

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

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What kind of parameters can we measure in laboratory?

concentration of:

- pigments
- chromophoric dissolved matter
- suspended particles

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

Basic steps in field work

On ship
board

water
sampling

filtration

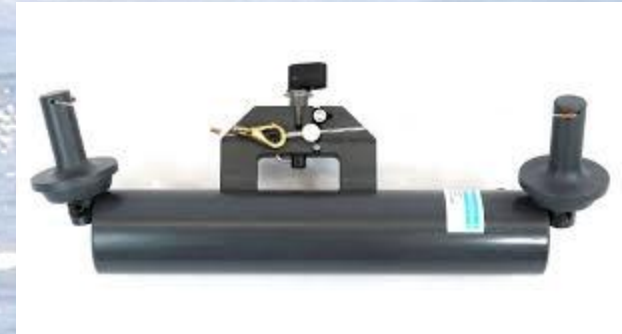
storage

laboratory
analysis

At the
institute



bathometers



filters: glass fibre GFF
and cellulose-acetate



dewar with liquid nitrogen



deep freezer (-80°C)



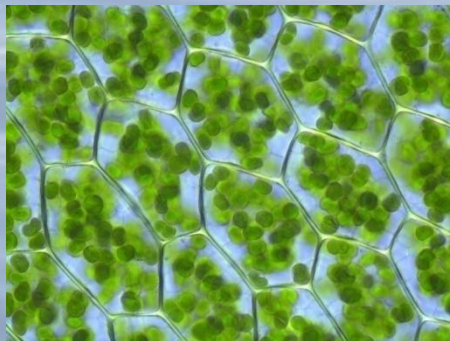
refrigerator



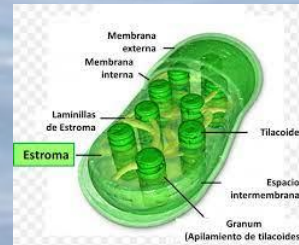
Concentration of chlorophyll a



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

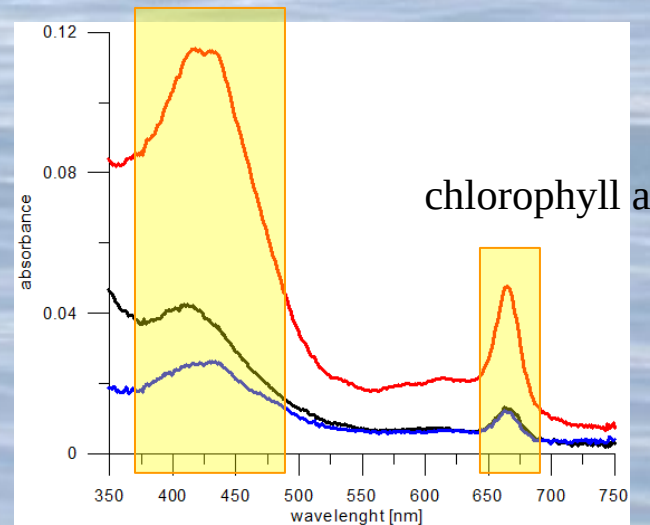
Concentration of chlorophyll a



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 [cm³],
 v_{filtr} – volume of filtered seawater [dm³],
 l – optical length [cm],
 83 – value of absorbance coefficient of chlorophyll a in ethanol
 (Sartory i Grobbelaar 1984, Lorenzen i Newton Downs 1986,
 Guideline for Baltic Monitoring Program 1988).

Chlorophyll a in 90% acetone

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

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

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

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

$A_{663-665b}$ - before acidification.

$A_{663-665a}$ - after acidification.

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

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

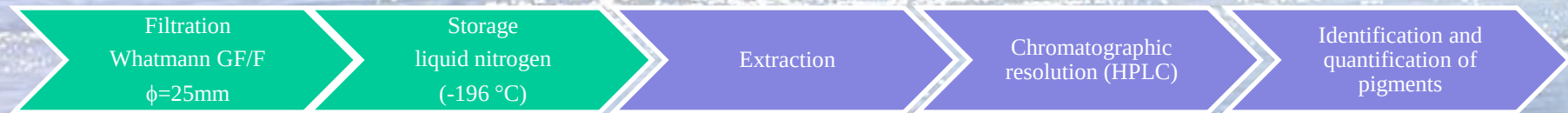
- **The equation of SCOR/UNESCO (1968) [SU] :**

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

References:

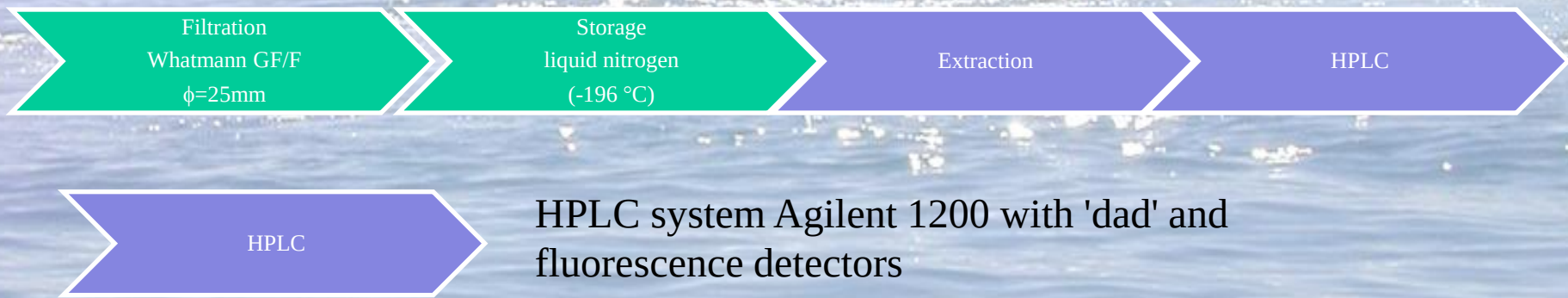
- BMB, 1979, *Recommendations on methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll*, [Ed. L. Edler], BMB, 5
- Guideline for Baltic Monitoring Program, 1988, Baltic Marine Environment Protection Commission, Helsinki, 116.
- Jeffrey S. W., Humphrey G. F., 1975, New 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

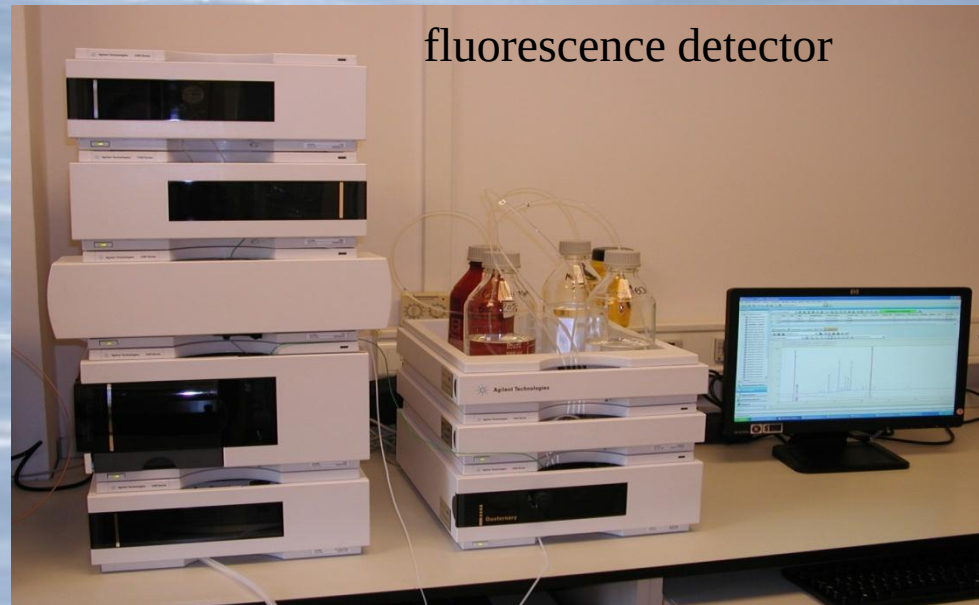


1. Unfreeze filters and placed them into plastic vials
2. Solvent – 90% acetone, 3 ml
3. Mechanical homogenization + sonication
4. Time of extraction – 2h
5. Conditions: darkness, 4 °C
6. Centrifugation of samples to clarify extract

Concentration of phytoplankton pigments: chlorophylls and carotenoids



absorbance detector
chromatographic column
autosampler

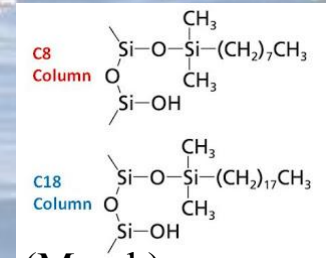


Heart - pump

Concentration of phytoplankton pigments: chlorophylls and carotenoids



HPLC system Agilent 1200



chromatographic column

LichroCART™ LiChrospher™ 100 RP18e (Merck)
Dimension 250 x 4 mm, particle size 5 μm, pore size 100 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 $\lambda_{ex} = 431 \text{ nm}$ i emisji $\lambda_{em} = 660 \text{ nm}$ to confirm presence of chloropigments in sample

method

Used solvents:

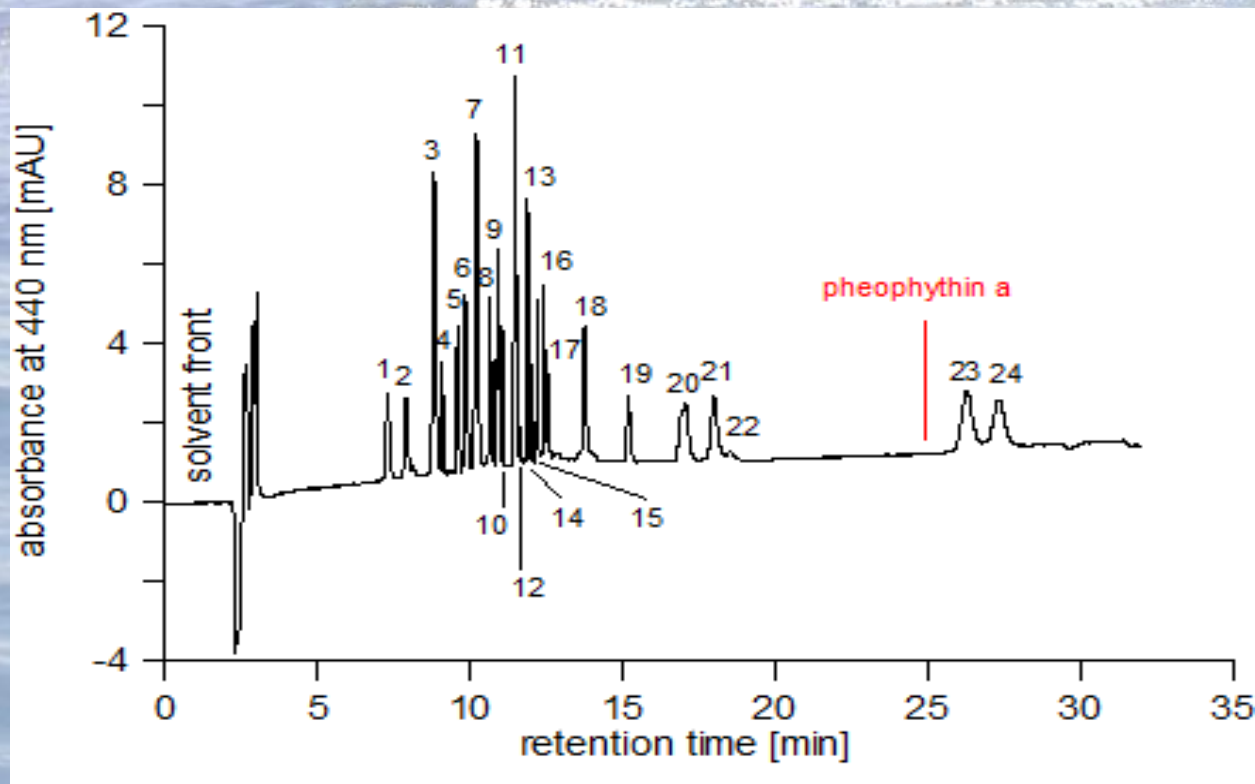
A - methanol: 1M ammonium acetate (80:20, v:v)

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 – diadinoxanthin
- 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

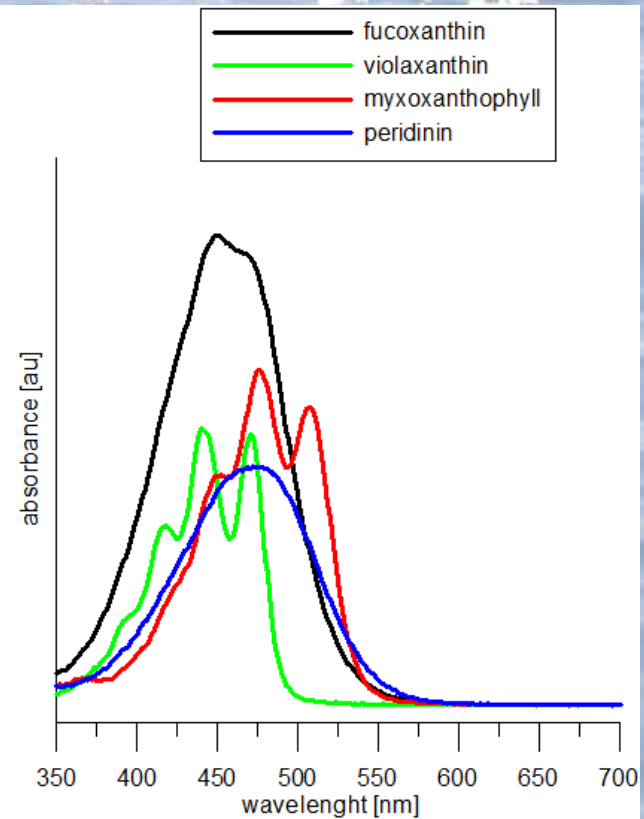
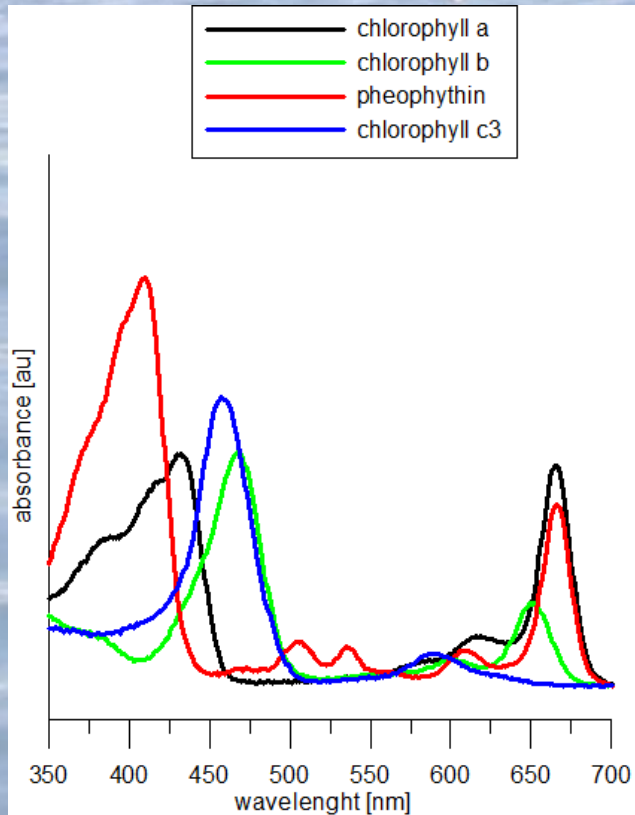
Stoń J., Kosakowska A., 2002, *J. Appl. Phycol.*, 14, 205-210

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

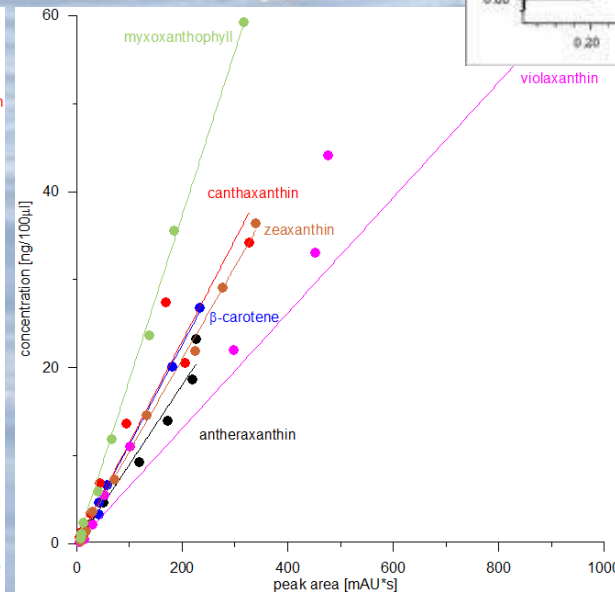
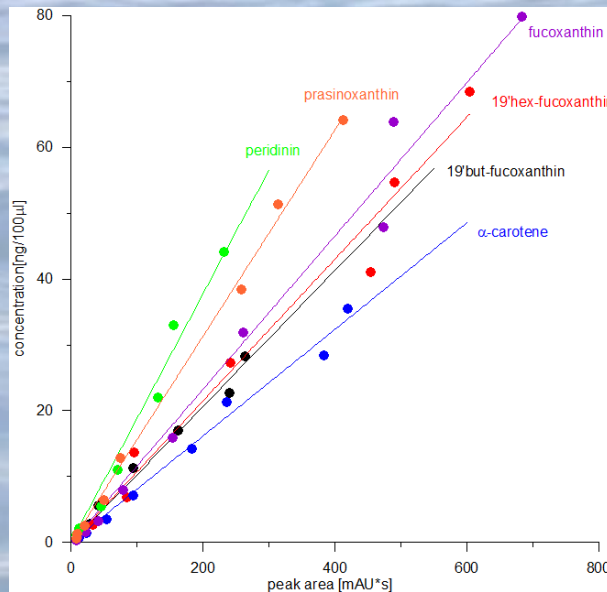
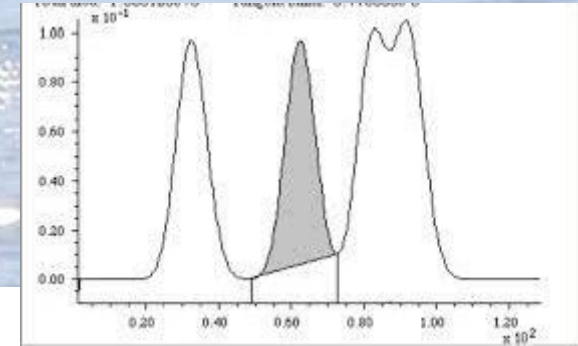
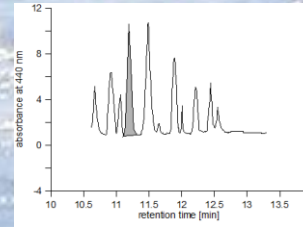
Pigment qualification

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



Pigment quantification

- peak area
- response factor obtained from calibration curves



External standardization equation
(Mantoura i Repeta 1997)

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

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

Concentration of phycobilins



1. Unfreeze filters and placed them into plastic vials
2. Solvent – MEDIUM, Trizma Base, Na_2EDTA $2\text{H}_2\text{O}$, 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 $2\text{H}_2\text{O}$, Lizozym , pH 5.5 !!

Concentration of phycobilins

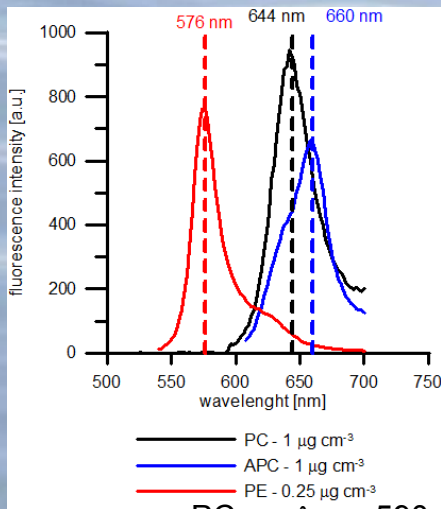


Cary Eclipse, Varian, Agilent Technologies

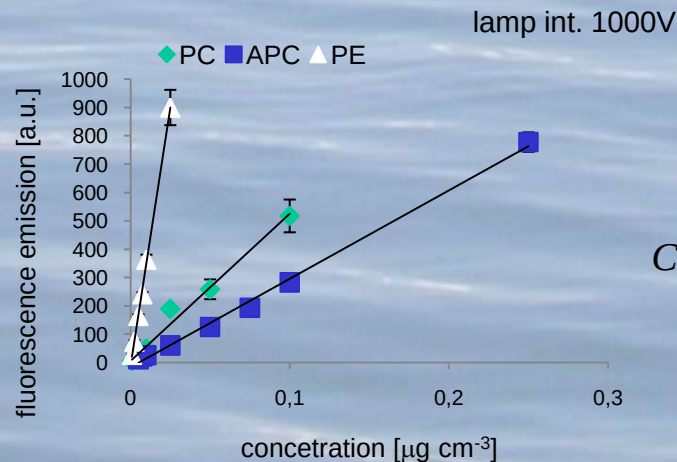


Spectrofluorometric
measurements

Identification and
quantification of
pigments

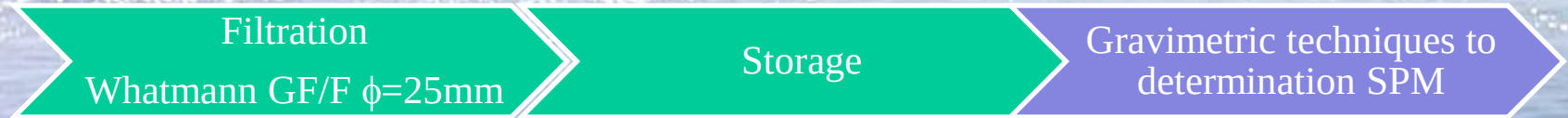


Phycobilins standards (ProZyme Inc. San Leandro, CA, USA)



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

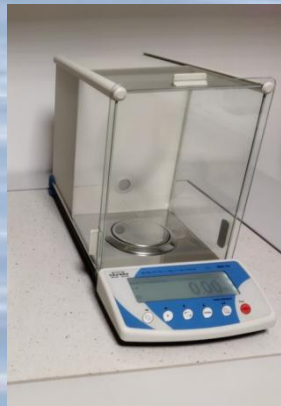
Concentration of suspended particular matter SPM, their organic and inorganic fractions



exiccator



Scales
WAX 110, RADWAG



Combustion in oven
450 °C during 4 h



calculations

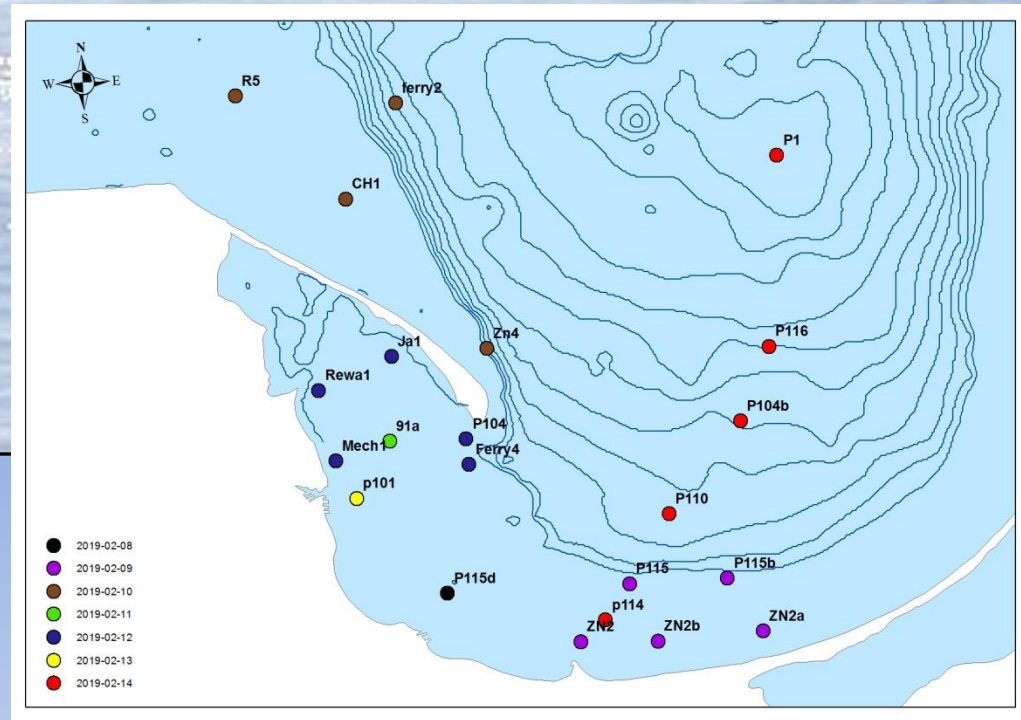
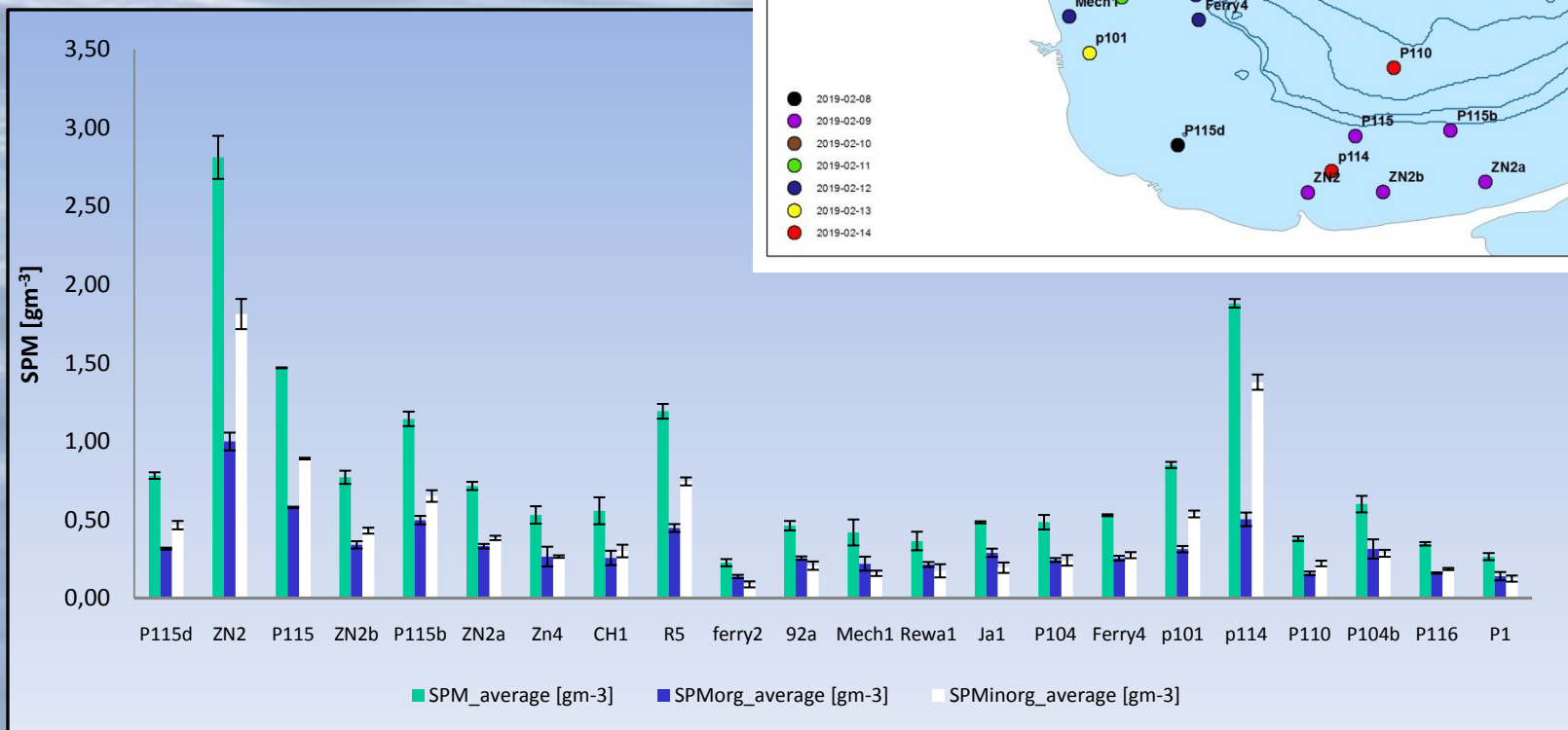
$$SPM = \frac{ms - mf}{V_{filtr}}$$

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

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

where – averaged weight of filters without suspension: mf [mg], with suspension: ms, with suspension, after combustion: msf;
 V_{filtr} [dm³] – volume of filtered seawater

SPM in February 2019



Particle size distribution PSD

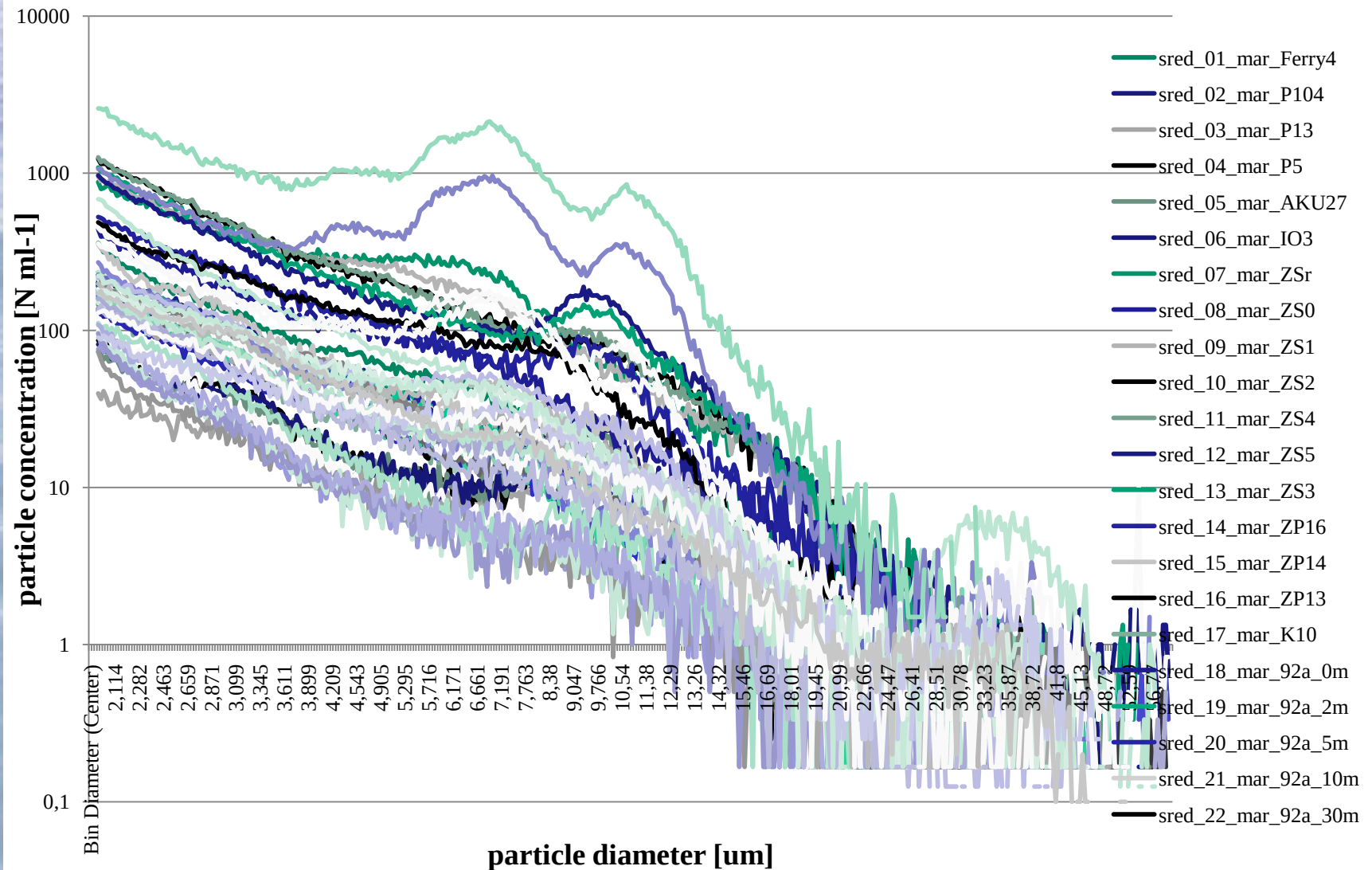
Multisizer 4 COULTER COUNTER



sample

Aperture 100
Size of measured
particles 2-60 μm

Particle size distribution PSD in March 2019



CDOM – chromophoric dissolved organic matter

Filtration

Storage

Spectrophotometric
measurements

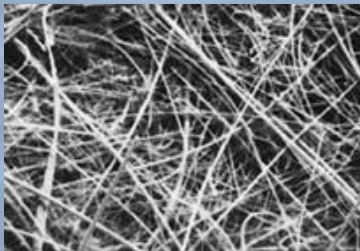
Filtration

Two-stage filtration

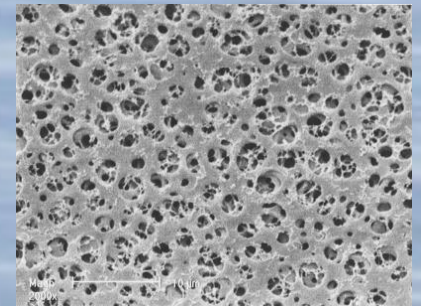
1



glass fibre GF/F
0.7 μm pore size



2



cellulose-acetate membrane filter,
0.2 μm pore size

CDOM – chromophoric dissolved organic matter

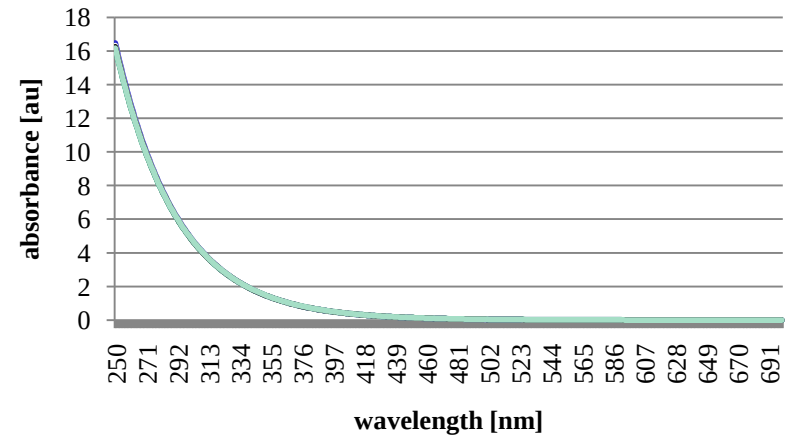
Filtration

Storage

Spectrophotometric
measurements

Spectrophotometric
measurements

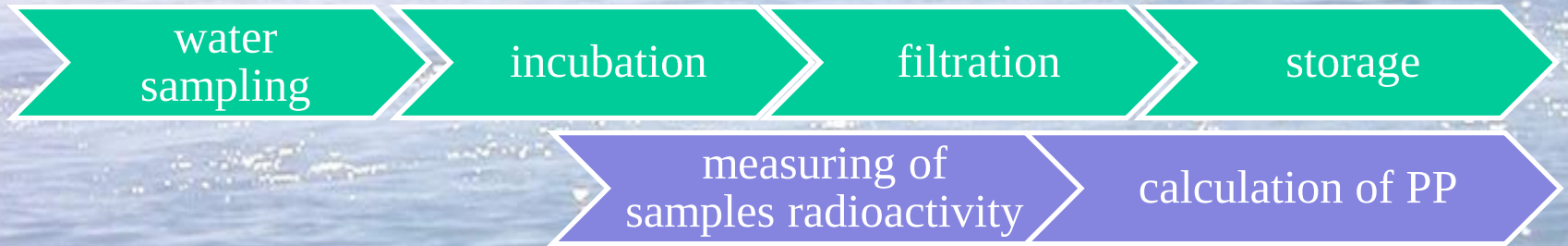
LAMBDA 650 UV/Vis
Spectrophotometer,
PerkinElmer, Inc



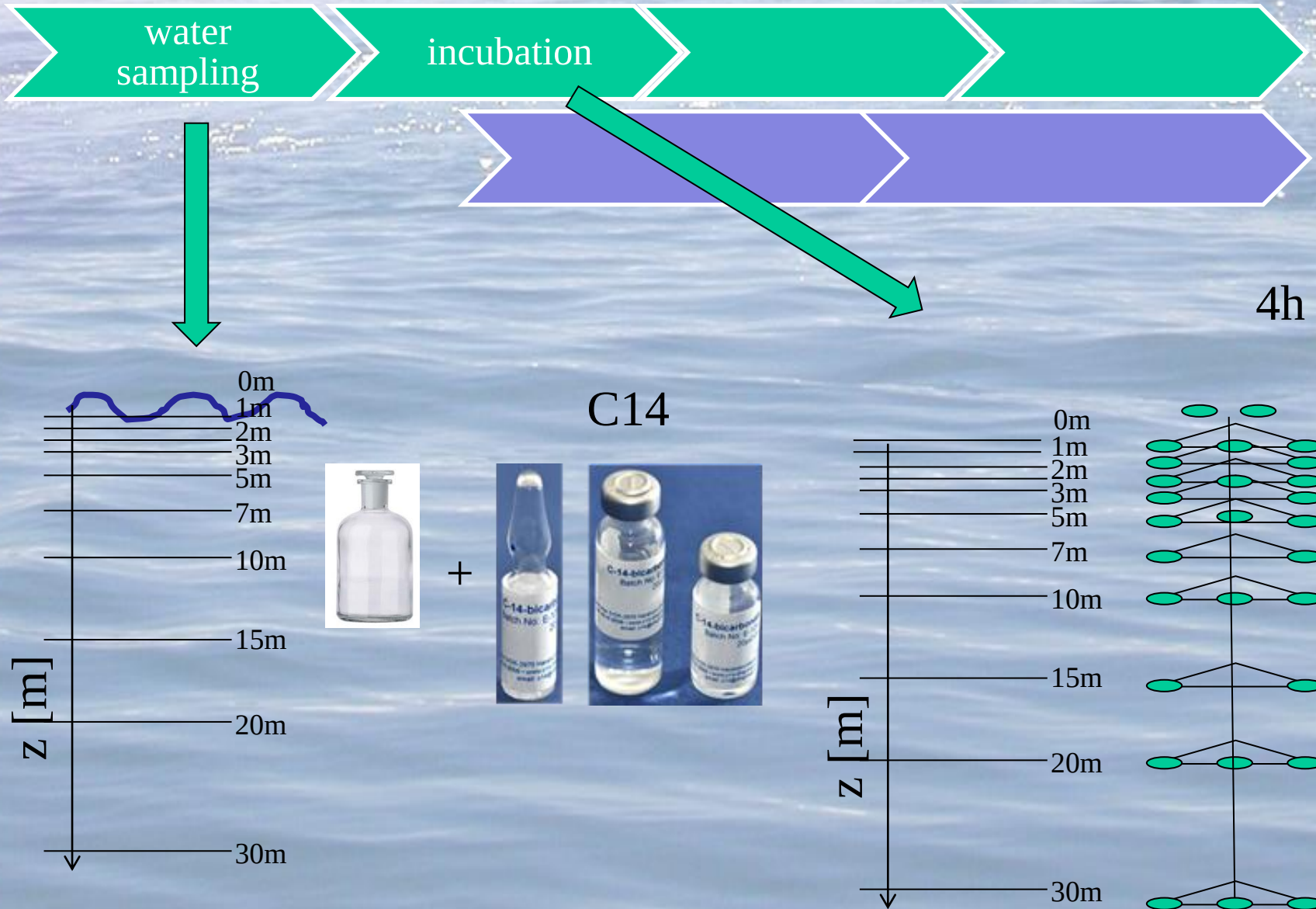
Coefficient of absorption by CDOM [m^{-1}]

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

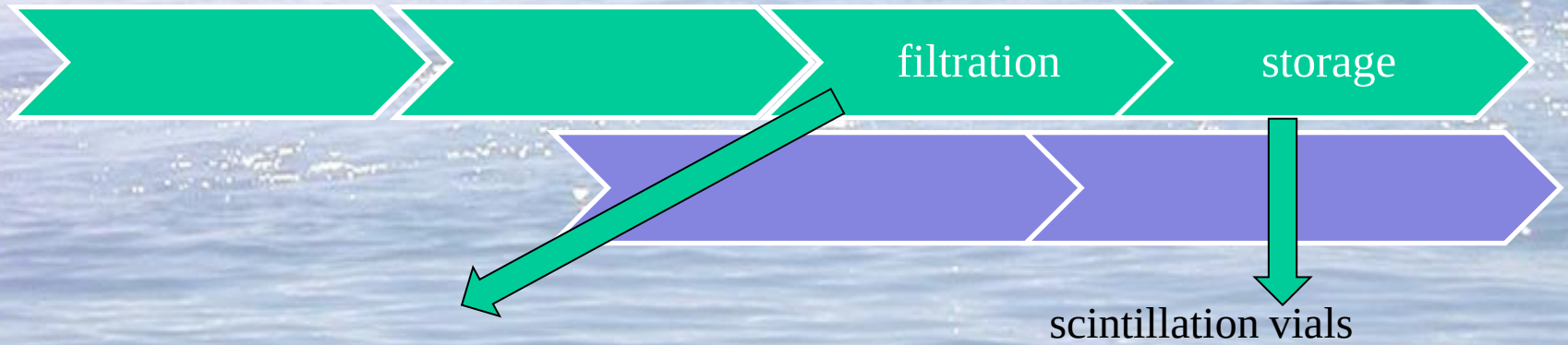
Primary production (PP)



Primary production (PP)



Primary production (PP)



cellulose-acetate membrane filter,
0.45 μ m pore size



Primary production (PP)

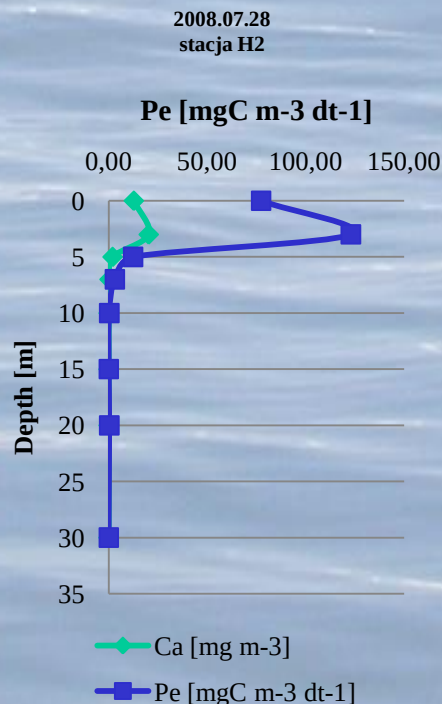
measuring of
samples radioactivity

calculation of PP

Beckman Model LS 6000LL Liquid
Scintillation Counter



$$PP(z) = \frac{\langle dpm_a(z) \rangle \cdot totalCO_2 \cdot 13.356 \cdot k_1 \cdot k_2 \cdot k_3}{dmp_b} \quad [mgCdm^{-3}h^{-1}]$$



$\langle dmp_a(z) \rangle$ 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