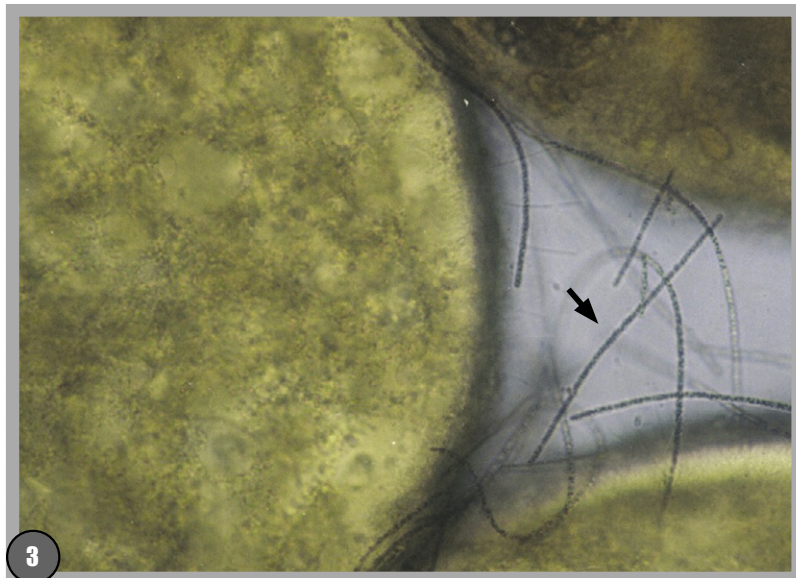
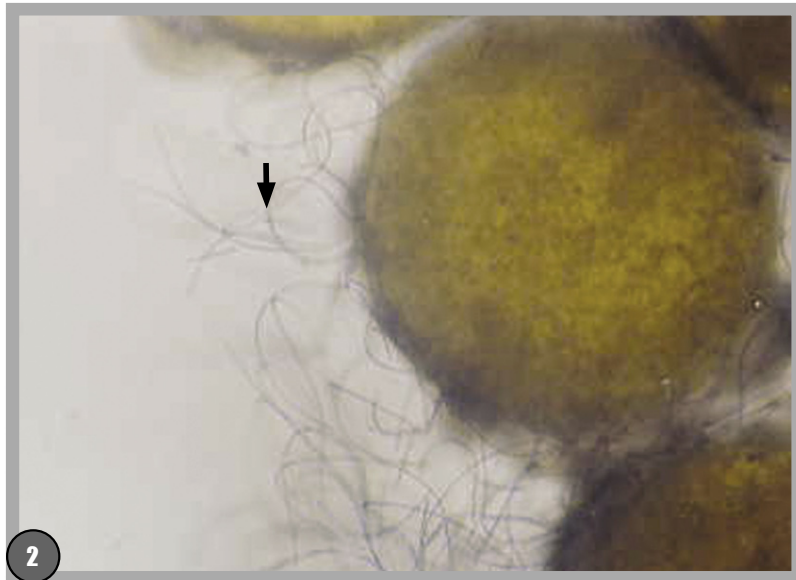
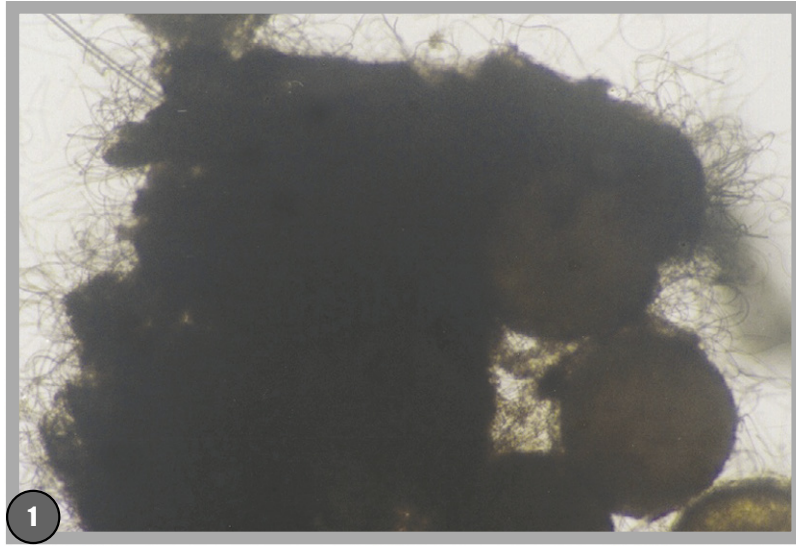
Methods of Prevention and Control:

- 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

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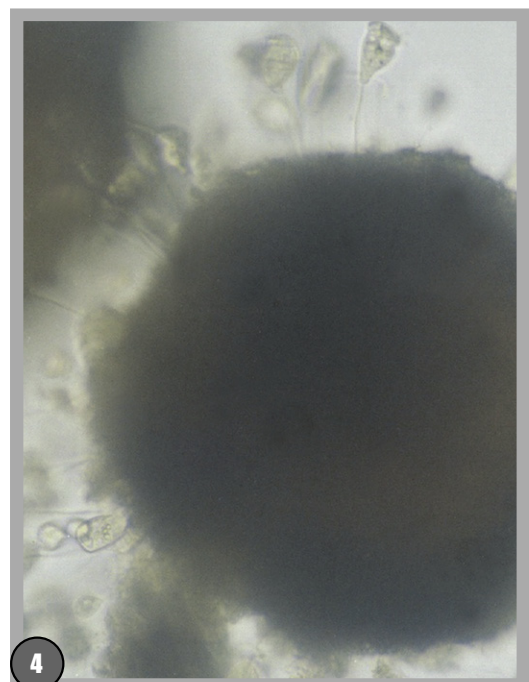
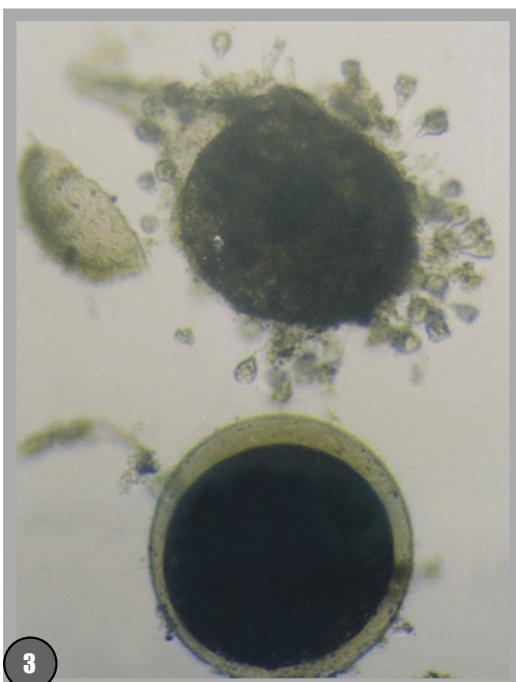
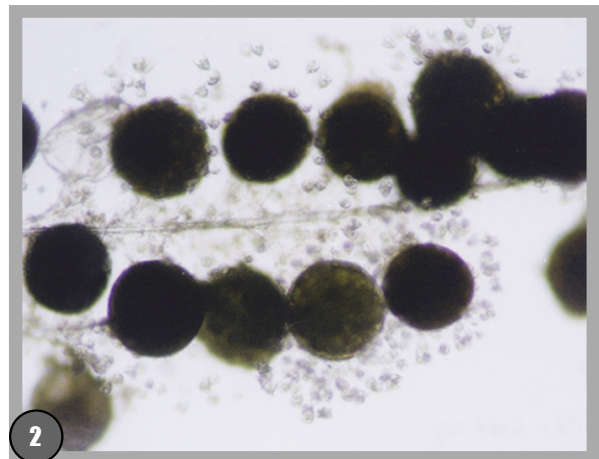
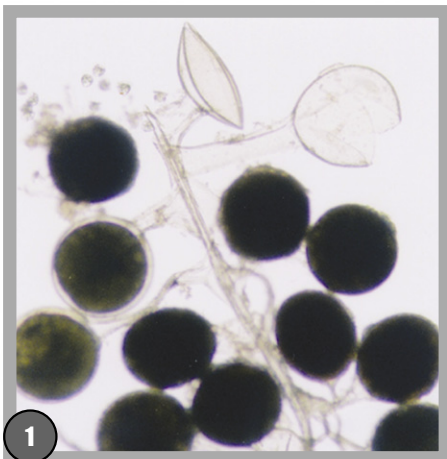
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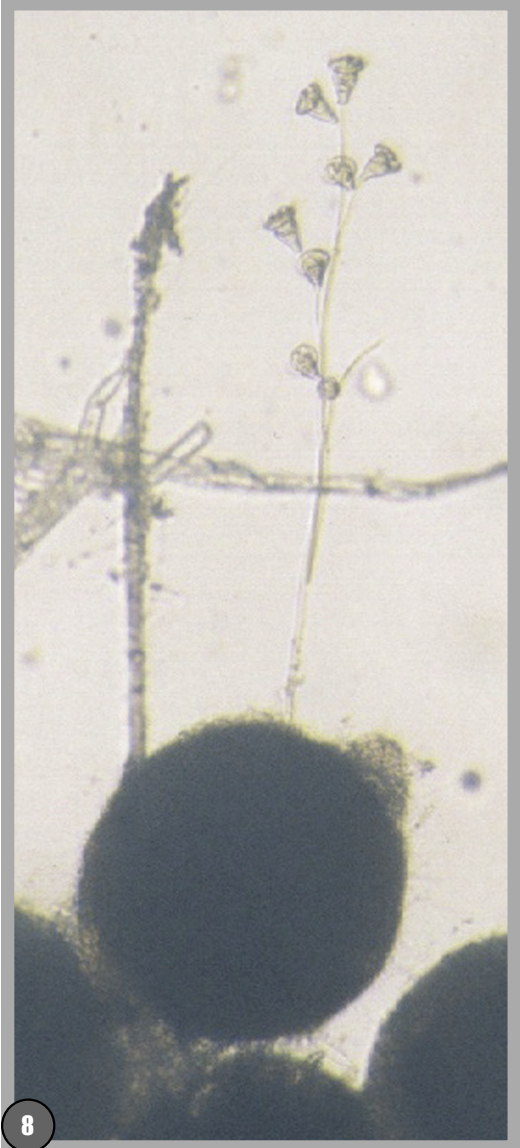
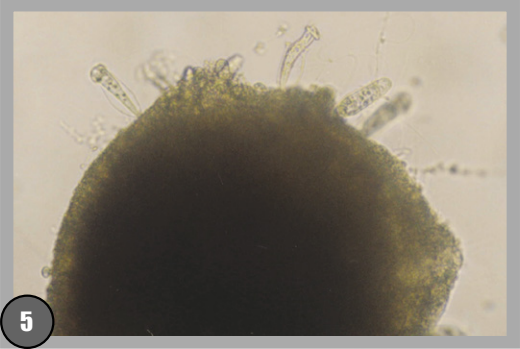
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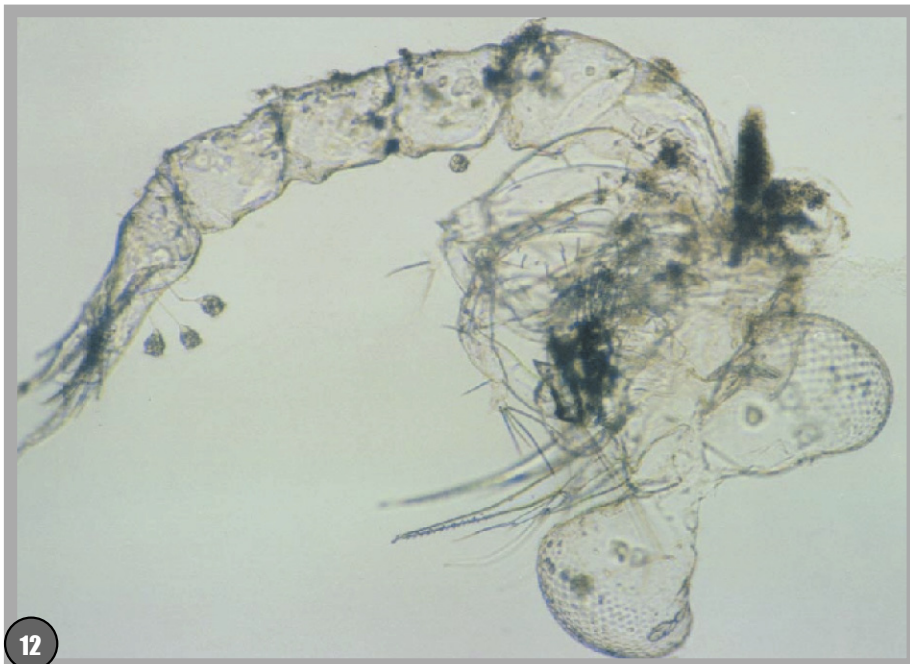
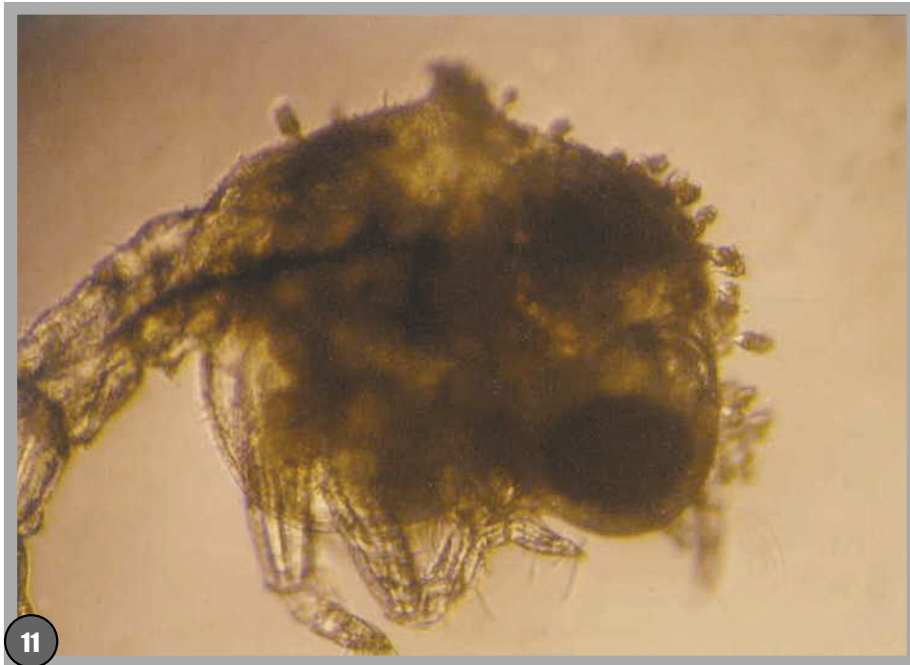
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Web-based Resources

<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

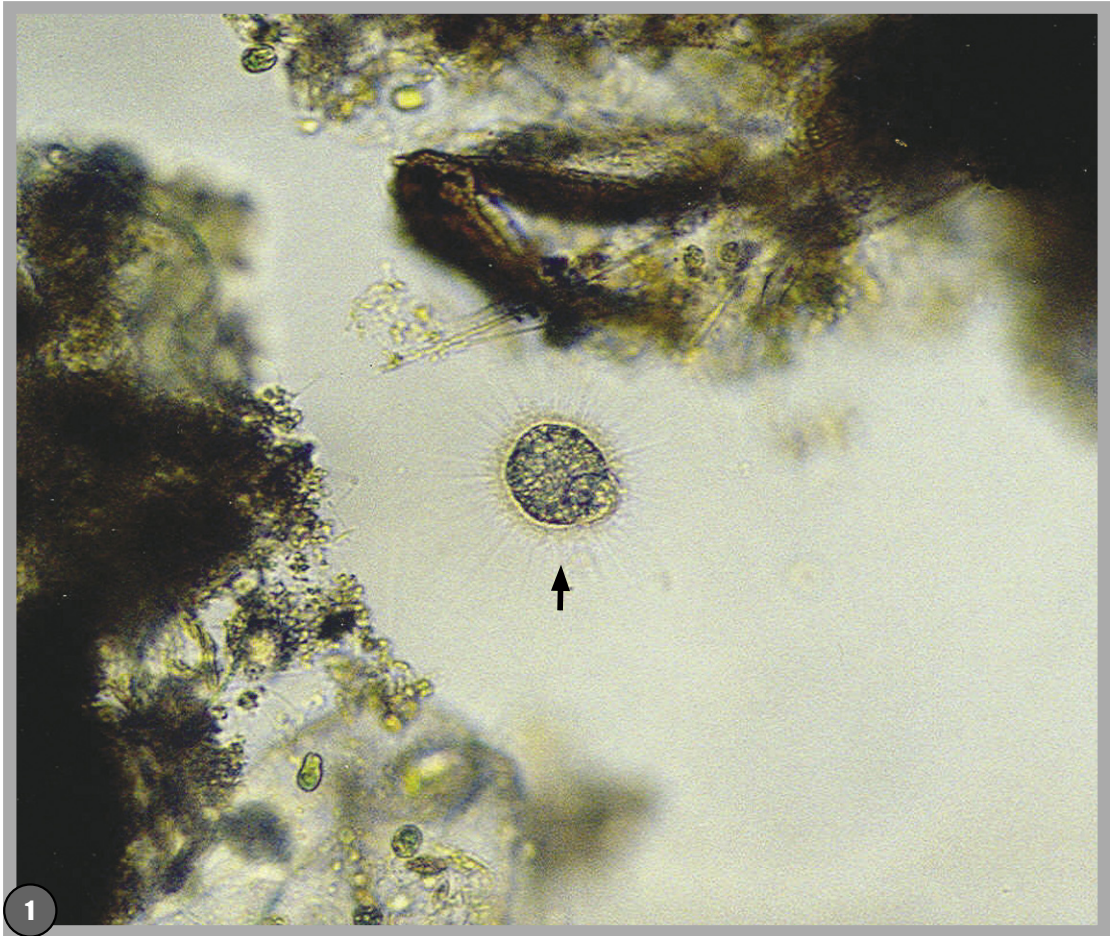
Methods of Prevention and Control:

- 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):

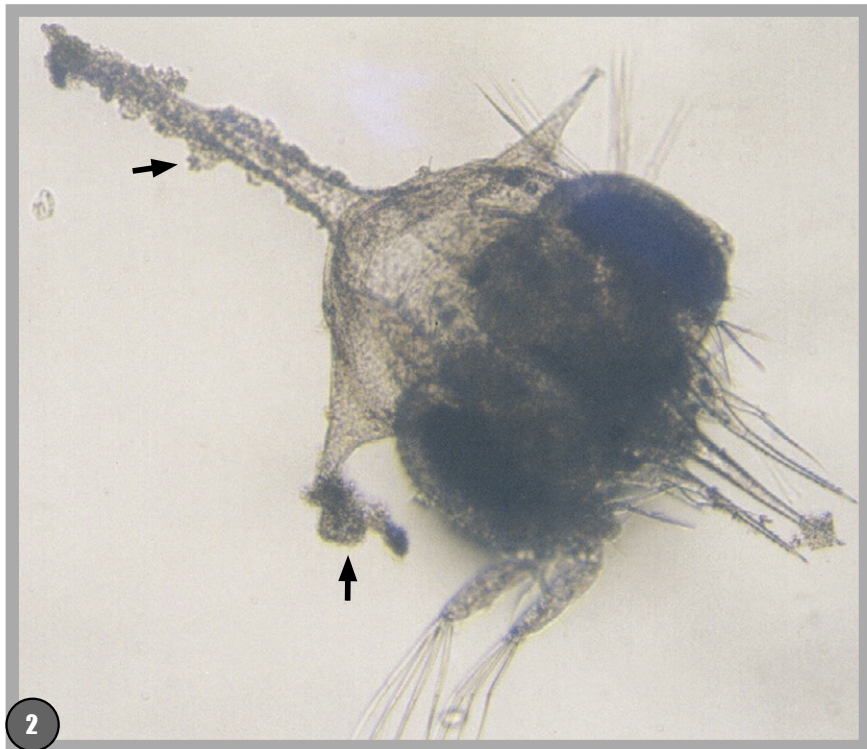
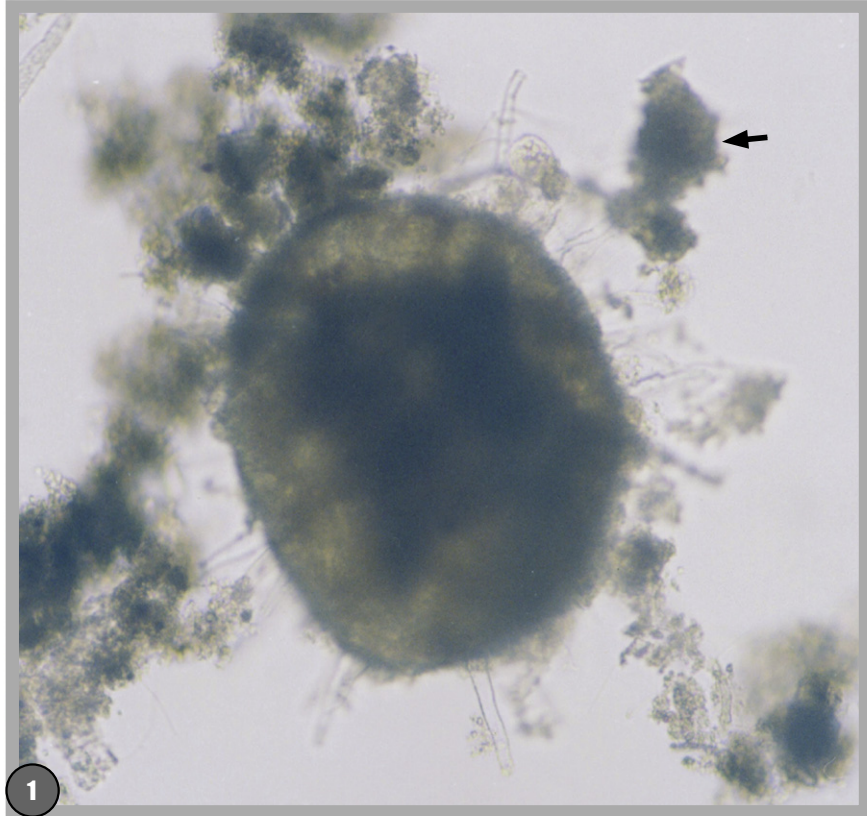
- Discoloration of egg mass different from its normal color change due to larval development
- Slow swimming behavior among larvae and a tendency for them to stay near the bottom

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>



