Methodology of measurements biogeochemical parameters of suspended and dissolved particles in seawater

Joanna Stoń-Egiert

Biophysics Laboratory
Instytute of Oceanology
Polish Academy of Science

lecture given in the framework of
activities promoting science DUN

What kind of parameters can we measure in laboratory?

concentration of:

- pigments
- chromophoric dissolved matter
- suspended particles

- absorption coefficients (phytoplankton and derytus)
- partice size distribution

Basic steps in field work

On ship board

water sampling

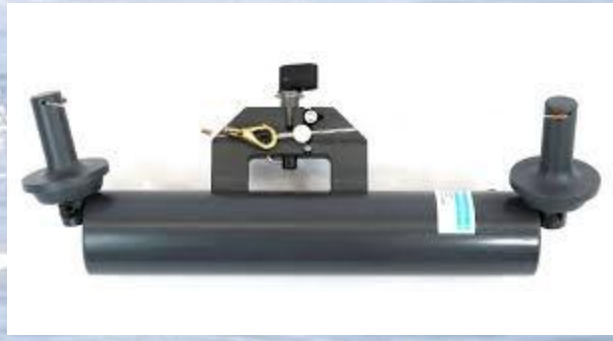
filtration

storage

At the institute

laboratory analysis

bathometers



filters: glass fibre GFF and cellulose-acetate

dewar with liquid nitrogen

deep freezer (-80°C)

refrigerator

Concentration of chlorophyll a

Filtration
Whatmann GF/F
ϕ=47mm

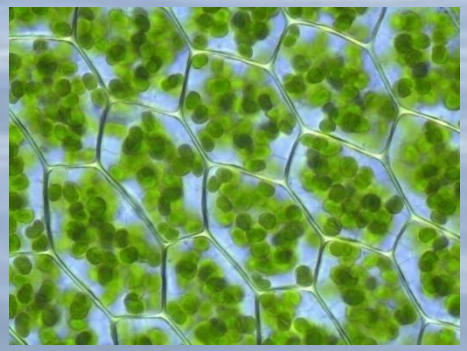
Storage
liquid nitrogen
(-196 °C)

Extraction

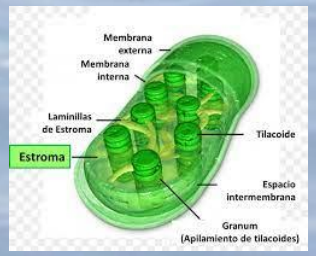
Spectrophotomeric measurements

Extraction

1. Unfreeze filters and placed them into plastic vials
2. Solvent – 96% ethanol, 8 ml
3. Time of extraction – 24h
4. Conditions: darkness, room temp.
5. Centrifugation of samples to clarify extract



Example of algae cells



Scheme of chloroplast

Chlorophyll a extracts

Concentration of chlorophyll a

Filtration
Whatmann GF/F
ϕ=47mm

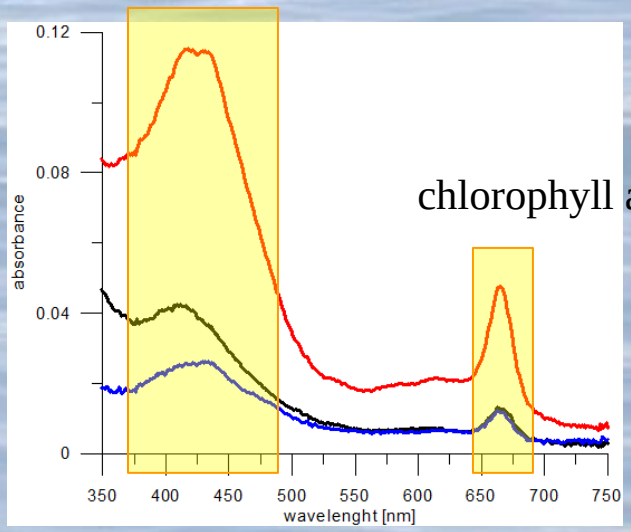
Storage
liquid nitrogen
(-196 °C)

Extraction

Spectrophotomeric measurements

LAMBDA 650 UV/Vis Spectrophotometer, PerkinElmer, Inc

other chlorophylls and carotenoids



$$Ca\,[mg\,m^{-3}] = \frac{10^3 \cdot (A_{665nm} - A_{750nm}) \cdot v_{extr}}{83 \cdot v_{filtr} \cdot l}$$

v_{extr} –volume of solvent used to extraction [cm^3],
v_{filtr} – volume of filtered seawater [dm^3],
l – optical lenght [cm],
83 – value of absorbance coefficent of chlorophyll a in ethanol (Sartory i Grobbelaar 1984, Lorenzen i Newton Downs 1986, Guideline for Baltic Monitoring Program 1988).

Guideline for Baltic Monitoring Program, 1988, Baltic Marine Environment Protection Commission, Helsinki, 116

Lorenzen C.J., Newton Downes J., 1986, *The specific absorption coefficients of chlorophyllide a and pheophorbide a in 90% aceton and comments on the fluorometric determinations of chlorophyll and pheopigments*, Limnology and Oceanography **31**, pp. 449–456

Sartory D.P., Grobbelaar J.U., 1984, *Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis*, Hydrobiologia 114, 177 - 187

Chlorophyll a in 90% acetone

- ***The equation of Jeffrey and Humphrey (1975)*** **[JH]:**

$$Ca\,[mg\,m^{-3}] = \frac{(11{,}85 \cdot A_{663\text{–}665nm} - 1{,}54 \cdot A_{647nm} - 0{,}08 \cdot A_{630nm}) \cdot v_{extr}}{v_{filtr} \cdot l}$$

- ***The equation of Lorenzen (1967)*** **[L]:**

$$Ca\,[mg\,m^{-3}] = \frac{26{,}7(A_{663\text{–}665bnm} - A_{647\text{–}665anm}) \cdot v_{extr}}{v_{filtr} \cdot l}$$

$A_{663\text{-}665b}$ - before acidification.
$A_{663\text{-}665a}$ - after acidification.

- ***The equation of Parsons and Strickland (1968)*** **[PS]:**

$$Ca\,[mg\,m^{-3}] = \frac{(11{,}6 \cdot A_{665nm} - 1{,}31 \cdot A_{647nm} - 0{,}14 \cdot A_{630nm}) \cdot v_{extr}}{v_{filtr} \cdot l}$$

- ***The equation of SCOR/UNESCO (1968)*** **[SU] :**

$$Ca\,[mg\,m^{-3}] = \frac{(11{,}64 \cdot A_{665nm} - 2{,}16 \cdot A_{645nm} - 0{,}1 \cdot A_{630nm}) \cdot v_{extr}}{v_{filtr} \cdot l}$$

References:

BMB, 1979, Recommendations on methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll, [Ed. L. Edler], BMB, 5

Guideline for Baltic Monitoring Program, 1988, Baltic Marine Environment Protection Commision, Helsinki, 116.

Jeffrey S. W., Humphrey G. F., 1975, New spectrophotometric equation for determining chlorophyll a, b, c1 and c2, Biochem. Physiol. Pflanz., 167, 194-204

Lorenzen C. J., 1967, Determination of chlorophyll and phaeopigments: spectrophotometric equations, Limnol. Oceanogr., 12

Strickland J. D. H., Parsons T. R., 1968, A practical handbook of seawater analysis. Pigment analysis, Bull. Fish. Res. Bd. Canada, 167

UNESCO, 1966, Determinations of photosynthetic pigments in seawater, Rep. SCOR/UNESCO WG 17, UNESCO Monogr. Oceanogr. Methodol., 1, Paris

Concentration of phytoplankton pigments: chlorophylls and carotenoids

Filtration
Whatmann GF/F
ϕ=25mm

Storage
liquid nitrogen
(-196 °C)

Extraction

Chromatographic resolution (HPLC)

Identification and quantification of pigments

Extraction

1. Unfreeze filters and placed them into plastic vials
2. Solvent – 90% acetone, 3 ml
3. Mechanical homogenization + sonication
4. Time of extraction – 2h
5. Conditions: darkness, 4 °C
6. Centrifugation of samples to clarify extract

Concentration of phytoplankton pigments: chlorophylls and carotenoids

Filtration
Whatmann GF/F
ϕ=25mm

Storage
liquid nitrogen
(-196 °C)

Extraction

HPLC

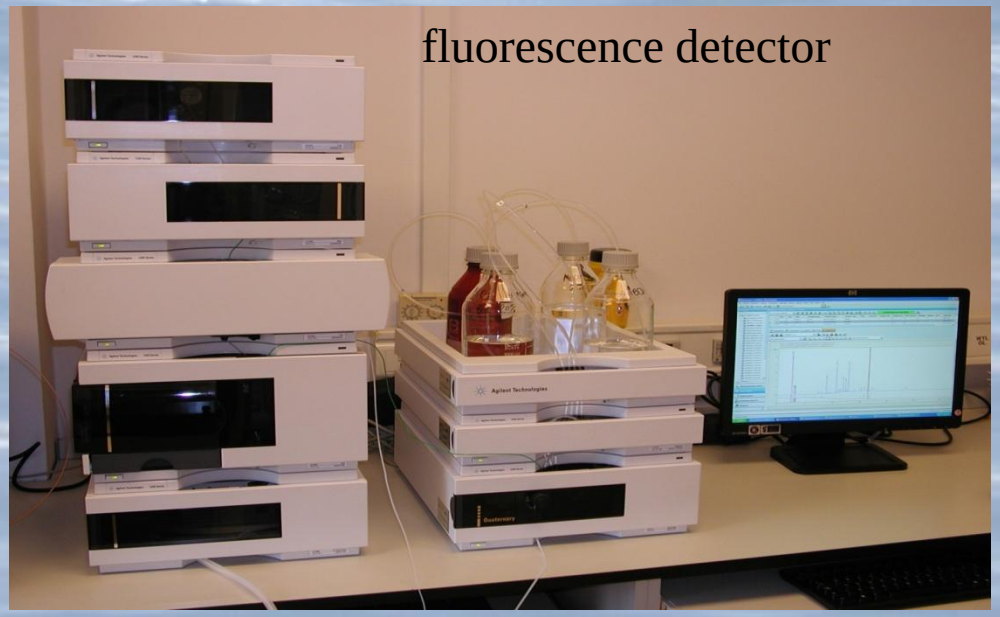
HPLC

HPLC system Agilent 1200 with 'dad' and fluorescence detectors

absorbance detector

chromatographic column

autosampler



fluorescence detector

Heart - pump

Concentration of phytoplankton pigments: chlorophylls and carotenoids

Filtration
Whatmann GF/F
ϕ=25mm

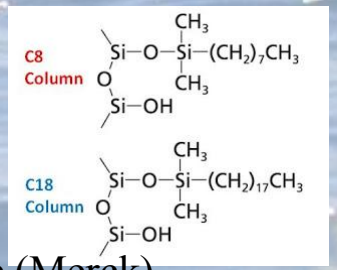
Storage
liquid nitrogen
(-196 °C)

Extraction

HPLC

HPLC

HPLC system Agilent 1200



chromatographic column — LichroCART™ LiChrospher™ 100 RP18e (Merck)
Dimenssion 250 x 4 mm, particle size 5 µm, pore size100 Å

absorbance detector — ‘dad’ – diode array detector, scanning spectrum in range 350nm-700nm with 1 nm step and 0.4 s of time resolution

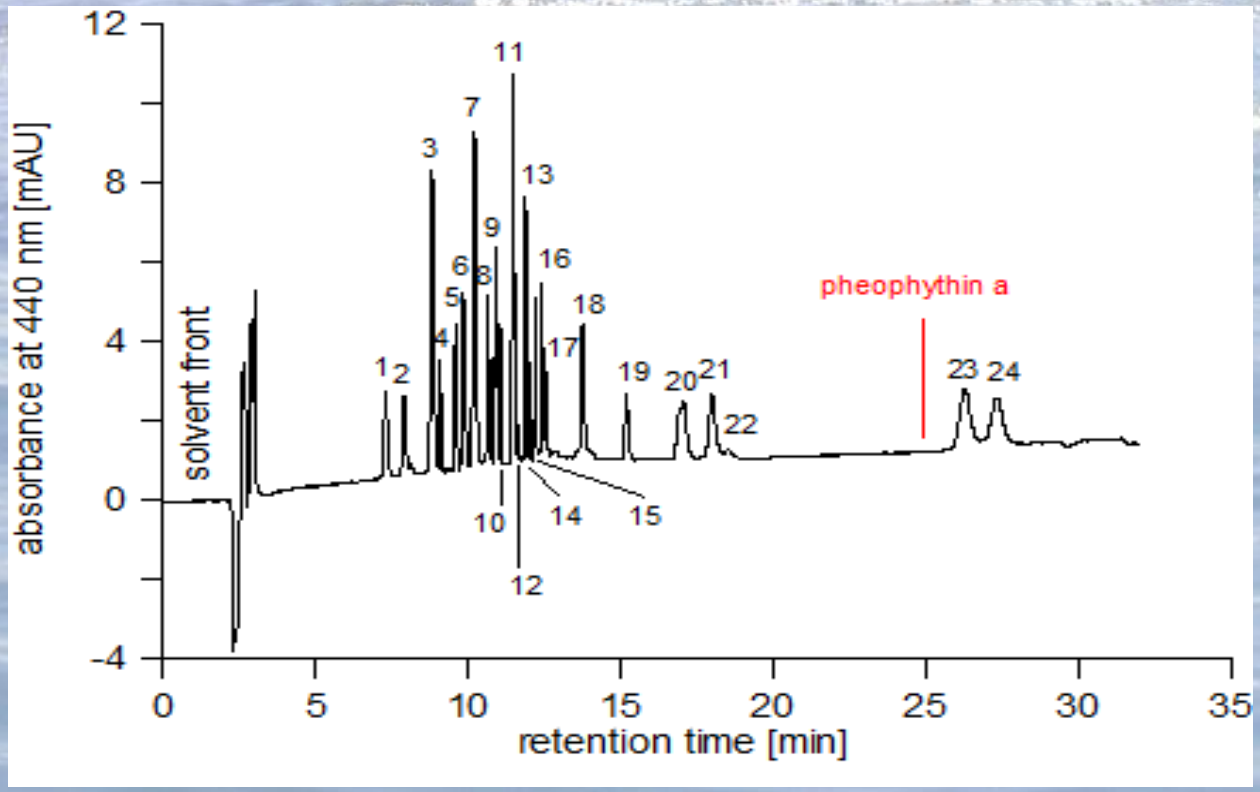
fluorescence detector — set at λ_{ex} = 431 nm i emisji λ_{em} = 660 nm to confirm presence of chloropigments in sample

method

Used solvents:
A - methanol: 1M ammonium acetate (80:20, v:v)
B – methanol : acetone (60:40, v:v)

Changing in time solvent composition:
from 100% of solution A to 100% of B within 10 min, 100% of mixture B to the end of analysis

Example of chromatograph



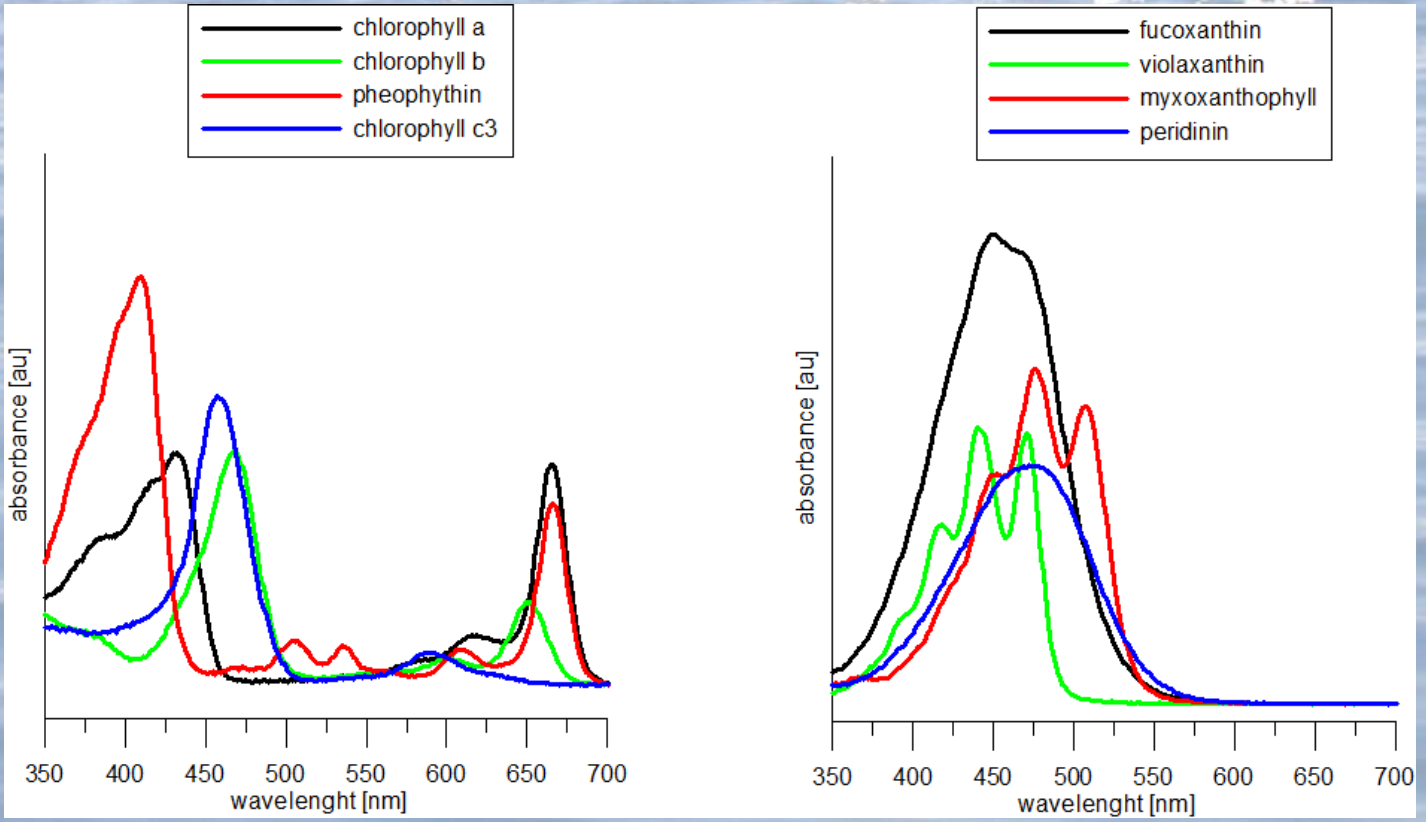
1 – chlorophyll *c3*
2 – chlorophyllide *a*
3 – chlorophyll *c1+c2*
4 – peridinin
5 – 19'but-fucoxanthin
6 – fucoxanthin
7 – 19'hex-fucoxanthin
8 – neoxanthin
9 – prasinoxanthin
10 – violaxanthin
11 – diadinoksantyna
12 – antheraxanthin
13 – alloxanthin
14 – myxoxanthophyll
15 – diatoxanthin
16 – lutein
17 – zeaxanthin
18 – kantaksantyna
19 – chlorophyll *b*
20 – divinyl chlorophyll *a*
21 – chlorophyll *a*
22 – echinenone
23 – α-carotene
24 – β-carotene
25 – pheophythin

Mantoura R.F.C., Llewellyn C.A.,1983, Anal Chim Acta 151: 297-314
***Stoń J.,** Kosakowska A., 2002, J. Appl. Phycol., 14, 205-210*
***Stoń-Egiert J**., Kosakowska A., 2005, Mar. Biol., 147, 251-260*

Standards - The International Agency for ^{14}C Determination DHI Institute for Water and Environment, Denmark

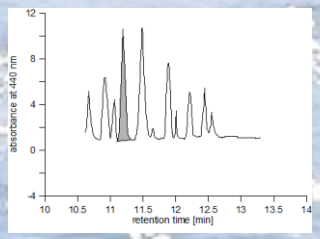
Pigment qualification

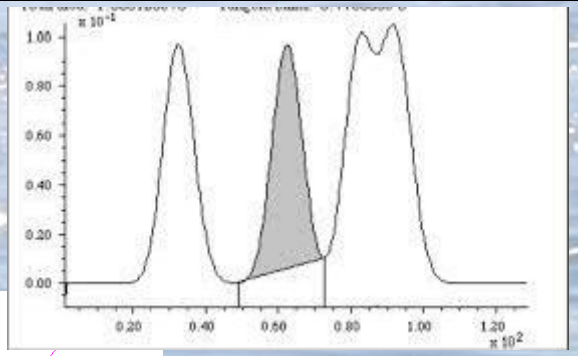
- retention time (relative retention time)
- spectrum shape
- coelution with standards

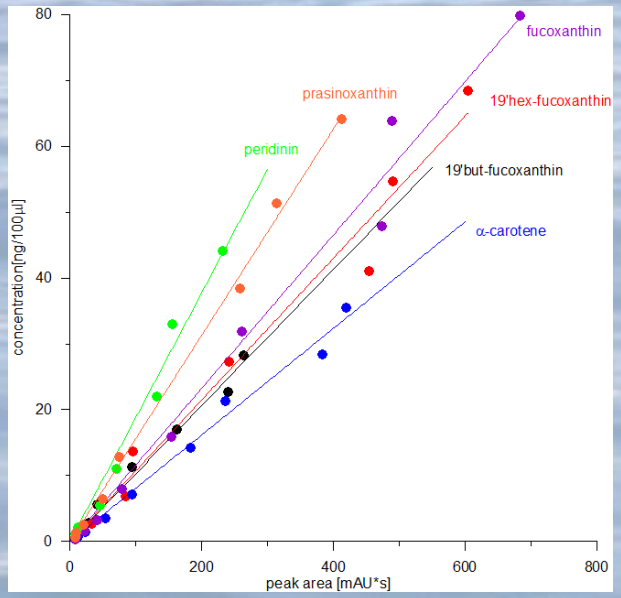


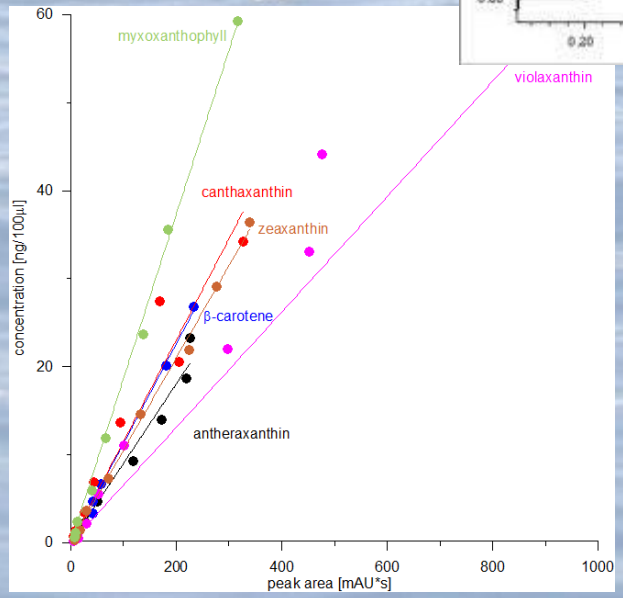
Pigment quantification

- peak area
- response factor obtained from calibration curves









External standarization equation
(*Mantoura i Repeta 1997*)

$$C_i = \frac{A_p f_p v_{ext} 10^3}{v_{inj} v_{filt} B}$$

$C_p \sim f(A_p, f_R, v_{extr}, v_{filtr}, v_{inj})$

Concentration of phycobilins

Filtration
Whatmann GF/F
ϕ=25mm

Storage
liquid nitrogen
(-196 °C)

Extraction

Spectrofluorometric measuremens

Identification and quantification of pigments

Extraction

1. Unfreeze filters and placed them into plastic vials
2. Solvent – MEDIUM, Trizma Base, Na_2EDTA $2H_2O$, Lizozym , pH 5.5, 4 ml
3. Mechanical homogenization + sonication
4. Time of extraction – incubation during 2h in 37 °C + 22h in 4 °C in darkness conditions
5. Centrifugation of samples to clarify extract

Modification and adaptation of methods:

Cobley J., Bennet A., Bogorad J. 1973. J. Cell Biology 58; 419 – 435

Steward D.E. Farmer F. 1984. Limnol. Oceanogr. 29 (2): 392 – 397

Abaldde J., Betancourt L. 1998. Plant Science 136: 108 – 120

Extraction medium: Trizma Base, Na_2EDTA $2H_2O$, Lizozym , pH 5.5 !!

Concentration of phycobilins

Filtration
Whatmann GF/F
ϕ=25mm

Storage
liquid nitrogen
(-196 °C)

Extraction

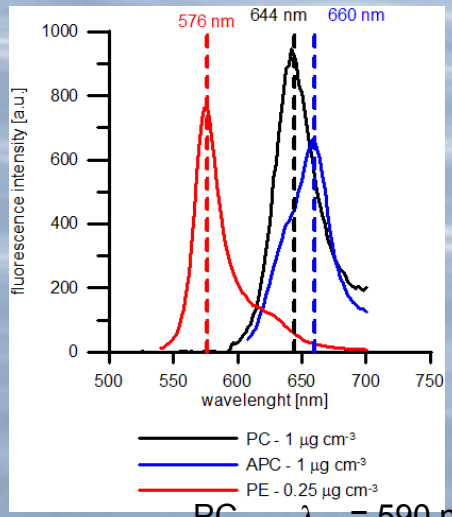
Spectrofluorometric measuremens

Identification and quantification of pigments

Cary Eclipse, Varian, Agilent Technologies

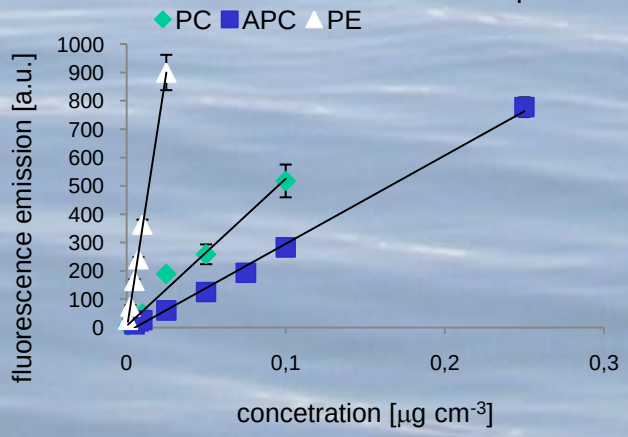
Spectrofluorometric measuremens

Identification and quantification of pigments



Phycobilins standards (ProZyme Inc. San Leandro, CA, USA)

lamp int. 1000V



$$C_{phyco} = \frac{Int_{fluo} * f_{a,fik,fluo} * v_{extr}}{v_{filtr}}$$

PC λ_{ex} = 590 nm
APC λ_{ex} = 600 nm
PE λ_{ex} = 530 nm

Concentration of suspended particular matter SPM, their organic and inorganic fractions

Filtration
Whatmann GF/F ϕ=25mm

Storage

Gravimetric techniques to determination SPM

exiccator

Scales
WAX 110, RADWAG

Combustion in oven
450 °C during 4 h

calculations

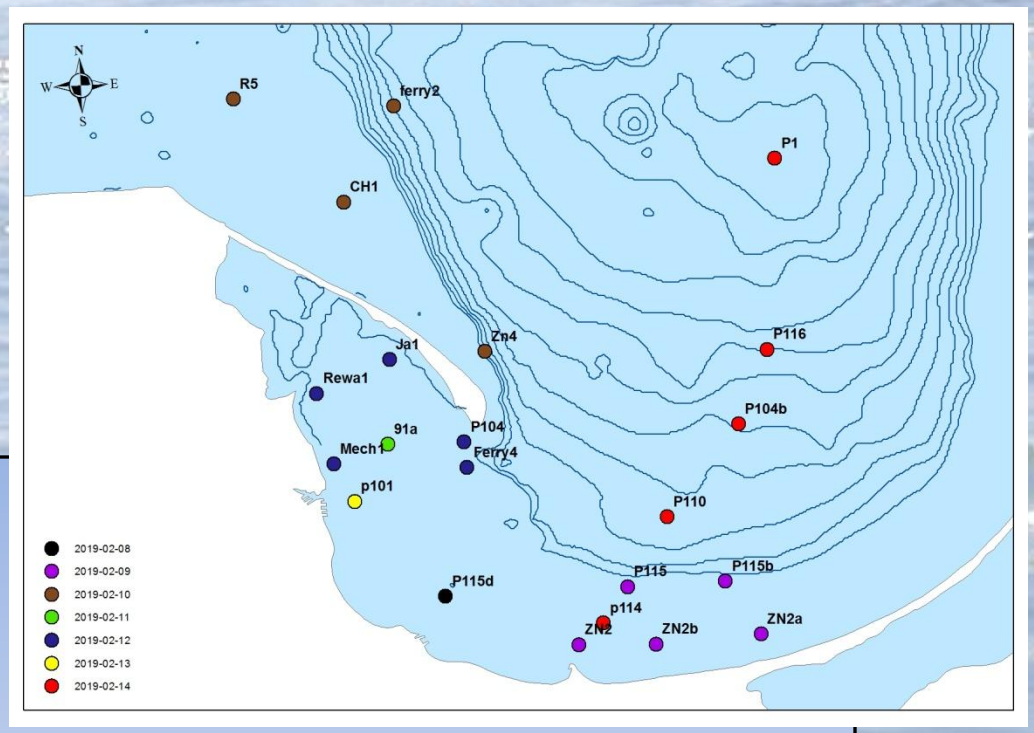
$$SPM = \frac{ms - mf}{v_{filtr}}$$

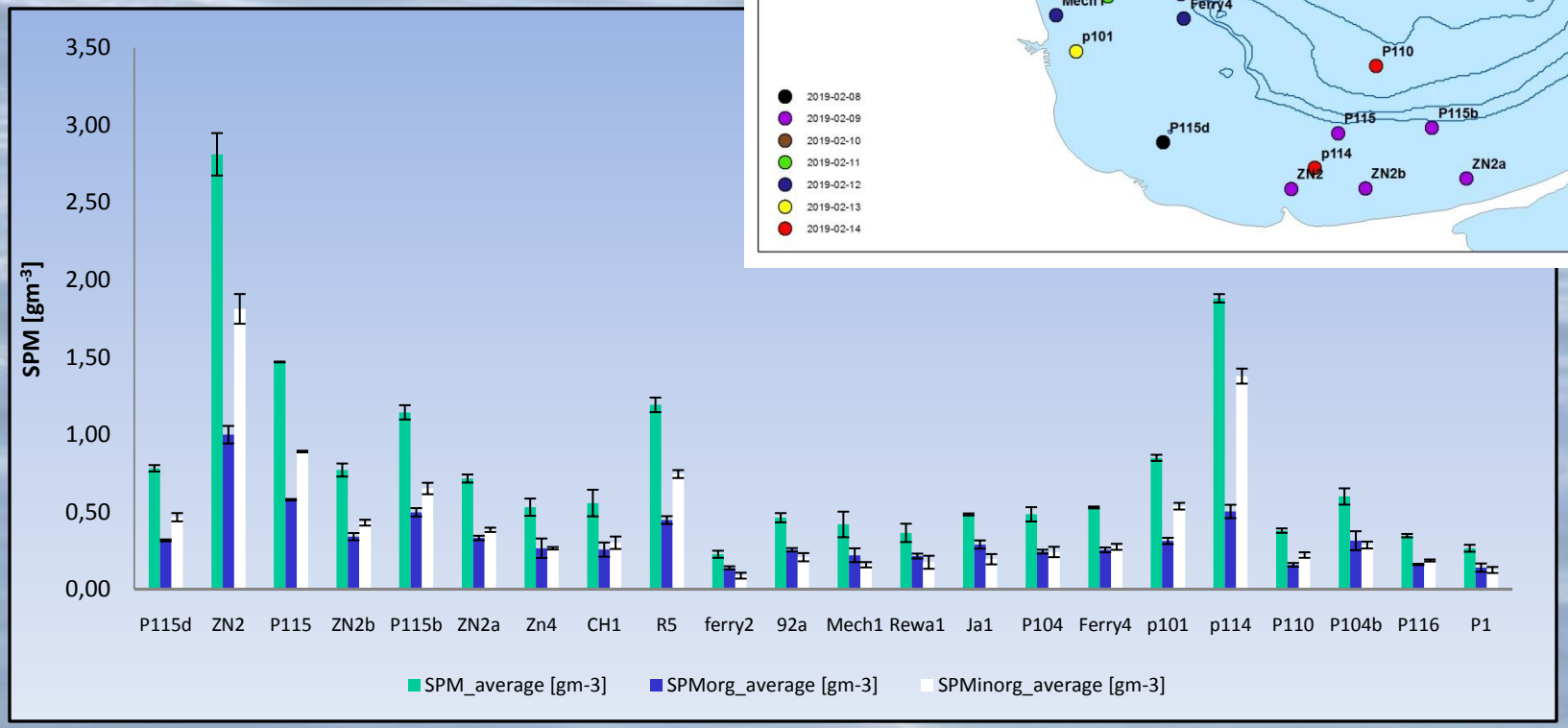
$$SPM_{org} = \frac{ms - msf}{v_{filtr}}$$

$$SPM_{inorg} = SPM - SPM_{org}$$

where – averaged weight of filters without suspension: mf [mg], with suspension: ms, with suspension, after combustion: msf;
V_{filtr} [dm^3] – volume of filtered seawater

SPM in February 2019





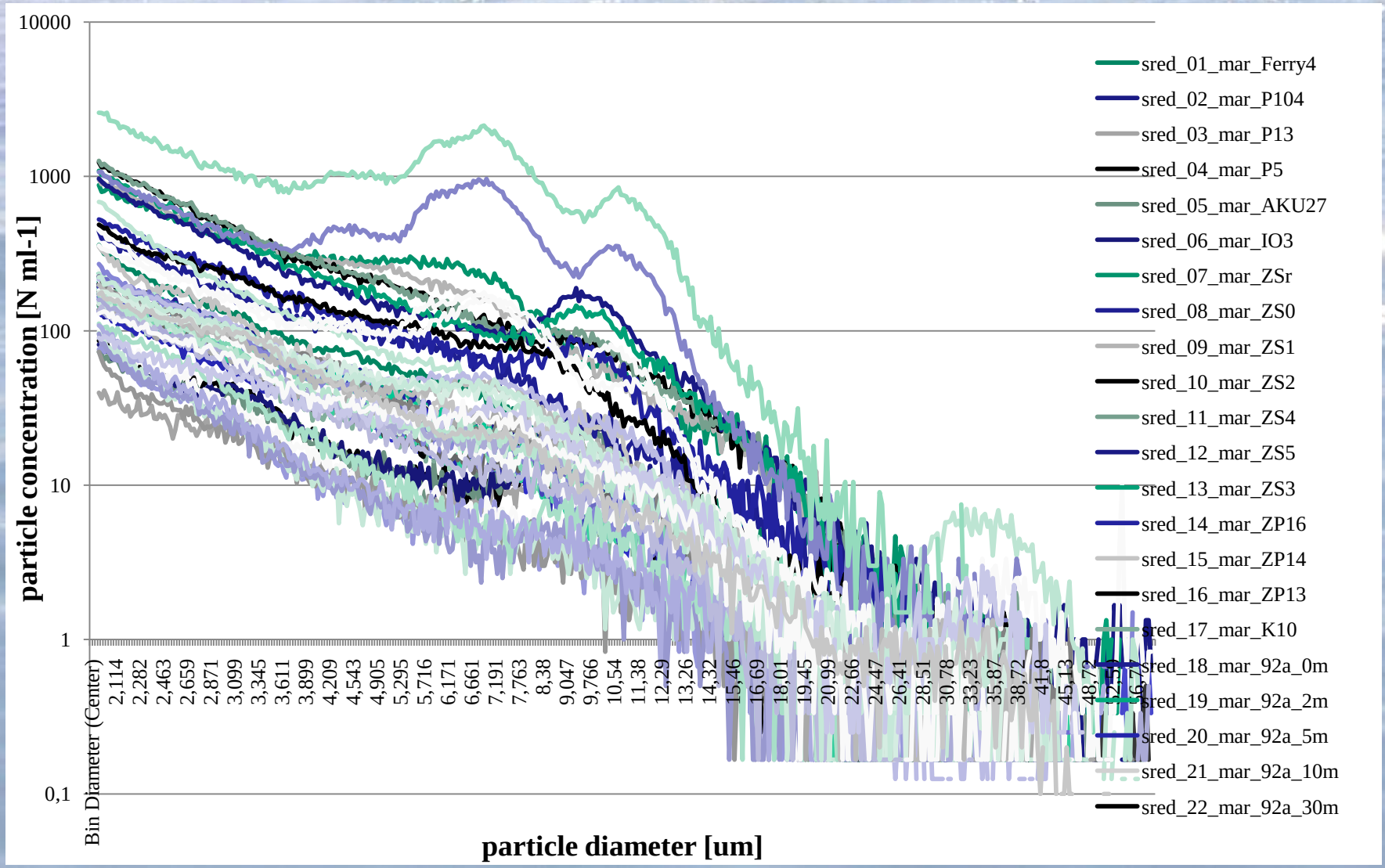
Particle size distribution PSD

Multisizer 4 COULTER COUNTER

sample

Aperture 100
Size of measured
particles 2-60 um

Particle size distribution PSD in March 2019



CDOM – chromophoric dissolved organic matter

Filtration → Storage → Spectrophotometric measurements

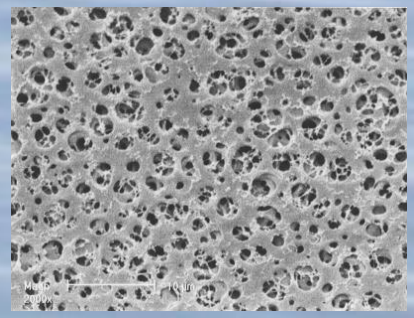
Filtration

Two-stage filtration

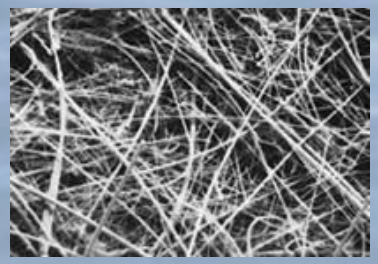
1

glass fibre GF/F
0.7 um pore size

2



cellulose-acetate membrane filter,
0.2 μm pore size

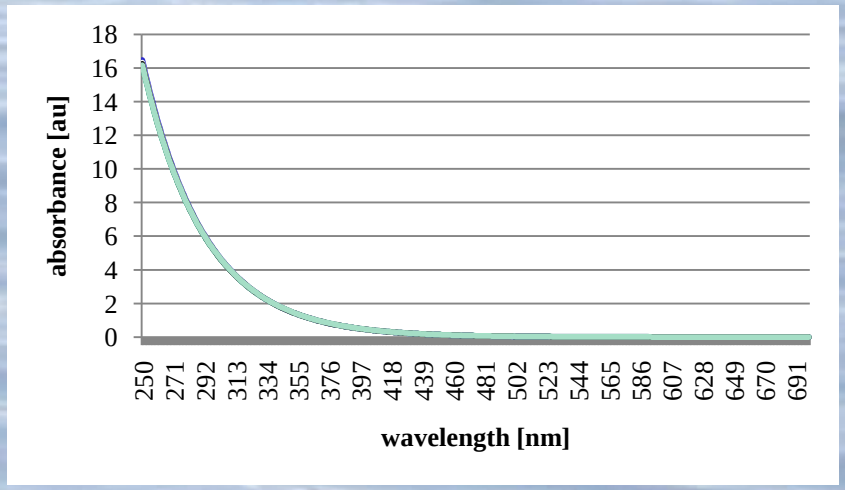


CDOM – chromophoric dissolved organic matter

Filtration → Storage → Spectrophotometric measurements

Spectrophotometric measurements

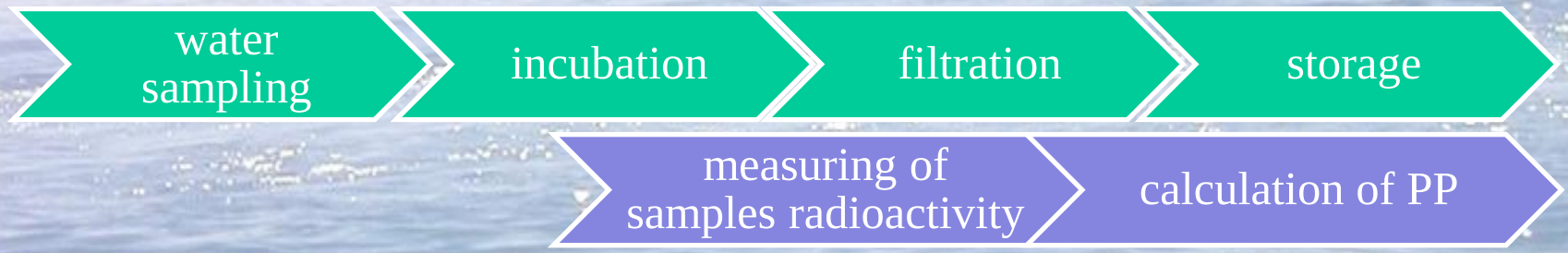
LAMBDA 650 UV/Vis Spectrophotometer, PerkinElmer, Inc



Coefficient of absorption by CDOM [m^{-1}]

$$a_{CDOM}(\lambda) = \frac{2.303 \cdot A(\lambda)}{l}$$

Primary production (PP)

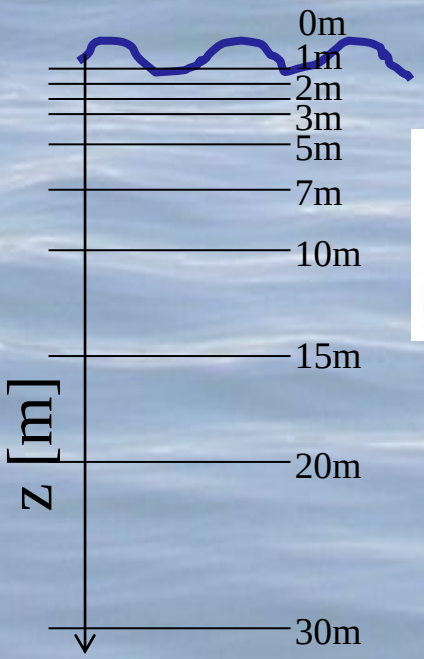


Primary production (PP)

water sampling

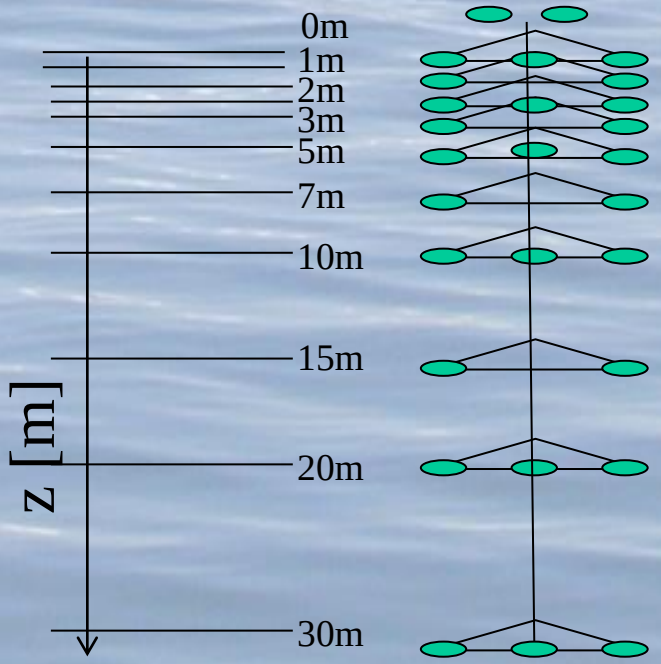
incubation

4h

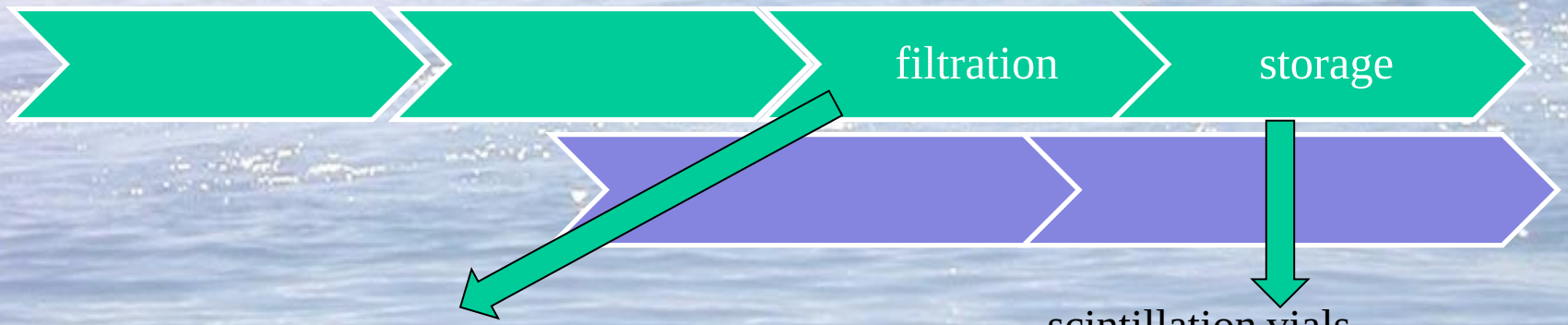


C14

+



Primary production (PP)



cellulose-acetate membrane filter,
0.45µm pore size

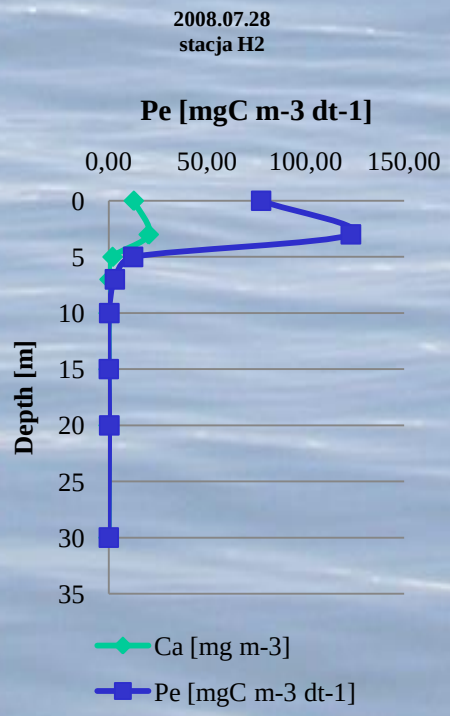
Primary production (PP)

measuring of samples radioactivity → calculation of PP

Beckman Model LS 6000LL Liquid Scintillation Counter

$$PP(z) = \frac{\langle dpm_a(z) \rangle \cdot totalCO_2 \cdot 13.356 \cdot k_1 \cdot k_2 \cdot k_3}{dmp_b}$$

[mgCdm^{-3}h^{-1}]



<dmp$_a$(z)> mean of *dpm(z)– dpm$_d$(z)*
dmp$_b$ activity of isotope C14

total CO2 – total content of inorganic carbon in water [mM dm^{-3}]

value 13.256 – multiplication of atomic mass of carbon, correction factor on respiration, discrimination

k1, k2, k3 – corrections on filtration, time of exposition, units


Thank you