

TRAINING WORKSHOP IN PLANKTON

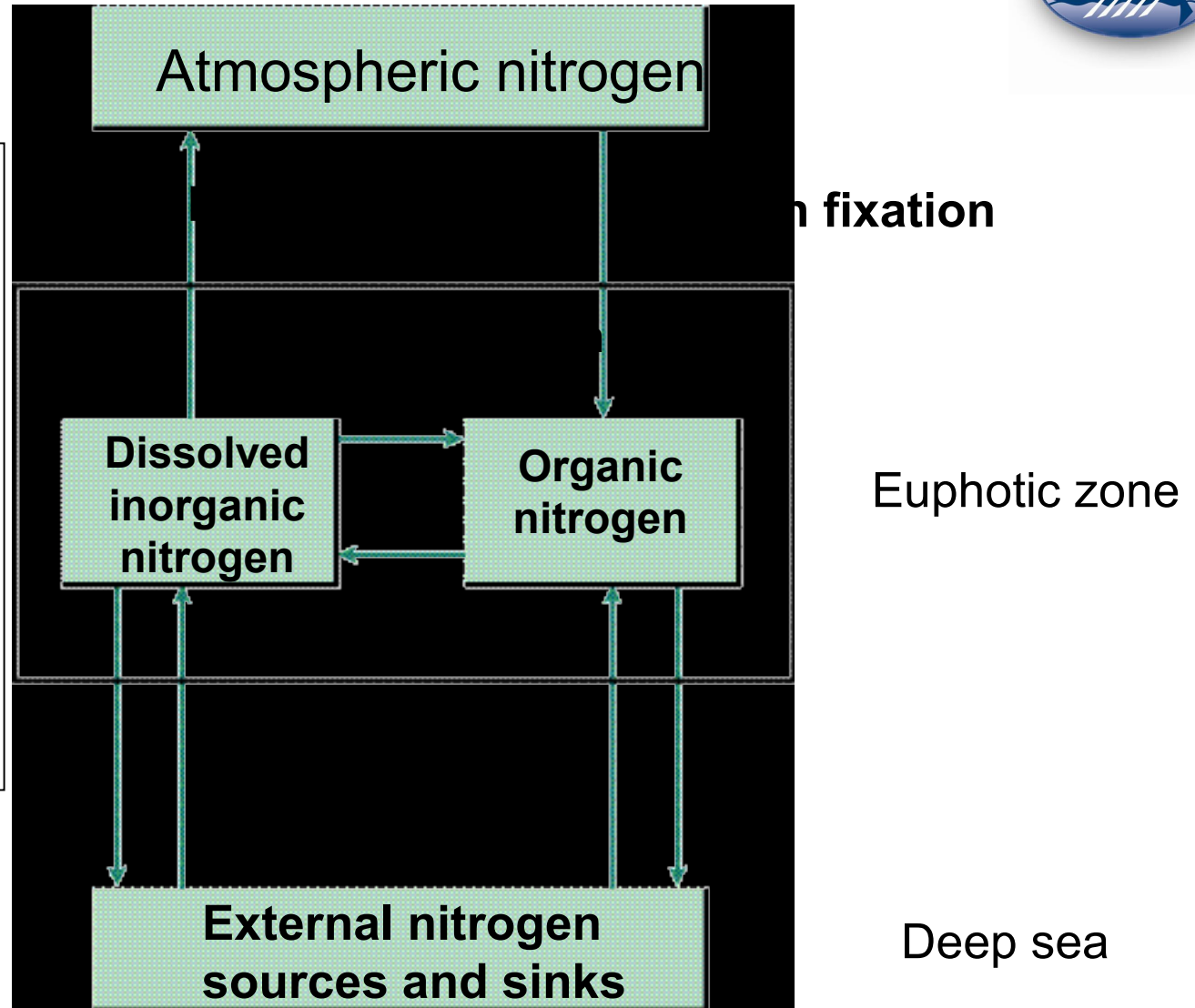
In the frame of SESAME IP

- **Primary Production rates measurements,**
- **And some examples**

Primary production

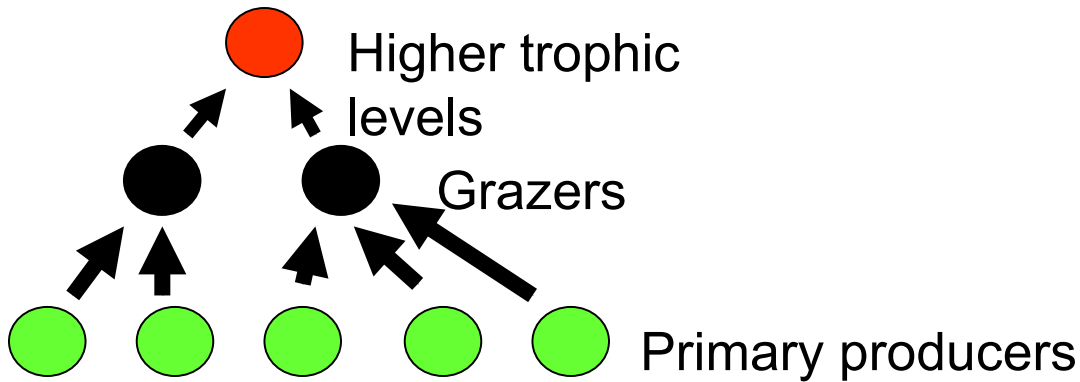
- Synthesis of higher energy organic compounds from lower energy organic compounds
- Photosynthesis- light energy
 - $n\text{CO}_2 + 2\text{H}_2\text{O} \rightarrow (\text{CH}_2\text{O})_n + n\text{O}_2 + \text{H}_2\text{O}$
- Basic respiratory (in dark) equation
 - $\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + 36\text{ATP}$
- Chemosynthesis- chemical energy
 - Oxidation of hydrogen sulfide (H_2S)
 - $\text{CO}_2 + \text{O}_2 + 4(\text{H}_2\text{S}) \rightarrow \text{CH}_2\text{O} + 4(\text{S}) + 3(\text{H}_2\text{O})$
- Measured as: weight carbon/area/time

Rate of primary production in the ocean plays key role in Global geochemical cycles



Nitrogen Cycle

Rate of primary production can determine the Structure of marine food webs



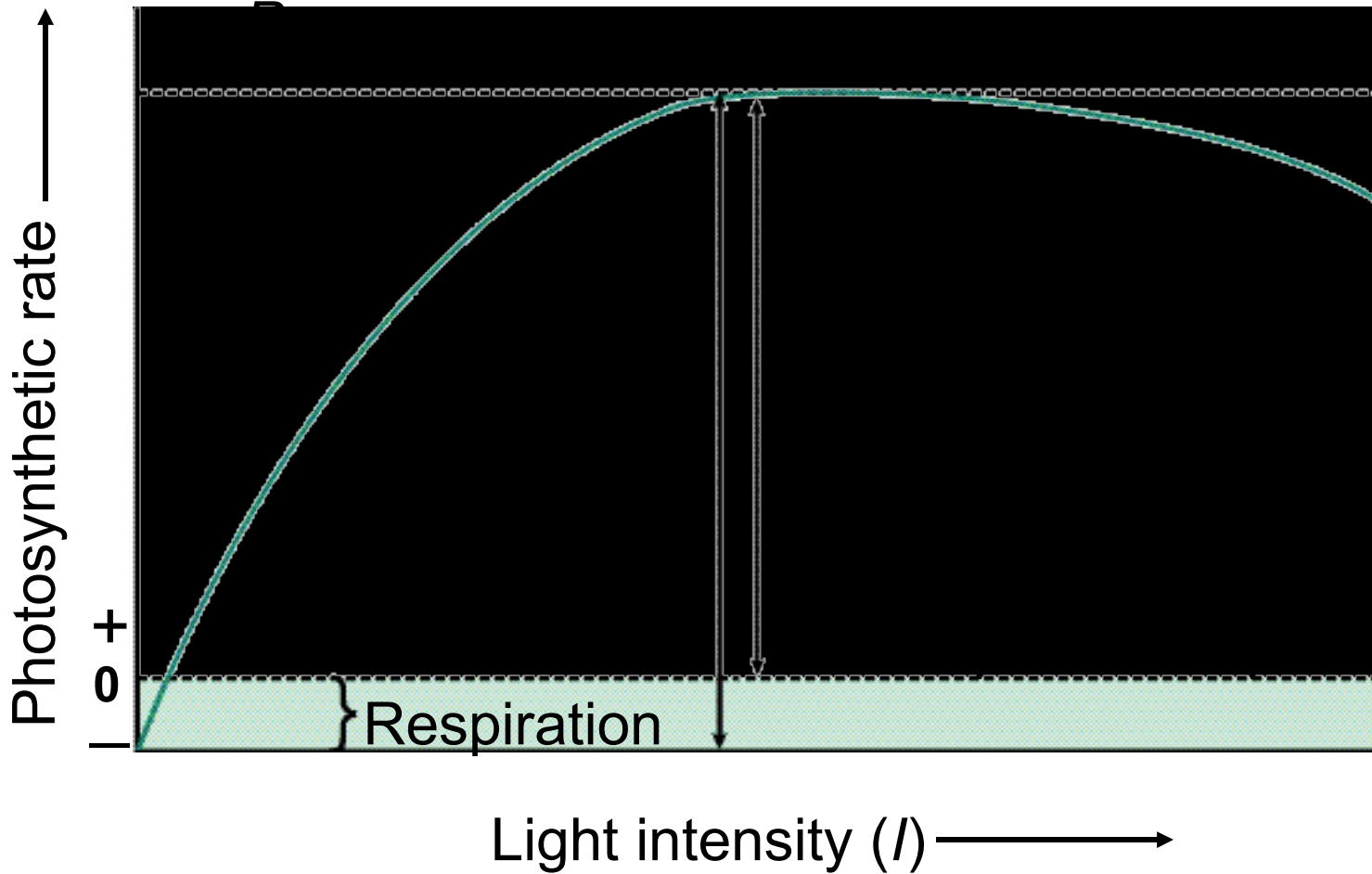
- “Bottom up” vs. “top down” controls of diversity
 - Bottom up- does the rate of primary production determine diversity at higher trophic levels?
 - Top down- do predators or grazers control diversity at lower trophic levels

Factors that influence phytoplankton growth:

- Light (amount, availability, quality)
- Nutrients
- Temperature

Factors that influence phytoplankton growth

Compensation point



Factors that influence phytoplankton growth

Compensation depth

- Depth where energy consumed by respiration = gross photosynthetic rate (therefore net photosynthesis = 0)
- Rule of thumb: Depth at 1% surface intensity
- Changes with season, latitude, turbidity
- Tropics-120-150 m, temperate= 2 m
- Strong shade-adaptation of tropical phytoplankton leads to maximal abundance at 100 m

Photosynthesis



This process can be quantified by measuring the increase of oxygen, the decrease of CO_2 , or the increase of organic carbon.

For practical reasons, oceanographers measure the incorporation of radioactive ^{14}C into organic compounds; some label water with ^{18}O (stable isotope) and measure its appearance in oxygen.

Primary Production by ^{14}C

Method for the determination of primary production in seawater, expressed as $\text{mg C/m}^3/\text{day}$ or integrated vertically to units of $\text{mg C/m}^2/\text{day}$. (or per hour or per year....)

Primary production is defined as the uptake of inorganic carbon into particulate matter as: **Primary production = $\text{mg carbon} / \text{m}^3 / \text{day}$**

A vertical profile of production measurements can be integrated to yield a production rate per unit area in units of: **Primary production = $\text{mg carbon} / \text{m}^2 / \text{day}$**

Principle of Analysis

The rate of carbon fixation (= primary production) by autotrophs in seawater is measured by tracing the uptake of radioactive ^{14}C from the dissolved inorganic form to the particulate organic form.

Radiocarbon is added at a known or assumed ratio to the total inorganic carbon content of the seawater sample.

The uptake of radiocarbon by the particulate phytoplankton is converted to total carbon uptake by conversion using this radiocarbon:total carbon ratio.

Inorganic carbon uptake into particulate inorganic carbon is not measured as the samples are acidified before analysis. The method is easily expanded to include measurements of size-fractionated particulate production or the net production of radio labelled dissolved organic carbon.

Photosynthetic activity during SESAME will be measured mainly *in situ* by the ^{14}C technique (as in Ignatiades, 1990; Ignatiades *et al.*, 2002, etc).

For each sampling depth $\sim 2L$ of seawater will be required. The same fractions as for size fractionated chl-a will be studied:

$\emptyset > 5$ or $10\mu\text{m}$

5 or $10\mu\text{m} > \emptyset > 2\mu\text{m}$

$2\mu\text{m} > \emptyset > 0.2 \mu\text{m}$

Apparatus, Reagents and Supplies

Scintillation Counter: The measurement of radioactivity is typically done by liquid scintillation counting (Beckman L56500). It measures the β -radiation emitted by the ^{14}C incorporated into the cells

Stock ^{14}C sodium bicarbonate (aqueous, specific activity 5 mCi/ml, 5 mCi lots)

Teflon bottles for holding stock ^{14}C solutions (100 mls) and for preparing the stock solutions (500 mls).

Working Solutions

A sodium [^{14}C] bicarbonate AQUEOUS SOLUTION of **1.0mCi** 37.0MBq (Amersham, Code number: CFA3)

Stock solution (**1.0mCi**) diluted in 200ml Milli-Q, adding pellets of NaOH

Make small aliquots (1ml) in cryovials. One cryovial of 1ml stock solution is **5 μ Ci**. Aliquots are stored so that a new aliquot can be used for each incubation.

Acid Cleaning Solution (0.5 N HCl) prepared using Milli-Q water. A small aliquot of this solution can also be used for the filter acidification steps.

Scintillation Cocktail: BCS (Biodegradable Counting Scintillation fluid) Code No NBCS104 (4liters) Amersham.

Sampling and *In Situ* Incubation Procedures

The method involves an *in situ* incubation of the productivity samples at the depths of collection.

In situ incubations allow the samples to be exposed to the natural temperatures and light levels (both intensity and spectral quality).

Deckboard incubators are also acceptable and in some instances are the only acceptable method. Neutral-density screens (e.g. perforated nickel) are usually employed (Lohrenz et al., 1992). Spectrally-corrected 'blue' screens have been recommended for 14C deck incubations (Laws et al., 1989). The most realistic conditions possible with regard to light quality and temperature are encouraged.

Outline:

- Photosynthetic productivity is performed *in situ*
- The photosynthetic productivity rates are measured according to STEEMAN- NIELSEN (1952)
- Seawater collected from standard depths and DCM of the euphotic zone.
- Seawater is dispensed in 250ml transparent polycarbonate bottles (three light and one dark for each depth, if size fractionated each light bottle corresponds to each fraction, otherwise 3 replicates of total PP)
- Each one is injected with $5\mu\text{Ci}$ ^{14}C - NaHCO_3
- Incubation at the respected sampling depths for 2 -3 hours

Field infrastructure: CTD, Rosette sampler, polyethylene bottles (i.e. CORNING or NALGENE, etc, 300ml, high transparency), syringes, ¹⁴C ampules, buoys, ropes, etc., filtering apparatus, filters (Millipore or Nuclepore polycarbonate 0.2, 2, 5 or 10 μm), glassware, vacuum pumps, scintillation vials, HCl, scintillation fluid, pH-meter.

Incubation Bottles

Polycarbonate 0.25 l bottles for productivity incubations. New bottles are soaked for 72 hours in a 5% solution of Micro detergent. Bottles are then rinsed thoroughly with deionized water, and subsequently soaked for 72 hours in the acid cleaning solution.

Once a new bottle has been cleaned as described above, then cleaning between cruises consists of soaking in the acid cleaning solution for several days and rinsing 3 times with Milli-Q.

In some applications it may be appropriate to use smaller bottles, however, there is a general feeling (and some published papers) that suggests that larger bottles are preferable.

Large bottles have a smaller surface-volume ratio and thus minimize contamination and biological problems associated with the container walls.

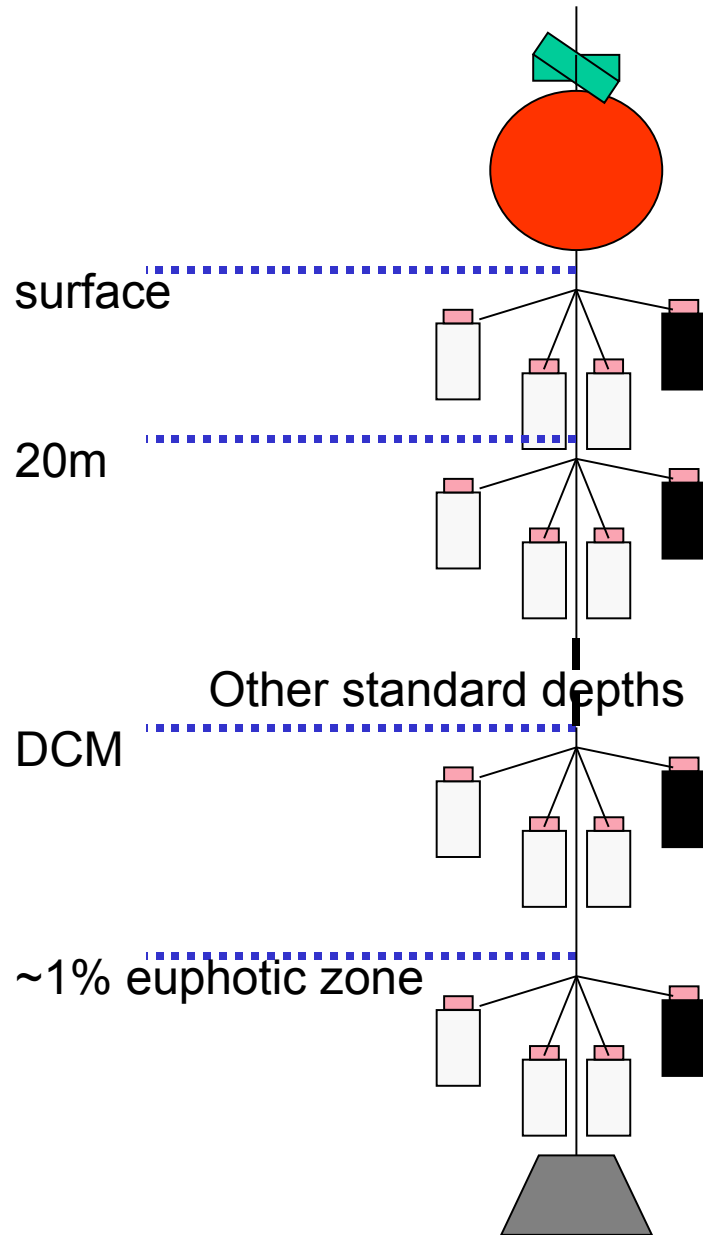
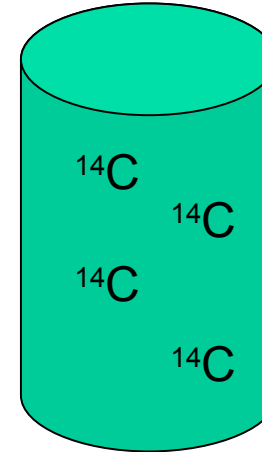
Larger bottles also result in much larger volumes of radioactive waste. For investigations in any environment, investigators should conduct their own experiments to determine the appropriate container volume.

For the measurement of productivity on consecutive days (as on a long transect cruise), it may be advisable to have two or three complete sets of incubation bottles to allow for adequate washing of each set between incubations.

Preparations

- The dark bottle and 3 light bottles are hooked together with an appropriate system for suspension on the *in situ* array.
- This can be a simple arrangement of plastic electrical cable ties or a complex plastic rack.
- The incubation bottles should be kept dark until deployment. The suspension apparatus should be tested for recovery under rough conditions.

^{14}C Addition



- Add radioactive carbon molecules (typically bicarbonate: $\text{H}^{14}\text{CO}_3^-$)
- Incubate in light conditions
- Filter out phytoplankton cells and measure amount of radio-labeled carbon incorporated in cells

Deployment

The bottom weight, attached to a pre measured polypropylene line, is lowered first.

Each group of bottles is then secured to hooks attached to the line at the depth that the sample was originally collected.

The entire productivity line is suspended from an orange plastic float, which is attached to a spar equipped with strobe flash and VHF radio beacon.

Time and position of deployment are recorded.

Recovery

Approximately after 2 hours the productivity array is recovered.

Sample bottles are detached from the line and placed in dark plastic or wooden containers until filtration.

Filtrations should be carried out as soon as possible since respiration and grazing continue once the bottles are onboard.

Time and position of recovery are recorded.

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Laboratory Analysis:

The incubated samples are filtered immediately after termination of incubation through 0.2 μ m, 2.0 μ m and 5 or 10 μ m polycarbonate Millipore filters (47 mm diameter) so the rates of primary production are estimated for the total autotrophic population and for the fractions of the photosynthetic populations.

Filters are placed in scintillation vials, acidified with 1ml 0.5N HCl

10ml scintillation fluid (BCS= BIODEGRADABLE COUNTING FLUID< AMERSHAM) is added.

the scintillations vials containing the filters will be stored in dark until measuring ^{14}C in the lab.

the samples are counted in the lab in a liquid scintillation counter (BECKMAN LS6500)

Available Irradiance on sea surface and/or the depth disappearance of Secchi disc are used to calculate the depth of the euphotic zone (depth where the 1% of the surface energy is reached).

Calculations

CPM values are converted (**mgC.m-3.h-1**) to productivity rates per hour using the following equation:

$$\textit{Production (mgC.m-3.h-1)} = ((R_s - R_b) * W * 1.05) / (R * N)$$

where:

R_s = CPMs in filtered sample

R_b = CPMs in blank bottle

R = CPMs in stock solution

W = **2.13.10⁻⁴mgC.m⁻³** (DIC concentration in samples)

1.05 = correction for the lower uptake of ¹⁴C compared to ¹²C

N = hours

Integrated Water Column Production

- The individual depth measurements of daily production are used to calculate water column integrated production ($\text{mg C m}^{-2} \text{ d}^{-1}$) by trapezoidal integration.
- The rate nearest the surface is assumed to be constant up to 0 m, and a zero rate is assumed for an arbitrarily deep depth (e.g. 200m).
- The production at each pair of depths is averaged, then multiplied by the difference between the two depths to get a total production in that depth interval.
- These depth interval values are then summed over the entire depth range to get the integrated production rate.

Some notes regarding ^{14}C fixation:

Strength of solutions for different types of water:

- $25\mu\text{Ci}$ for most open ocean waters
- $5\mu\text{Ci}$ for moderately productive inshore waters
- $1\mu\text{Ci}$ for coastal areas during a bloom

Use of black bottles because even in total darkness there is some uptake by plants, animals and a little true fixation. The black bottle is treated as the light one. CPM measurement of the dark bottle is subtracted from the light bottle CPM from the same depth

Filters are treated by acid to remove the inorganic labelled C

Filters can be stored for several weeks if adequately protected

Primary Production rates in Greek Seas:

Coastal areas (as Saronikos Gulf)

Elefsis Bay: 4-200mgC/m³/h, mean integrated value: 40 mg/m²/h

Sewage area: two times less

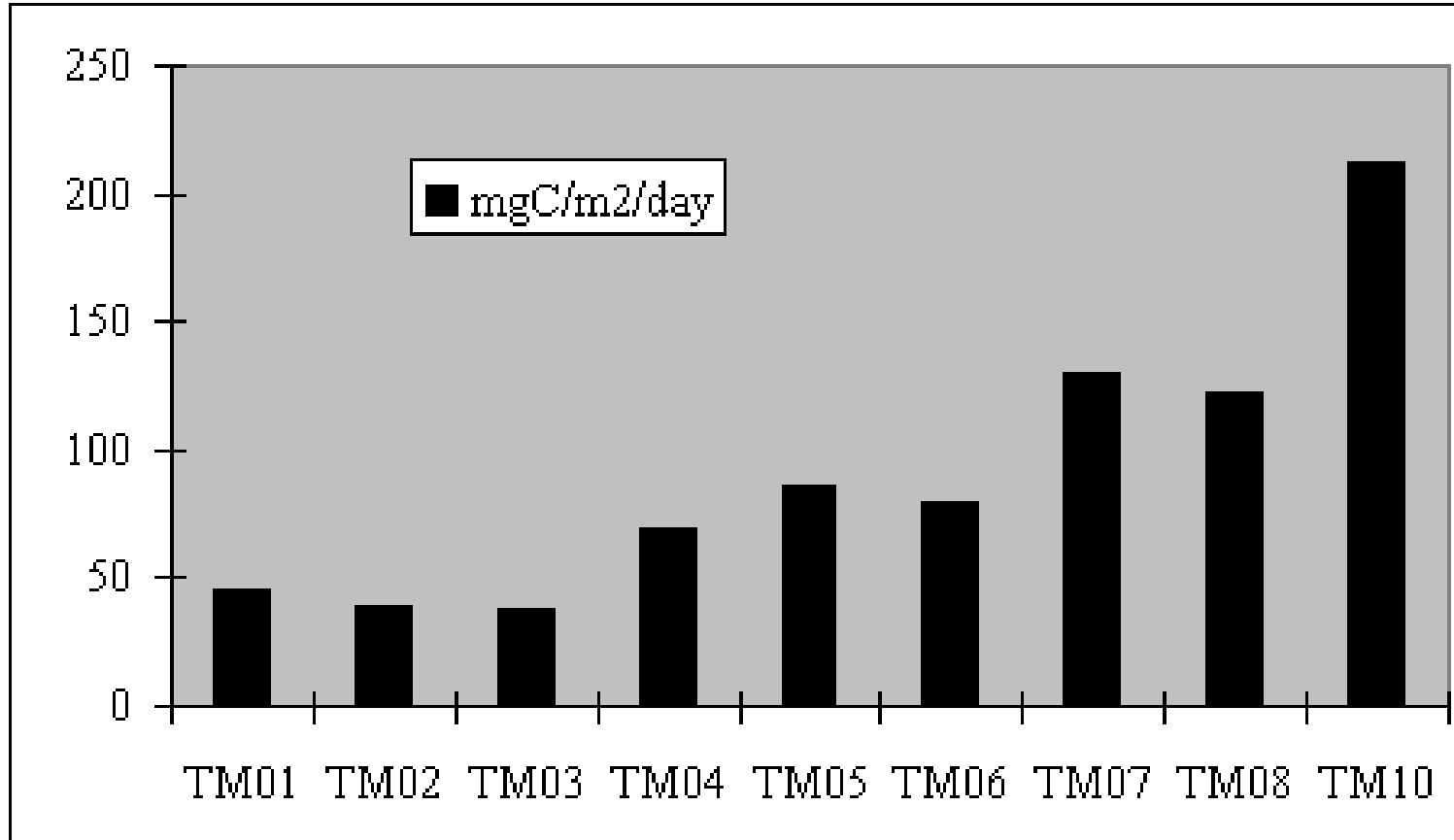
Not affected areas: 5-7 mgC/m²/h

Open Sea Waters:

0.05-0.80 mgC/m³/h

Values typical in the pelagic waters of Levantine Basin

TRANSMEDITERRANEAN CRUISE





EU project MATER

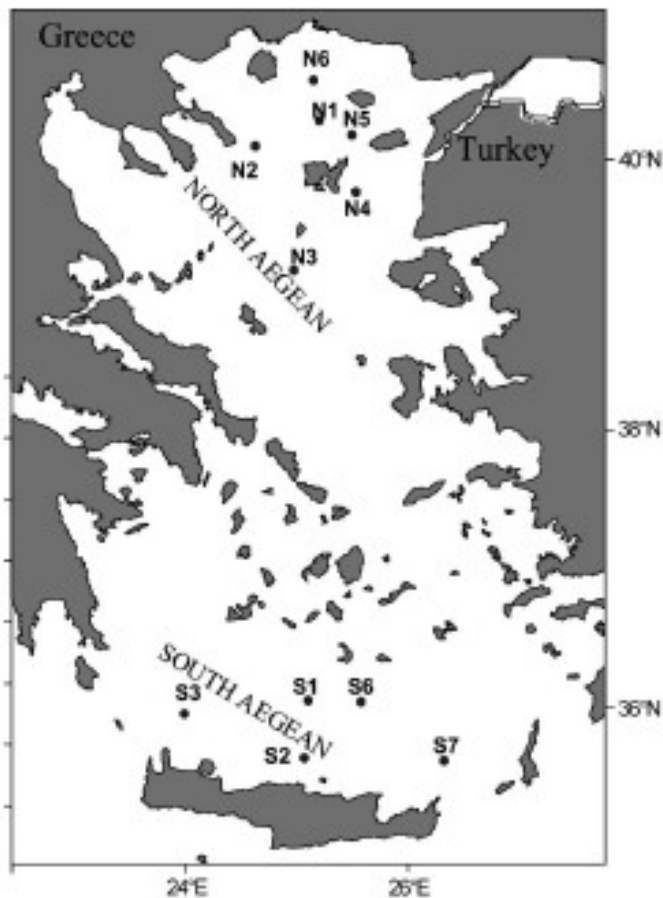
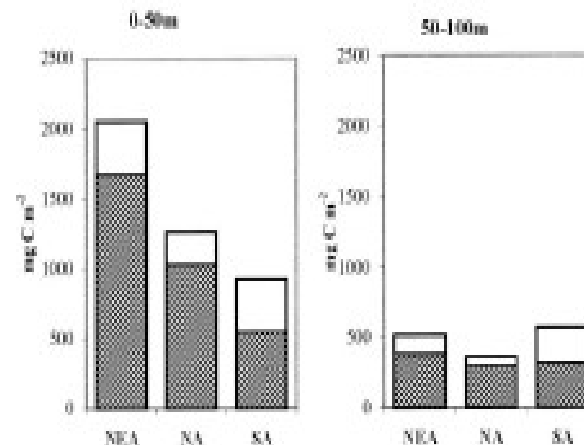


Fig. 1. Location of stations.

MARCH 1997



SEPTEMBER 1997

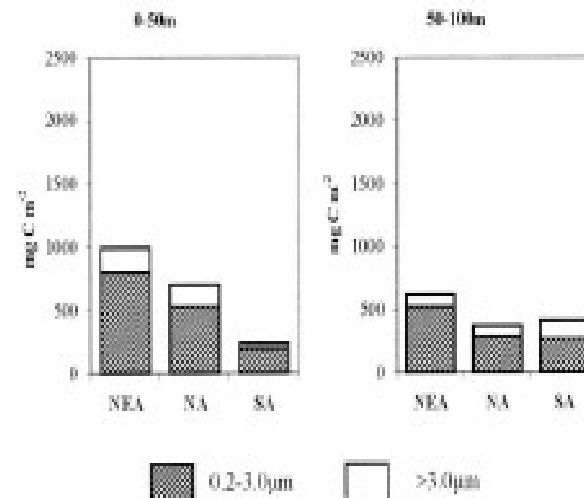


Fig. 4. Biomass distribution of autotrophs in the 0-50 and 50-100 m layers during March and September 1997.

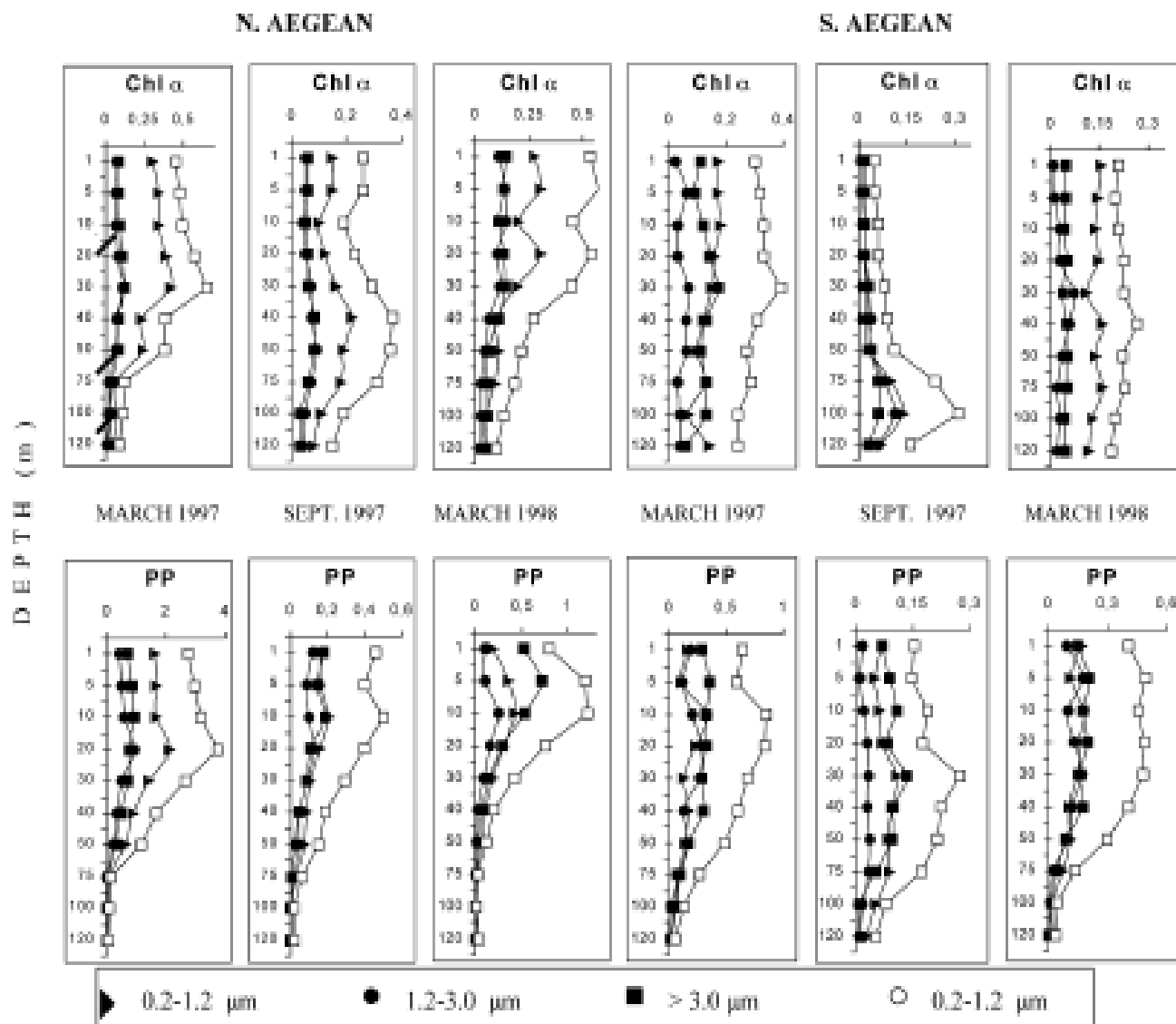


Fig. 4. Vertical distribution of chlorophyll a ($chl\ a$, $\mu g\ m^{-3}$) and primary production (PP, $mg\ C\ m^{-3}\ h^{-1}$) fractions in the N. and S. Aegean Sea. [picoplankton (0.2–1.2 μm), ultraplankton (1.2–3.0 μm), nano/micropkton (>3.0 μm)]

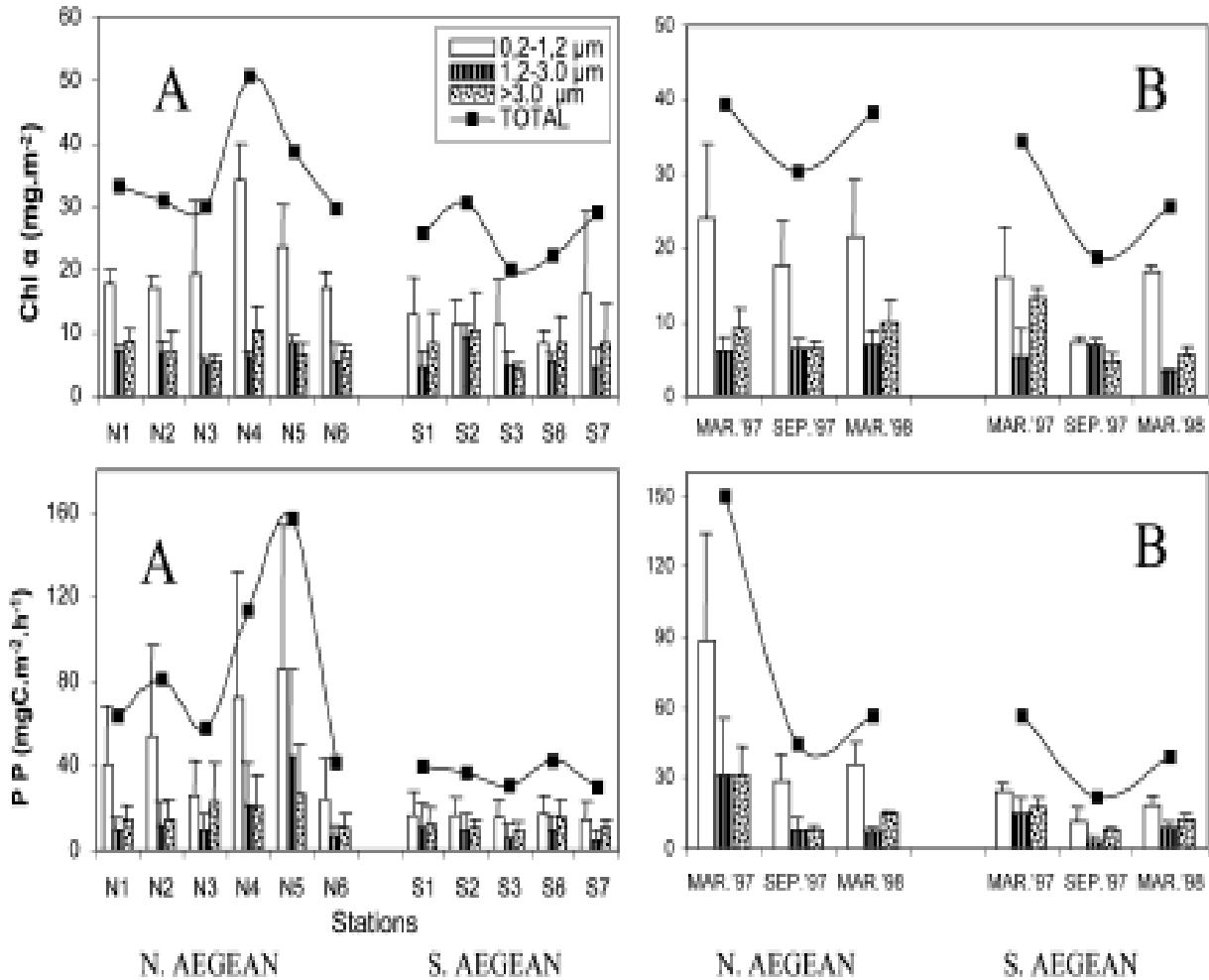
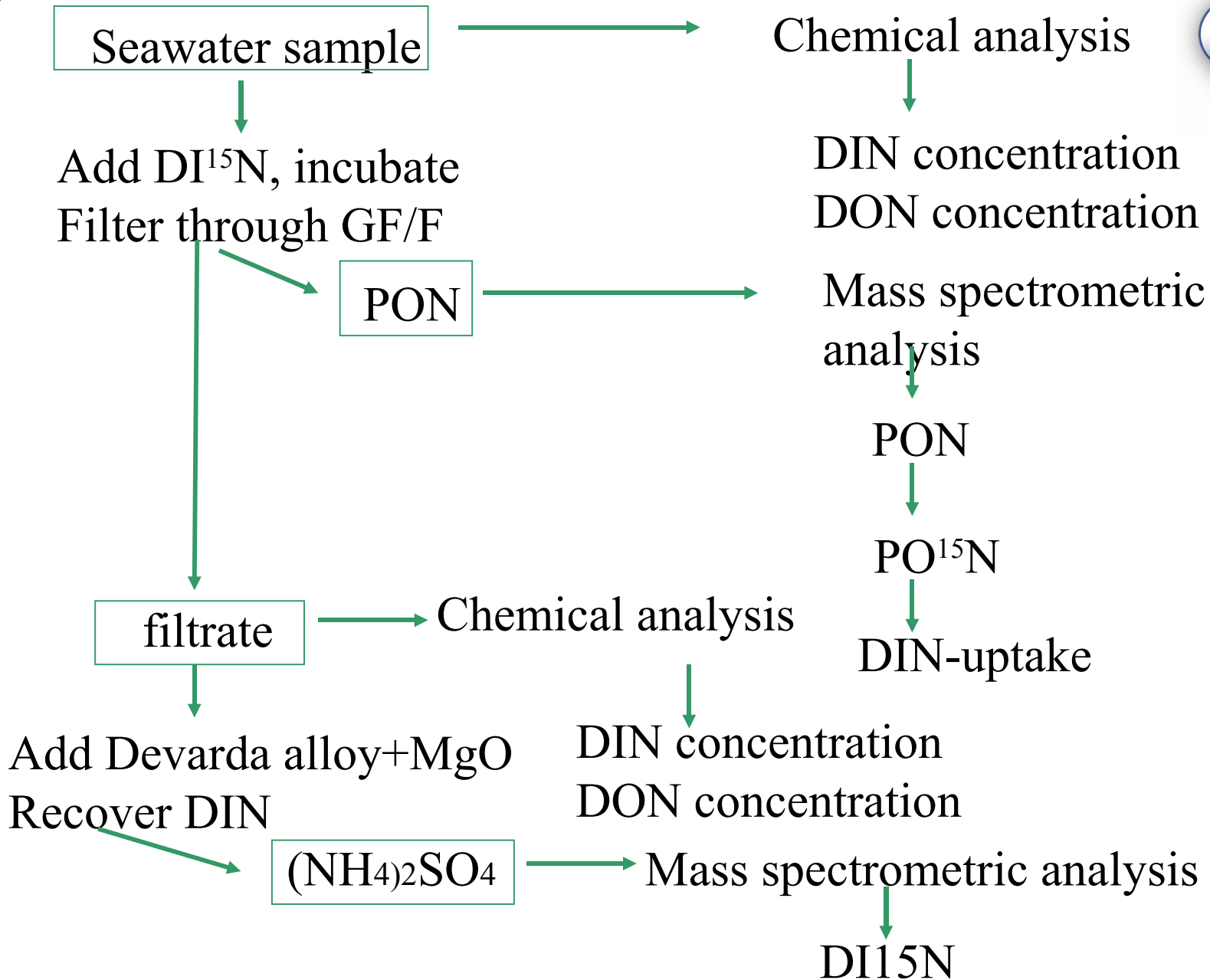


Fig. 3. Spatial (A) and temporal (B) distribution of chlorophyll a (chl a) and primary production (P.P.) stations in the N. and S. Aegean Sea. (picoplankton (0.2-1.2 μm), ultraplankton (1.2-3.0 μm), nanomicroplankton (>3.0 μm)).

Primary Production measurements using ^{15}N -tracers

The ^{15}N -tracer method for measuring PP in terms of N is based in a 2-compartment model, which includes inorganic or organic N (DIN or urea, the source pool of ^{15}N -labelled N) and particulate organic N (PON the target pool of ^{15}N -labelled N) and in which the net flux of N added as a tracer depends on the relative importance of assimilative (on the filter) and regenerative processes (filtrate)

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The method:

Total primary production in terms of ^{15}N is measured at the same stations as for the ^{14}C experiments, according to the method described by Slawyk & Raimbault (1995).

Water samples from the same standard depths are dispensed into two 500 ml acid-clean transparent polycarbonate bottles and each one is inoculated with the ^{15}N -tracer as K^{15}NO_3 or $^{15}\text{NH}_4\text{Cl}$ (99 atom % ^{15}N).

Since initial NO_3^- and NH_4^+ concentrations are not immediately determined on board prior to the ^{15}N experiments, the additions of $0.05\text{-}0.1\mu\text{g at }^{15}\text{N.l}^{-1}$, which is expected to be 10-20% of the ambient concentration, results in enrichment greater than 50%, in some occasions, in nutrient-impooverished oligotrophic waters.

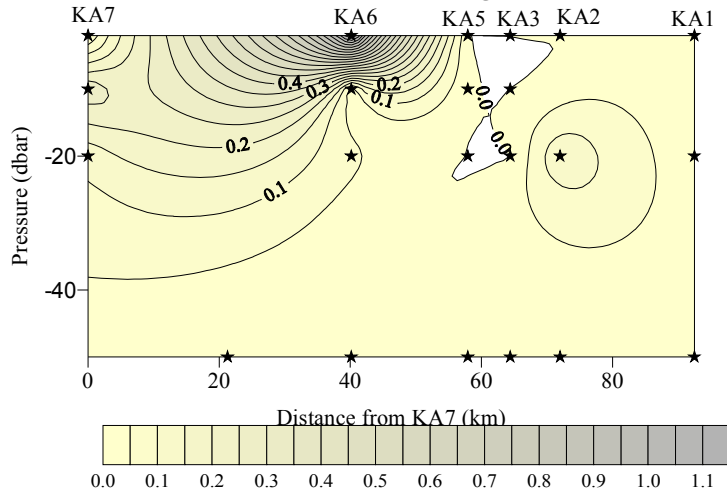
It should be noted that these ^{15}N addition above tracer amount could significantly alter the nitrogen environment of phytoplankton and thus the measured uptake rates (Allen *et al.*, 1996; Harrison *et al.*, 1996).

However these results can still be informative regarding uptake rates.

The bottles are incubated *in situ* at the chosen depths, for 4 hours around noon.

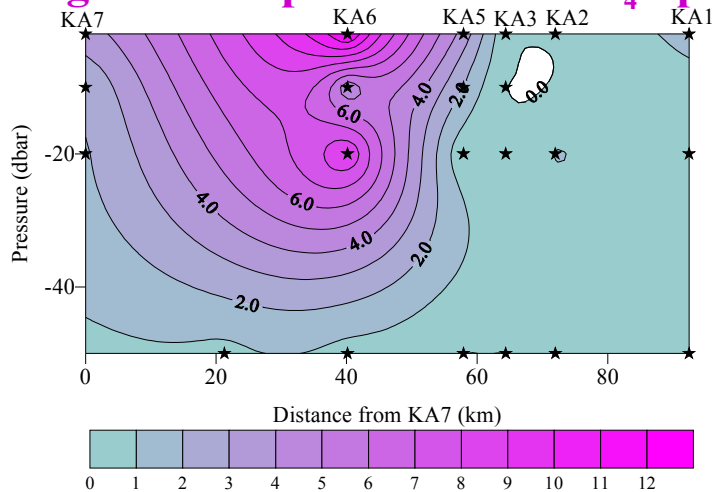
Following incubation, samples are filtered onto 25mm precombusted GF/F filters, dried at 60°C and stored with desiccant until mass spectrometric analysis

New production: $^{15}\text{NO}_3$ uptake rates = ρNO_3 (nM/h)

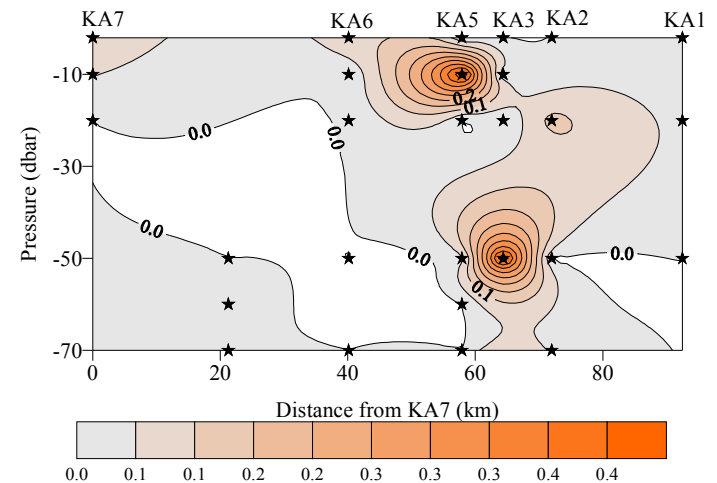


*N. Aegean Sea
September 1999*

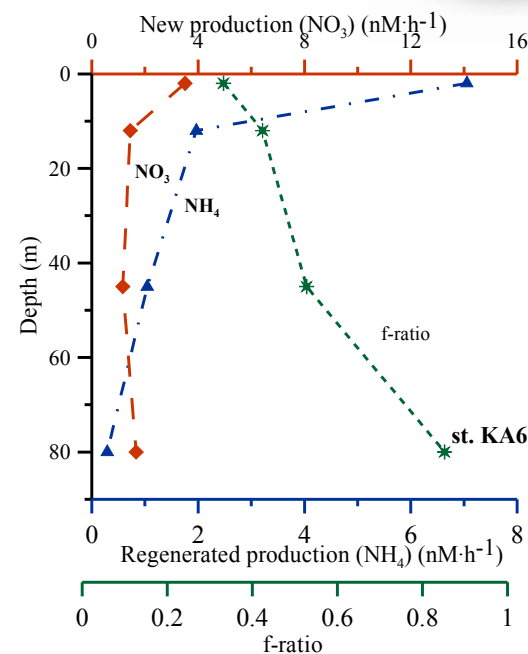
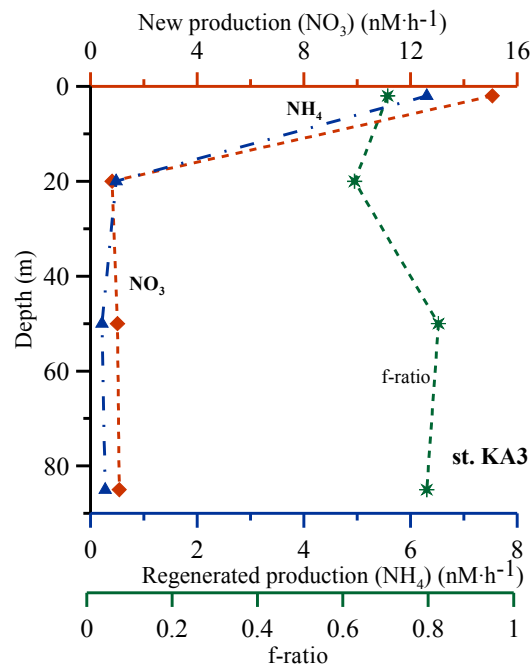
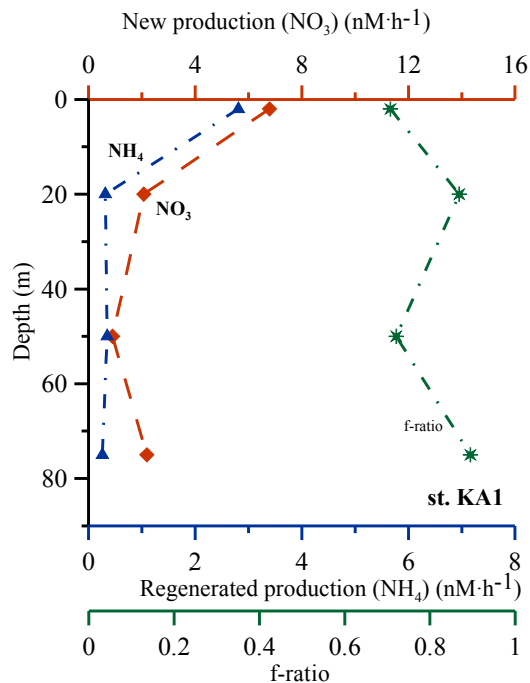
Regenerated production: $^{15}\text{NH}_4$ uptake rates = ρNH_4 (nM/h)



"f" ratio: new / total production



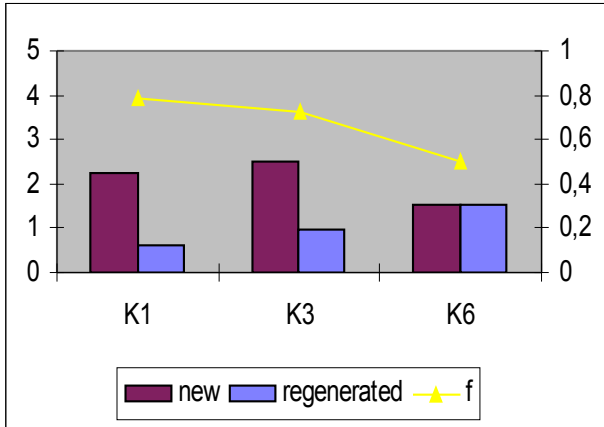
N. Aegean Sea - April 2000



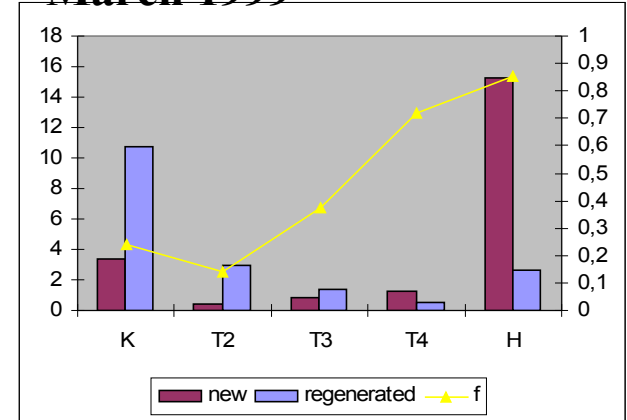
- "f" ratio, new/total
- ^{15}N uptake rate, ρNO_3 (nM/h)
- ^{15}N uptake rate, ρNH_4 (nM/h)

N. Aegean vs Skagerrak

April 2000



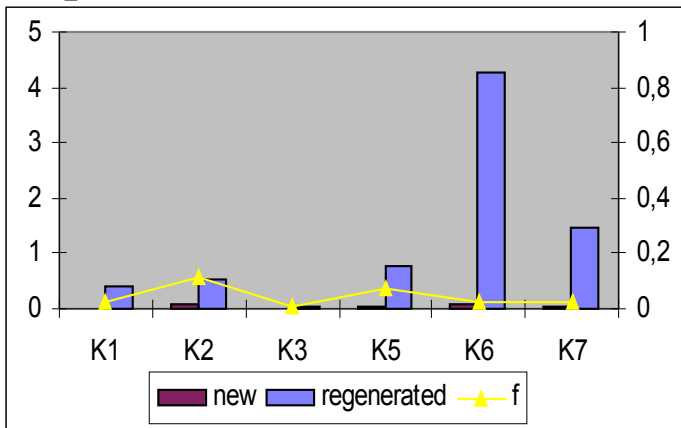
March 1999



N uptake rates and "f" ratio

Mean values for the water column

September 1999



August 2000

