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Dynamics of phytoplankton communities in eutrophying tropical shrimp ponds affected by vibriosis

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ABSTRACT

Tropical shrimp aquaculture systems in New Caledonia regularly face major crises resulting from outbreaks of *Vibrio* infections. Ponds are highly dynamic and challenging environments and display a wide range of trophic conditions. In farms affected by vibriosis, phytoplankton biomass and composition are highly variable. These conditions may promote the development of harmful algae increasing shrimp susceptibility to bacterial infections. Phytoplankton compartment before and during mortality outbreaks was monitored at a shrimp farm that has been regularly and highly impacted by these diseases. Combining information from flow cytometry, microscopy, pigment and phylogenetic analysis, the presence of Picocyanobacteria, Prasinophyceae and Diatomophyceae were detected as dominant phytoplankton groups and Cryptophyceae, Prymnesiophyceae and Dinophyceae as minor components. At the onset of the first shrimp mortalities, Bacillariophyceae increased while Cyanobacteria, Prymnesiophyceae and Dinophyceae decreased in the water column, followed by proliferation of Prasinophyceae. Several taxa were identified as potential harmful algae (Cyanobacteria, dinoflagellates and *Phaeocystis*).

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1. Introduction

The world shrimp industry regularly faces major crises due to outbreaks of serious bacterial or viral infections (Flegel, 2012; Tran et al., 2013). In the literature, it is increasingly agreed that diseases will be the limiting factor for future food supply from the crustacean aquaculture sector (Stentiford et al., 2012). As observed in various shrimp species and locations elsewhere in the world, shrimp aquaculture in New Caledonia (*Litopenaeus stylirostris*) is affected by epizooties. During the warm and cold seasons, two types of vibriosis induce shrimp mortalities in growing ponds, threatening the economic viability of the industry. Epidemiological studies have revealed that the first type of vibriosis results from the highly pathogenic (HP) *Vibrio nigripulchritudo* (Vn) strain and that the colonization of the shrimp pond ecosystem by this particular pathogenic cluster occurs at the onset of the disease outbreak (Goarant et al., 2006a, 2006b; Reynaud et al., 2008; Le Roux et al., 2010; Goudenège et al., 2013). Known as “Summer Syndrome”, this disease is currently restricted to one particular area of New Caledonia (St Vincent Bay). Regarding this epizooty, three risk factors were identified for shrimp mortality occurrence and strength: fast shrimp growth, early eutrophication of the pond ecosystem, and the presence of the HP Vn strain (Lemonnier et al., 2006). The second type of vibriosis is related to *Vibrio penaeicida* (Vp) and is known as “Syndrome 93” (Costa et al., 1998; Goarant et al., 1999; Saulnier et al., 2000). This pathogen is frequently detected in waters pumped from the lagoon, which are suspected to be the main source of pond bacterial contamination (Goarant et al., 1999; Goarant and Merien, 2006). Syndrome 93 disease is observed in all shrimp ponds of New Caledonia when temperature suddenly drops (Mermoud et al., 1998). High shrimp density at the onset of the disease is the main risk factor of this vibriosis (Lemonnier, 2007). Whatever the disease, mortality appears in specific environmental conditions characterized by strong phytoplankton and bacterioplankton oscillations and abiotic parameters (pH, ammonia, etc.) close to stressful values (Lemonnier et al., 2006, 2010; Lucas et al., 2010). Such conditions, which may occur in combination with another unknown triggering factor, weaken the host physiological status, parameters, increasing shrimp susceptibility to bacterial infections (Mugnier et al., 2013). The sudden collapse of some phytoplankton populations suggested weak ecosystem stability and the loss of homeostatic

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mechanisms. This lack of stability may promote the development of toxic algal species increasing the risk of infection. The close relationship between the incidence of shrimp diseases and the surrounding microbial community has been well established by several authors (Kautsky et al., 2000; Zhang et al., 2014). However, our knowledge of phytoplankton community diversity and dynamics in ponds, particularly harmful algae, is still too fragmentary to fully understand the mechanisms triggering and/or promoting disease outbreaks (Casé et al., 2008).

With the help of a farmer, a study was conducted in industrial ponds to monitor phytoplankton composition and dynamics before and during mortality outbreaks. The farm concerned has been regularly and severely impacted by both types of vibriosis for several years. Flow cytometry (FCM) was used to describe the phytoplankton community changes, because this technique is one of the most efficient for monitoring rapid microbial population changes within the water column (Courties and Boeuf, 2004). The statistical reliability of abundance is better than microscopic cell counts as greater proportion of cells (thousands) can be analysed over shorter period (seconds) (Troussellier et al., 1993). Potentially toxic phytoplankton species were searched in all samples collected during mortality outbreaks, using optical microscopy. To describe more accurately and specifically the phytoplankton communities, complementary approaches were combined on several samples. Taxonomic groups of phytoplankton were identified by HPLC analysis according to their marker pigments. Various photopigments characterize specific phytoplankton taxonomic groups (chlorophytes, cryptophytes, cyanobacteria, diatoms and dinoflagellates) (Paerl et al., 2003; Jeffrey et al., 2011). This technique has also been shown to be useful for the detection of fragile flagellates, which are destroyed by procedures used for microscopic observations. This technique, known as chemotaxonomy, has been widely and successfully used in marine, estuarine and freshwater environments (Jeffrey et al., 1997; Descy et al., 2009), but has been applied less frequently to tropical aquaculture ponds (Burford, 1997; Gárate-Lizárraga et al., 2009). Metagenomic analysis of flow-sorted selected cells was conducted on one fresh sample to estimate the diversity of two dominant cytometric groups: picoeukaryotes, which are highly abundant, and an unknown prokaryotic group regularly observed in shrimp ponds in New Caledonia (Courties and Boeuf, 2004; Lucas et al., 2010). The combination of cell sorting by FCM with molecular tools allows the molecular taxonomic identification of specific planktonic cells (Guillebault et al., 2010).

2. Material and methods

2.1. Field survey

The field survey was conducted during the 2009–2010 austral summer, in two ponds, named C and D. These ponds have been regularly impacted by vibriosis for several years. The stocking date chosen for this field survey was particularly favourable to the occurrence of the diseases (Lemonnier et al., 2006). The ponds were managed by the farmer according to his usual techniques without advices from scientific team. The ponds (around 8 ha) were dried for more than one month before breeding shrimps and supplied with blue shrimp (*L. stylirostris*) postlarvae (0.03 g) at 22 individual.m-2 on the 19 October 2009. Shrimps were fed on a daily basis with commercial food containing between 35 and 40% protein. Daily water renewal increased in pond D from 5 to 30% between the beginning and the end of the rearing. To test the effect of water exchange rates (WER) on disease outbreak, WER applied by the farmer were voluntary lower in pond C than in pond D. There was no mechanical aeration. During rearing, shrimp mortality was estimated by counting dead and moribund shrimp at the pond edges or on the filters located on the effluent gates. This estimation should be viewed in this study as a qualitative method. Isolation of the pathogen strains was conducted from hemocultures of 30 moribund shrimp. The different strains isolated were phylogenetically identified using molecular-based tools (Goarant et al., 2006b, 2007). The survival rate (%) calculated by the farmer was estimated by the ratio of the number of harvesting shrimp at the end of the rearing to the number of stocking shrimp at the beginning of the rearing.

2.2. Sampling and field data

The sampling strategy was based on previous studies that showed a good mixing of the water column (Lemonnier et al., 2010). One sampling station was representative of the whole pond. Thus, water sampling was carried out early in the morning (between 6 am and 8 am, local time) by the farmer at a station located near the pond discharge-gate. Sampling was scheduled three times a week over a period ranging from the 30th rearing day (d30) to the end of the rearing (d180). Surface water samples were collected using a 2-L polyethylene bottle. Procedures used in this study to measure water physico-chemical parameters (salinity, temperature, dissolved oxygen (DO), Secchi) have already been fully described in Lemonnier et al. (2006).

2.3. Laboratory analysis

2.3.1. Chlorophyll a

Water samples (20 to 50 ml) were filtered through a Whatman GF/F filter then stored frozen at −20 °C prior to analysis. Total chlorophyll *a* (TChl *a*) and pheophytin were determined using a fluorimeter (Turner Desings TD700, Sunnyvale, CA, USA) in accordance with the method described by Holm-Hansen et al. (1965). To estimate the size fractioned chl *a*, a first sub-sample was filtered through 2 μm Nucleopore to obtain biomass >2 μm (1). The size fractions <20 μm were obtained using 20 μm nylon sieve pre-filtered sub-samples filtered onto Whatman GF/F filter (2). The size fractioned chl *a* (<2 μm; 2 to 20 μm; and >20 μm) were calculated using data from (1), (2) and TChl *a*. Picoplankton is classified as having cell sizes smaller than 2 μm, nanoplankton as having cell sizes between 2 and 20 μm, and microplankton as having cell sizes greater than 20 μm.

2.3.2. Optical microscope analysis

This approach aimed to detect potential toxic phytoplankton species in the samples collected during mortality outbreaks. Preservation of 100 ml of five samples was directly implemented on site with 1% Lugol's iodine solution (final concentration) stored in the dark. Microscopic screening was achieved at the Ifremer laboratory using a counting chamber and inverted microscope ($N = 5$). Five other samples were collected at the same farm in 2010 and 2011 during mortality outbreaks to complete our sampling campaign and to identify more precisely certain taxa. After microscopic screening, cells were further identified using single cell PCR methodology (Auinger et al., 2008).

2.3.3. Flow cytometry analysis (FCM)

Freshwater 2 ml subsamples were preserved with 1% glutaraldehyde (final concentration) stored in liquid nitrogen pending flow cytometric analysis (Vaulot et al., 1989). Quickly thawed at room temperature, samples were then analysed using a FACSCan flow cytometer (BD-Biosciences, San Jose, CA) equipped with an air-cooled argon laser (488 nm, 15 mW). Phytoplankton cells were discriminated and enumerated on the basis of their right-angle light scattering properties (SSC, roughly related to cell size), and orange (560–620 nm) and red (>670 nm) fluorescence due to phycoerythrin and chlorophyll pigments, respectively. Data acquisition was performed using CellQuest software (BD-Biosciences). Fluorescent 1.002 μm beads (Polysciences Inc., Europe) used as an internal standard were systematically added to each analysed sample. Mean cell fluorescence and light scatter were standardized by dividing them by the homologue signals of beads, thus making results comparable. Accurate analysed volumes – varying between 225 and 400 μl – and subsequent estimations of cell concentrations were calculated by measuring the remaining volume and subtracting it from the initial subsample volume (1 ml). Accuracy and

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