Alkaline phosphatase activity and regulation in the North Pacific Subtropical Gyre.

Presenters

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Why measure alkaline phosphatase activity?

- Phosphorus (P), is an **essential element for life** (Karl 2000) and its availability has a **predominant role in controlling planktonic biomass and production in the ocean** (Karl et al. 2001; Van Wambeke et al. 2002; Moutin et al. 2008).
- Among the P reservoirs, **dissolved inorganic P (DIP) is directly incorporated** by microorganisms while **dissolved organic P (DOP) must generally be enzymatically hydrolyzed** to release DIP that can be transported and incorporated into the cell (Figure 1).
- There are many enzymes that hydrolyze DOP but **alkaline phosphatase is the best studied and arguably the most important for nutrition**, since it has the potential to hydrolyze a broad spectrum of DOP compounds and is produced by a majority of marine heterotrophic bacteria and phytoplankton (Cembella et al. 1984; Hoppe 2003).
- Thus, **alkaline phosphatase plays a prominent role in the recycling of organic P**.
- Expected increases in CO_2 levels and temperature in the future ocean may lead to an increase in global dinitrogen fixation, potentially driving some oceanic regimes, such as the North Pacific Subtropical Gyre (NPSG), towards **P stress and increasing demand for DOP** (Karl 1999; Karl et al. 2001; Hutchins et al. 2007).

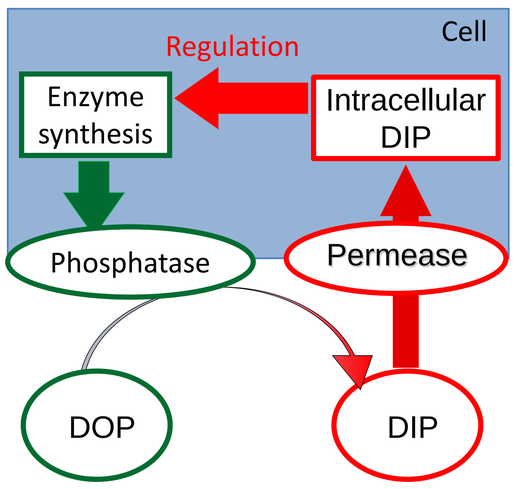


Figure 1: Schematic representation of two major phosphate incorporation pathways in heterotrophic or autotrophic prokaryote and eukaryote cells.

- It is thus necessary to **assess DOP bioavailability** and the **microorganisms potential dependency and utilization characteristics of DOP**

Alkaline phosphatase activity at station ALOHA

We examined the **vertical** (5 to 125 m - i.e., euphotic layer) and **seasonal variability of the kinetic parameters of alkaline phosphatase activity** (APA) at station ALOHA over 2 years (Figure 3).

We used a transformation of the Michaelis–Menten model to determine the kinetic coefficients:

$$S/v = (K + S_n)/V_m + S/V_m$$

Where:

- v = velocity of hydrolysis,
- S = substrate (MUFP) concentration
- V_m = maximum velocity of hydrolysis: provides the enzymatic potentialities of microorganisms
- $K + S_n$ = sum of the half-saturation constant plus the natural concentration.

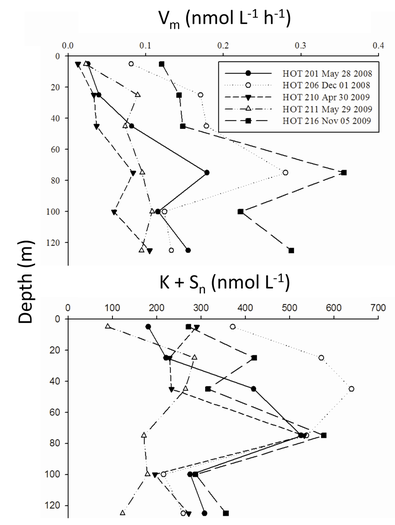


Figure 3: Variability of the kinetic parameters of APA at station ALOHA measured in series of MUFP concentrations ranging from 0.25 to 2 µmol L^{-1}.

- $K + S_n$ varied between 90 and 640 nmol L^{-1} (Figure 3), which is the signature of **high-affinity of the enzymes for the substrate**.
- V_m varied between 0.01 and 0.36 nmol L^{-1} h^{-1} (Figure 3). Vm often increases with increasing P-limitation, but these values are in the lower range of those found in the literature, and likely indicate a **lack of community P-limitation**.
- Nevertheless, this enzymatic activity could potentially release between 0.24 and 8.64 nmol L^{-1} d^{-1} of DIP from the alkaline-phosphatase-hydrolysable fraction of DOP, which corresponds to 0.4 to 30% of the DIP measured in the corresponding samples.
- These results suggest that **the alkaline-phosphatase-hydrolysable fraction of DOP could provide a significant source of labile P to sustain production in the NPSG**.

Methods to study alkaline phosphatase activity

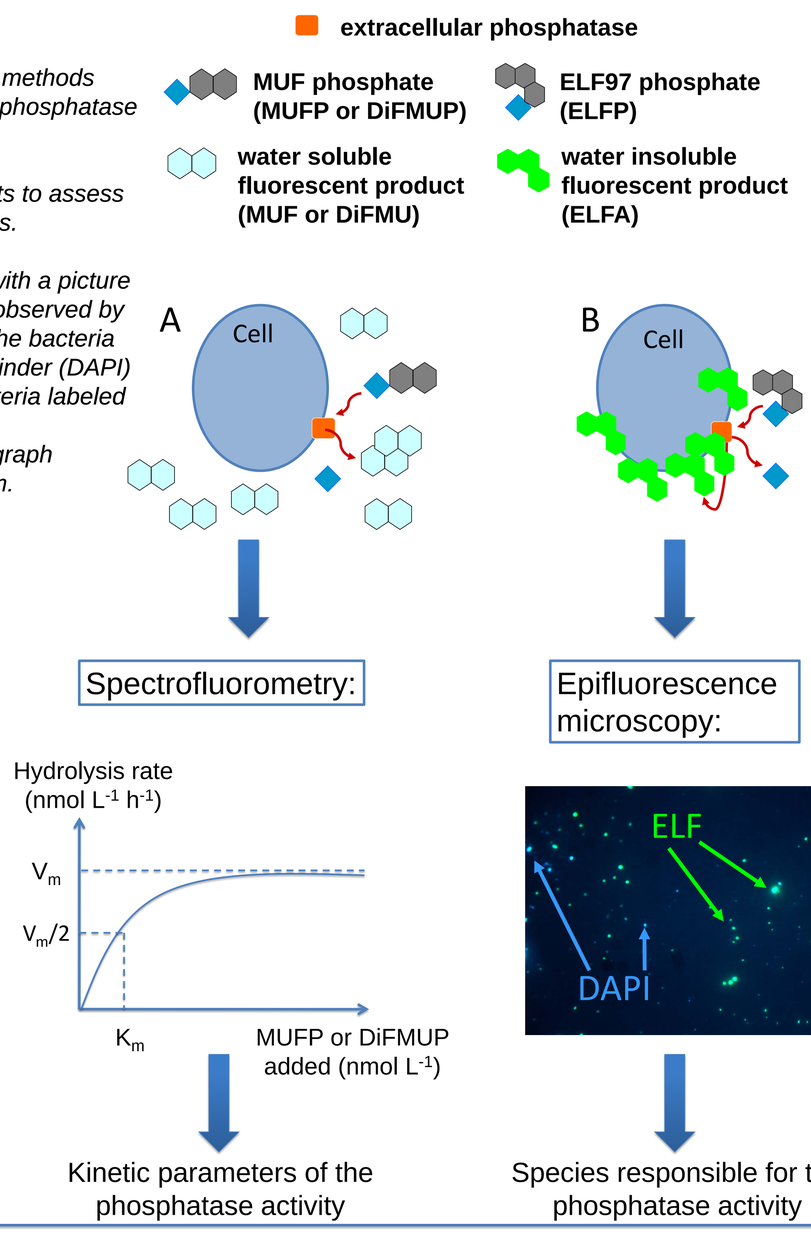
- **Bulk activity:** with 4-methyumbellyferyl phosphate (**MUFP**) or 6,8-difluoro-4-methylumbelliferyl phosphate (**DiMUFP**) - upon enzymatic hydrolysis, these soluble substrates provide a soluble fluorescent product used to **quantify the kinetic parameters of phosphatase activity**. They provide global information at the community level but do not provide information about the origin of the activity (Figure 2A).

- **Cell-specific activity:** withELF-97 [2-(59-chloro-29-phosphoryloxyphenyl)-6-chloro-4-(3H)-quinazolinone]-phosphate (**ELF-P**) - upon enzymatic hydrolysis, this soluble substrate rapidly provides an insoluble fluorescent precipitate of ELF alcohol (ELFA) **marking the site of the activity** (Figure 2B).

Figure 2: Schematic representation of the methods use to study alkaline phosphatase activity:

***A**: bulk measurements to assess the kinetic parameters.*

***B**: single-cell assay with a picture of ELF-labeled cells observed by microscopy (in blue the bacteria labeled with a DNA binder (DAPI) and in green the bacteria labeled with ELFA).*
White scale in photograph corresponds to 10 µm.



Regulation of alkaline phosphatase activity

- **Goals:** study APA regulation and evaluate the capacity of picoplankton organisms (i.e., 0.2-2-µm size range) to access the alkaline-phosphatase-hydrolysable fraction of DOP
- **Method:** APA was measured at several stations in the NPSG in July 2008, and in a series of nutrient addition experiments: nitrate plus ammonium (+N) or phosphate (+P) (Duhamel et al., in press).
- **Results:**
  - primary limitation of the biomass by nitrogen.
  - Both total (using DiFMUP) and cell-specific (using ELFP) APA were enhanced in the +N samples and reduced in the +P samples, **suggesting that DOP is an important resource for picoplankton nutrition and species composition**.
  - In the +N treatment, <5% and up to 96% of the cells in the heterotrophic bacteria-enriched and picophytoplankton-enriched fractions, respectively, were ELFA-labeled after 5 d (Figure 4C,D).
  - Following N enrichment, the microbial assemblage shifted from cell-free phosphatase dominated under N-limitation and P-stress (i.e., physiological response) to picophytoplankton-based phosphatase dominated under P-limitation (i.e., production or growth rate limitation).
- **Conclusion:** If, as predicted, the ocean evolves towards P-limitation, **DOP availability would become of major importance to sustain productivity**.

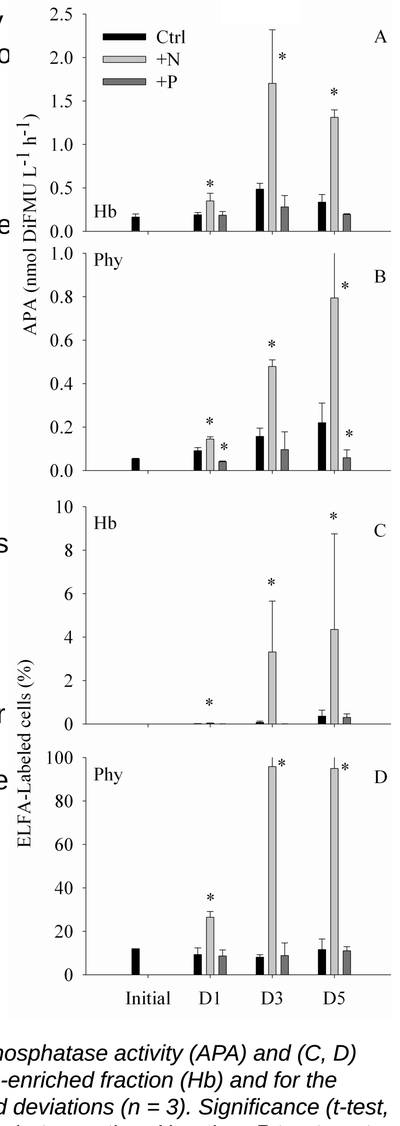


Figure 4: Plots from a nutrient addition experiments. (A, B) alkaline phosphatase activity (APA) and (C, D) percentage of ELFA-labeled cells, given for the heterotrophic bacteria-enriched fraction (Hb) and for the picophytoplankton-enriched fraction (Phy). Error bars denote standard deviations (n = 3). Significance (t-test, dF = 11, p ≤ 0.05) is indicated by an asterisk for pairwise comparisons between the +N or the +P treatments and the control.

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