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The relationships between particulate and soluble alkaline phosphatase activities and the concentration of phosphorus dissolved in the seawater of Toulon Bay (NW Mediterranean)

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ABSTRACT

The activities of particulate and soluble phosphatase were analyzed monthly for 1 year in the coastal ecosystems of the NW Mediterranean Sea. The mean contribution of the particulate activity increased from 56% at an *MUF-P* concentration of 30 μM to 77% at 0.04 μM. This particulate activity was negatively correlated with the DIP, DOP and TDP concentrations when the activities were related to the seawater volume, chlorophyll *a* or the protein concentration. The TDP correlations were highly significant (p: 0.001). The DOP correlations were significant (p: 0.04) and became highly significant (p: 0.009) at low DIP concentrations (<0.13 μM). The DIP correlations were significant (p: 0.04) only at low DOP concentrations (<0.18 μM). Thus, the effects of seawater DIP and DOP were found to be linked. The soluble activity exhibited distinct phosphatase fractions with high (0.5–29.5 μM) and low (0.02–2 μM) *Km* values, but none exhibited significant correlations with phosphorus compounds.

1. Introduction

In oligotrophic marine environments such as the Mediterranean Sea, dissolved inorganic phosphorus concentrations (DIP) are often low with the phosphorus required for plankton growth and metabolism deriving from dissolved organic phosphorus (DOP) (Thingstad and Rassoulzadegan, 1995; Dyhrman et al., 2007). Dissolved organic phosphorus consists of low and high molecular weight compounds whose compositions have recently been determined: 85% are esters (phosphoamino acids, sugar phosphates and/or small nucleotides), 8–13% are polyphosphates and 5–10% are phosphonates (Young and Ingall, 2010). They are primarily of biological origin (membrane phospholipids, nucleotides and nucleic acids) and were released following the decomposition of dead plankton cells, phytoplankton exudation, zooplankton grazing (predation) and excretion, and bacterial secretions, among others (Benitez-Nelson, 2000; Kolowitz et al., 2001). A portion of these compounds derives from external inputs, such as coastal river flooding, soil leaching, or from the resuspension of sediment (Labry et al., 2005; Paytan and McLaughlin, 2007; Llebot et al., 2010).

The conversion of phosphate esters into DIP is carried out by hydrolases, the most studied being alkaline phosphatase (Jansson et al., 1988; Hoppe, 2003). Alkaline phosphatase has a broad specificity and hydrolyzes numerous substrates including nucleoside polyphosphates, deoxynucleoside polyphosphates, or p-nitrophenyl phosphate (Suzumura et al., 1998). This activity is produced by phytoplankton (pico and nano), bacterioplankton and zooplankton (Jansson et al., 1988; Sebastian et al., 2004) and derives from ectoenzymes inserted into the cell membranes of phytoplankton or concentrated in the periplasmic space of bacteria (Jansson et al., 1988; Martinez and Azam, 1993). The activity associated with the living matter constitutes the "particulate activity". Numerous studies have been carried out in the natural environment to clarify its role in the recycling of DOP and in the regulation of the C:N:P equilibrium (Hoppe, 2003; Nausch and Nausch, 2004). Another aspect of the bulk activity (the "soluble activity") derives from the "free" enzymes dissolved in seawater. "Free" phosphatases are generally defined as enzymes that can pass through 0.45-μM filters (Jansson et al., 1988). This soluble activity arises from bacterial secretions, dead cells, grazing by zooplankton or virolysis (Koch et al., 2009; Duhamel et al., 2010). The synthesis of alkaline phosphatase is derepressed when the DIP concentrations are low, leading to increased activity (Jansson et al., 1988; Hoppe, 2003). However, in the natural environment, this negative correlation between phosphatase activity and DIP concentrations is uncertain, and the role of dissolved compounds on phosphatase

Abbreviations: DIP, dissolved inorganic phosphorus; DOP, dissolved organic phosphorus; TDP, total dissolved phosphorus; SRP, soluble reactive phosphorus; MUF-P, Methyl umbelliferyl phosphate; MUF, methylumbelliferone.

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activity is not fully understood in coastal and oceanic waters (Sebastian et al., 2004; Tanaka et al., 2006).

This work was carried out during 2005–2006 in a coastal ecosystem of the NW Mediterranean Sea (Toulon Bay, France), where a monitoring program was implemented between 2002 and 2007 to restore water quality ("Le contrat de baie de la rade de Toulon"). In a previous study, we found that the bulk phosphatase activity was negatively correlated with the total dissolved phosphorus (TDP) in the seawater, not with the DIP or DOP alone (Bogé et al., 2012). This work aims to analyze the influence of seawater DIP and DOP on the particulate and soluble components of the bulk activity to provide additional insight into the control of alkaline phosphatase and to present the biochemical characteristics of these activities and their respective contributions.

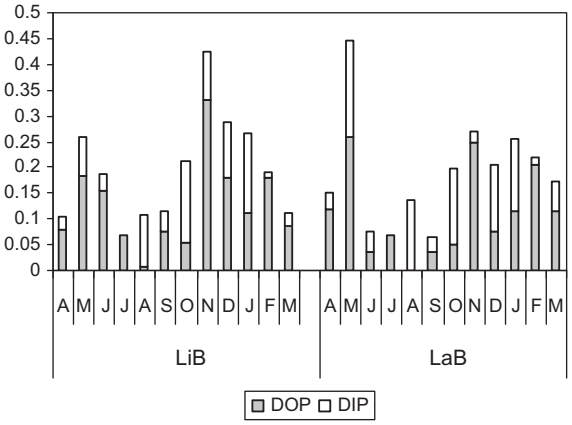

Fig. 2. DIP, DOP and TDP (DIP + DOP) concentrations (in μm) in Little Bay (LiB) and in Large Bay (LaB).

2. Methodology

2.1. Study sites

Toulon Bay is located on the southern coast of France on the Mediterranean Sea (lat. 43°5′ N and long. 6°0′ E) (Fig. 1). Toulon Bay is subdivided into two zones: "Little Bay" and "Large Bay," which are separated by an artificial breakwater (Fig. 1). Little Bay is semi-enclosed and shelters the sea port of Toulon. Large Bay is open to the sea. The Eygoutier river empties into Large Bay and the Las river into Little Bay.

2.2. Sampling procedure

Seawater was collected monthly throughout 2005 (19.04, 30.05, 15.06, 05.07, 24.08, 12.09, 04.10, 14.11, 15.12) and 2006 (10.01, 28.02, 14.03) between 8 AM and 10 AM at a depth of approximately 3 m using a Niskin bottle.

2.3. Analysis

2.3.1. Phosphorus

50-ml samples were immediately passed through 0.45-μM filters. The DIP was analyzed as soluble reactive phosphorus (SRP) according to the method of Murphy and Riley (1962). The DOP was quantified as the SRP released following DOP oxidation by acidic peroxodisulfate (AFNOR NF-EN 1189) at a pressure of 35 bar and a power of 900 W for 15 min using a microwave mineralizator (Multiwave 3000 Digesteur, Anton Paar). Three replicates were performed in 100-mm quartz cells.

2.3.2. Biomass

The protein concentrations of the particulate material collected on the 0.45-μM filters were measured to estimate the total planktonic biomass (Lowry et al., 1951). The chlorophyll *a* content of the

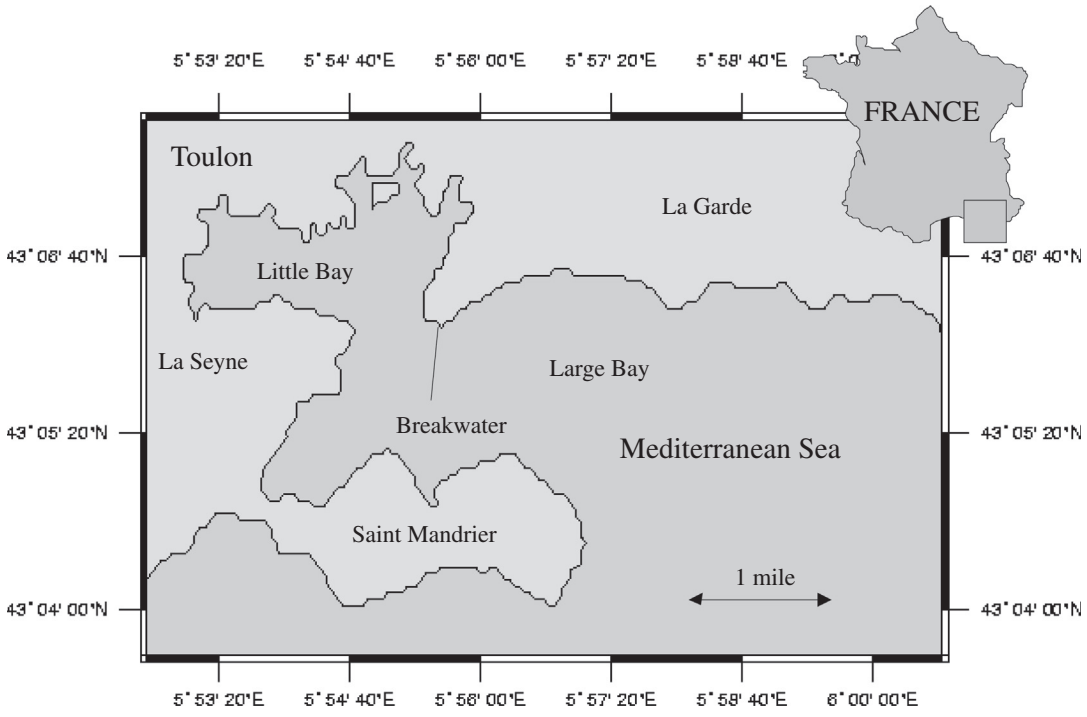

Fig. 1. Map of Toulon Bay.