Disease problems in farmed penaeids in Italy

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Abstract — The industrial farming of penaeid shrimps began in Italy in the early eighties with the introduction of Penaeus japonicus, that replaced Penaeus kerathurus.

In Italy there are no farms for producing eggs and fry on a commercial basis, the restocking material is usually imported from abroad at the stage of post-larval 22-25.

The growing normally takes place from May to October in earth ponds at shrimp concentrations ranging from 1 to 30/m².

Various pathologies were found that appeared to be connected with husbandry techniques, such as overfeeding with low water exchange, overcrowding and poor water quality.

Our laboratory diagnosed diseases caused by:

1. — VIRUS. Infectious pancreactic necrosis (IPN) virus was isolated on EPC-RTG2 cells from spawners without apparent mortality. Biological tests were carried out placing post larvae into infecting baths, with $7 \times 10^7$ cfu for 60 min. The infected individuals presented locomotor ataxy without mortality; histological examination showed cell vacuolization of the tubules of the hepatopancreas.

2. — BACTERIA. The most frequently isolated bacterium was Vibrio anguillarum, on TSA, marine agar and TCBS. Vaccination trials were carried out utilizing a commercial vaccine, with infection tests after 30 days. Post-larvae were bathed for 60 min. in an infected solution of $66 \times 10^7$ cells. The vaccine seems to give a good protection.

3. — PARASITES. Serious infections of the gills were seen caused by zootamnium, a peritrichous ciliated protozoan that was satisfactorily controlled with permanent baths of formalin at a concentration of 20 ppm.

4. — FUNGI. Very few infections by Fusarium solani, causing lesions in the eye, were diagnosed.

INTRODUCTION

A great interest in sea water crustacean rearing has developed in Italy in the last few years. After some experiences on Penaeus kerathurus the...
interest has now shifted to *Penaeus japonicus* and *Penaeus monodon* because they appear to be more suitable for our climatic and environmental conditions. In fact, *P. japonicus* in manured earth ponds without artificial feeding can grow up to 28-30 g in 4 months (from June to September), *P. monodon* 10-20 g more in the same time.

Since in our country it is not possible to produce restocking material for production purposes, we depend totally on foreign countries. In 1988 we have imported *P. japonicus* and *P. monodon* postlarvae especially from France, Spain and China. The sanitary control at the customs is non-existent practically based on the health certificate of the country of origin. This involves the danger of importing any kind of diseases. We know that baculovirus disease has caused serious problems in some foreign areas of production and therefore our attention must always be directed to importation of juvenile stages only from surely disease free areas or farms.

**PURPOSE OF THE STUDY**

The main purpose of this study has been to know the actual sanitary situation as regards shrimp culture in order to programme a suitable plan of prophylaxis. In the activity of our laboratory we have tried to locate the pathological conditions which occur in any kind of shrimp farming. Where possible, we have tried to programme a study of virus and bacteria pathogenicity.

In particular we have studied:
1. the pathogenicity of infectious pancreatic necrosis virus (IPN) isolated from spawners on juvenile stage;
2. the pathogenicity of *Vibrio anguillarum*, the most widely encountered bacterium in sea water fish and shrimp farms; we have also tried to plan an active immunization (vaccination) against this agent; we must recognize that there is little experience on the resistance mechanisms of the invertebrata; our trail had no scientific object but only a practical one to assist shrimp farmers in their problems.

**STUDY ON KNOWLEDGE OF SANITARY SITUATION:**

**MATERIAL AND METHODS**

We planned a series of visits to 11 shrimp farms located throughout Italy in the period 1986 to 1988. Farming is carried out in earth ponds, manured or not, almost always without artificial feeding, at shrimp density varying from 0.5 to 3-4 for m$^2$; water height is 80-120 cm, water exchange very poor and depending on the tide. Some trials were also tried at higher density, up to 30 shrimps for m$^3$ in small tanks but were unsuccessful.

During each visit to the farms we tried to spot weak animals; anyway, at least 20 shrimps were sampled and subjected to necroscopic tests in order to find type and location of possible external lesions. Water samples were analysed to determine the main parameters (temperature, dissolved oxygen, BODS, salinity, phosphates, nitrites, turbidity, etc...) to find
possible relationships between environmental conditions and pathological aspects.

The sampled shrimps were immediately placed into thermic containers to bring them still alive to the Fish Disease Laboratory, where
microscopic tests were carried out on gills for parasites and cultural tests were carried out from hepatopancreas for the isolation and identification of bacteria and virus.

Microbiological tests for bacteria were carried out on the following media:

- TSA
- TSA NaCl 3.5%
- TSA NaCl 7%
- TCBS
- Ordal
- Mac Conchey
- Marine Agar
- Pseudomonas Agar Base
- Vibrio Selective Agar
- Antibiotic Sulphonamide Sensitivity Test Agar
- O-F Basal Medium

Microbiological tests for mycetes were carried out on the following media:

- Sabouraud glucose 4%
- Selective Agar for mycetes
- Mycosel
- Mycosel Agar
- Littman Agar

Virus isolation was carried out on RTG2 and EPC cells, virus identification through indirect immunofluorescence.

**Results**

The following pathological forms were found.

1. *Chitinolitic bacteria* were present almost in all samples with external lesions, with prevalence of *Vibrio* sp. and *Alcaligenes* sp.. The lesions caused by these bacteria can be of different seriousness, and are normally restricted to modest area of erosion in the abdominal part of the exoskeleton.

These lesions were almost constantly reported during the whole period of the study, but usually they were not serious. We did not think it was necessary to treat this pathology, because of the low percentage of affected animals and also the low number of individuals per unit volume.

2. *Ciliate protozoa* were responsible for heavy losses in the farms at higher stocking density (up to 30 shrimps per m³) and where water exchange was almost inexistent, depending only on the tide. Microscopic test on gills of examined individuals showed numerous colonies of *Zoanthamnium* sp.. This is a peritrich ciliate protozoan with branched colonies, which interferes in gaseous exchanges.

Shrimp behaviour was the main sign of the condition; many individuals swam on the surface of the water during the day or remained still near the edges of the pond during the night. In this pathology we found that the water had high pH and BOD values and there was notable presence of nitrogen and phosphorus.
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We also carried out disinfections with formalin (20 ppm for permanent baths) which proved effective to eliminate these parasites.

3. *Vibrio* sp. was more frequently isolated from stressed shrimps, sometimes in association with other bacteria, such as *Aeromonas* sp., *Alcaligenes* sp., and other opportunistic bacteria.
The lesions consisted of reddened areas on the caudal part of animal; blacksening areas were also sometimes present on the gills.

4. *Virus*: IPN virus was sometimes isolated from adult individuals; the presence of *Baculovirus* was never detected.

Environmental factors were of remarkable importance in epizoology; in fact in some cases excessive salinity (more than 40 %) and high number of individuals (more than 30 shrimps per m³) greatly impaired shrimp growth.

Still more important was the oxygen level which, in some particularly eutrophized basins, reached levels of 1-2 ppm in critical hours. This caused survival problems and favoured occurrence of *ciliate protozoa* and *chitinolitic bacteria* on shrimps.

**Discussion**

Tests carried out during the study period have not showed big generalized pathological problems. Although at the moment these problems can be controlled with a suitable environmental management, we can foresee an increase of their incidence with the spreading of intensive culture methods and therefore with higher stocking density per unit volume.

**BIOLOGICAL TESTS**

*Pathogenicity test of IPN virus on shrimp larvae*

IPN virus isolated from *Penaeus japonicus* spawners was used. This virus is frequently encountered in aquatic environments and is of remarkable importance in salmonid juvenile stages.

**Material and methods**

We have used 2000 postlarvae PL30 provided by the laboratory of the Italian National Research Council (NRC) and acclimatized in tanks of the Fish Disease Laboratory: salinity 25 %, temperature 25°C, recycle with Eheim filters, feeding an experimental feed B 1000 µ supplied by the Italian NRC.

The shrimps were divided into two groups:

1) the *control group* was placed for 60 min. into a container with 1 l of water to which 100 ml of Eagle's MEM medium for virus growth were added.

2) the *infected group* was placed 60 min. into a container with 1 l of water to which 100 ml of Eagle's MEM medium containing $7 \times 10^4$ vfp (+)/ml of virus, with a final concentration of $7 \times 10^4$ vfp (+)/ml, were added.

In the following days no mortality was noted in any tanks. A particular form of locomotory ataxy of the infected shrimps was seen, characterized by long periods of stasis on one side, followed by sudden movements with return to the normal position.
After 12 days, some of the infected individuals were fixed in 50% glycerin and others in Davidson's for virus isolation and histological preparations, respectively.
Results

Investigations carried out by indirect immunofluorescence after growing in EPC and RTG2 cells never showed presence of virus, which evidently was reproduced and eliminated in a short time.

From the histological point of view we found differences in the structure of the hepatopancreas between infected and control animals. In the infected shrimps we noted a slight vacuolization of the tubuli cells.

Discussion

On the basis of infection tests on adult shrimps carried out with the same virus and the data reported in literature on crustacean virosis, we think that IPN virus has a limited pathogenicity. It is largely conditioned by environmental factors and can be of pathological significance in juvenile individuals, in which it can cause serious structural and functional degenerations of the hepatopancreas. It may be that the reduced pathogenicity of the IPN virus was caused by the high water temperature (25°C) during the trial.

TEST OF INFECTION BY *VIBRIO ANGUILLARUM*

We wanted to verify the pathogenicity of *Vibrio anguillarum* in shrimps; this facultative pathogen is the most frequently encountered bacterium in brackish and sea waters, and can be responsible for serious pathological conditions in sea water fish and shrimp culture.

Material and Methods

A stock of *Vibrio anguillarum* isolated from shrimp and reactivated through intra-abdominal injection in adult shrimps was used to achieve infection. After 24 hours shrimps treated in this way were dead and pure *Vibrio anguillarum* were reisolated by cultures from hepatopancreas.

We used 2000 postlarvae PL25 acclimatized in tanks of the Fish Disease Laboratory: salinity 25 %, temperature 26°C, recycle with Eheim filters, feeding an experimental feed B 1000 µ.

Shrimps were divided into two groups:

1. the control group was placed for 30 sec. into a container with 9 l of water to which 1 l of Tryptone soya broth were added.

2. the infected group was placed for 30 sec. into a container with 9 l of water to which 1 l of Tryptone soya broth with a culture of *Vibrio anguillarum* containing $35 \times 10^7$ cfu (+)/ml, with a final concentration of $35 \times 10^6$ cfu (+)/ml were added.

In the following days we noted a serious mortality in the infected shrimps, which in the period of one week reached up to 80 % of the individuals.

The main signs of the condition were non-feeding, motory ataxy, lethargy and finally death, with sometimes reddening of the caudal part of the abdomen and telson.
Photo 7. — Branchial blackening in individual with marked infection by *Zoothamnium*.

Photo 8. — White individuals, probably fed in an ascorbic acid deficient diet.

From the hepatopancreas of dead shrimps microbiological cultures were carried out on Tryptone soya agar, Marine agar and TCBS.

All cultural, microscopic and biochemical tests always confirmed the purity reisolation of *Vibrio anguillarum*. 
The biochemical characteristics of the isolates are schematized in Table 1.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
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<tbody>
<tr>
<td>TSA 22-25°C</td>
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<tr>
<td>TSA 37°C</td>
<td>+</td>
</tr>
<tr>
<td>TSA NaCl 3,5 % 22-25°C</td>
<td>+</td>
</tr>
<tr>
<td>TSA NaCl 7 % 22-25°C</td>
<td>−/+</td>
</tr>
<tr>
<td>TCBS 22-25°C</td>
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<tr>
<td>Gram</td>
<td>−</td>
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<tr>
<td>Motility</td>
<td>+</td>
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<tr>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
</tr>
<tr>
<td>Glucose (gas)</td>
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</tr>
<tr>
<td>Nitrate reduction</td>
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</tr>
<tr>
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<td>S</td>
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<tr>
<td>Galactosidase</td>
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<tr>
<td>Arginine dihydrolase</td>
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</tr>
<tr>
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<tr>
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<tr>
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<td>−/+</td>
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<tr>
<td>Arabinose (A)</td>
<td>−/+</td>
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</table>

Table 1. Biochemical characteristics of *Vibrio anguillarum* used for the test.

**Discussion**

The result of the infection test and the demonstration of the actual pathogenicity of *Vibrio anguillarum* in shrimps, show the particularly significant role that this bacterium can have in this kind of shrimp farming.

**VACCINATION TEST AGAINST VIBRIO ANGUILLARUM**

Considering the demonstration of the pathogenicity of *Vibrio anguillarum* and the difficulties which may arise from the need of therapeutic treatments with chemioantibiotics in pond rearing, we have thought it useful to try active immunization (vaccination) of postlarvae.

There are few bibliographic data on the immune system of shrimps which report on the type of immunity and period during which this immunity persists.

We based our trials on personal communications by foreign colleagues who applied the same method of immunization normally used on fish.
and which in shrimps would give an immunity limited in time but sufficient to cover the period of 4 months required in our country for bringing shrimps to market size.

**Photo 9.** — Stalked colonies of *Zoothamnium* (scraping from exoskeleton).

**Material and methods**

We used 500 postlarvae PL30 acclimatized in tanks of the Fish Disease Laboratory: salinity 25‰, temperature 24°C, recycle with Eheim filters, feeding an experimental feed B 1000 ℮ supplied by the Italian NRC, subsequently replaced by mussel meat.

Shrimps were divided into two groups:

1) the **control group** was dipped for 60 sec. in a container with 1.5 ℮ of water.

2) the **vaccinated group** was dipped for 60 sec. in a vaccinal solution consisting of 1575 ℮ of water and 175 ℮ of a commercial vaccine against vibriosis; we followed the method used for trout vaccination.

After 30 days, time sufficient for the formation of antibodies in trout, we carried out infection test using the same stock of *Vibrio anguillarum* used for the preceding test.

Both vaccinated and control individuals were then placed for 30 min. in a container with 4.5 ℮ of water to which 500 ℮ of infectant broth (Tryptone soya broth with *Vibrio anguillarum* culture) were added containing $66 \times 10^6$ cfu (+)/ml, with a final concentration of $66 \times 10^6$ cfu (+)/ml.

Mortality of control group began on the first day after infection and reached 30% after 9 days. By cultures carried out on dying and dead shrimps it was always possible to reisolate *Vibrio anguillarum*. 
Mortality of 14% occurred also in the vaccinated group, but *Vibrio anguillarum* was reisolated only sporadically.

**Discussion**

In this experiment the results are incomplete and need further verifications. Infection was only partially successful and we were not able to understand the 14% mortality in the vaccinated group.

**RECOMMENDATIONS AND SUGGESTIONS**

The type of penaeid farming carried out in Italy has made an exhaustive study of the pathologies usually occurring in intensive culture impossible. Nevertheless, some pathological conditions were observed, the one caused by bacteria being the more serious.

Farmers are greatly interested in this culture which offers remarkable profits, limited biological risks and very short production cycles. Unfortunately, Italy, like many other countries interested in the culture of these crustaceans, cannot produce juveniles and all restocking material must be imported.

That being stated, strict sanitary inspections on restocking material are necessary at the borders. It is desirable as well that protected areas be arranged where shrimp cultures for the production of livestock for restocking are free from contagious diseases material should be transmissible disease transferred.

This meeting could be the starting point for planning the necessary measures to obtain protected areas, bearing also in mind what the Commission of European experts is doing in view of the elimination of the frontiers among the European Economic Community Countries in 1992.

Similar diagnostic techniques with standard antigens and sera should be adopted by the reference laboratories of the various countries. This could be a good opportunity to start an International Reference Centre on sea crustacean pathology.

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