Cell-based measurements to assess physiological status of *Pseudo-nitzschia multiseries*, a toxic diatom

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Abstract

Diatoms of the genus *Pseudo-nitzschia* are potentially toxic microalgae, whose blooms can trigger amnesic shellfish poisoning. The purpose of this study was to test and adapt different probes and procedures in order to assess the physiological status of *Pseudo-nitzschia multiseries* at the cell level using flow cytometry. To perform these analyses, probes and procedures were first optimized for concentration and incubation time. The percentage of dead *Pseudo-nitzschia* cells, the metabolic activity of live cells and their intracellular lipid content were then measured following a complete growth cycle. In addition, chlorophyll autofluorescence and efficiency of photosynthesis (quantum yield) were monitored. The concentration and viability of bacteria present in the medium were also assessed. Domoic acid (DA) was quantified as well. Just before the exponential phase, cells exhibited high metabolic activity, but low DA content. DA content per cell became most abundant at the beginning of the exponential phase when lipid storage was high, which provided a metabolic energy source, and when they were surrounded by a high number of bacteria (high bacteria/*P. multiseries* ratio). These physiological measurements tended to decrease during exponential phase and until stationary phase, at which time *P. multiseries* cells did not contain any DA nor store any lipids, and started to die.

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1. Introduction

*Pseudo-nitzschia* is a potentially toxic diatom genus with a worldwide distribution. Some species are able to produce domoic acid (DA), an amnesic shellfish toxin leading to food poisoning (Sierra-Beltrán et al., 1998) with a few cases of mortality reported in humans (Wright et al., 1989), and hundreds of cases of sea bird (Sierra-Beltrán et al., 1997; Work et al., 1993) and marine mammal mortality (Scholin et al., 2000; Fire et al., 2009; de la Riva et al., 2009). These poisonings often occurred following a bloom of *Pseudo-nitzschia* spp. The reasons why these blooms occurred are poorly known. Some studies tried to create models to predict their occurrence (Anderson et al., 2009; Lane et al., 2009), but the determinism of each bloom seems different. Although factors enhancing or decreasing *Pseudo-nitzschia* cell toxicity have been intensively studied, they remain unclear. The study of *Pseudo-nitzschia* spp. physiology may help to understand why a bloom appears and becomes toxic. Tools to assess the physiological status of microalgae are still fairly rare. Photosynthetic capacities of *Pseudo-nitzschia* spp. have been studied under different conditions (Ilyash et al., 2007; El-Sabaawi and Harrison, 2006), but do not provide enough information to assess cell physiological status. In addition to photosynthetic parameters and chlorophyll content, other parameters have sometimes been studied in diatoms, e.g. silicification (Leblanc et al., 2005; Kroger and Poulsen, 2008) and carbohydrate levels (De Philippis et al., 2002; Magaletti et al., 2004), but these are also insufficient to characterize physiological processes occurring inside the cell. It is therefore important to develop and