Lab 8:
Nutrient Limitation in Coastal Phytoplankton Communities

Introduction

In the field, the growth of organisms may be limited by the availability of essential resources to fuel the energy and metabolic demands of growing and dividing cells. For phytoplankton, such limiting resources can be light as the primary energy resource to fuel photosynthesis or nutrients. Among nutrients, nitrogen and phosphate are the major limiting resources in marine and freshwater systems. For diatoms, silicate is another potentially limiting nutrient, because diatoms require silicate for frustule formation upon cell division.

Deep mixing during winter storms reduce the light availability to phytoplankton particularly in temperate and polar seas. Light decreases exponentially with water depth, and by deep mixing, the average light energy a single cell experiences over a day is decreased. Integrated over the light period of a day, deep mixing can cause algal cells to experience less light, thus less energy input, than required for active growth. Light limitation is less likely in clear, shallow waters and in tropical oceans, which are mostly stratified and exhibit shallow mixing.

Carbon dioxide is another essential resource for phytoplankton growth as it presents the major carbon source for biomass formation by photosynthesis. Whereas CO_2 can become limiting to photosynthesis in terrestrial plants and freshwater systems, the carbonate reservoir of seawater mostly prevents limiting CO_2 concentrations.

There are special regions in the oceans where other essential elements are limiting phytoplankton growth. For example, iron has been shown to limit phytoplankton production in the Southern Ocean around Antarctica, but also in the central Pacific Ocean. Such elements that are required for cell growth and division but occur in minor concentrations are referred to as micronutrients, in contrast to macronutrients such as nitrogen (N), phosphorus (P) and silicate (Si).

Whereas most freshwater systems are P-limited, most marine systems are N-limited. In coastal areas and estuaries, the influence of land and rivers can shift nutrient limitation to P as well. Studies in the late 1980's revealed that Biscayne Bay phytoplankton is primarily P-limited. Our experiments shall revisit this hypothesis and reveal the degree of nitrogen or phosphorus limitation at different stations within and outside the Bay.

The principle of nutrient limitation experiments is to incubate sample volumes from the same station at identical conditions (light, temperature). Nutrients are added in surplus to all but one (control) sample, and algal growth is followed and compared to the control. To test whether a phytoplankton community is P- or N-limited, nutrient additions are provided in the form of nitrate, phosphate, or both. If algal growth is nutrient limited, it will be higher in the nutrient amended sample(s) than in the control (Fig. 1).

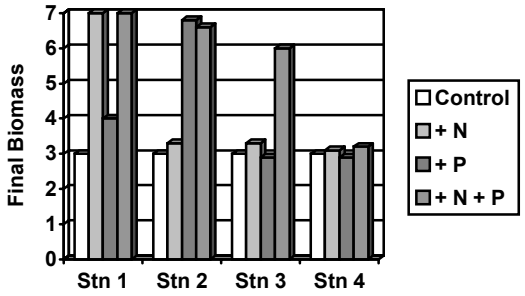

Fig 1: Nutrient enrichment experiments. Stn 1 is N limited, Stn 2 is P limited, Stn 3 is N/P co-limited, Stn 4 is neither limited by N or P.

Lab Work

From your sample, fill 4 1-L Erlenmeyer flasks with 500 ml of sample water. Label each flask as "control", "N", "P", "NP", respectively. Add nutrients to each flask according to the below scheme (nutrient stock solutions will be provided):

Control	no addition
N	0.5 ml nitrate solution
P	0.5 ml phosphate solution
NP	0.5 ml nitrate + 0.5 ml phosphate

Close all flasks with aluminum foil to prevent contamination and excess water evaporation and place your labeled flasks in the light incubator. Make sure that all flasks are placed on the same shelf and at the same distance from the door (light source).

From the original sample, measure 500 ml and filter onto a Whatman GF/F glass fiber filter under –300 mbar vacuum on the filtration manifold for later chlorophyll analysis. Place your filters in labeled glass scintillation vials with the filtered (green) surface up and store vials in the freezer at –20°C until analysis during the next lab class.

In the next class, retrieve your culture flasks from the light incubator. Filter the content of each flask (500 ml) onto Whatman GF/F filter under –300 mbar vacuum, and place filter in labeled glass scintillation vials.

One after the other, place the GF/F filter in a mortar (filtered side up) and add 10 ml ice-cold 90% acetone by bottle dispenser. Grind the filter and fill the acetone extract with ground filter debris into a labeled glass tube. Place glass tube on ice in benchtop cooler. Keep your labeled scintillation vials for later use! After all filters are extracted, place all 6 glass tubes into the centrifuge and spin for 10 min at 70% setting. Then carefully remove one tube from the centrifuge and decant the supernatant carefully into the corresponding scintillation vial. Close vial and put on ice. Repeat for all tubes.

Warning: Label all vials and tubes with label tape and ball pen or pencil. Do not use marker, because acetone might dissolve your labeling if drops are running along the vial or tube surface.

Chlorophyll extracts will be measured in the Turner 111 fluorometer. One after the other, fill your chlorophyll extract from the vial into the fluorometer cuvet and note your reading. Convert fluorometer readings to chl.a in units of $\mu g\ l^{-1}$ by the provided conversion factor (see instructor for conversion factor).

For your final lab report, analyze chlorophyll concentrations in comparing the final numbers of each flask with the initial number. Plot increase or decrease of phytoplankton biomass (final – initial value) against the sample treatment as depicted in Fig. 1. From your results for your field station, which nutrient (N, P) does limit phytoplankton production?