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Eutrophication in a tropical pond: Understanding the bacterioplankton and phytoplankton dynamics during a vibriosis outbreak using flow cytometric analyses

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ABSTRACT

In tropical shrimp ponds, the increasing of feed input, concomitantly with the stocking shrimp biomass, induces an eutrophication of the ecosystem. Although difficult to maintain, its stability is required to guarantee the success of the culture. A 110-day period of phytoplankton and bacterioplankton stock and dynamics in an earthen pond (1.2 ha area, 1 m depth) was monitored using flow cytometry to provide baseline information on community characteristics and ecosystem instability. Seven autotrophic cell types were identified over the whole sampling period. Prokaryotic cells included *Synechococcus sp*., a group named UNK which presented an atypical new flow cytometric signature and picoeukaryotes (PEUK). Nanophytoplankton cells were represented by 4 groups: NAN1, NAN2, NAN3 and Cryptophytes. During the first part of the survey, picophytoplankton dominated the phytoplanktonic assemblage. The mean abundance of total cells (up to 8×10^6 cells mL^{-1}) was among the highest recorded in marine and brackish waters. Bacterial abundance and production ranged from 0.8 to 5.1×10^7 cells mL^{-1} and from 30 to 110 μg C L^{-1} h^{-1}. A shift from pico to nanophytoplankton abundance was observed for a few days from d 96. During this period, heterotrophic bacteria production and abundance suddenly dropped, implying a change in the functioning of the microbial loop. This shift was concomitant with a significant shrimp mortality outbreak due to *Vibrio penaeicida*, the etiological agent of a disease known as Syndrome 93, which affects the shrimp industry in New Caledonia. This survey suggests that flow cytometric analysis could be used for the monitoring of aquaculture systems to improve our understanding of the complex phytoplankton and bacterial dynamics of these systems and its potential influence on disease development.

1. Introduction

During an aquaculture production cycle, feed supply in shrimp ponds increases concomitantly with the stocking biomass, which can induce an increasing eutrophication level in the pond ecosystem (Burford et al., 2003a). Subsequently there is an increase in algal biomass, whereas phytoplankton communities are characterized by a continuous succession of dominant species due to dynamic changes of growth factors such as light, temperature and nutrient concentrations (Burford, 1997; Yusoff et al., 2002; Casé et al., 2008). Phytoplankton communities are primary producers and consumers of dissolved oxygen and maintaining the stability in the stocking biomass and metabolic activity of phytoplankton communities is essential to provide a suitable environment for cultured animals. Changes in the phytoplankton community due to the increase in nutrients may result in outbreaks of harmful algal blooms (Cloern, 2001; Alonso-Rodriguez and Paez-Osuna, 2003; Zimba et al., 2006). Bacteria populations also rapidly respond to physical, chemical and biological changes in eutrophying systems. This issue is of particular interest when endemic and pathogenic bacterial populations, such as certain *Vibrio*, may pose a health risk to reared populations (Moriarty, 1997).

In New Caledonia, the grow-out shrimp industry is affected by two vibriosis. One is known as "Summer syndrome" and is caused by *Vibrio nigripulchritudo*. The other, known as "Syndrome 93," results in high shrimp mortality during the transition periods between the two main seasons (Mermoud et al., 1998). Epidemiological and bacteriological studies revealed that moribund shrimp always suffered from severe bacterial septicaemia due to *V. penaeicida* (Costa et al., 1998; Goarant et al., 1999; Saulnier et al., 2000). This pathogen demonstrated high pathogeneicity and is considered by several authors (Saulnier et al., 2000; Goarant and Merien, 2006) as a specific rather than opportunistic agent, in contrast to many other *Vibrio* species that produce disease only in stressed shrimp. *Vibrio penaeicida* is frequently detected in water pumped from the bays, and might therefore be an infection source for grow-out ponds (Goarant et al., 1999; Goarant

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and Merien, 2006). Stocking shrimp density is the main risk factor, so, an increase of the density from 15 to 40 shrimp m^{-2} was shown to grow the probabilities of the appearance and the severity of the epizooty in the ponds (Lemonnier, 2007). The highest mortalities occurred in ponds following sudden temperature drops (Mermoud et al., 1998) and might be related to eutrophication intensity (Lefevre et al., 2004). However, our knowledge of the dynamics of the ecosystem and its relationship to the epizooty is very limited.

One of the problems in describing and understanding disease development in aquaculture grow-out ponds is the monitoring of the microbial communities. Time series observations are critical to assess the direct and indirect effects resulting from the microbial population dynamics (Li, 2009). Flow cytometry (FCM) has emerged as a powerful analytical tool that allows fast access to cell numbers or densities, cytometric biomasses, and size classes as well as enabling diversity assessment within a biological community. Flow cytometry facilitates the processing of a large number of samples and microbial population monitoring over wide temporal scales, revealing the heterogeneity present in the populations or communities. This technique is now communally used in oceanography from 25 years to study phytoplankton and bacterioplankton (Troussellier et al., 1993; Legendre et al., 2001). However, FCM application in ecological studies dealing with marine eutrophic aquaculture system remains very infrequent (Endo et al., 2000). Although flow cytometry does not provide "taxonomic information," it can specify sub-groups based upon flow cytometric parameters such as fluorescence and relative side scatter. For phytoplankton species, flow cytometry can distinguish community structure at very broad phylogenetic resolution, for instance by separating *Synechococcus sp.*, *Prochlorococcus sp.* and picoeukaryotes within the picophytoplankton compartment (Legendre et al., 2001). This technique is also regularly used to discriminate sub-populations in the heterotrophic bacterial community within natural marine communities through the nucleic acid content and cell scattering properties of individual cells (Troussellier et al., 1995; Marie et al., 1997). It is well established that heterotrophic bacteria are important in the structure and dynamics of marine food webs and biogeochemical cycles (Cole et al., 1988). At least 2 sub-populations of bacterial cells, High Nucleic Acid content (HNA) and Low Nucleic Acid content (LNA) bacteria, can be discriminated. The findings of several studies have supported the idea that HNA bacteria can be considered as the most active component of marine bacterioplankton assemblage (Gasol et al., 1999; Lebaron et al., 2001a; Morán et al., 2007). This discrimination is an important step towards the identification of the most important micro-organism groups and their functions inside the whole community (Lebaron et al., 1999, 2001b; Schäfer et al., 2001). The correlation among bacterial variables and other biological variables could help to understand which factors control bacterial dynamics in the pond ecosystem (Andrade et al., 2003).

In this study, eutrophication of the pond ecosystem was investigated by characterizing pond conditions during a shrimp grow-out period using high frequency FCM measurements concomitantly with traditional environmental monitoring (i.e. oxygen concentrations, Chlorophyll *a* survey). Moreover, the overall flux of bacterial production was evaluated as an index of available organic matter for bacteria, in order to characterize the trophic status (Ducklow and Carlson, 1992). Besides providing a baseline information of community characteristics and ecosystem variability associated to vibrioses, our study aims to assess the use of flow cytometry technique as a tool to improve the process understanding and early detection of unbalanced ecosystem for further pond management.

2. Materials and methods

2.1. Field survey

The field survey was conducted over a 4-month period in 2004 in a 1200 m^2 pond at the Saint-Vincent (IFREMER) aquaculture research centre, located on the West Coast of New Caledonia (South Western Pacific—21°55′36 S, 166°05′04 E). The pond was dried out 82 days before filling. Ten days later, at day 0 (d 0), the pond was stocked with *Litopenaeus stylirostris* post-larvae PL20 at an initial stocking density of 19 shrimp m^{-2}. For the whole duration of the survey, shrimp were fed on a daily basis with a commercial feed having a 35–40% protein concentration using usual feeding strategy described by Della Patrona and Brun (2008). The fed input increased from 0.5 g m^{-2} at the beginning to about 6.0 g m^{-2} at d 60 and stabilized after that until the end of the survey. Fertilization was carried out by adding a total amount of 70 kg of urea starting 6 days before the post-larvae deployment and lasting 41 days. Daily water exchange in the pond varied from 5% to 20% of the total pond volume depending on the shrimp biomass, calculated from shrimp weight and estimated survival rate (Della Patrona and Brun, 2008). From d 91 to d 119, oxygen levels were maintained by mechanical aeration (total power applied: 3.4 hp ha^{-1}). During the rearing, shrimp mortality was estimated by counting dead and moribund shrimp at the pond edges. At d 119, all the shrimp were harvested including buried shrimp to calculate the final survival.

2.2. Sampling and field data

Pond water samples were collected at 07:00 am and 4:00 pm every 7 days from d 0 (beginning of the rearing) until d 41, and every 2 days from d 41 to d 119 (end of the rearing). Samples were collected at three stations: in the middle of the pond, near to the discharge gate and at the opposite end from the discharge gate. At each station, samples were collected from two depths: sub-surface samples were collected from 15 cm below the water surface and bottom samples from 5 cm above the sediment. For sub-surface samples, water was collected by opening and closing a submerged 2 L polyethylene bottle. Bottom sub-samples (5 cm above sediment) were pumped (manual pump) into a 2-L polyethylene bottle through silicone tubing, avoiding sediment disturbance. Dissolved oxygen (DO), pH and salinity were recorded *in situ* at each station (bottom and surface) with portable oxygen (WTW Oxi 315i), pH (WTW pH 340i) and conductivity (WTW Cond 315i) meters. Temperature was automatically and continuously (30-minute steps) recorded using three optic StowAway Temp Loggers (Aquatic Eco-systems, USA) deployed at the three stations close to the pond bottom. Input water samples were collected in the morning every 7 days in the first part of the survey and every 2 days from d 41 to d 119. Daily rainfall data were obtained from the local Weather Forecast Service (Météo-France, Nouméa).

2.3. Laboratory analysis

The following parameters were analyzed on pond water samples (surface and bottom) collected near the discharge gate only at 07:00 am and in the input water: nitrate and nitrite (N_{ox}), silicates (Si), size fractioned chlorophyll *a* (Chl *a*) and bacterial production (BP). Flow cytometry measurements were conducted on all samples collected at 07:00 am.

2.3.1. Nutrient analysis

Prior to nutrient analysis, water samples (500 mL) were immediately filtered through a GF/F (nominal pore size 0.45 µm) Whatman filter. Colorimetric analyses for total ammonia nitrogen $[NH_4^+ + NH_3) - N]$ (TAN), dissolved inorganic phosphorus (DIP) and silicates (Si) were carried out on fresh samples on 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 (Murphy and Riley, 1962). Concentrations are expressed in µmol N L^{-1} and in µmol P L^{-1} for TAN and DIP, respectively. Silicates were analyzed on thawed samples as previously described (Mullin and Riley, 1955). Nitrate and nitrite