ADVANCES IN TROPICAL AQUACULTURE. Tahiti, Feb. 20 - March 4, 1989.
AQUACOP. IFREMER. Actes de Colloque 9 pp. 233-236.

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Infectious pathology in mollusc and shrimp hatcheries

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Abstract — *Development of aquaculture has been linked to zootechnical mastering of larval production in hatcheries.*

Now, expected progression will depend upon rearing new species or strains (hybrids, polyploïds, etc.) and reducing the risk of introducing pathogens (closed-circuit, biological filter, etc.). But, like in all animal or vegetal production, pathogens must always be taken on account as a potential risk. The importance and frequency of infectious diseases in hatcheries of molluscs and shrimps are poorly estimated in broodstocks and larval productions because of general lack of data on epidemiology and exact determination of the causes of mortalities. In some cases, viral, bacterial and fungal agents were involved in severe mortalities. It appears therefore necessary to develop methods suitable for qualitative and quantitative diagnosis of pathogens and for use in hatcheries (miniaturized systems for marine bacteria identification and quantification; enzymatic and fluorescent virus-immunoassays). The availability of these diagnostics as « kits » may be of major importance for zootechnicians in order to quickly detect and identify a pathogen. By this way, they would be able to elaborate prophylactic measures and regularly check sensitive points in hatcheries.

Regular and excessive use of antibiotics (antifungal or antibacterial) may thus also be reduced, permitting to limit release of these products in sea-water and, consequently, avoiding progressive selection of resistant-strains. At the same time, sea-water adapted formulations (microemulsions, etc.) of active products may lead also to decrease used-amounts and to improve efficiency of treatments.

Development of aquaculture has been linked to zootechnical control of larval production in hatcheries. Thus, for most economically important species, have become more independant of environment for regular supply of larvae. But this independance is relative, in so far as the hatcheries are more or less directly connected with their surroundings. This feature, which is essential concerning physico-chemical quality of sea water, appears primordial relating to pathogens due to the risk of epidemic outbreaks. Besides this direct sea-water pathogen introduction, broodstock, algae and *Artemia* may be a secondary contamination route and even an amplification step. Epidemiological investigations of mortalities occurring in hatcheries are still limited but sufficient to point out the involvement of infectious agents. Their actual impact is probably underestimated due

to the lack of adapted methods suitable for pathogen diagnostic by non-trained staff. It must also be emphasized that some « psychological » reticence expressed by stock-breeders and zootechnicians to take into account the pathological hypothesis during mortalities.

Most frequent and drastic pathogens involved in hatcheries are fungi, bacteria and viruses.

Among fungal agents, *Lagenidium* and *Fusarium* are worthy of notice for their pathogenicity in shrimp larvae but also juveniles and broodstocks. The spreading of spores by sea water and by aerosols and a supposed wide hosts range ensure fungi continuance.

Bacteria are ubiquitous microorganisms present in sea water, broodstocks, algae, *Artemia* (dessicated cysts and cultured nauplii) and finally in larvae tanks. *Vibrio* and *Aeromonas* (Vibrionaceae) are the most frequently determined species associated with mortalities. Unlike human and veterinary medicine, where some true pathogenic species are identified and alone to be considered, the bacteriological data are more confused in aquaculture. This fact is due to the absence of adapted tools for standardized biochemical identification and for rapid and automatized enumeration of bacterial flora in normal and abnormal breeding cycles. Moreover, when bacteriological investigations are performed in hatcheries, they generally concern sea water samples and thus may be unreliable, as some *Vibrio*-like bacteria need to be associated with a biological support like the cells of intestine. This binding capacity must be related to oligomeric protein production, by some *Vibrio* species, these proteins including toxic and binding subunits.

Among mollusc mortalities in hatcheries, only the oyster velar disease of *Crassostrea gigas* has a known viral etiology. This supposed iridovirosis was discovered through obvious macroscopical infection symptoms and easy light microscopic detection of viroplasms and even viral particles (340 nm). The potential risk of this disease for worldwide oyster farming must be seriously considered, keeping in mind, first the total disappearance of the Portuguese oyster *C. angulata* from atlantic coasts subsequently due to an Iridovirus epidemic, and secondly the transovarian transmission demonstration for similar viruses of insects. It may be assumed that more systematic and more sophisticated analysis (electron microscopy) performed during severe mortalities will lead to detection of other virus types yet identified in different molluscan species adult stages.

The role of viruses in crustacean hatchery mortalities is better studied. Baculoviruses, easily detected by light microscopy after inclusion bodies formation, are responsible for large epidemics, sometimes on a nation-wide basis in productive countries. Picornaviruses and Parvoviruses are also involved in mortalities. Transmission modalities are poorly understood because of the lack of experimental pathology methods and qualitative and quantitative diagnosis techniques. Nevertheless, transovarian transmission is supposed for Baculoviruses and alternate hosts, such as *Artemia* which are associated with several virus types, are possible.

A common opinion stated by hatchery zootechnicians concerns the dependance on stress activation of viral infection triggering. This feature, more assumed than experimentally demonstrated, is however essential to consider because it suggests that infected hatchery produced larvae, when

taken out of hatcheries, become healthy carriers or an infectious source in breeding areas.

Confronted to epidemics, hatchery conceptions and zootechnical processes have quickly evoluted trying to develop prophylaxis : individualization of units and tanks to limit infection spreading; systematic antibiotic treatments to prevent bacterial proliferation. More recently, progresses aiming at increasing the environment-independance have been related to biological filters and broodstock management.

In order to insure more reliable aquaculture productions, it is now advisable that zootechnicians and pathologists devote together their energies to the improvement of larval productions in hatcheries which are the initial steps of the breeding system.

Fungal diseases due to *Lagenidium* are relatively well-controlled using Treflan preventive treatment, and a recently elaborated microemulsified formulation is effective against *Fusarium conidia* propagation. A macroscopic and individual examination of breeders is useful to avoid hatchery introduction of highly infected and contaminating animals.

The prioritary researches in the bacteriology field aim at developing adapted tools for routine bacteriological hatchery surveys. Classical methods, using Petri dishes with solid media, appear indeed expensive, time consuming (preparation, inoculation) and reading is much too delayed (one to a few days) considering to bacterial proliferation time. Thus, a bacteriological unit in hatcheries is in practise non-operating. Miniaturized systems ready for use are in progress for quick total and *Vibrio*-like bacteria quantification according to the most probable number method. Such systems must permit a daily control of larvae tanks and of different crucial points (filters, algae, etc.). Any change in bacterial populations could be precocely detected, allowing a prompt intervention. In such a perspective of available « bacteriometer », it would be possible to conceive some changes in antibiotherapy. Applying antibiotics only when and where necessary would be of particular interest in reducing antibiotic use. This concept becomes urgent facing the aquaculture development and political will for antibiotic and chemical regulation.

Such antibiotic decrease may also be facilitated by the elaboration of special formulations with the following items :

- microemulsified formulations permitting easy and quick solubility in sea water whatever the spontaneous solubility of antibiotics. These kinds of formulations are well adapted to simultaneous disinfection of tanks and contaminated animals (external and also inside the digestive tract of molluscan and crustacean larvae). Moreover these formulations allow precise adjustment of selected concentrations avoiding any excess of non-soluble and residue-forming antibiotics.
- availability of a panel of antibiotics with different modes of action in order to reduce constant and similar selection pressures for resistance. It is important to keep in memory the extraordinary adaptation capacity showed by bacteria to resist antibiotics (detoxifying enzymes, transposon-mediated amplification, intra and interspecific plasmid-mediated transfer).

— suitability of bactericidal antibiotic formulations, alone or in association. Such formulations, carried out when an abnormal bacterial outbreak is detected (bacteriometer), are adapted to a true elimination of bacteria. Indeed, bacteriostatic treatments promote growth of resistant bacteria, leading thus to a progressive selection of more and more resistant bacteria populations.

Progresses in aquaculture bacteriology will have to take inspiration from human medicine. Microsystems for exact antibiotic sensitivity determination would be easier for zootechnicians, compared to classical disc antibiogramme method. Elaboration of miniaturized systems, specially conceived for biochemical identification of marine bacteria, would also be useful tools for standardized characterisation of pathogenic strains. Worldwide exchange of epidemiological information would lead to identification of opportunistic and perhaps true pathogenic bacteria. Specific and sensitive diagnostic methods could then be elaborated.

Concerning virological problems, the only way of investigate is prophylaxis. Close relationships will be necessary between zootechnicians and pathologists, first to develop experimental pathology methods (isolation and purification of viruses, laboratory reproduction of diseases, qualitative and quantitative diagnosis methods). Then, it will be possible to develop hybrid production and individual selection programmes, refering specially to insect or plant virology. Indeed, taking into account the molecular basis of virus specificity (cell receptors) and pathogenicity, some highly-resistant or refractory strains can be selected in short time.

More long-term studies concern gene transfer methodology by which viral DNA-sequences integration in host chromosomes may lead to resistant transformation of the host (parasite-derived host resistance concept).

Awaiting resistant-strain selection and in order to avoid large hatchery epidemics and international virus spreading, adapted-diagnostic methods must quickly be elaborated (immunodiagnostics using monoclonal antibodies, DNA probe methods).

MOLLUSC PATHOLOGY
DISCUSSION

Hill — How do we speed up selection for resistance ? Also what are MAB's being developed for ? Another priority is development of molluscan cell lines. Is anyone considering transgenic oysters or genetic engineering for resistance ? First can we discuss black pearl oysters and their problems. Have you done any pathology ?

Weppe — We have sampled 800 animals for pathology. (Histology, bacteriology and virology).

Hill — What was the mortality rate ?

Cabral — About 50 %.

Hill — Over what time ? It could be slow mortality over a long time.

Weppe — It is very difficult to say. We have sampled with Grizel all the stages of diseased animals before they died; we have not found any histological modification but always the large lysosomes with some autolysis in the digestive gland. But we do not know if we sampled normal and healthy animals to make the difference.

Hill — Did you sample black pearl oysters in other country without mortality problems ?

Weppe — I sampled wild specimens in Fiji, where no mortality was reported. Histological aspect was exactly the same.

Mialhe — What do you think of the viral infection of *Pinctada maxima* ?

Grizel — The disease in Australian oysters is noticeable. We have observed large lysosomes, autosomes, and autolysis. Mussels under stress show the same abnormalities in the digestive gland. We have examined oysters after the graft and have found mal-formation of the shell. After 1986 we have not observed shell abnormalities. *Ruditapes philippinarum* have a brown stain and this can be reproduced by injecting bacterial isolates.

Mialhe — It is, however, impossible to see bacteria in shells with brown ring; they may be destroyed by fixation.

Lester — A brown nacre stain has been reported in oysters in Western Australia.

Elston — There is the possibility of a water-borne toxin. Mucus is a response to irritation. Perhaps that happened in 1985. I agree with Grizel you should concentrate on the grafting.

Weppe — Yes, the grafting is certainly responsible of an increasing of the mortality. But a large part of the mortality occurs before grafting.

Hill — What about genetic factors ?

Cabral — Madame Blanc from Montpelier did a genetic survey for polymorphism. No possibility of larvae crossing with different lagoons. There is a possibility it is inbreeding but she showed it is not the case.

Michel — She found a strong difference between the Marquesas and Tuamotu. May be the larval viability is low thus maintaining diversity in any lagoon.

Hill — Has spat collection altered and is this a selective process in itself ?

Michel — Yes. But in lagoons where there is no spat collection there are few wild larvae.

Hill — May be you are selecting for weakness. What is the economic cost ?

Cabral — 3 000 of 10 000 people on Tuamotu are involved, with up to $25 m just for exportation. In grafted oysters there is 40-60 % mortality with only 40 % pearl-formation rate and only 10 % of those good quality.

Hill — What sort of increase in production could be achieved if this mortality problem was overcome ?

Cabral — It could be more than doubled.

Hine — Have you considered zinc toxicity ?

Cabral — Yes we have considered it, but data do not suggest it is the cause of mortality.

Hill — Can we discuss selection for resistance ?

Elston — There is resistance to Malpeque Bay disease after 40-50 years. We should look at speeding up the development of resistance. If you have to wait a year to determine resistance it takes a long time to breed resistant strains. We should determine immunocompetence.

Hill — You could determine phagocytosis index, bactericidal capacity, and chemoluminescence.

Mialhe — Chemoluminescence is different in molluscs. There are few hemocytes. In *Crassostrea gigas* it can vary greatly. We still have to determine immunological factors. First selection cases involve protozoans, it is more difficult with viruses. *Bonamia*/oyster relationships are complex; what for example is the function of blood enzymes ? May be we should consider humoral factors such as interferon.

Hill — We need virus models.

Mialhe — We, in the United States, Europe and New Zealand, have a common problem and should work on this.

Hill — Ralph (Elston) what have we learned about Malpeque Bay disease immunology ?

Elston — It has not been properly studied. We should look at resistance by selecting after challenge.

Lester — Could different species of oyster be crossed ?

Grizel — We have tried it without success, we must now try cytogenetics.

Mialhe — Between genera it is not easy.

Lester — As the parasites are so specific it might be quicker than using selection.

Grizel — It has taken seven generations to develop resistance against protozoans.

Elston — Natural processes are too long.

Mialhe — We have found *Ostrea angasi* is more susceptible than *O. edulis* to infections, so crossing may not be an advantage. In short term we need resistance.

Elston — If you can change management, in the short time, this is what must be done. We should look at linkage or (genetic) traits.

Hine — Susan Ford at Rutgers has noticed MSX-resistant *Crassostrea virginica* are slow-growing, and our, apparently, *Bonamia* resistant oysters are also slow-growing. These could be undesirable linkages.

Mialhe — We must remain optimistic.

Grizel — We should try cytogenetic techniques, polyploidy triploidy, introducing the sperm head of one species into the egg of another.

Hill — Does anyone know if there are genetic studies in molluscs ? There are in fish. One group is trying to alter a gene that controls haemoglobin and the ability of trout to tolerate low oxygen levels.

Mialhe — There are many such techniques with insects, such as mosquitoes. Insect geneticists are trying to develop mosquitoes that are bad vectors for *Plasmodium.* There are similar developments in chickens and plants. It will be harder with protozoans and rickettsias. There are also many studies on the genetics of *Drosophila.*

Hill — There is obviously a gap in research.

Grizel — We are working to find molecules to fight pathogens. But it will take 2 years, 3 years...

Hill — Can we move to cell culture. Is anyone claiming and showing progress ?

Mialhe — Lee Ellis at VIMS has taken embryonic cells from *Crassostrea* and tried to insert ras genes, oncongens, into them. We have to know what genes are involved in neoplastic transformation.

Hill — How can we encourage more work on this ?

Elston — How would you (Hill) justify it ?

Hill — We have to fund « fire-fighting » at the moment. We need the problem before we can act.

Mialhe — It will be easier in shrimps. It has been easy to establish insect cell lines.

Elston — You need a whole variety of molluscan lines; we may not need advanced technology, we need to understand basics like nutrition better.

Hill — Can we move to diagnostic kits ? These techniques can be done by technicians and are rapid.

Grizel — We must focus on the most important pathogens like *Bonamia.* Haplosporidans in the United States and Europe are also important. *Marteilia* is also important. *Minchinia* is being worked on but we need better purification. The same problem exists with iridoviruses. DNA probes may be better than ELISA techniques.

Hill — What will be the next kit from France ?

Grizel — May be *Marteilia.*

Mialhe — We need to determine the pathogen's role of rickettsias first. There are marketing problems. The kits must be available at the right time. MAB's from neoplastic mussels are not a commercial prospect.

Hill — Kits are important as they standardize certification procedures. It gives greater uniformity.