DEDUCTION: A Research Project for Shrimp Farming Sustainability in New Caledonia

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ABSTRACT

New Caledonian shrimp farming feasibility studies started in the mid-seventies. Today ca. 2000 tons of *Litopenaeus stylirostris*, introduced in 1978, are semi-intensively produced each year. Since fifteen years, production has been impacted by two seasonal vibriosis, the "Summer" syndrome caused by *Vibrio nigripulchritudo* in relation to pond bottom and water column quality, and the "Winter Syndrome" caused by *V. penaeicida* in relation to drastic temperature drops . With the ambition of elucidating processes possibly leading to mortality outbreaks in ponds, Ifremer has been conducting a research program since 2003 under the DESANS project (2003-2006) and the present DEDUCTION project, equally funded by the South and North Provinces and by the Government of New Caledonia. This program has been designed in a multidisciplinary approach integrating the suspected multifactorial origin of shrimp mortalities. Environment quality in ponds (either sediment or water column) plays a major role in the shrimp ecophysiological status, influencing sensitivity to pathogens . DEDUCTION focuses on a better knowledge of phytoplankton composition and biogeochemical fluxes at the sediment-water interface in ponds. Impact of farm discharges is also investigated. In addition, a more fundamental work is conducted to understand the pathogenicity mechanisms of these two *Vibrio* species but also to prevent the occurrence of viral infections through a RNA interference approach. Markers of the ecophysiological status of broodstock, larvae, post-larvae and reared animals, such as enzymes testifying from an oxidative stress, are developed. Gain of performance (resistance to pathogens and growth) were obtained on hybrids of the New Caledonian and introduced Hawaiian strains. Finally the database Stylog is exploited in view of discriminating "good" from "bad" rearings on the basis of relevant indicators.

Keywords

Shrimp farming, Technical and scientific support, Vibriosis, Water quality, Pathology, Ecophysiology, Genetics, Zoosanitary monitoring and procedures, Multifactorial and multidisciplinary approach

1. INTRODUCTION

Blue shrimp *Litopenaeus stylirostris*, was introduced 30 years ago in New Caledonia by Ifremer (French Institute for Sea Research and Exploitation). Around 2000 tons of this top quality shrimp, with the three quarters exported, are semi-intensively produced annually by the 18 farms scattered along the West coast. After a period of fast growth, the production is now stabilized, far from the 5000 tons envisioned 20 years ago. The New Caledonian shrimp is indeed affected since the early nineties by two seasonal vibriosis. The first one, named Syndrome 93, is a cool season vibriosis caused by *Vibrio penaeicida*, that induces high mortalities in juvenile shrimp and whose geographical extension has led the industry to avoid winter crops to limit the impact of this syndrome [1] [2] [3] [4]. The second one, called "Summer syndrome" has occurred during the warm season in penaeid shrimp farms since 1997. This septicemic vibriosis, due to *V. nigripulchritudo*, has caused severe and recurring mortalities ever since [5] [6]. Since 2003, Ifremer has developed a research program to better understand the determinism of these vibriosis affecting the New Caledonian shrimp production. In a necessary multidisciplinary approach, results obtained between 2003-2006 showed that syndromes are due to a disequilibrium imbalance between the host (shrimp) and the pathogens (vibrios) under various environmental constraints [7]. Consequently better zootechnical practices, as compiled by Della Patrona and Brun [8] should minimize production risks. Beside syndromes, in 2007 the industry faced high mortalities in the New Caledonian hatcheries that severely affected the quantity of post-larvae provided to farms and thus mechanically the New Caledonian production by 20%. Therefore the DEDUCTION project 2007-2010, in line of DESANS for several aspects, reinforced studies conducted on larval stages also adopting a multi-disciplinary approach. In this paper we describe the main components of the project providing some concrete results and presenting perspectives.

2. RESULTS AND PERSPECTIVES

2.1 Shrimp farming and Environment

2.1.1 Eutrophication process in tropical shrimp aquaculture ponds and in coastal water receiving organic wastes

In shrimp ponds, feed supply raises with animal biomass and induces an increased eutrophication level in pond ecosystem. Several results indicate that the dynamic of this process could play a role in the vibriosis responsible for the summer syndrome mortalities which affect shrimp aquaculture in New Caledonia [9]. A higher variability of phytoplanktonic compartment, possibly implying a modification of the linkage between bacteria and phytoplankton compartments, was observed in a farm affected by this disease compared to another farm where the pathogen had been observed without any mortality event occurring. Thus, studying phytoplanktonic population compositions can provide valuable insight in understanding fine process in the pond environment.

Flow cytometry (FCM) has been used for almost three decades to investigate and determine rapidly phytoplankton abundance, structure and diversity. But its application to ecological studies dealing with aquaculture is very scarce [10]. Several studies recently conducted in New Caledonia with FCM have shown that picophytoplankton (< 3 µm) dominated the autotrophic compartment during the first part of shrimp rearing. Picophytoplankton was mainly represented by three populations, *Synechococcus*, picoeukaryotes and an unknown cell type showing an atypical new flow cytometric signature, with very low red fluorescence and light scatter properties. Abundance of each picophytoplankton population were exceptionally high, reaching concentrations well beyond 10^6 ml^{-1}. After around 50 days of shrimp rearing, picophytoplankton abundance in ponds suddenly decreased, as far as disappearingas the unknown type. Generally, after day 50, several abundant and short (2-4 days) nanophytoplancton blooms occurred until shrimp harvesting. Nanoplankton blooms were made of three different FCM cell types, showing increasing red and light scatter properties, and which were clearly related to highest chlorophyll biomasses measured in ponds [11] [12] [13].

Shrimp farming in New Caledonia typically uses a flow-through system with water exchange rates as a tool to maintain optimum hydrological and biological parameters for the crop. Moreover, the effluent shows hydrobiological characteristics (suspended matter, phytoplankton biomass and organic matter) significantly higher than those of the receiving environment [14]. Discharge effects were spatially limited and clearly restricted to periods of effluent release. However, high residence time favoured the installation of a feedback system in which organic matter was not exported [15]. Mineralization of organic matter led to the release of nutrients, which in turn, caused an increased eutrophication of this ecosystem. The study of the pico- and nanophytoplankton assemblages showed (i) a shift in composition from picophytoplankton to nanophytoplankton from offshore towards the coast and (ii) a shift within the picophytoplankton composition with the disappearance of *Prochlorococcus* and the increase of picoeucaryotes towards the shoreline. These community changes may partially be related to a nitrogen enrichment of the environment by shrimp farm discharges. Our results suggest that FCM is a straightforward method to monitor changes in water quality and determine if the management measures taken on land are having a noticeable impact on the ecosystems and water quality of the New Caledonia lagoon.

A molecular approach has been recently tested on a pondsample filtered through 5 µm, to investigate the heterotrophic and autotrophic diversity of the cell assemblage below 5 µm. This global method, consisting in DNA extraction, cloning, and ARNr 16S and ARNr 18S sequencing, allowed to highlight an unknown cell diversity within shrimp ponds. As an example, for autotrophic eukaryotes, nine taxa were shown among which 3 from Chlorophytes and 3 from Dinoflagellates belong to unknown or not described genus.

2.1.2 Sediment quality and fluxes at the sediment-water column interface

Rearing ponds are built on ca. ten hectare clayey grounds more or less productive and heterogeneous. When in operation unfavourable areas for shrimps may develop in ponds. Very important field sampling operations have been conducted since 2006 in order to determine the reliability of parameters that can be used in order to qualify the pond bottom quality status. Finding a pool of relevant indicators will help in discriminating a well managed pond from a pond where zootechnical procedures were not optimal. Ideally scale values of these indicators should be categorized to provide a classification of ponds as an incentive for better management such as food control, water renewal, oxygenation and dry-out. The operational feasibility of the potential biosedimentary indicators will be examined in view of their transfer on a routine basis.

To complete a synoptic view of the main processes taking place in a pond, a qualitative and quantitative description of the biogeochemical interactions between sediment and water and at the interface will be performed. To this end, oxygen and other relevant chemical species transfers will be quantified at the interface, and ideally modelled. Experiments will be conducted in mesocosms, aiming at defining the envelopes of physico-chemical conditions allowing to maintain the water-sediment interface in oxic and thus in favourable rearing conditions.

2.2. Characteristics of vibrios virulence and research on ARN interference for viral infection prevention

In New Caledonia, shrimp farmers face diseases of bacterial but also viral origin. In marine invertebrates, the lack of an adaptive immune system as well as the rearing conditions greatly reduce the zoosanitary measures that can be enforced to avoid disease expansion. To date, risk management mainly relies on preventative measures which include a better knowledge of these pathogens.

An approach aimed at 1) characterizing the toxic factors secreted by *V. penaeicida* as well as their molecular bases and 2) at investigating the molecular mechanisms implicated in the emergence of *V. nigripulchritudo* pathogenic strains is currently being developed through functional and comparative genomic analyses as well as host-pathogen interaction studies. Genotyping methods combined with experimental infection tests, performed on a collection of *V. nigripulchritudo* strains isolated from animals and shrimp farm environment, have brought to light a clustering of highly (HP) and moderately pathogenic (MP) isolates, excluding all the nonpathogenic (NP) ones. This correlation between taxonomic markers and pathogenicity suggests that, at least, some virulence genes are chromosomally localized. In addition, a suppressive subtractive hybridization approach comparing two strains of different virulence status allowed the identification of a plasmid (designated pSFn1) evidenced only in the HP isolates, suggesting that this element played a role in the emergence of HP [16]. Taken together, these results have opened several research perspectives and contribute to greatly advance our understanding of shrimp vibriosis.

In addition to vibriosis, shrimp farming industry is confronted to a viral disease due to infectious hypodermal and hematopoietic necrosis virus (IHHNV). First detected in *Litopenaeus stylirostris* shrimp from Hawaii in 1981, where it caused acute epizootics and mass mortality, IHHNV appears to have a world-wide distribution in either wild or cultured penaeid shrimp [17] [18]. The *L. stylirostris* variety cultivated in New Caledonia has developed tolerance to IHHNV. However, morphological deformities and size disparities have recently been reported in new caledonian shrimp, corresponding to a high viral load. To date, strategies to control the occurrence and spread of viral diseases in marine invertebrate remain scarce. However, recent studies have demonstrated that injections of viral gene-specific dsRNA/siRNA into shrimp inhibited viral replication and/or protected shrimp from viral infections [19] [20]. This phenomenon relies on RNA interference, a highly conserved nucleic acid-based mechanism [21]. Because this strategy has proven to be effective against three unrelated viruses, we are currently attempting to develop a similar approach to prevent/reduce IHHNV replication in shrimp.

2.3 Adulte and juvenile shrimps physiological status assessment

All living organisms are under constant attack from reactive oxygen species (ROS), which, if produced in excess, can lead to “oxidative stress” (OS) resulting to serious cellular damage. ROS are naturally produced in animals during normal aerobic metabolism [22]. Many stress conditions like temperatures at the edge of thermal windows of the species, hypoxia and/or pathogens (vibrio or virus) lead to an increased production of ROS resulting in OS inside the cell [23] [24] [25]. However, ROS production also comes as part of the immune defence system and plays an important role in microbicidal activity [26].

Our laboratory, in New Caledonia, measures OS by analyzing both antioxidant defences and damages as consequences of radicular attacks. Analyzed biomarkers are for (i) antioxidant defences: enzymes superoxyde dismutase (SOD), catalase (CAT) and glutathionperoxidase (Gpx), total and oxidized glutathiones, and TAS (total antioxidant status), and for (ii) oxidative damages: malondialdehyde (MDA) and carbonyl protein. The analysis of oxidative damages of nucleic acids is being developed by the Ifremer ecotoxicology laboratory in Nantes (Loire-Atlantique, France).

Measurement of antioxidant defences status and OS-related damages allows to assess physiological status and to study effects of rearing conditions (temperature, oxygenation, …) and/or effects of treatment (food, probiotics, …) on farmed shrimps.

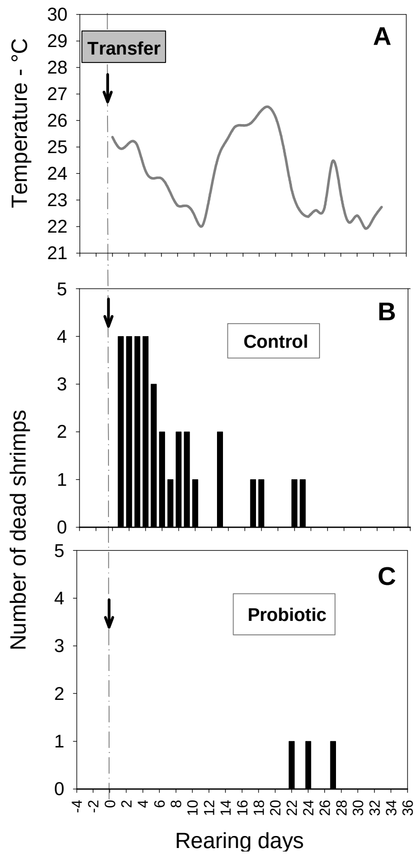

Figure 1: Evolution of temperature in experimental *tanks* (A), recovered dead shrimps after transfer into tanks for control (B) and for the probiotic treatment (C).

References [27] and [28] thus showed that infection by pathogenic vibrios which are incriminated in shrimp mass mortality in New Caledonia [5] [29] produce significant alterations in the antioxidant defence system and results in increased OS in *L. stylirostris*. However, these adverse effects are significantly reduced and shrimps showed better resistance to deseases when they are fed with probiotic *Pediococcus acidilactici* (Figure 1).

Beside probiotics our laboratory is interested in dietary antioxidants and rearing systems that could reinforce antioxidant status of the animal which in turn could better manage ROS attacks caused by various rearing stresses.

More recently, our work showed that antioxidant defences are not fully developed in early shrimp larval stages [30]. Therefore, egg and nauplii appear to partially depend on antioxidant defences transmitted by their mother through vitellus. Among these antioxidants we are studying some vitamins (C, E, and asthaxantine) and glutathiones. The aim of this research is to be able to enforce egg and nauplii antioxidant status *via* specific diet enrichment of female during their vitellogenesis.

Whichever considered stage, from larvae to broodstock through juveniles, physiological comfort of animals is a key condition for their optimal growth and feed use. Our OS researches should allow to define the conditions of farming the shrimp *L. stylirostris* in physiological comfort and thus to improve the output and the sustainability of this industry in New Caledonia.

2.4 New "blood" to improve survival and growth

Shrimp farming in New Caledonia relies on the culture of a strain of *Litopenaeus stylirostris* introduced from Mexico and then domesticated at a time when genetic principles were of little or no consideration. The strain appeared to be very inbred and with low allelic variability. Another population of *L. stylirostris* available in Hawaii was identified as an interesting source of domesticated genetic variability [31].

Since then, advances in agriculture and for some aquatic species of importance led caledonian shrimp farmers to reconsider the appropriateness of a genetic improvement strategy adapted to local biotechnical and economical constraints. The Hawaiian population was introduced in 2005 through quarantine facilities: as no pathogens were found in quarantine [32], Hawaiian animals were allowed to be transferred in earthen ponds to become breeders. The genetic strategy chosen to be tested in priority was based on the cross of the two different strains of *L. stylirostris* which were maintained separately. This conceptually simple approach aimed at eliminating inbreeding, the first genetic limiting factor of improvement in captive populations. Tests were conducted on pure Hawaiians, F1-hybrids and pure Caledonians during 3 years in multiple conditions with mixed tagged populations: grow-out in earthen ponds, grow-out in cage cultures, experimental infection with vibrios in controlled biosecure facilities.

The main results obtained in ponds during the first two years were consistent with those obtained in floating cages and following bacterial challenge: F1-hybrids demonstrated better growth and survival than the 2 pure lines, and this could be interpreted as the demonstration of either a heterosis effect or inbreeding in the 2 parental populations (Figure 2, [33]). Nevertheless, these results could not be reproduced during the

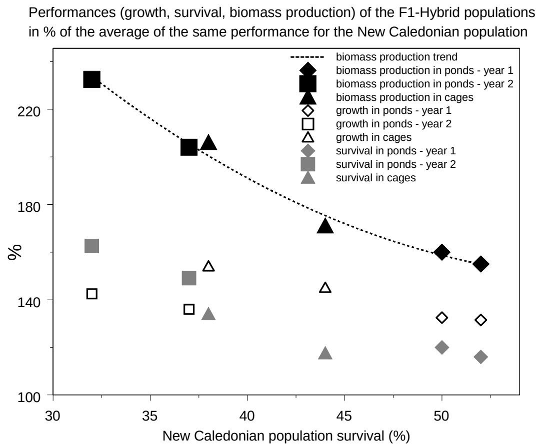

Figure 2. Relative performances of Hybrid-F1 population compared with the Caledonian population. Average values for survival, growth rate and biomass, obtained in ponds and cages, is expressed in % of the values observed in Caledonian shrimps (CC) for the same criteria [33].

third year of testing during which IHHN Virus induced unexpectedly very poor growth and survival rates (near 0%) in the Hawaiian line, while the F1 hybrids were mainly affected in growth. In the meantime the Caledonian line confirmed its tolerance to IHHNV. An hypothesis is that the Hawaiian line, which was checked free of virus at the time of importation, may have been contaminated during the first two generations and vertical transmission of the virus may have led to a very high prevalence and disease during the third year of testing.

The genetic strategy tested here which was efficient as long as the prevalence of the virus was low could not be transferred to the industry. Its implementation will need the development of a strategy of seed quality through an SPF program which will also protect the Caledonian line from any new viral disease which could arise locally. This strategy and organization, tested in New Caledonia, could possibly be of benefit to other small scale aquaculture activities in the Pacific islands.

2.5 Technical support

2.5.1 Health surveillance and monitoring programme

Nineteen shrimp farms, five hatcheries, and one experimental station are in operation in New Caledonia to date. In order to both meet the needs of the farmers and monitor the shrimp health status in New Caledonia, a health surveillance and monitoring program has been set up by the Direction des Affaires Vétérinaires, Alimentaires et Rurales de la Nouvelle Calédonie (DAVAR) in association with Ifremer. The aim of this network is to check up the health status of the industry, particularly regarding the diseases notifiable to the OIE, World Organization for Animal Health, and to investigate any abnormal mortality outbreak. In this plan of action, Ifremer is in charge of the clinical watch of the aquaculture operations. It acts as point of entry and conduct diagnostic investigations in relation with the New Caledonian government veterinary laboratory. In case of suspicion of notifiable disease outbreak, the official veterinary service takes the appropriate biosecurity measures. The information to farmers which is essential to ensure a good operation of the program is carried out by DAVAR in relation with the Shrimp Farmer Association (GFA). Beside this sustained health surveillance, the official agriculture services in New Caledonia (DAVAR) has carried out a shrimp virus monitoring programme since 2002 in accordance with OIE recommendations Covering all shrimp farms, this regular survey has allowed to draw a health status regarding diseases notifiable to the OIE. The implementation of this network allows an efficient surveillance of the New Caledonian shrimp health status by centralising the information and improving dialogue and communication between laboratories.

2.5.2 Development and valorisation of Stylog database

The Stylog software which gathers and displays crop information has been recognised as an essential tool for real time monitoring and has the potential to identify risks factors. Two sub-activities are derived from Stylog:

- Database management is essential with availability of technical support for use, maintenance and evolution of the system. It has developed for instance a “health watch” module gathering information on regular health status surveillance and abnormal mortality outbreaks. Stylog has slowly established itself among farmers management tools panel and a dedicated personnel has recently been employed by the Prawn Farmers Association (GFA) to relay the Ifremer staff on the field and to work jointly on the development of Stylog as part of a network.
- Data valorisation aims at cleaning datasets and finding crop quality indicators in order to give the farmers technical crop bulletins at the farm and industry levels. Automatically generated, these bulletins display raw data or graphs for several productivity and quality indicators comparing them to reference crops identified by the farmers. The possibility also exists to carry out specific analysis on particular data sets.

2.5.3 Promotion of biosecurity strategies

Latest developments on the new caledonian health status regarding IHHN Virus has led the industry and more particularly the Ifremer experimental station to seriously consider biosecurity principles and strategies. The aim of this program is to lower the IHHNV incidence and to produce healthy shrimp for research. To this end, the risk analysis and HACCP principles are used to set up and implement biosecurity measures, starting with the experimental shrimp hatchery. It also supports the industry in taking decisions and orientations towards several biosecurity strategies.

3. CONCLUSION

Assessing biogeochemical fluxes at the pond bottom-water column interface is one of the piece of the puzzle lacking to understand the main processes in shrimp rearing ponds. Controlling phytoplanktonic biomass production is one of the main farmer leverage to minimize mortality risk but a deeper insight in microalgae composition/succession currently under study might also help, especially if some toxic species can be identified. Acquiring a better understanding of vibrio pathogeneicity mechanisms in particular through genomics techniques is also fundamental in terms of epidemiology. Preventing viral infections using innovative techniques such as RNA interference is essential not only facing present IHHNV infections but also in view of emergent viruses. Qualifying the ecophysiological status of broodstock larvae, post-larvae and adults in rearing is the only way of assessing environmental stresses and conducting rational nutrition studies. Genetics has led to a spectacular increase in shrimp performances using the Hawaiian strain as new blood. This should lead the industry to adopt a genetic program within 2-3 years conditionally to proper infrastructures. Finally, crunching data in Stylog, *via* appropriate statistical techniques, should help in drawing indicators which ideally could act as on-line warnings during rearing. Here above are summarized the main research topics and the linkage with the industry problems. DESANS first and DEDUCTION now, meeting high international research standards as testified by numerous publications, do not provide any magic tool but a synthesis of research results and technical recommendations, addressing the main stake holders issues, and potentially useful to the industry sustainability in a broader context.

4. ACKNOWLEDGMENTS

The authors are grateful to the technical staff of the Saint-Vincent station for providing high quality biological material for research purposes. They also thank farmers and institutional partners for participating to the project set up and evolution through the *ad hoc* committees.

5. REFERENCES

[1] Costa, R., Mermoud, I., Koblavi, S., Morlet, B., Haffner, P., Berthe, F., Le Groumellec, M. and Grimont, P. 1998. Isolation and characterization of bacteria associated with a *Penaeus stylirostris* disease (Syndrome 93) in New Caledonia. Aquaculture 164, 297-309.

[2] Mermoud, I., Costa, R., Mari J., Bonami, J. R., Hasson, K., Lightner, D. V. 1998. Investigations of *Penaeus stylirostris* disease (Syndrome 93) in New Caledonia exploring a viral hypothesis. Aquaculture 164, 311-322.

[3] Goarant, C., Mérien, J., Berthe, F., Mermoud, I., Pérolat, P. 1999. Arbitrarily primed PCR to type *Vibrio spp*. Pathogenic for shrimp. Appl. Environ. Microbiol. 65, 1145-1151.

[4] Saulnier, D., Avarre, J.-C., Le Moullac, G., Ansquer, D., Levy, P., Vonau, V. 2000. Rapid and sensitive PCR detection of *Vibrio penaeicida*, the putative etiological agent of Syndrome 93 in New Caledonia. Diseases of Aquatic Organisms 40, 109-115.

[5] Goarant, C., Ansquer, D., Herlin, J., Domalain, D., Imbert, F., de Decker, S. 2006a. “Summer Syndrome” in *Litopenaeus stylirostris* in New Caledonia: pathology and epidemiology of the etiological agent, *Vibrio nigripulchritudo*. Aquaculture 253, 105-113.

[6] Goarant, C., Reynaud, Y., Ansquer, D., de Decker, S., Saulnier, D., le Roux, F. 2006b. Molecular epidemiology of *Vibrio nigripulchritudo*, a pathogen of cultured penaeid shrimp (*Litopenaeus stylirostris*) in New Caledonia. Syst. Appl. Microbiol. 29, 570-580.

[7] Herbland, A. and Harache, Y., coord. 2008. Santé de le Crevette d’Elevage en Nouvelle-Calédonie. Ed. Quae, Versailles, 142 p.

[8] Della Patrona, L. and Brun, P. 2008. Elevage de la crevette bleue Litopeaneus stylirostris en Nouvelle Calédonie. Bases zootechniques et Biologie. Ed. Ifremer *available on request*, 320 p.

[9] Lemonnier, H., Herbland, A., Salery, L. and Soulard, B. 2006. “Summer syndrome” in *Litopenaeus stylirostris* grow out ponds in New Caledonia: zootechnical and environmental factors. Aquaculture 261, 1039-1047.

[10] Courties, C. and Boeuf, G. 2004. Mesure en cytométrie en flux du picoplancton autotrophe et hétérotrophe : un outil de contrôle de la qualité des eaux de fermes d’élevage de crevettes. *In* : Styli 2003. Trente ans de crevetticulture en Nouvelle Calédonie. Nouméa-Koné, 2-6 juin 2003. Ed. Ifremer, Actes Colloq., 38, 147-150.

[11] Courties, C., Bourrain, M., Chrétiennot-Dinet, M.-J. and Escande M.-L. 2005. Diversité du peuplement planctonique d’un bassin d’élevage de la ferme de Saint-Vincent, résultats préliminaires obtenues par l’analyse des ARNr 16S et 18S par observation en microscopie

électronique. Résumé du séminaire Ecosystèmes et crevetticulture en Nouvelle-Calédonie, Nouméa 22-24 juin 2005, Ifremer/DAC ed., Nouvelle-Calédonie.

[12] Lemonnier, H. 2007. Effet des conditions environnementales sur le développement des pathologies à *Vibrio* dans les élevages de crevettes en Nouvelle Calédonie. Discipline Océanologie biologique, Université de La Rochelle, 203 p.

[13] Lucas, R., Courties, C., Lemonnier, H. and Herbland, A. 2008. A flow cytometric approach to follow eutrophication in ponds. “The International conference and exhibition of EAS” Krakow, Poland, September 15-18. Oral comunication.

[14] Lemonnier, H. and Faninoz, S. 2006. Effects of water exchange rate on effluent and sediment characteristics and on partial nitrogen budget in semi-intensive shrimp ponds in New Caledonia. Aquaculture Research. 37, 938-948.

[15] Thomas, Y., Courties, C., El Helwe, Y., Herbland, A. and Lemonnier, H. In Press. Spatial and temporal extension of eutrophication originated by shrimp farm wastewater discharges in New Caledonia lagoon. Marine Pollution Bulletin.

[16] Reynaud, Y., Saulnier, D., Mazel, D., Goarant, C., le Roux, F. 2008. Correlation between the detection of a plasmid and high virulence of *Vibrio nigripulchritudo,* a pathogen of the shrimp *Litopenaeus stylirostris,* Appl. Environ. Microbiol. 74, 3038-47.

[17] Lightner, D. V., Redman, R. M., Bell, T. A. 1983. Infectious hypodermal and hematopoietic necrosis, a newly recognized virus disease of penaeid shrimp. J. Inv. Pathol. 42, 62-70.

[18] Flegel, T. W. 1997. Major viral diseases of the black tiger prawn (*Penaeus monodon*) in Thailand. World J. Microbiol. Biotechnol. 13, 433-442.

[19] Robalino, J, Bartlett, T. C., Chapman, R. W., Gross P. S., Browdy C. L., Warr G. W. 2007. Double-stranded RNA and antiviral immunity in marine shrimp: inducible host mechanisms and evidence for the evolution of viral counter-responses. Dev. Comp. Immunol. 31, 539-47.

[20] Shekhar, M. S., and Lu, Y. 2009. Application of nucleic-acid-based therapeutics for viral infections in shrimp aquaculture. Mar Biotechnol 11, 1-9.

[21] Fire, A. S., Xu, M. K., Montgomery, S. A., Kostas, S., Driver, E. and Mello, C. C. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391, 806-11.

[22] Livingstone, D. R. 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull. 42, 656-666.

[23] Temple, M. D., Perrone, G. G., Dawes, I. W. 2005. Complex cellular responses to reactive oxygen species. Trends in Cell Biology 15, 319-326.

[24] Lemaire, P. and Chim, L. 2007. Effect of experimental temperature fluctuations on some “oxidative stress” bioindicators in the digestive gland of the shrimp *Litopenaeus stylirostris*. Third workshop in Comparative Aspects of Oxidative Stress in Biological Systems. Annual meeting, 9-13 May 2007, Mexico.

[25] Castex, M., Lemaire, P., Wabete, N., Chim, L. In Press. Effect of dietary probiotic *Pediococcus acidilactici* on antioxidant defences and oxidative stress status in *Litopenaeus stylirostris* shrimp. Aquaculture.

[26] Muñoz, M., Cedeño, R., Rodríguez, J., Van der Knap, W.P.W., Mialhe, E. and Bachère, E. 2000. Measurement of reactive oxygen intermediate production in haemocytes of the penaeid shrimp, *Penaeus vannamei*. Aquaculture 191, 89-107.

[27] Castex, M., Chim, L., Pham, D., Lemaire, P., Wabete, N., Nicolas, J.-L., Schmidely, P. and Mariojouls, C. 2008. Probiotic *P. acidilactici* application in shrimp *Litopenaeus stylirostris* culture subject to vibriosis in New Caledonia. Aquaculture 275, 182-193.

[28] Castex, M. 2009. Evaluation du probiotique bactérien *Pediococcus acidilactici* MA18/5M chez la crevette pénéide Litopenaeus stylirostris en Nouvelle-Calédonie. Thèse de Doctorat de l’AgroParisTech. Spécialité Physiologie, Nutrition. Ecole Doctorale ABIES, 400 p.

[29] Le Groumellec, M., Goarant, C., Haffner, P., Berthe, F., Costa, R., Mermoud, I. 1996. Syndrome 93 in New Caledonia: investigation of the bacterial hypothesis by experimental infections, with reference to stress-induced mortality. SICCPPS book of abstracts, SEAFDEC, Iloilo City, Philippines, p. 46.

[30] Pham, D., Lemaire, P., Wabete, N., Meralikan, M., Chim L. 2008. World Aquaculture 2008, May 19-23, 2008. Busan, Korea. Book of abstracts, p. 589.

[31] Goyard, E., Arnaud, S ., Vonau, V., Bishoff, V., Mouchel, O., Pham, D., Wyban, J., Boudry, P., Aquacop 2003. Residual genetic variability in domesticated populations of the Pacific blue shrimp (*Litopenaeus stylirostris*) of New-Caledonia, French Polynesia and Hawaii and some management recommendations. Aquat. Living Resour. 16, 501-508.

[32] Patrois, J., Goyard, E., Peignon, J.-M., Dufour, R., Ansquer, D. 2007. Sécurisation des souches de crevettes d’élevage en Nouvelle-Calédonie : résultats de la quarantaine et du conservatoire expérimental et éléments pour la définition d’une stratégie de sécurisation des souches de crevettes en Nouvelle-Calédonie. Ifremer/DAC/RST. 2007-02, 43 p.

[33] Goyard, E., Goarant, C., Ansquer, D., Brun, P., de Decker, S., Dufour, R., Galinié, C., Peignon, J.-M., Pham, D., Vourey, E., Harache, Y. and Patrois, J. 2008. Cross breeding of different domesticated lines as a simple way for genetic improvement in small aquaculture industries: heterosis and inbreeding effects on growth and survival rates of the Pacific blue shrimp *Litopenaeus* (*Penaeus*) *stylirostris*. Aquaculture 278, 43-50.