DISEASES OF PRAWNS
(Pests & Diseases of Sugpo)

Rogelio Q. Gacutan
SEAFDEC Aquaculture Department

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Fungal Diseases

The most prevalent cause of larval mortality are comycetous/ phycomycetous fungi belonging to the genera Lagenidium, Sirolpidium and Haliphthoros. Lagenidium callinectes Couch is the most pathogenic and virulent of the three. In 1976, 35 of 51 hatchery production runs were affected by fungal infections, 22 of these were severely hit and therefore discarded. In 1978, due to safeguards instituted against infection, only 9 of 43 experimental runs were affected. Production increased from 2.6 M P_5s in 1976 to more than 8 M in 1978.

L. callinectes, its occurrence

Since its isolation from diseased crab eggs (Couch, 1942) it has been reported to affect (be transmitted, harbored by) a variety of marine animals including 4 species of penaeids, 5 species of crabs and a species each of lobster, barnacle and red alga. These are Penaeus aztecus Yves (Cook, 1971); P. setiferus (Lightner and Fontaine, 1973); dungeness crab Cancer magister Dana (Rogers-Talbert, 1948; Armstrong et al., 1976); blue crab Callinectes sapidus Rathbun (Couch, 1942; Sandoz et al., 1944; Bland and Amerson, 1973); American lobster Homarus americanus (Nilson et al., 1976); P. merguiensis de Man (AQUACOP, 1975); P. monodon Fabricius (SWUSVOP, 1977; Baticados, et al., 1978); barnacle Chelonibia patula Ranzani (Johnson and Bonner, 1960); oyster crab Pinnotheres ostreum (Rogers-Talbert, 1948); mud crab Neopanope texana (Rogers-Talbert, 1948) and the alga Ectocarpus (Fuller et al., 1964).

Morphology, Zoosporogenesis and Pathogenecity

Despite wide variations in sizes of the vegetative and reproductive structures, the fungus may be recognized on the basis of the characteristic hyphal system, the discharge vesicles formed, and the manner of sporulation. Isolates from P. monodon zoea (Baticados et al., 1978) produced (a) a mycelial system wherein the hyphae measured 2.5-6.25 u wide (b) discharge vesicles ranging from 14.4 to 25 u in diameter, the latter containing from 14 to 32 spores, 3.75 to 6.25 u wide and 5-6.25 u long.

Larvae infected callinectes in P. monodon replaces the larval tissues with an expensive hyphal system and produces extramatrical germ tubes. These would develop terminal discharge vesicles from which zoospores develop. The zoospores are released into the water after the vesicle collapses.

The time factor is short and may end in less than 30 minutes. A discharge vesicle is formed when the cytoplasm of the hypha flows into the extramatrical tube. This occurs in 5 to 10 minutes. Spore formation then proceeds for 10 to 20 minutes after which the spores are seen continuously moving inside the vesicle. The spores then become active and are released 10 to 15 minutes later.

Approaches to the control of the pathogen

Physiological experiments show that the P. monodon isolate has a wide range of tolerance for water temperature, pH, and salinity so that its life cycle may not easily be disrupted by these parameters. In fact, growth and sporulation are known to approach or coincide with the optima for larval rearing. Ultraviolet irradiation has been shown to be ineffective against isolates.

Being phycomycetous, the pathogen may either be air-, water- or soil-borne. One would therefore expect the zoospores or the hyphal system to gain entry via (a) water or (b) spawners, and, judging from long history of infections in some of the tanks used, to have been established deeply in the tank crevices. It was therefore necessary on account of a to have an efficient water filtration system and as a matter of protocol to subject the tanks to chemical treatment with a suitable disinfectant and to drying before and after an experimental run.

The search for a suitable chemotherapeutic

That much effort is spent for screening for a suitable chemotherapeutic does not come as a surprise. Bland et al., (1976) did in vitro toxicity studies against 2 L. callinectes isolates from P. setiferus and C. sapidus using 12 compounds. He identified two -- malachite green and DS 9073 (Experimental bactericide/fungicide, ICI America, Inc. -- to be particularly effective. At the same time Armstrong et al., (1976) came up with two, namely, Trifluralin (L,L,L-trifluoro-2, 6-dinitro-N, N-dipropyl-p-toluidine) and Benomyl ("Benlate", methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate) which were effective against L. callinectes from C. magister. In SEAFDEC, Po et al., (1978) screened 34 antimycotic compounds against L. callinectes isolates from P. monodon and Scylla serrata. Based on the results, six loomed as potential chemotherapeutants. These are clotrimazole, crystal violet, econazole nitrate ("Pevaryl," 1/2-(2,4-dichlorophenyll-2(4-chlorobenzyloxyl)-ethyl/-inidazole nitrate), malachite green, trifluralin and Treflan R (23.1% Trifluralin).

Animal toxicity tests

The ideal chemotherapeutic in aquaculture situations is one which should inhibit the growth of the fungus and causes no harm to the crustacean larva. Whether or not a chemical is suitable may be borne out by results of bioassay experiments.

Results of experiments by Bland et al., (1976) shows that malachite green at concentrations of 0.006-0.012 ppm added for 24 hr had very little effect on larval survival for either P. californiensis, P. stylirostro or P. vannamei. Toxic effects were in fact not evident until a treatment of more than 0.06 ppm was used. P. stylirostris zoea were most sensitive. Similar bio-assays by Po et al., (1978) on P. monodon reveal that treatment level of 0.006 may be applied only in mysis stages. The other compound Bland et al., (1976) found effective, DS 9073, was not subjected to a bioassay but believed to be potentially suitable as it rapidly incapacitate zoospores even at concentrations lower than the toxic level.

Armstrong et al., (1976) considered trifluralin to hold promise for fungal control on the basis of a low effective concentration of 0.0015 mg/L. Furthermore it was toxic to Cancer magistrate larvae at a concentration 100 times larger for a selectivity ratio of 117.1 over 96 hrs.* Benomyl had an SR of 100 after 48 hrs and may be used over that time duration and not beyond.

Of the chemicals screened by Po et al., (1978) only malachite green and Treflan R were bioassayed. Results indicate the possibility for use of malachite green only in mysis. Treflan R (Gacutan, 1978; unpublished observations) concentrations higher than 0.1 mg/L were well-tolerated by M_1 for 96 hrs.

Lessons from other hatcheries

Chemotherapeutics in larval rearing should be used in conjunction with physical methods of excluding the propagule or agent of infection from the water such as filtration and/or sterilization of all seawater used during the rearing. Bland et al., (1978) believed that these methods entail considerable expense. Moreover, Fischer et al., (1975) used a malachite green dip in combination with ultraviolet irradiation in successfully preventing Lagenidium infection in Homarus americanus larvae. In one of the more successful P. monodon larval rearing facility in the tropics, Tahiti, AQUACOP (1978) uses as a routine protocol chlorinated water and a preventive antibiotic at 2 ppm and the antifungal Treflan EC**at 0.01 ppm, the latter dispensed from a dilute solution in a bottle continuously for 6 hours. It is claimed that this procedure assures an 80 percent survival to P_4 from N_1 at 28°C.

Arguments against addition of a phophylactic chemical

The exact or even appropriate time when infection starts is not commonly known. The experience in this laboratory indicates that histopathology in hosts and signs of the pathogen are apparent only when the disease is already widespread. This has also been observed in Tahiti (AQUACOP, 1977). Armstrong et al., (1976) observed that it was often

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*SR = EC_{50}larvae/Cone Toxic to fungus.
**Effective component is Trifluralin.

too late to save the portion of the larvae due to the endobiotic nature of the fungus. Everything thus points to a preventive approach.

The Tahiti group (AQUACOP, 1977) observed that fungal spores are attracted to dead eggs or dead larvae where they (spores) settle, germinate and reproduce rapidly. It was observed earlier (Bland and Amerson, 1973) that zoospores swim readily toward Callinectes sapidus eggs when placed in a glass slide. The same mechanism is believed to occur in penaeids. In addition, researchers postulate a naturally-occurring source of zoospore like the gills of the spawner which release the zoospores precisely for a collision between the two bodies (Bland, 1978 as cited by Lightner, 1978 on a visit to SEAFDEC) leading to infection.

The SEAFDEC Experience

The above postulates of Bland led us to try a different approach to Lagenidium. In numerous instances over the past 3 years, unhatched eggs were observed to have been infected when the larvae have already metamorphosed to the naupliar (N_6) and zoeal stages (Z_1). This convinced us to disinfect spawners with very high concentrations of Treflan R for an hour after an equal duration of thorough washing. None of the 28 experimental runs conducted showed signs of either Lagenidium or Sirolpidium infections (Gacutan, 1978). Of the 28, twelve (12) runs were successfully reared to the 5th day of the postlarval stages (P_5) for a total production of more than 6.224 M P_5's.

Future researches on Lagenidium, recommendations

Treflan R is better known as an herbicide. More of this kind of chemical must be tried. Another herbicide, 2,4-D (2,4-dichloro-phenoxy-acetic acid) was subjected to bioassays, and its effect on egg hatching and its ability to arrest epizootics caused by Lagenidium was assessed. It was well tolerated considering that the LD_{50} at 96 hrs was 0.6 mg/L for M_1. These values compare favorably to that for Treflan. Furthermore it was shown to be equally effective in arresting epizootics and minimally affected the hatching rate (Gacutan, unpublished data).

Bacterial Diseases

Locally, studies on this group of pathogens had been relegated in priority on account of the search for temporary solutions to fungal diseases. Lately however two kinds of minor diseases were recognized from a few hatchery runs of P. monodon.

Necrosis of appendages

The earliest symptoms ("browning" in the exoskeleton, pleopods, pereiopods, or telson/uropods were noticed as early as the Z_1. The browning spread progressively towards the base of the extremities leading

to necrosis and finally to erosion of the affected areas. Deaths have been attributed to invasion by secondary/opportunistic organisms after considerable cuticular injury and breakdown, or to the inability of the larvae to molt, as the old carapace is still adpressed to the musculature. The disease is seldom lethal but it could be serious if it starts early in the larval stages.

The etiology of the disease in P. monodon has not yet been worked out. However, shell diseases of this nature as in other penaeids were shown to have been caused by three genera of bacteria namely, the Chitinoclastic, Beneckea, Vibrio and Psuedomonas (Cook and Lofton, 1973).

Locally, the disease plays a minor role in hatchery mortalities presumably due to the ability of the larvae to tolerate the lesions. In Tahiti (AQUACOP 1977) where the disease is reportedly rampant, control is brought about by the application of an antibiotic mixture consisting of streptomycin-bipenicillin (2 ppm of the active product (AP), 2 IU/ml), erythromycin phosphate (1 ppm AP), tetracycline chlorhydrate (1 ppm AP), sulfamethazine (3 ppm AP), and furanace (0.1 ppm AP). At present only erythromycin phosphate at 1 ppm is being used for prevention to preclude possible toxic effects of the other antibiotics. Right after application the larvae react to the treatment; their feeding activities are resumed. Recovery is quick, and no trace of the necrosis is left after metamorphosis.

Frequent renewal of the rearing water, chlorination, or ultraviolet treatment of the water were ineffective as control measures. Filamentous bacteria, Leucothrix mucoz.

This is an ectocommensal bacterium which affects penaeids particularly those held in crowded volumes of water rich in organic and inorganic substrates such as phosphates and nitrates (Fisher, 1976) and with optimum temperature. It has been shown to be widespread, epiphytic in many marine animals (Overstreet, 1973; Johnson et al., 1971; Nilson et al., 1975; Steenbergen and Schapiro, 1976; Fischer and Nelson, 1977).

In P. monodon, L. mucoz has so far exhibited a distinct seasonality, affecting cultures towards the months of October to December. Its primary effect upon the host lies obviously on asphyxiation when attached to the gills, or on impairment of the molting process as the setae may be entangled by the filaments. The animal can cast off the infestation by molting.

Morphologically, the filaments are unbranched, attached singly to the cuticle, have a basal diameter of 2 mu and consist of septage chains of almost square-shaped bacteria (Couch, 1978). The bacterium lacks a differentiated holdfast; it does not penetrate the epicuticle and is apparently secured by an electron opaque mucouslike substance presumably secreted by the bacterium.

The use of antibiotics to prevent and treat the disease is a very popular alternative. The Tahiti group (AQUACOP, 1977) routinely uses an antibiotic mixture to control the disease. Cultures are treated every 3 days as preventive measure and with twice the dosage for treatment. Leucothrix in rock crab eggs and larvae responded to 4 mg/L streptomycin (Johnson et al., 1971); those in H. americanus to 10 mg/L neomycin (Steenbergen and Schapiro, 1976); those in C. sapidus eggs to 1 mg/L streptomycin and 1 mg/L penicillin (Fisher, 1976); and C. sapidus larvae to 100 mg/L each of streptomycin and penicillin (Fisher and Nelson, 1977).

Sudden mortalities; Vibrio/Aeromonas

Isolation studies from moribund shrimps in the SEAFDEC laboratory point to the prevalence of Vibrio-caused mortality. This genus and Aeromonas are widely known to cause many diseases in fishes.

The role that Vibrio plays in shrimp health is not clear. Vanderzant et al., 1970 for example isolated V. parahaemolyticus from white shrimp from the Gulf of Mexico. The pathogenicity of the organism on shrimp is not established although it is known to cause human gastro-enteritis in Japan and, possibly, in the United States (Krantz et al., 1969).

Natural waters especially those located in inshore regions are veritable depositories of bacteria or "gram-negative bacterial soups" (Couch, 1978) and therefore Vibrio and Aeromonas are expected to abound. Vibrio parahaemolyticus isolated from water have very short generation times (12-14 min) at high temperatures (39°C). It is believed that warm environments such as ponds might enhance the pathogenecity of bacteria to shrimps (Ulitzur, 1974).

Diseases Caused by Protozoans, Algae

Extensive daily monitoring of all hatchery runs of P. monodon larvae was carried out for 3 years. The most common protozoans encountered are the ciliates Zoothamnium, Vorticella and Espistylis. A gregarine, presumably Gregarina, and a green alga, Licmophora abbreviata Agardh are the other common pathogens. A suctorian, Ephelota gemmipara, a very powerful pathogen wrought havoc on cultures from March through June, 1976 and has not occurred thereafter.

Ciliates are observed to foul up the external surfaces of P. monodon by egg membranes or larval gills as was observed by Johnson (1974a) and Nilson et al., (1975) in C. magister and H. americanus. They are much more harmful to the larval stages as they can become entangled with the setae and may prevent ecdysis. In many instances the infestation were cast off by successful molt, but the hosts were reinfected, probably by contact with animals or exuviae.

These being not really lethal in local P. monodon hatchery operations, no control measures have so far been adapted. However, Johnson (1976) screened and found quinacrine hydrochloride (0.6 mg/L) to be most effective against Epistylis in juvenile P. setiferus. Three other chemicals -- chloramine T, quinine bisulfate, and quinine sulfate were also therapeutic.

More often than not, the type and extent of microbial fouling results from prior water quality (high nutrient load, high siltation, turbidity, low O_2 tension) rather than from activity of a specific microbial pathogen. Fisher (1977) went to the extent of suggesting the use of activated charcoal if only to reduce the nutrient load so as to limit microbial growth and avoid heavy epibiotic fouling.

Recapitulation

There are a few aspects that need to be dealth with especially now that there is a headway in the control of Lagenidium callinectes. Firstly, nothing much is known of diseases and causes of mortality the moment the P_5's are stocked into ponds. Secondly, the spawners should be viewed as a potential source of infection. These two areas of researches have to be explored further.

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