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Nutrient and microbial dynamics in eutrophying shrimp ponds affected or unaffected by vibriosis

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ABSTRACT

A field survey was conducted on two intensive shrimp farms using similar technical practices: one (DF) historically affected by a vibriosis, the other (HC) in which the pathogen has been observed although no mortality event has occurred. Because historical data suggest that eutrophication process may directly or indirectly play a role in the disease outbreak, we focussed our research on its dynamics. A higher variability of the phytoplanktonic compartment linked to an imbalance in the molar N:P ratio was observed in farm DF compared to farm HC, implying a modification on the linkage between the bacteria and phytoplankton compartments at DF. The beginning of the mortality outbreak at DF followed a shift from pico- to nanophytoplankton. The organic matter mineralization process at the water–sediment interface may explain the disturbance observed in the water column during eutrophication. The consequences of this disturbance on shrimps' health status and pathogen ecology are discussed.

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1. Introduction

Disease in marine aquaculture is described as the end result of complex interactions between the host, its environment, and the pathogen itself. This is illustrated by Snieszko's (1974) famous diagram of three intersecting circles. In this "epidemiological triad", the host status may vary (species, strain, age, life stage, nutritional status, etc.); the pathogen may vary in virulence, concentration, ability to circumvent the host's defences, etc.; and the environment may vary from the ideal to one that more favours the development of the disease. The factors of pathogen(s), host(s) or agent(s), and environment must all be considered by diagnosticians in determining the cause of a disease (Lightner and Redman, 1998).

In New Caledonia, the disease known as Summer Syndrome is responsible for seasonal mortalities affecting *Litopenaeus stylirostris* shrimp reared in earthen ponds. It was first identified in December 1997 in an intensive farm (called diseased farm, DF) and has been enzootic ever since, subsequently affecting a second (2003) and third farm (2005), both close to DF. Regarding the pathogen, experimental pathology and molecular epidemiology studies have demonstrated that this disease is caused by a single emerging cluster of a highly virulent strain of *Vibrio nigripulchritudo* (Goarant et al., 2006a,b; Reynaud et al., 2008). However, as observed for many pathogenic *Vibrio*, the presence of this strain in the ecosystem does not necessarily lead to disease (Goarant et al., 2007). Regarding the host, historical analysis of DF grow-out data shows that the first dead shrimps appear after 58 ± 8 days of rearing for an average shrimp weight of about 5 g, and disappear after about 120 days (Lemonnier et al., 2006). During an aquaculture production cycle, feed supply in shrimp pond increases proportionally to the stocking biomass and induces an increasing eutrophication level in the pond ecosystem. Mortality outbreaks occurred during particular phases of the eutrophication process (Lemonnier et al., 2006). We hypothesise that this process could play a role by directly or indirectly inducing stress for shrimp and/or increasing the growth and/or virulence factors of *V. nigripulchritudo*.

In order to describe the eutrophication process and events related to shrimp mortality, a high frequency field survey was conducted in two ponds located in two grow-out intensive farms, one a farm historically affected by the disease (DF), the other a "control" farm (HC) where the pathogen had been observed without mortality. In eutrophying ponds, the analysis of this process implies to study the role of bacteria and phytoplankton (Burford et al., 2003a). Stability in the stocking biomass and metabolic activity of phytoplankton communities is critical to maintain a suitable environment for cultured animals. Heterotrophic bacteria are an important component of the food web structure and biogeochem-

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ical cycles. Aerobic and anaerobic microbial processes can affect the community respiration, pH levels and ammonia concentrations. Furthermore, heterotrophic bacterial activity mediates the recycling of nutrients such as nitrogen and phosphorus which can subsequently stimulate primary production. The study was designed to follow by means of flow cytometry the dynamics of phytoplankton and bacterioplankton communities in the two ponds concomitantly to a classical environment monitoring (oxygen, nutrients, chlorophyll *a*). Bacterial production was also used as an index of available organic matter for bacteria to characterize the trophic status of the ponds (Ducklow and Carlson, 1992). Several indicators proposed by Hussenot and Martin (1995) and Avnimelech et al. (2001) were also followed to assess the eutrophic state of sediment in field conditions. This research had three objectives: (i) to describe the eutrophication dynamics in a healthy intensive pond, (ii) to compare the environmental conditions between the "healthy" and the "disease" ponds, and (iii) to discuss briefly the consequences on the ecophysiology of reared shrimp and the pathogen ecology.

2. Materials and methods

2.1. Field survey

During the 2002–2003 austral summer, a field survey was conducted on two farms, the first considered as a "control" farm (HC), the second affected by the disease since 1997 (DF). The two farms surveyed are close to each other (about 15 km). They have similar pond sizes between 3 and 4.5 ha, and similar intensive culture practices, for a total surface of 29 ha (HC) and 34 ha (DF), respectively. The ponds were dried out 5 months before filling and were stocked at day 0 (d0) with *L. stylirostris* post-larvae, originating from the same hatchery batch. Initial stocking density was 35 shrimps m^{-2} at HC and 28 shrimps m^{-2} at DF. For both farms, shrimps were fed daily with the same local commercial feed (including 35–40% protein). The ponds were managed by the two farmers according to their usual techniques without advice from the scientific team. At the outset, urea was added to the ponds to fertilize the water column. At HC, 6.7 g m^{-2} were added to the pond over a 15-day period. This was stopped 1 day before stocking with post-larvae. At DF, 7.0 g m^{-2} were used over a 40-day period, starting 10 days before stocking with post-larvae. Daily water exchange in the ponds was implemented, ranging from 5% to 40% of the total pond volume depending on the shrimp biomass. Mechanical aeration was regularly used in both ponds in relation to the shrimp biomass in the ponds. On each farm, daily pond mortalities were evaluated by counting dead and moribund shrimps at the pond edges.

2.2. Field sampling

2.2.1. Water column

Weekly water samplings were carried out in the morning (06:00) and the afternoon (16:00) from the beginning of the rearing until the 32nd rearing day (d32) in both farms and, subsequently, every other day alternately on one farm and the other. Sampling was conducted for each pond in three stations, located variously in the middle of the pond, near the discharge gate and opposite the discharge gate, and at two depths. Spatial analysis of a number of parameters (temperature, salinity, turbidity, dissolved oxygen, pH, total ammonia nitrogen, nitrite, mineral matter, total suspended matter and chlorophyll *a*) was carried out at six stations, including the three stations described above, once a week during the survey. These specific results are not presented. Subsurface (15 cm deep) samples were collected using a 2-l polyethylene bottle. Bottom sub-samples (5 cm above sediment) were carefully pumped into a bottle through silicone tubing, avoiding disturbance of the sediment. In sum, no significant spatial variability in the water column was observed except for mineral matter concentrations, suggesting that water column was well mixed and that a single sampling at two depths was representative of the whole pond (Lemonnier, 2007). Dissolved oxygen (DO) and pH were recorded *in situ* at each station (bottom and surface) with portable oxygen and pH meters (WTW oxi 315i and WTW pH 340i). Temperature was automatically and continuously measured using an Optic StowAway Temp Logger (Aquatic Eco-systems, USA). Located near the ponds' discharge gate, this operated at two depths (15 cm below the surface and 5 cm above the bottom) with a 15-min step.

2.2.2. Sediment

Sediment samplings were carried out in six stations per pond. Samples were taken at the end of the pond dry-out, just before filling it, and weekly during grow-out. The first cm layer of soil was sampled using a 40 cm long PVC tube of 10 cm diameter by scuba diver to minimize sediment disturbance. pH and redox potential (Eh) were assessed at each station according to the method described by Hussenot and Martin (1995). Eh was measured with a combined platinum electrode (PT5700A, Schott Instruments, Mainz, Germany). pH was measured directly in the wet sediment.

2.3. Laboratory analysis

2.3.1. Water column

The following parameters were analysed on samples (surface and bottom) collected near the discharge gates only: soluble reactive phosphorus, nitrate and nitrite, dissolved organic carbon, dissolved organic nitrogen, particulate organic carbon, particulate nitrogen, bacterial abundance and production.

2.3.1.1. Physico-chemical parameters. In the field laboratory, salinities were measured using a refractometer. Waters samples (250 ml) were immediately filtered through a GF/C Whatman filter to analyse total suspended solid (TSS). Mineral matter (MM) and particulate organic matter (POM) were evaluated by loss on ignition at 450 °C during 4 h.

2.3.1.2. Nutrients. Waters samples (500 ml) were immediately filtered through a GF/F Whatman filter. Colorimetric analyses for total ammonia nitrogen (TAN) and dissolved inorganic phosphorus (DIP) were carried out on fresh samples using a Spectronic Genesis 5 spectrophotometer (Thermo Electron Corporation) equipped with a 10 cm cell. TAN was measured using methods described by Koroleff (1976) and DIP according to the molybdenum blue reaction described by Murphy and Riley (1962). Nitrate and nitrite (N_{ox}) were determined on thawed samples using standard colorimetric techniques on a Bran + Luebbe autoanalyser III system according to Raimbault et al. (1990). N_{ox} and TAN concentrations were added to calculate dissolved inorganic nitrogen (DIN).

2.3.1.3. Dissolved organic matter. Water samples (100 ml) were immediately filtered through a muffled GF/F Whatman filter. Filtrates were collected in muffled tubes and stored frozen until analysis. Dissolved organic nitrogen (DON) was analysed following oxidation described by Raimbault et al. (1999). Pre-oxidation DIN concentrations were subtracted from the post-oxidation total dissolved nitrogen (TDN) to derive the DON concentrations. DOC concentrations were measured by high temperature catalytic oxidation on a Shimadzu TOC-V Total organic carbon analyzer.

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