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Penaeid pathology in Israel : problems and research

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Abstract — A comparative search for the shrimp most suitable for mariculture in Israel has led to the import of exotic species. Unfortunately, these shrimp were carriers of at least three pathogenic viruses. Infectious Hypodermal and Hematopoietic Necrosis (IHHN) virus, Hepatopancreatic Parvo-like Virus (HPV) and Monodon-type Baculovirus (MBV) caused cross-infections among both imported and local penaeids. Since these episodes, general disinfection operations were carried out and quarantine measures were strictly enforced for any newcomers. However, penaeid shrimp culture is to date subject to persistent MBV infections. Aiming at developing more rapid and sensitive diagnostic methods, trials to initiate cell cultures from *Penaeus semisulcatus* were carried out. This in order to produce a suitable substrate for the *in vitro* replication of MBV and possibly other viruses and the production of a fast developing cytopathic effect. Explants derived from hemopoietic and ovary tissues and the lymphoid organ, which are regarded to possess high generative capacity, were used. Primary cultures of cells, presumably hemocytes or lymphocytes, were established and maintained for 3 weeks. Sub-culturing attempts, however, failed. Sporadic fusariosis was also diagnosed in *P. semisulcatus* cultures. Macroconidia and hyphae typical of *Fusarium solani* were observed encapsulated within a large number of hemocyte clots produced by the host. *F. solani* is not unknown in Israel where it occasionally affects vegetables and causes keratomycoses in humans. Whether any of these strains, that all fit the same taxonomic location, can be, or become under particular conditions, pathogenic to such a variety of distantly related hosts remains to be seen. The hazard shrimp farmers stand when handling infected shrimp is discussed.

The climatic conditions in Israel's southern region, where most of the mariculture industry is located, are characterized by a sunny weather for most of the year and a very short and mild winter. The Gulf of Eilat is the northernmost part of the Red Sea and represents a very particular tropical biotope, with water temperatures between 23 and 26°C all year round and salinity of 40 ± 1 ‰.

The need to compare different species of shrimp in the continuous search for those most suitable for mariculture in these environmental conditions has led to the import of exotic species of penaeid shrimp. Although the potential danger of introducing infectious diseases into Israel and the concomitant hazard of spreading them in the local aquatic habitat were considered, the etiology of most viral diseases was unrecognized until

a few years ago, and no particular precaution had been taken against them. In 1980 *Penaeus stylirostris* was imported from Hawaii and *Penaeus monodon* from South Africa. In 1981 another shipment of *P. monodon* was imported from the Philippines.

Their growth rate was often erratic and in general they performed poorly. Weak and unable to preen properly, the shrimp were frequently infested with fouling epibionts such as algae (Fig. 1), both benthic (*Enteromorpha sp.*) and planktonic (in particular diatoms), peritrich protozoans



Figure 1. — Fouling epibiontic algae growing on a diseased *P. semisulcatus*.

(*Zoothamnium sp.*) and filamentous bacteria (*Leucothrix*, prob. *mucor*). Repeated treatments with $KMnO_4$ occasionally alleviated the pressure on the shrimp by these epibionts. *Leucothrix* was found to be able to decompose urea. Its urolytic ability gives this bacterium a physiological and ecological advantage. In fact, in conjunction with the relatively high pH of the sea water, the requirement for urea can be replaced by ammonia. It can be assumed that *Leucothrix* requires ammonia as a nitrogen source for its respiration and growth. Since both substances are secreted by the shrimp, a logical explanation can be suggested for its rapid spreading as an epibiont pest whenever environmental conditions in the shrimp tanks deteriorate. *Leucothrix* growth was inhibited by 1 ppm of Flumequine. It was eventually realized that all these organisms were secondary invaders and the shrimp were actually infected with pathogenic viruses. *Penaeus monodon*-type baculovirus (MBV) was diagnosed in *P. monodon* and In-

fectious Hypodermal and Hematopoietic Necrosis (IHHN) virus was diagnosed in *P. stylirostris*. By then, however, IHHN virus had also infected both the MBV-infected *P. monodon* and *Penaeus semisulcatus*, the latter a local species, which were cultured in the same facilities. The entire stocks of MBV-IHHN infected shrimp were destroyed and the system was dried, cleaned, and re-stocked 6-8 months later with *P. semisulcatus*, which was successfully cultured for three consecutive generations (Colorni *et al.*, 1987).

The last shipment of exotic shrimp (*P. monodon*) arrived in Eilat from Kenya in January 1986. This time a quarantine unit, specially designed to prevent the possibility of releasing the shrimp or their associated pathogens into the environment, had been set up. It was located downstream in relation to all the other facilities, and outlet water that would normally reach the main sewer system and return to the sea was diverted to an earthen seepage well. The use of hand-nets and other maintenance tools was limited to this unit. A basin with disinfectant solution was placed at the entrance for immersing footwear, and strict instructions were given to the personnel in charge of handling the shrimp to wash with antiseptic soap before attending to other shrimps. All these precautions proved fully justified.

Single basophilic inclusion bodies, typical of Hepatopancreatic Parvo-like Virus (HPV), a virus pathogenic to many penaeids including *P. semisulcatus*, were detected in the epithelial cells of the hepatopancreatic tubules of the shrimp. Besides HPV, which causes atrophy and necrosis of the hepatopancreas, other pathological conditions were observed in these shrimp, such as the so called « Red Disease », characterized by massive inflammation and necrosis of the hepatopancreas.

At present, only *P. semisulcatus* is reared in our facilities. This species was caught as brood stock off Haifa Bay in the Mediterranean Sea. While no viral infections were ever detected in samples of shrimp freshly caught, once in Eilat (on the Red Sea), the shrimps often develop MBV. Its origin is unclear, and two theories have been formulated to explain its persistent appearance in our system. According to the first, the disease is caused by a local viral strain, present in a latent form in the wild shrimp and becoming symptomatic in the stressful culture conditions. According to the second theory, it is still the same virus introduced into the system by diseased exotic shrimp, that survived perhaps carried by other Crustaceans, such as barnacles, cirripeds, copepods, etc. which cohabit our shrimp tanks and ponds, or possibly due to its resistance in the environment.

Also, some peculiar inclusion bodies somewhat similar to, but not typical of, MBV, severe Hemocytic Enteritis, and abnormal hepatopancreas structures were occasionally observed in *P. semisulcatus*. Butylated hydroxytoluene (BHT), a dietary antioxidant widely used as a food preservative for its antioxidizing properties, has been found to be a potent inactivator of lipid-containing mammalian and bacterial viruses (Snipes *et al.*, 1975) and to protect chickens exposed to Newcastle Disease Virus (Brugh, 1977). Since MBV also contains a lipidic component, an experiment was set up with MBV-infected *P. semisulcatus* to evaluate the antiviral potential of BHT. Amounts of 0.5, 1.0, and 2.0 ppm of this substance were incorporated in the regular feed and the shrimp were observed for over

6 weeks during which periodical samples of hepatopancreas were taken for histological examination. BHT, however, apparently made the pellets so unpalatable that a high rate of cannibalism occurred in the experimental tanks, and no definite trend could be observed.

All of the viruses detected in Eilat are characterized by the formation of intranuclear inclusion bodies in the target cells. This finding is consistent and typical enough in location, morphology and staining characteristics to be considered a good diagnostic criterion. However, formation of inclusion bodies is not a constant characteristic for every virus. Furthermore, diagnostics of MBV through histology, malachite green staining, acridine orange fluorescence (Fig. 2), or simple smear, although useful when intranuclear bodies become detectable (at medium or high intensity infections), are inadequate when screening for early or carrier state infections (Diamant and Colorni, 1987).

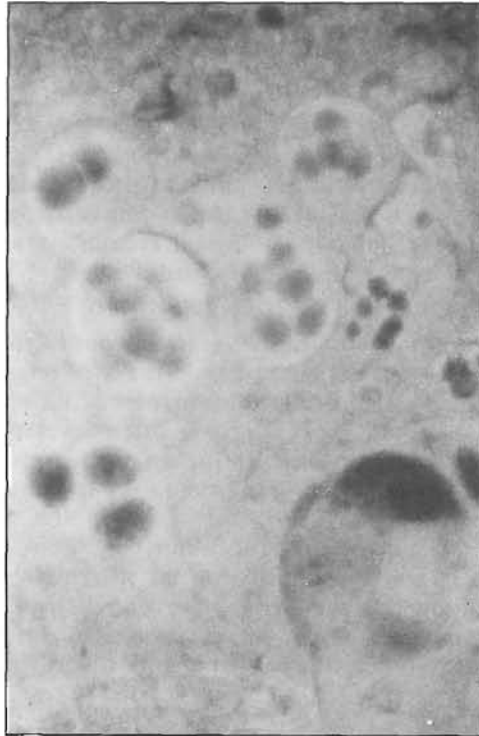


Figure 2. — MBV occlusion bodies as revealed by the acridine orange fluorescence method (courtesy of Dr. A. Diamant).

Development of sensitive detection methods for shrimp viral latent infections depends largely on the availability of purified viral particles. Crustacean *in vitro* cell culture which could support virus replication is still at a very early experimental stage. However, some preliminary trials to

initiate cell culture from our stock of *P. semisulcatus* in Eilat have yielded promising results (Rosenthal and Diamant, in press). Cultures were initiated from hematopoietic tissue/lymphoid organ and from ovarian tissue. Although these tissues do not support, at least *in vivo*, the growth of MBV as hepatopancreatic epithelium does, they typically possess high generative potential. Hepatopancreas in any case was found unsuitable for *in vitro* culture, due to its high content of lytic enzymes. The best results were obtained using cell culture medium M-199 supplemented with 15 % fetal bovine serum and 5 % heat inactivated shrimp hemolymph.

Streptomycin and kanamycin effectively prevented bacterial contamination. The cells migrated from the explants and formed fairly dense, though not confluent, monolayers (Fig. 3). However, no mitotic figures could be discerned. The cultured cells were maintained for 3 weeks, but attempts to sub-culture them were unsuccessful.

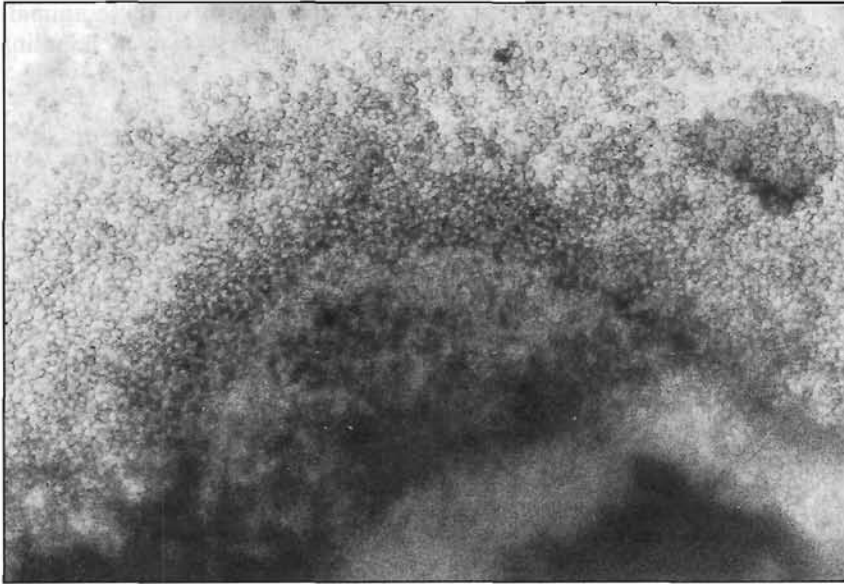


Figure 3. — Migrating cells from an explant of *P. semisulcatus* hematopoietic tissue/lymphoid organ (courtesy of Dr. A. Diamant).

The first case of shrimp fusariosis was diagnosed in Israel in 1988, in *P. semisulcatus* (Colorni, in press). An adult male from one of the rearing ponds displayed a large melanized lesion on one side affecting the cephalothorax and first abdominal segment.

The chitinous cuticula appeared degraded and the ulceration ran deep into the underlying tissues. Fungal hyphae and canoe-shaped macroconidia (Fig. 4) typical of *Fusarium solani* were observed, often encapsulated within a large number of hemocytic clots produced by the

inflammatory response of the host. Hyphae embedded in the underlying muscular fibers were surrounded by somewhat less or no inflamed tissue, suggesting their more recent growth and a gradual failure by the host to resist mycelial invasion. The report of this sporadic case in Israel extends the range of this mycosis to this geographical area. *F. solani* is not unknown in Israel where it has been associated with wilts and rots of dozens of plants. *F. solani* is also one of the most widespread causes of fungal eye infection in humans throughout the world. At least 3 cases of keratomycosis have been reported in Israel in recent years. Two cases were severe to the point that the infected eye had to be enucleated. Whether any of the strains above mentioned, which all fit the same taxonomic location, can become under particular conditions, pathogenic to such a variety of distantly related hosts remains to be seen. *F. solani* is a classic opportunist, and at least with regard to human pathology, is capable of producing disease only when carried into susceptible ocular tissues through an accidental injury to the eye or when the natural resistance to infection in the individual is abnormally low. In any case, since shrimps struggle and splash vigorously when they are harvested and superficial scratches and punctures by the prickly protuberances and appendages of these animals are a common occurrence, the hazard shrimp farmers face in handling infected shrimp should not be underestimated.



Figure 4. — Typical macroconidium of *F. solani* in *P. semisulcatus*.

Another fungus was detected in the eye stalk of a second shrimp from a different tank. The organ was reduced to a necrotic stump after experimental ablation had been performed to induce ovarian maturation and spawning. Histological sections stained with periodic acid-Schiff (PAS) clearly show presence of hyphae. However, the canoe-shaped macroconidia typical of *F. solani*, were not observed in this sample. Inoculation of pathological material onto Sabouraud, oatmeal and tellurite agars prepared with 25 % filtered sea water produced a slow growing salmon-colored mycelium similar to that of *F. solani*. Later though, basidia were produced and the fungus was thus identified as a basidiomycete. Fragments of mycelium grown on Sabouraud agar were inserted underneath the cuticula through a small cut between the first and second abdominal segments, but attempts of reinfesting the shrimp in this way all failed. Thus, the possibility of an external contamination during culture cannot be ruled out in this case, but it is noteworthy that, similarly to *Fusarium spp.*, the basidiomycetes also include many plant and insect pathogens.

Another frequent disease affecting the eyes of shrimp in Eilat appears as a « white-grayish patch ». Histological sections of the lesions show a

« shaft ». of atrophic tissue down to the reticular layers (Fig. 5). Larger lesions show a thickening of the eye cuticle and tissue atrophy down to the medullar layers (Fig. 6).

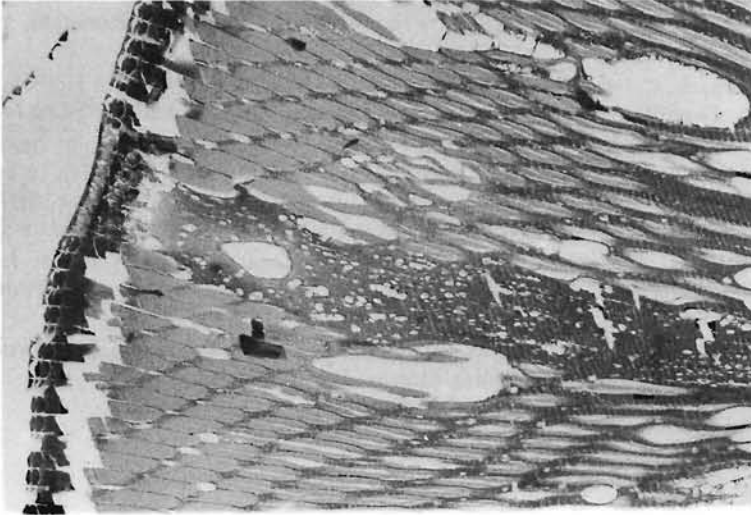


Figure 5. — « White-grayish patch » eye lesion, early stage.

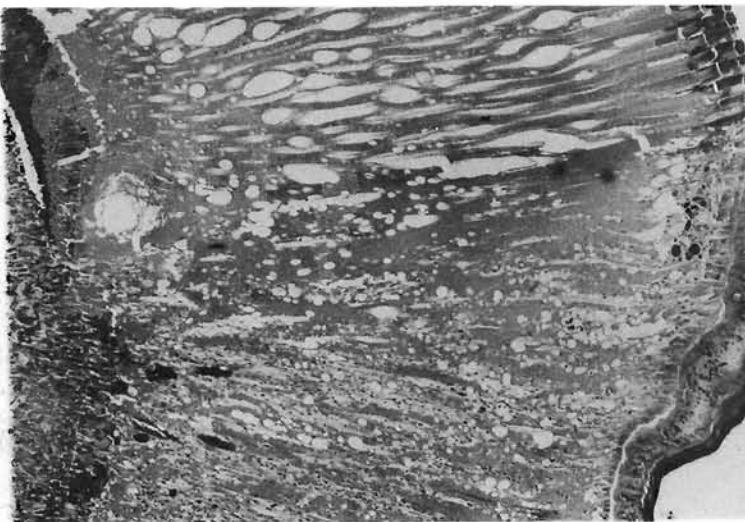


Figure 6. — « White-grayish patch » eye lesion, advanced stage.

This condition is similar to that described by Laramore et al. (1977) in *Penaeus vannamei*. The etiology of these lesions is still unclear, but apparently these atrophic tissues present a suitable substrate to secondary infections by *Fusarium*.

- Brugh, M. Jr., 1977.** Butylated hydroxytoluene protects chickens exposed to Newcastle disease virus. *Science*, **197** : 1291 - 1292.
- Colorni, A., (in press).** Fusariosis in the shrimp *Penaeus semisulcatus* cultured in Israel.
- Colorni, A., Samocho, T., Colorni, B., 1987.** Pathogenic viruses introduced into Israeli mariculture systems by imported penaeid shrimp. *Bamidgeh*, **39 (1)** : 21 - 28.
- Diamant, A., Colorni, A., 1987.** Viral infections of penaeid shrimp in Israel and use of acridine orange fluorescence as a rapid diagnostic method. Third Internatl. EAFP Conference, Bergen, Norway, 31 Aug. - 3 Sept., 1987.
- Laramore, C.R., Barkate, J.A., Persyn, H.O., 1977.** *Fusarium* infection in eyes of mature shrimp (*Penaeus vannamei*). Texas A&M University, Texas Agricultural Extension Service, Fish Disease Diagnostic Laboratory, Publ. # S9.
- Rosenthal, J., Diamant, A., (in press).** *In vitro* primary cell cultures from *Penaeus semisulcatus*. Proc. 3rd Internatl. coll. on Pathol. in Mar. Aquacult. (PAMAQ), VIMS, Gloucester Point, VA, U.S.A.
- Snipes, W., Person, S., Keith, A., Cupp, J., 1975.** Butylated hydroxytoluene inactivates lipid-containing viruses. *Science*, **188** : 64 - 65.