5th FerryBox Workshop - Celebrating 20 Years of Alg@line
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JOINT EUROPEAN RESEARCH INFRASTRUCTURE NETWORK FOR COASTAL OBSERVATORIES

An overview of spectral *in vivo* fluorescence methods for phytoplankton taxonomy

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AN OVERVIEW OF SPECTRAL *IN VIVO* FLUORESCENCE METHODS FOR PHYTOPLANKTON TAXONOMY

Pigment taxonomy

Spectral fluorescence
Taxonomic spectral groups

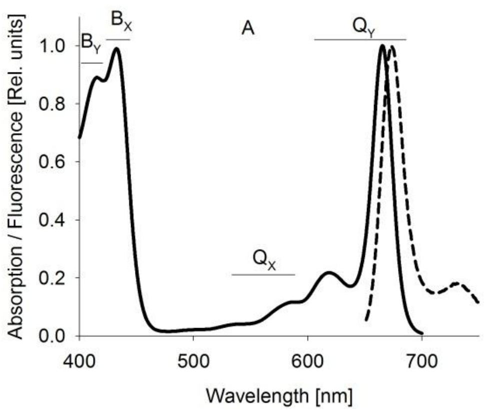
Phycobilin fluorescence

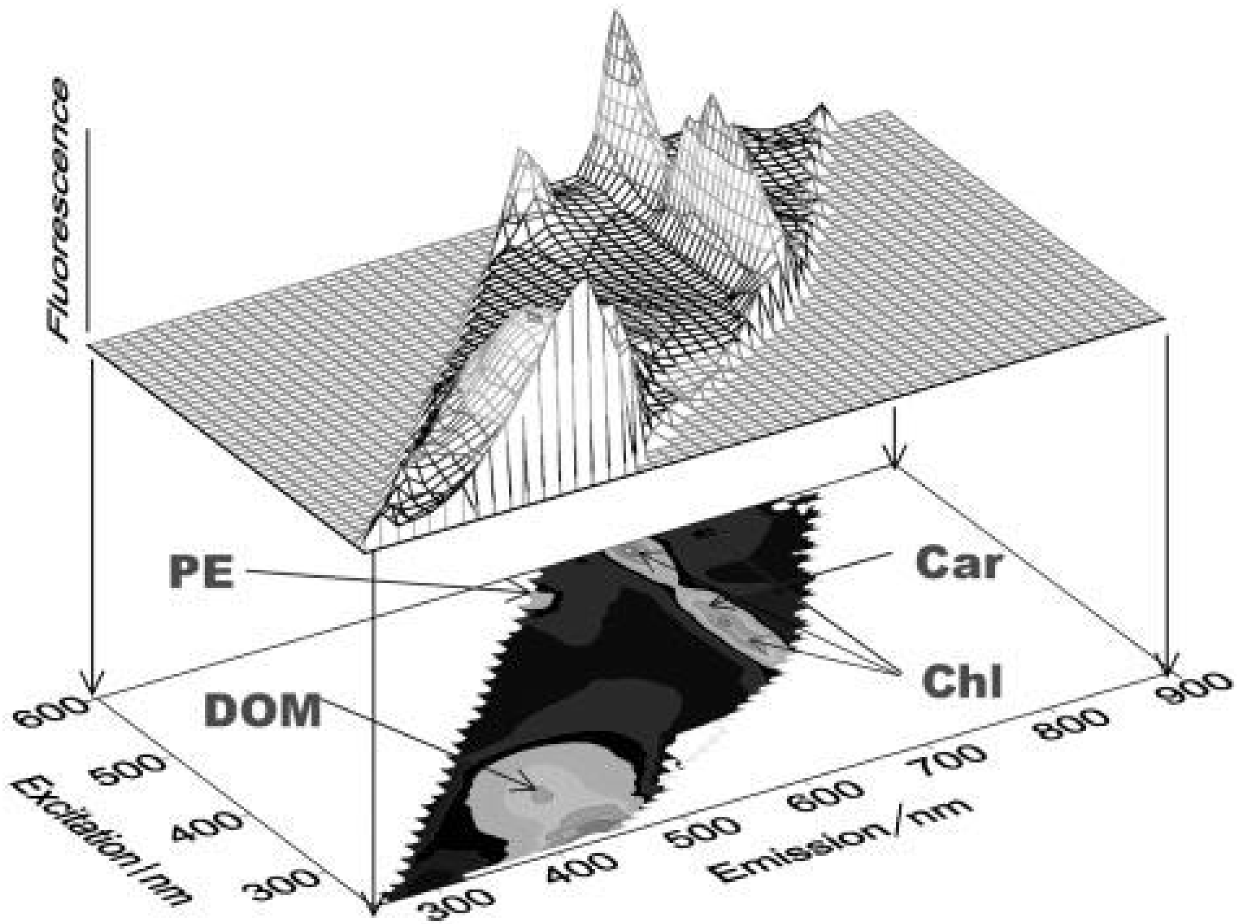
Origins of Phycocyanin / Phycoerythrin fluorescence
Instrumentation
Examples for Baltic Sea

Spectral fluorescence

Measuring techniques
Instrumentation
Data analysis
Examples

SPECTRAL FLUORESCENCE: two wavelength dimensions - excitation and emission - allowing better separation of various substances.

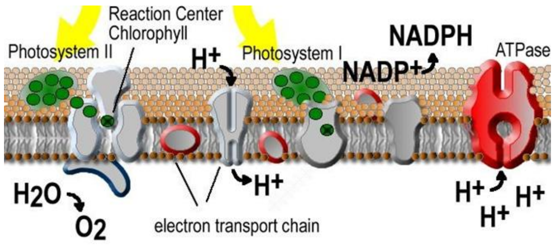




Babichenko et al 2000

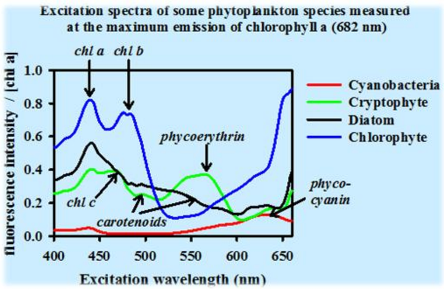
TAXONOMIC SPECTRAL GROUPS: In living phytoplankton, accessory pigments in PSII transfer energy to Chla but do not emit fluorescence, only Chla in PSII and phycobilin pigments emit fluorescence

	Absorption peaks (nm)	Fluorescence emission maximum (nm)
Chl*a*	435-440, 620-635, 672-676	682
Chl*b*	465-470, 650	
Chl*c*	455-465, 590, 625-643	
Alloxanthin	488[a]	
Diadinoxanthin	440-490	
Fucoxanthin	515-545	
Peridinin	440-540	
PE	490-575	570-580
PEC	570-595	625-635
PC	615-640	635-645
APC	620-655	660-675

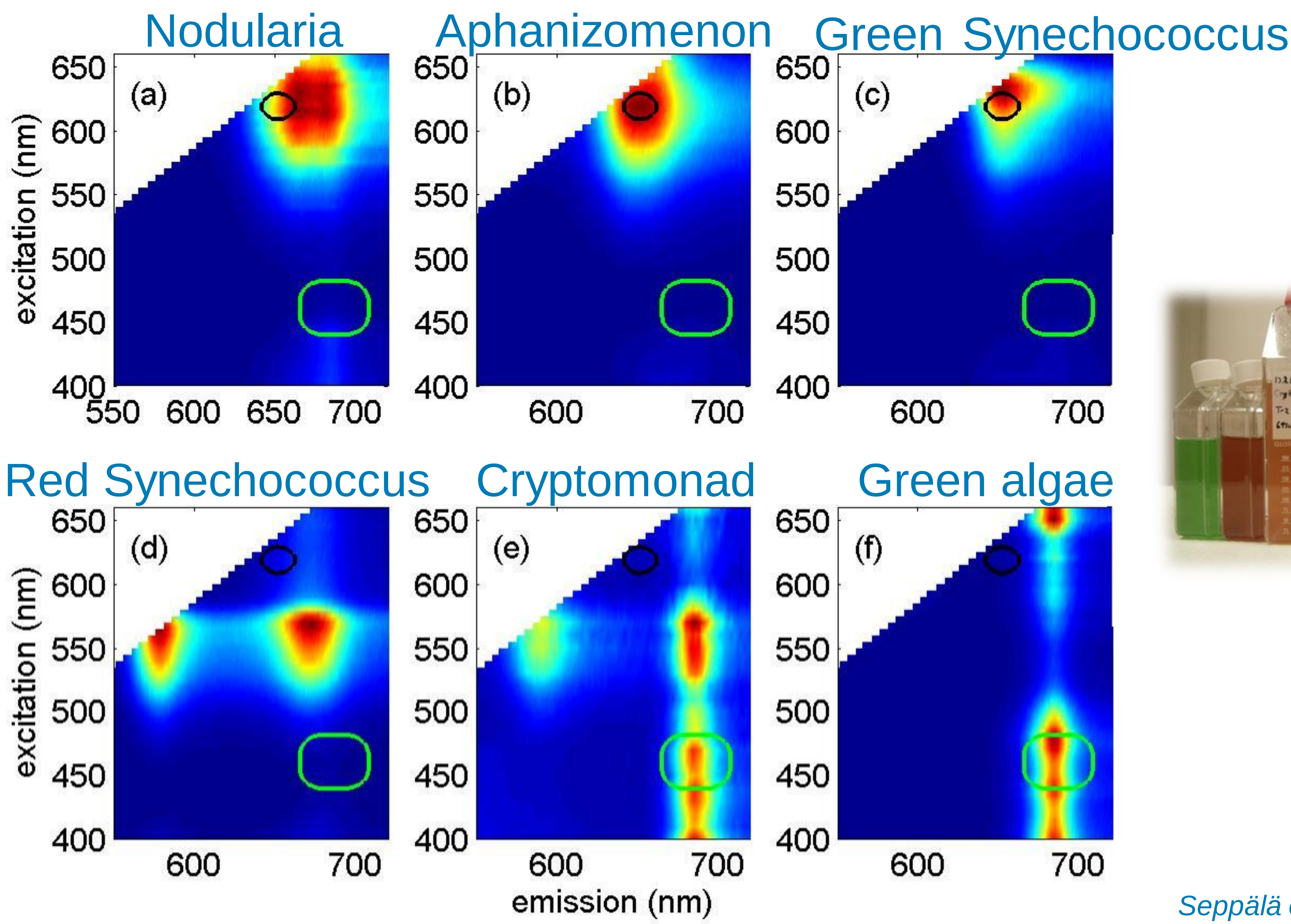


Algal group	Light harvesting antenna for PSII
Cyanobacteria	Phycobilisomes
Cryptophyta	Alloxanthin - Chl*a*/*c*; phycobiliproteins
Dinophyta	Peridinin - Chl*a*/*c*
Haptophyta	Fucoxanthin - Chl*a*/*c*
Chrysophyta	Fucoxanthin - Chl*a*/*c*
Eustigmatophyceae	Violaxanthin/vaucheriaxanthin - Chl*a*
Euglenophyta	Diadinoxanthin/diatoxanthin/neoxanthin - Chl*a*/*b*
Chlorophyta	Lutein/neoxanthin/zeaxanthin/violaxanthin - Chl*a*/*b*

Seppälä 2009



TAXONOMIC SPECTRAL GROUPS: Group-specific Excitation-Emission Matrix is the basis for fluorescence based taxonomy

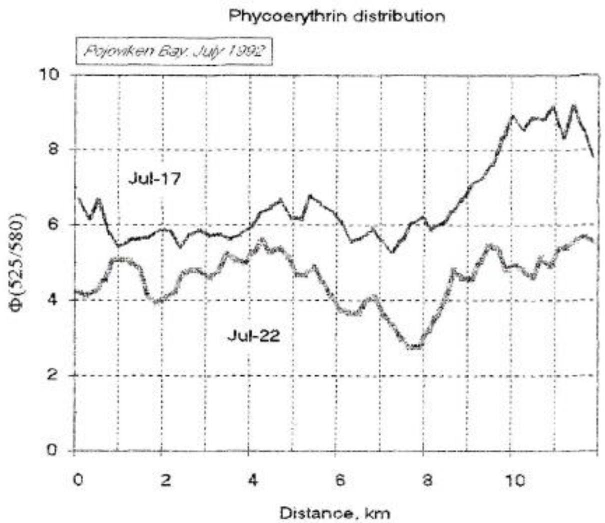


Seppälä et al 2007

PHYCOBILIN FLUORESCENCE, EARLY YEARS

"We may not know exactly what we are measuring, but the patterns observed are too strong to ignore" Cullen & Renger 1979.

Babichenko, Porvykina, Kaitala 1995



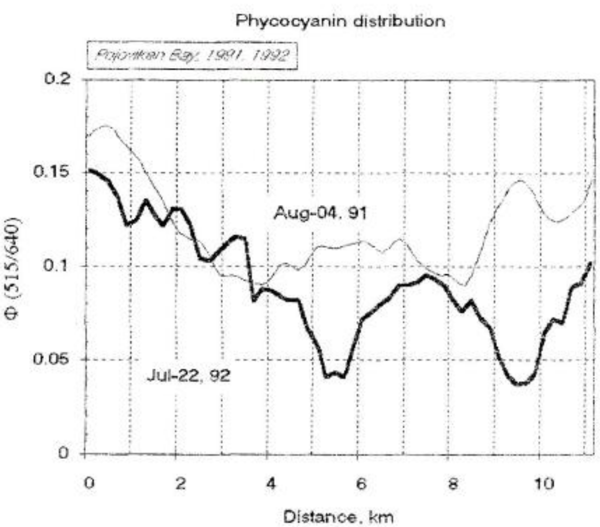
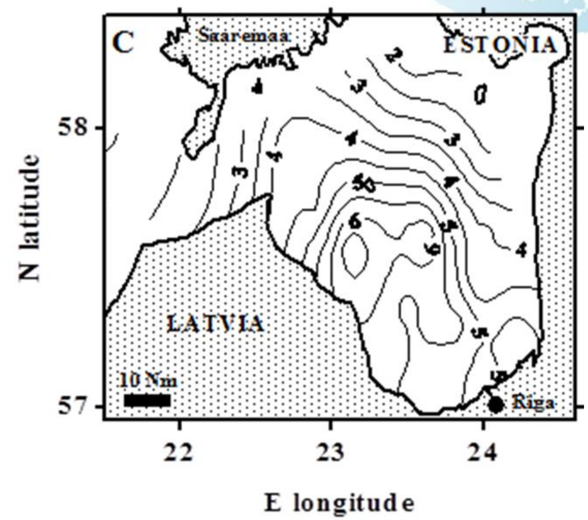
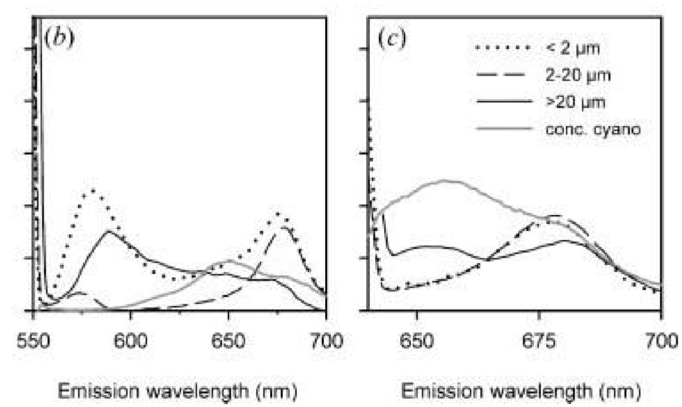


Fig. 2 - Spatial distribution of Phycoerythrin (a) and Phycocyanin (b).

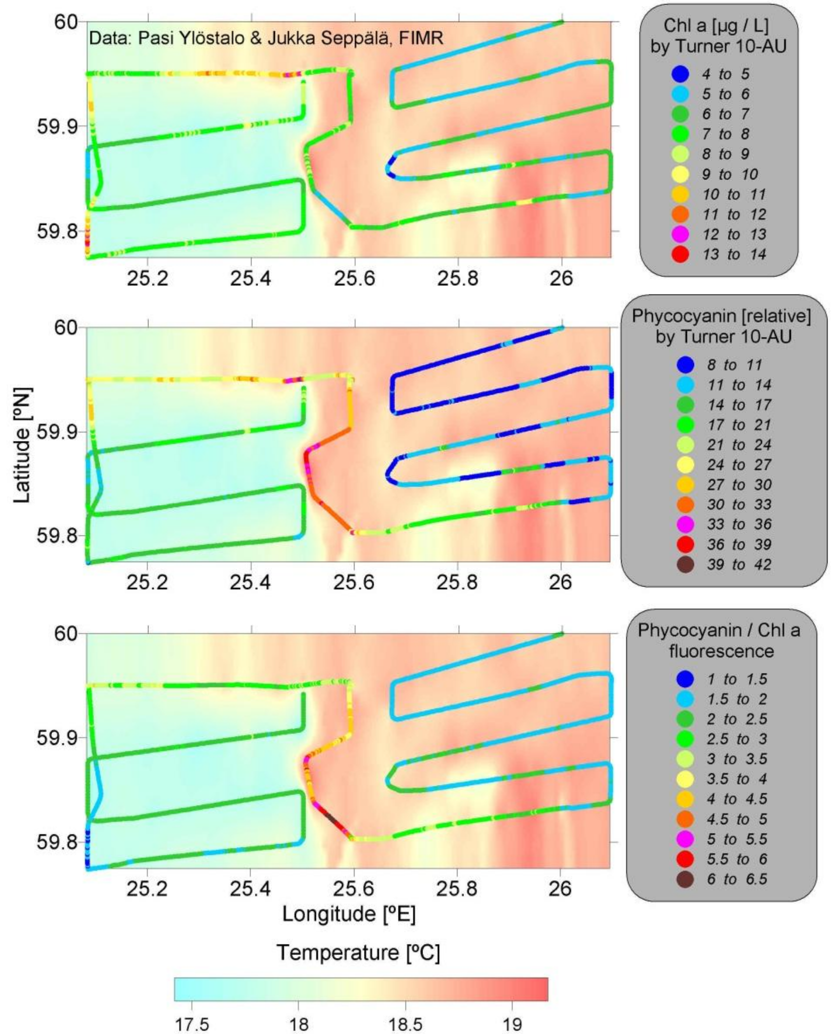


Seppälä & Balode 1998



Seppälä, Ylöstalo, Kuosa 2005

PHYCOCYANIN FLUORESCENCE, Operational detection of filamentous cyanobacteria started 2005



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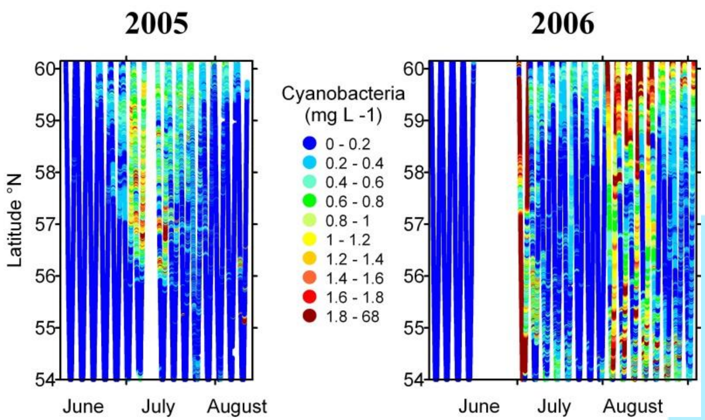
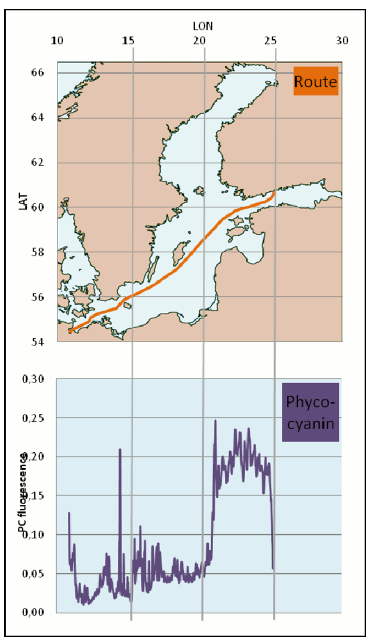
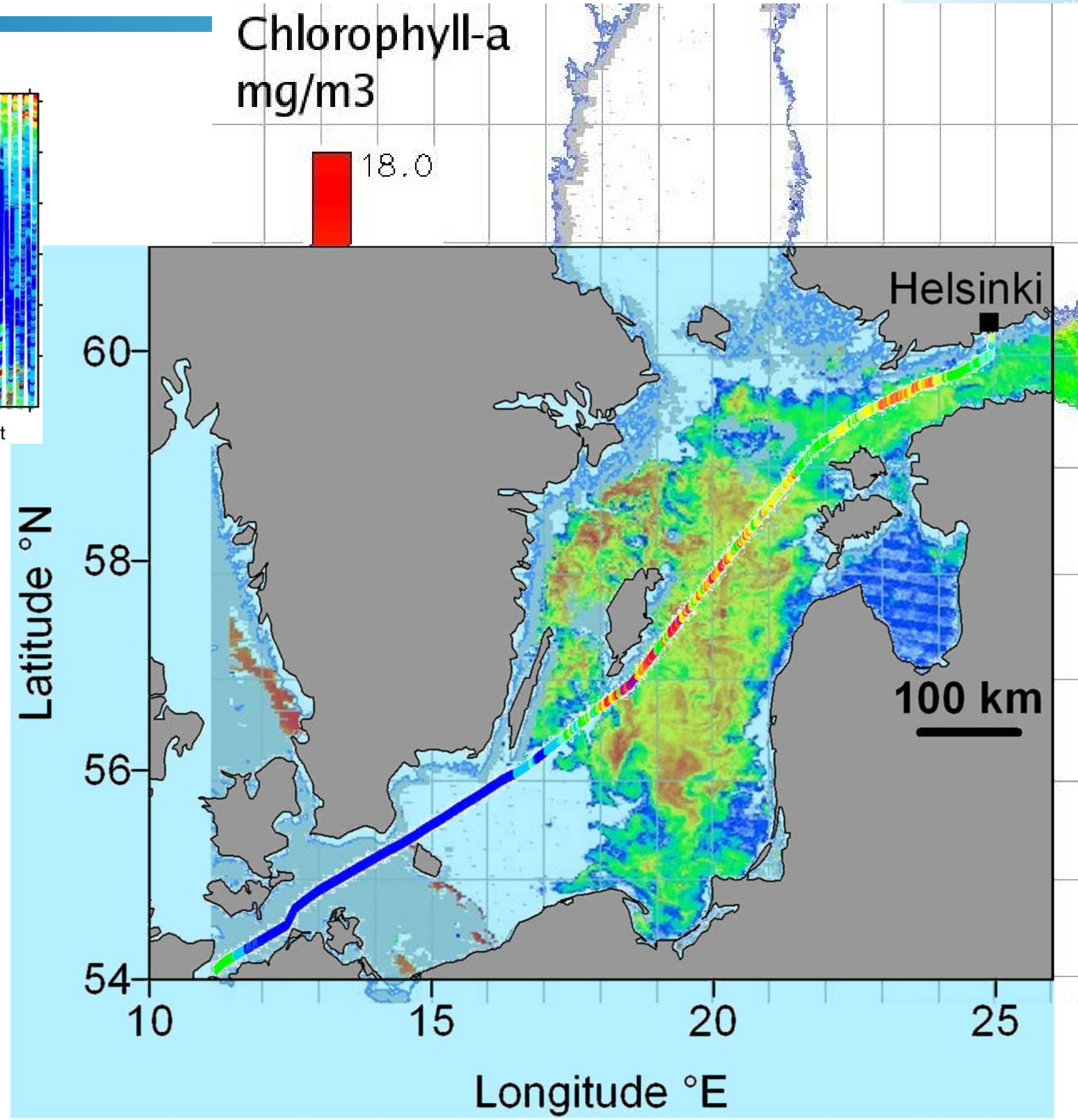
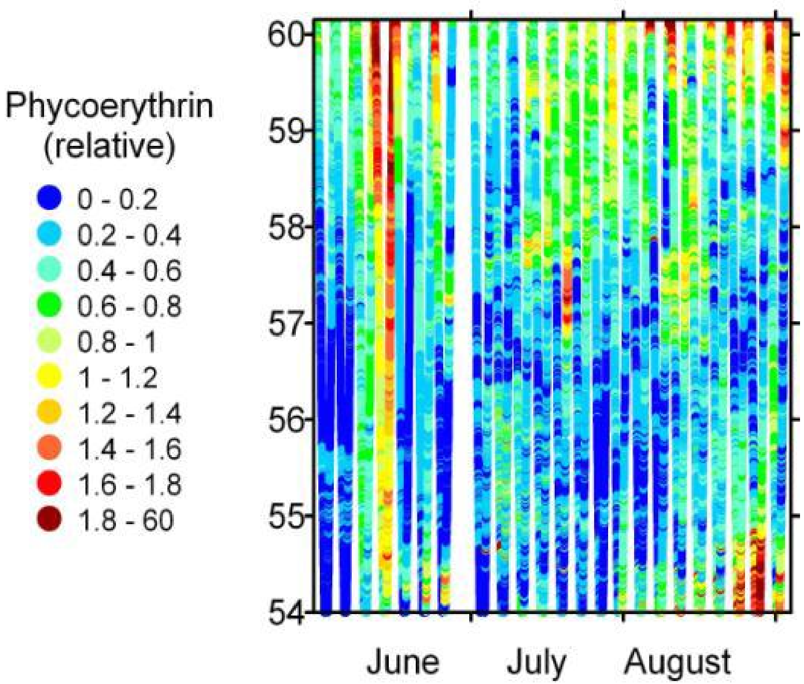
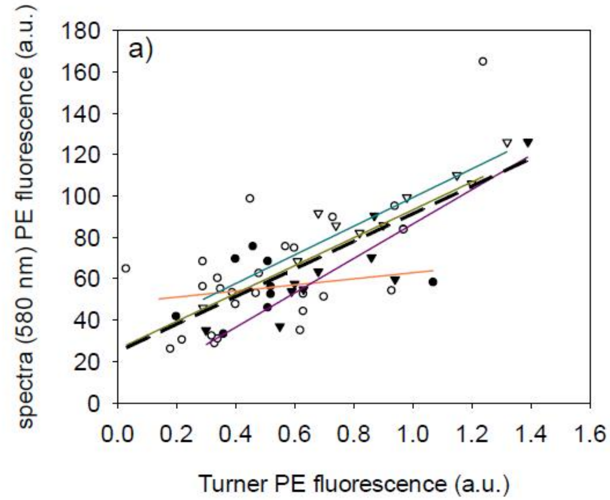


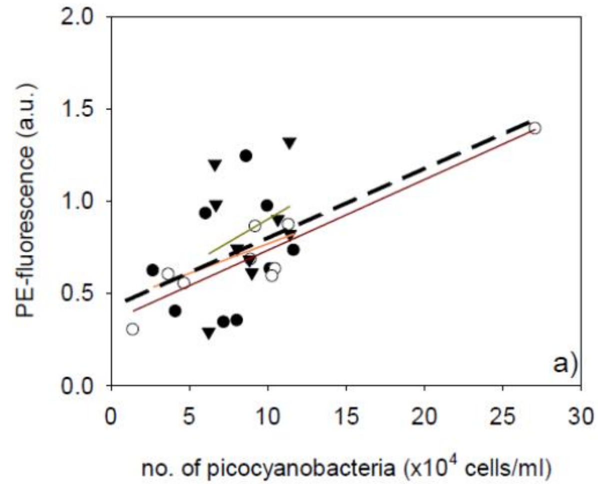




PHYCOERYTHRIN FLUORESCENCE, First tests in 2006 with old Turner 10-AU





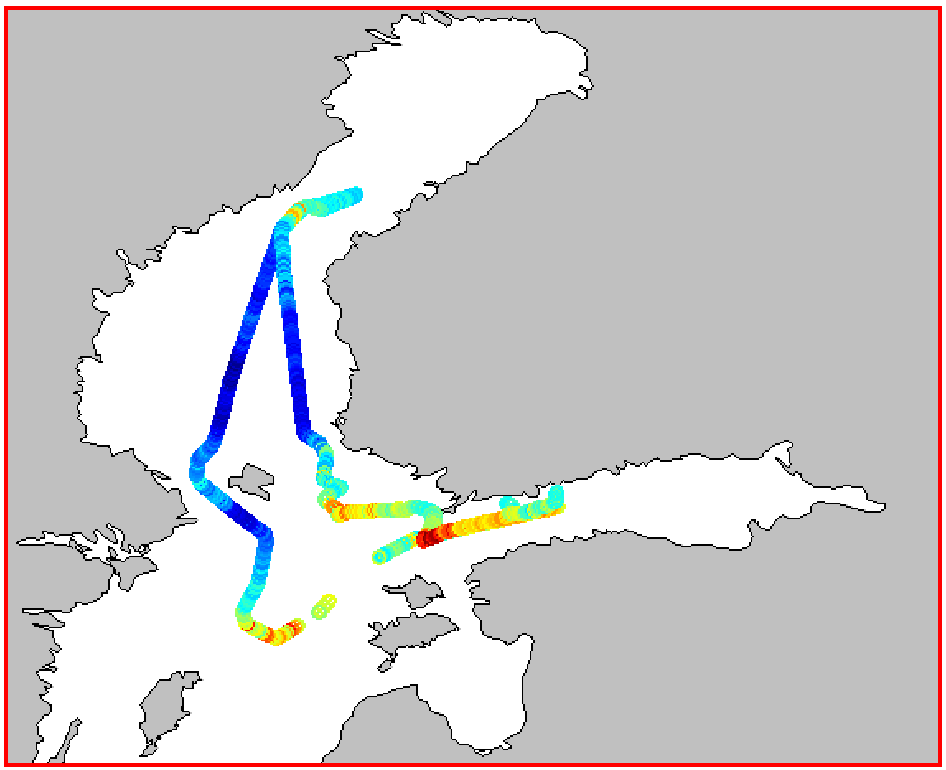


Niiranen 2008

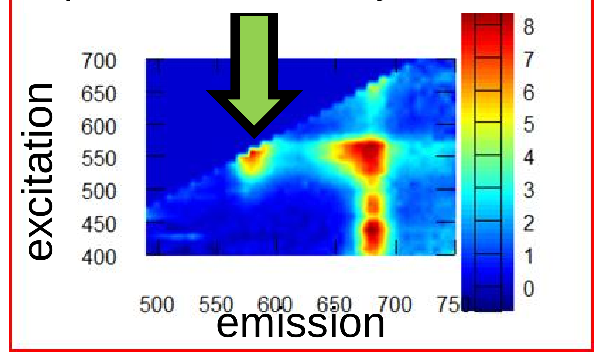
Phycoerythrin fluorescence, New trials with LED fluorometer in 2012

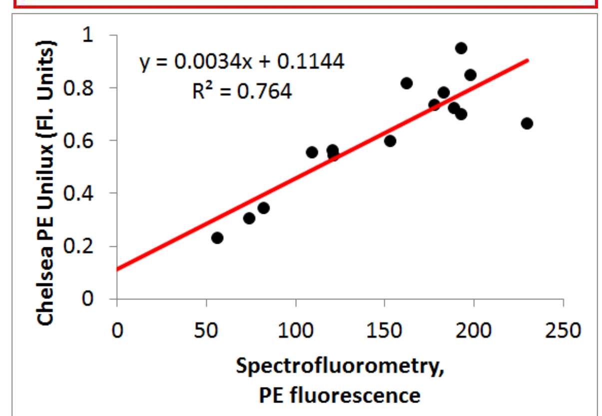
Testing fluorometers for phycoerythrin (PE) detection:

- Chelsea Instruments PE Unilux in flow-through system during summer cruise



Phycoerythrin signal from spectrofluorometry



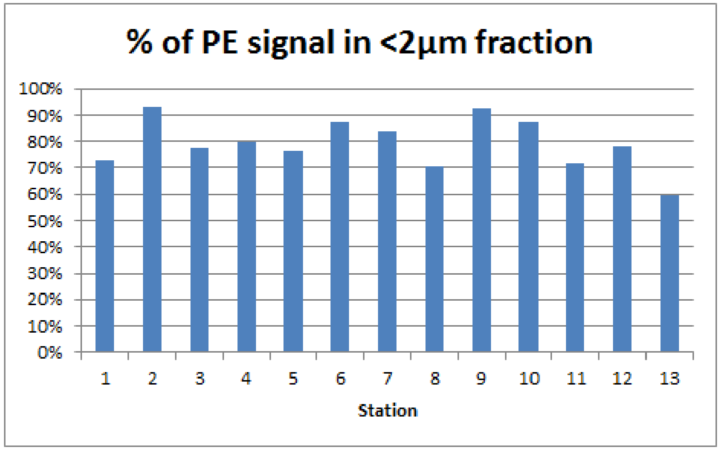


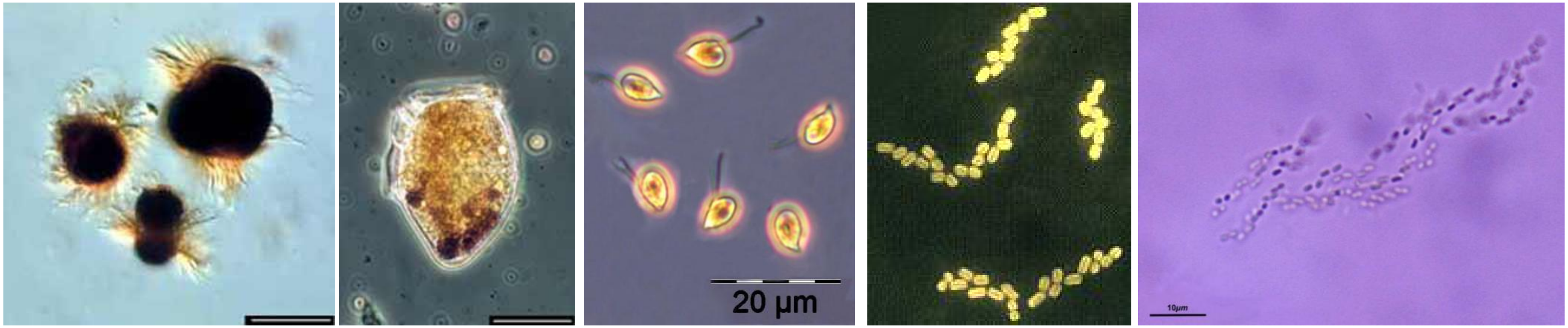
PHYCOERYTHRIN FLUORESCENCE, New trials with LED fluorometer in 2012 → 2013 FerryBox.

FlowCAM to identify and count PE containing larger cells (>5 µm)

PE containing species: *Mesodinium rubrum*, *Dinophysis norvegica*, Cryptomonads, colonial cyanobacteria

Picocyanobacteria counts (microscopy, flow cytometry)

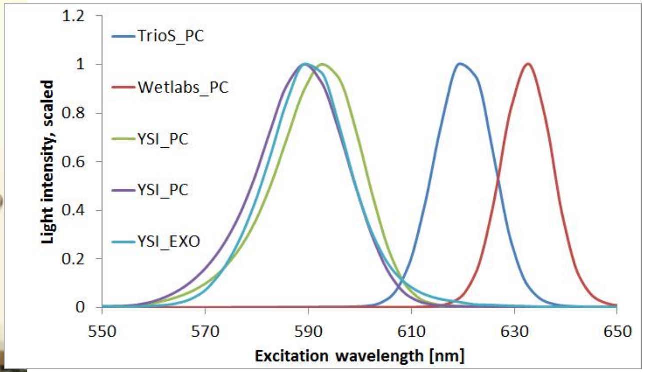


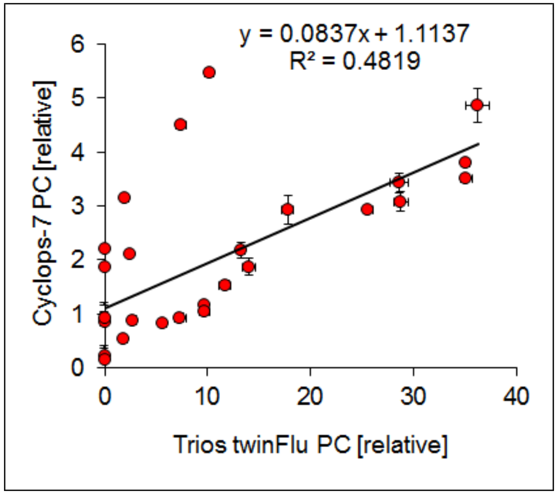


Photos: Baltic Sea Portal; nordicmicroalgae.org

PHYCOBILIN FLUORESCENCE: Current issues

- **Instrument selection**: Excitation/emission wavelengths vary between instruments. Not all are strictly specific for Phycocyanin/Phycoerythrin





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- **Instrument selection**: Excitation/emission wavelenghts vary between instruments. Not all are strictly specific for Phycocyanin/Phycoerythrin
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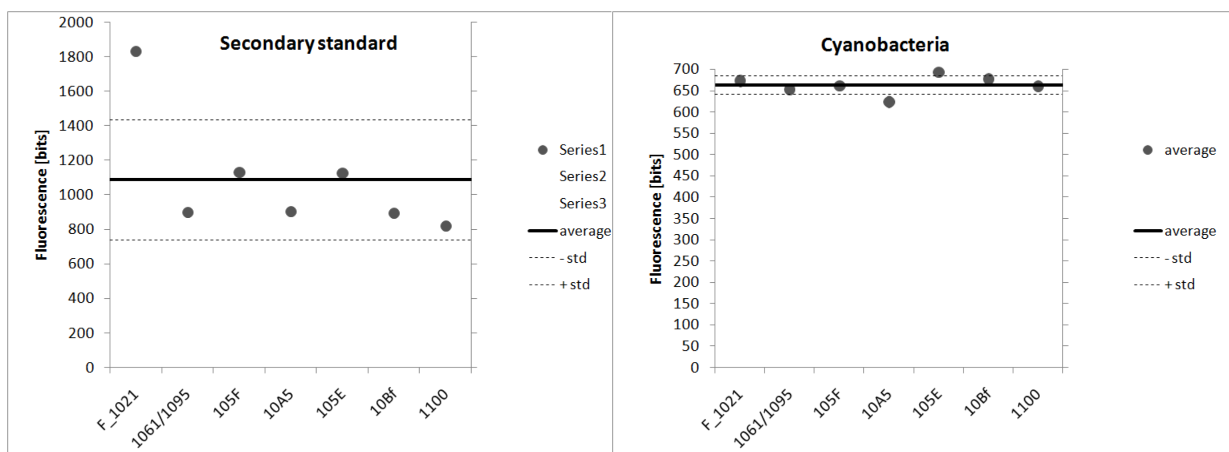

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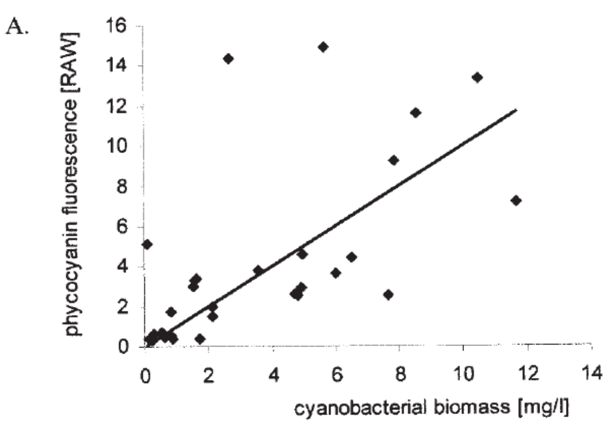
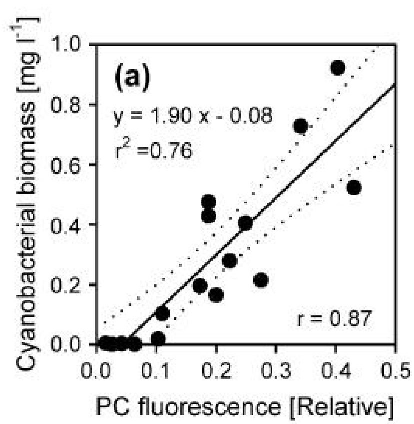
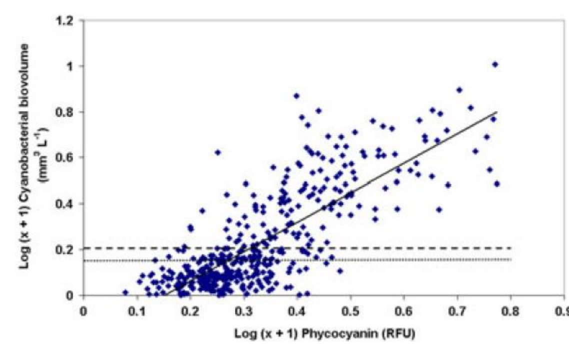
- **Validation**: More information is needed for biomass-fluorescence relationship



Izydorczyk etal 2005



Seppälä et al 2007



Bowling etal 2010

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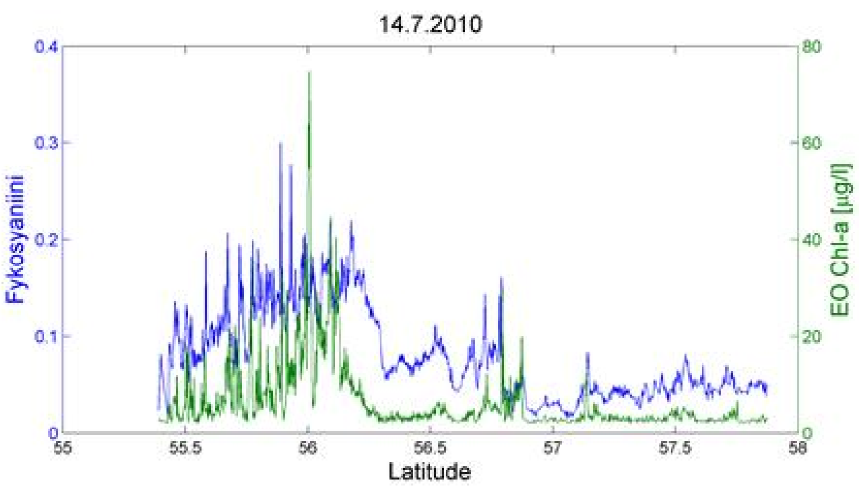
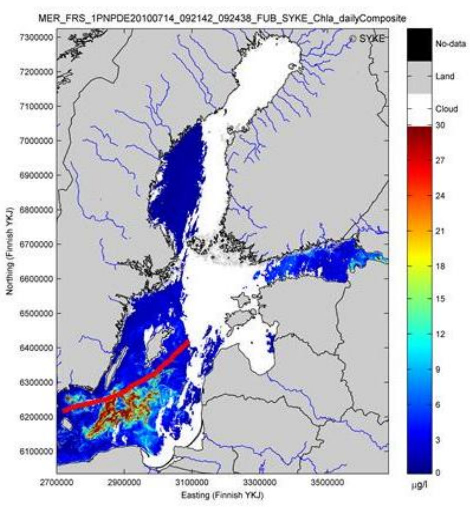
- **Validation**: More information is needed for biomass-fluorescence relationship
- <u>**Use of data**</u>: EO validation, Ecosystem model validation, visualization

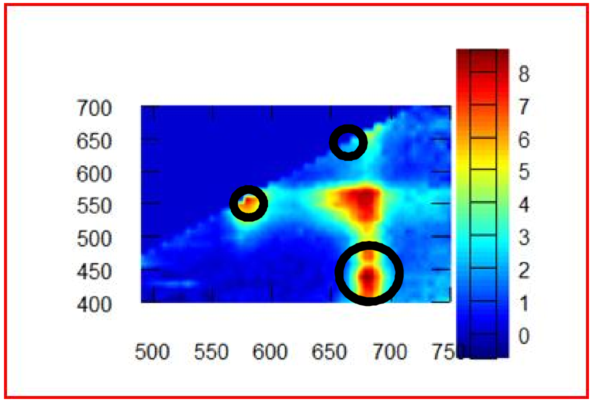




Attila et al. unpubl.

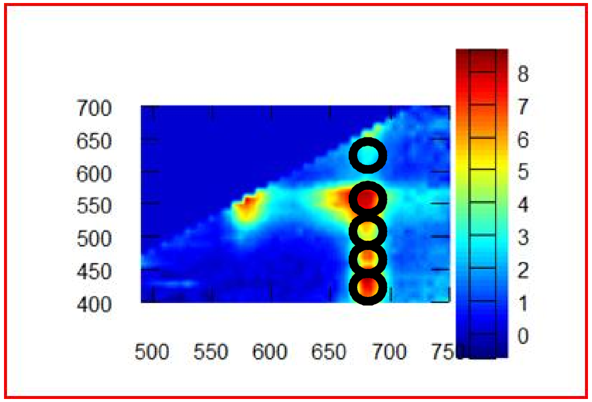
SPECTRAL FLUORESCENCE: Measuring techniques

Several fluoroprobes



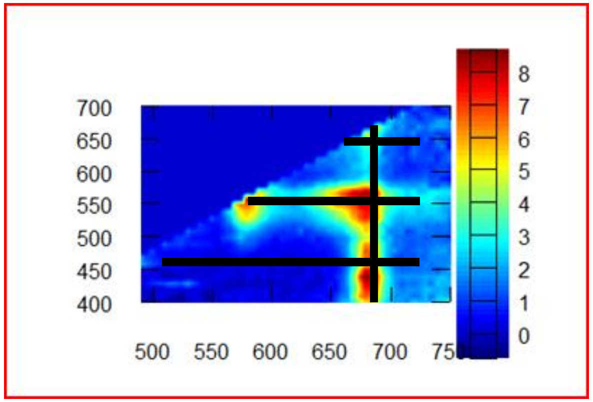
- 1-4 k€/channel
- Limited taxonomic discrimination
- Sensitive to background noise
- Only simple statistics possible
- Several manufacturers

Multichannel fluorometers



- >20 k€
- Limited detection of phycobilins
- Correction for background noise
- Multivariate statistics possible
- Bbe-Moldaenke,JFE Advantech

Spectral fluorometers



- Lab instruments <20 k€
- EEM measurement time consuming
- Background correction
- Multivariate statistics possible
- No commercial FB devices

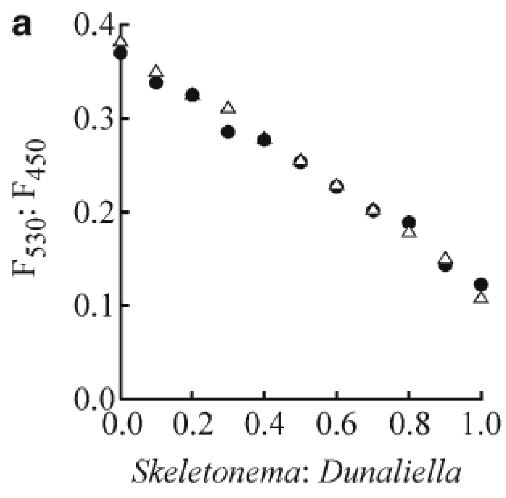
SPECTRAL FLUORESCENCE: How to retrieve taxonomic signal from fluorescence spectra

Ratio plots, clustering
Similarity indices
Principal component analysis
Wavelets
Linear unmixing, multivariate regression

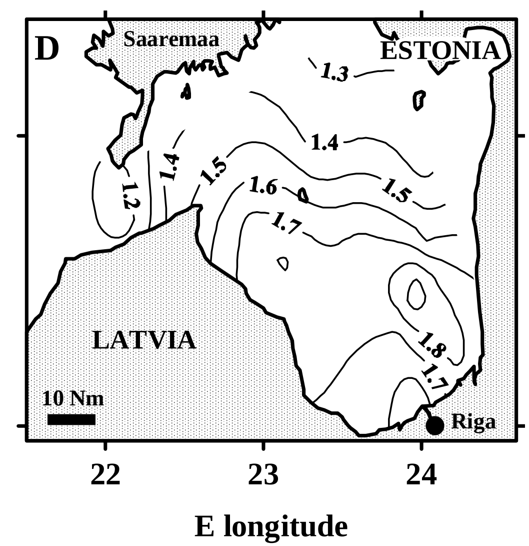
$$\mathrm{SFS}(\lambda) = \sum_{i=1}^{n} c_i k_i(\lambda)$$

$$\mathrm{SFS} = CK + E$$

MacIntyre etal 2010



Seppälä & Balode 1998



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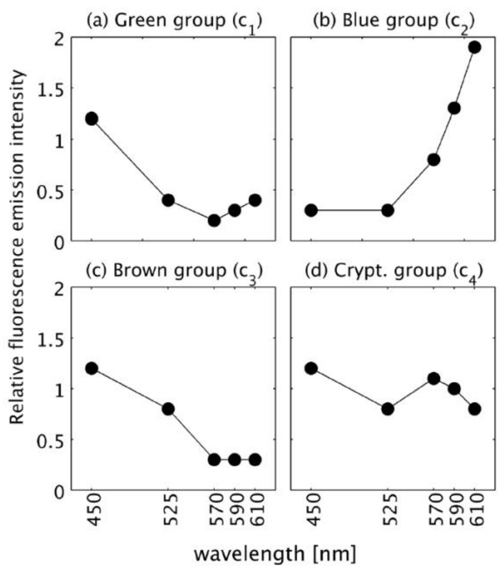
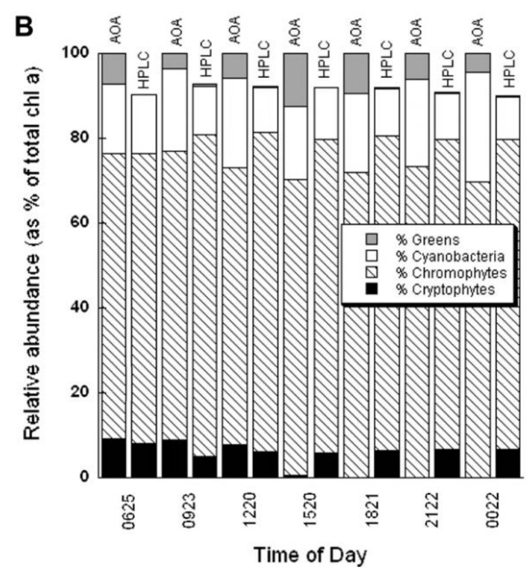
Alexander et al 2012



Richardson et al 2010



Spectral fluorescence: How to retrieve taxonomic signal from fluorescence spectra

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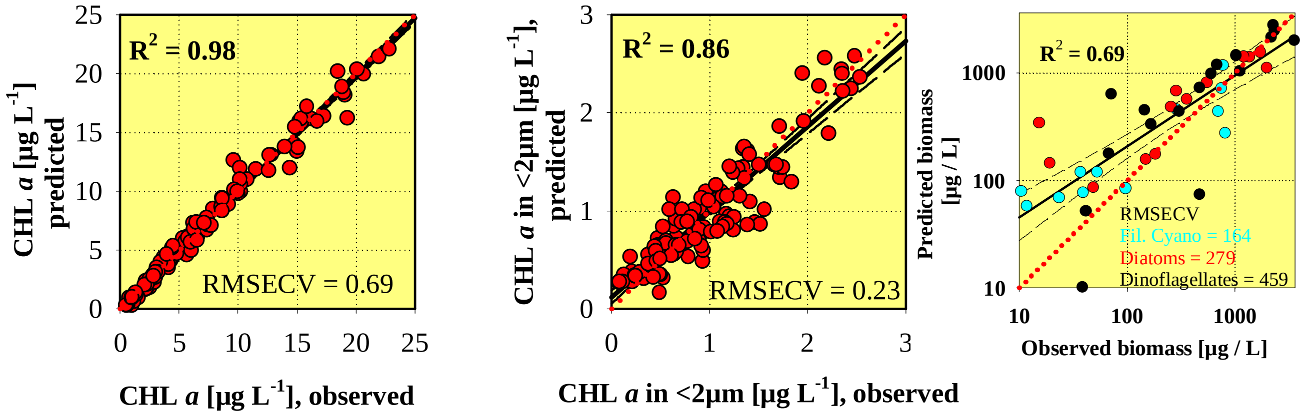
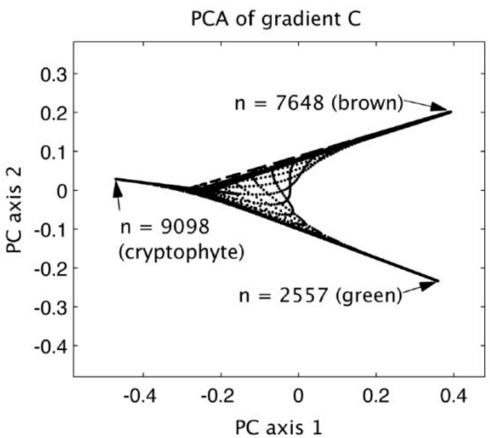
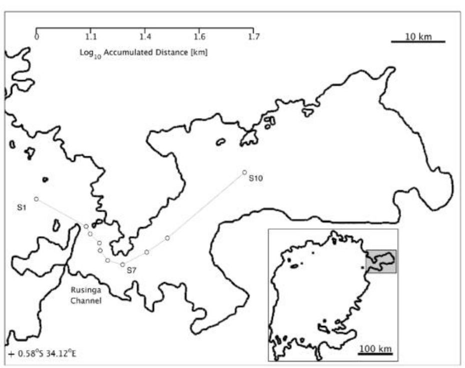
Seppälä 2009



SPECTRAL FLUORESCENCE: Identifying spatial structures to locate patches and facilitate sampling

Study at Lake Victoria;

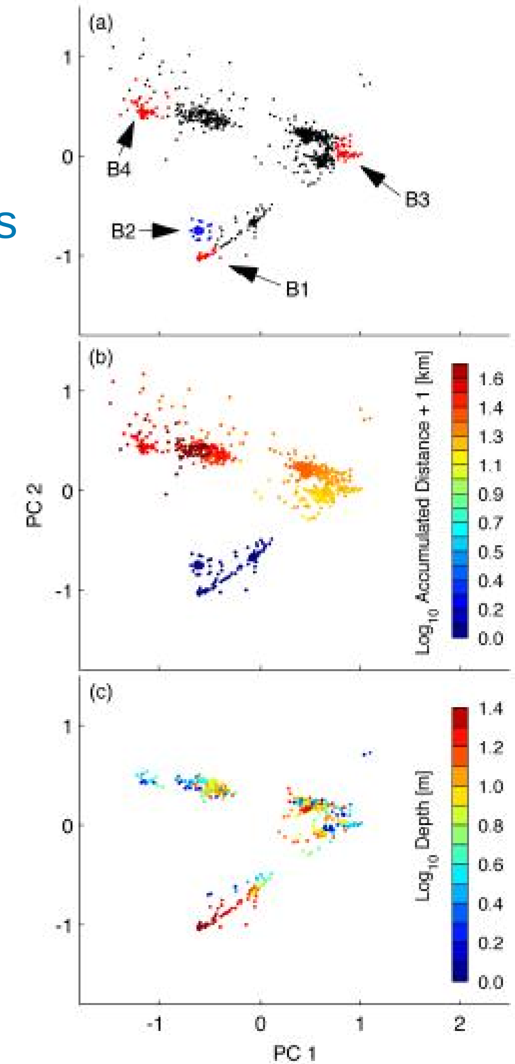




PCA identifies 4 spectral clusters, or species groups

Clusters are horizontally separated (color coding)

…as well as vertically (color coding)

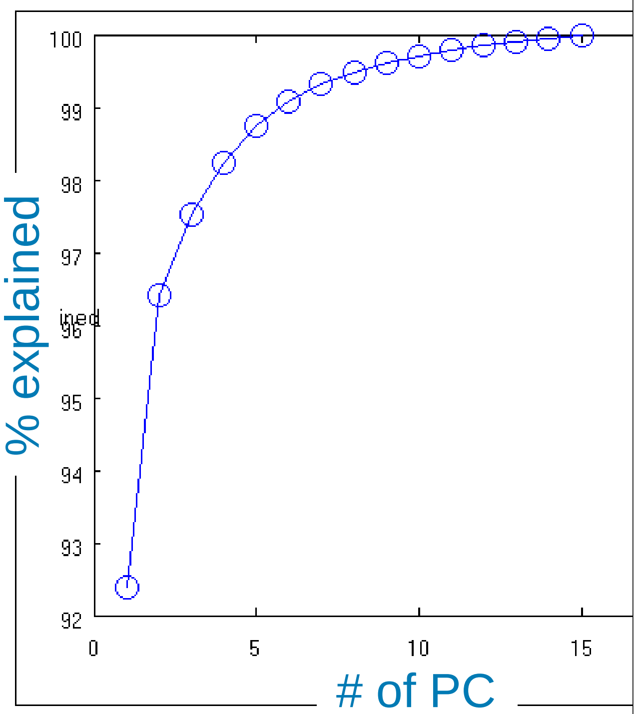


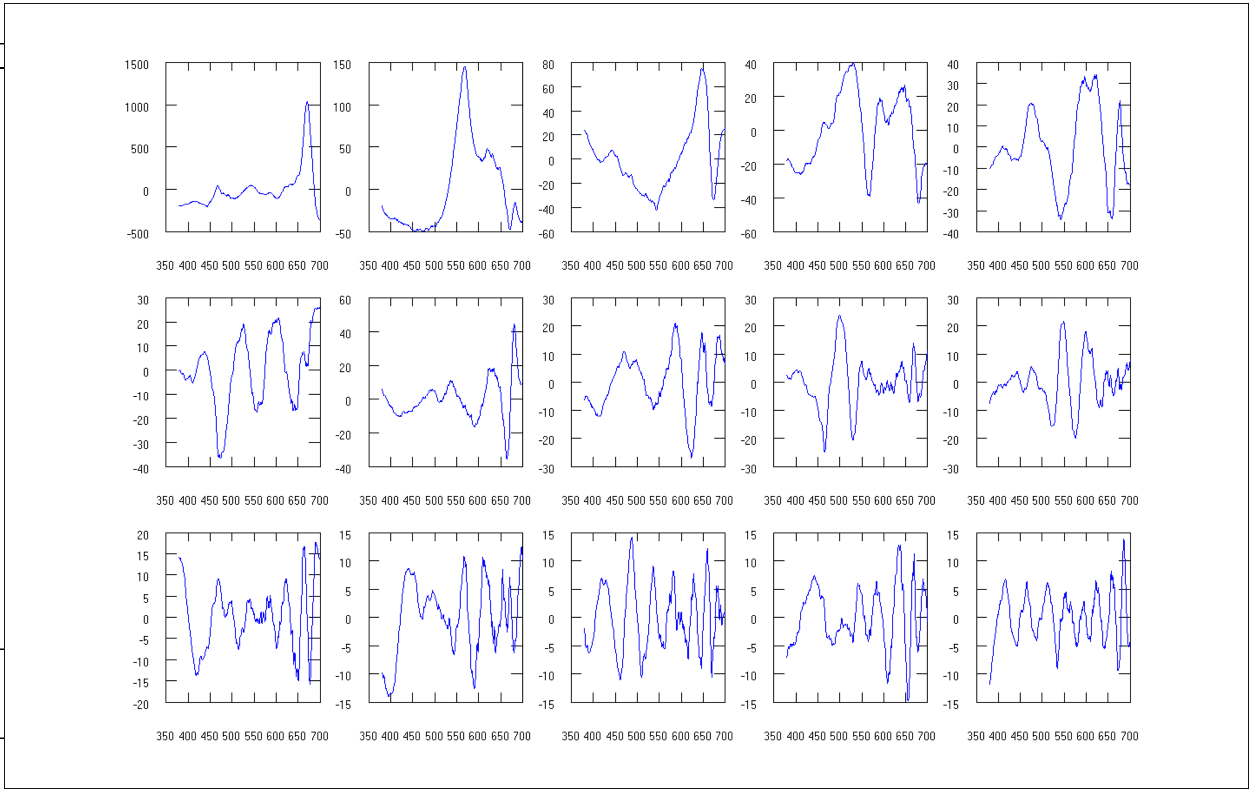
Alexander et al 2012

SPECTRAL FLUORESCENCE: Identifying seasonal structures, Gulf of Finland

Chla a excitation spectra (380-700nm / 730 nm)

PCA





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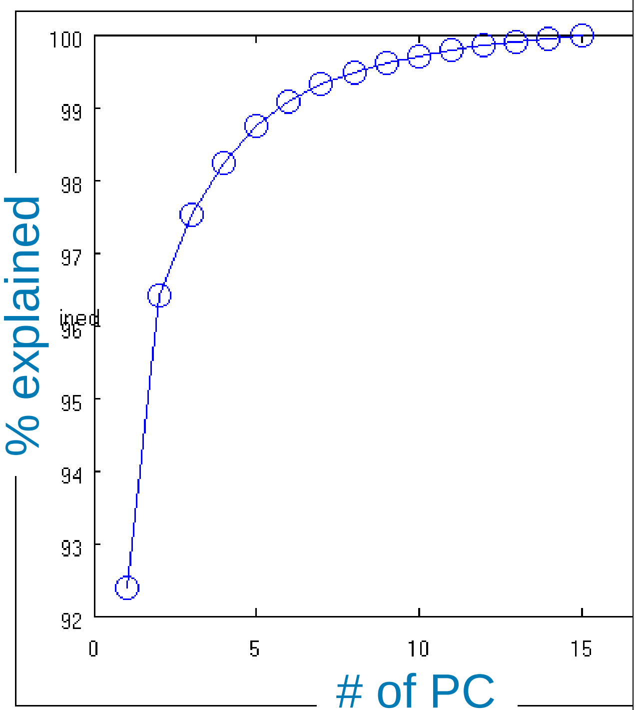

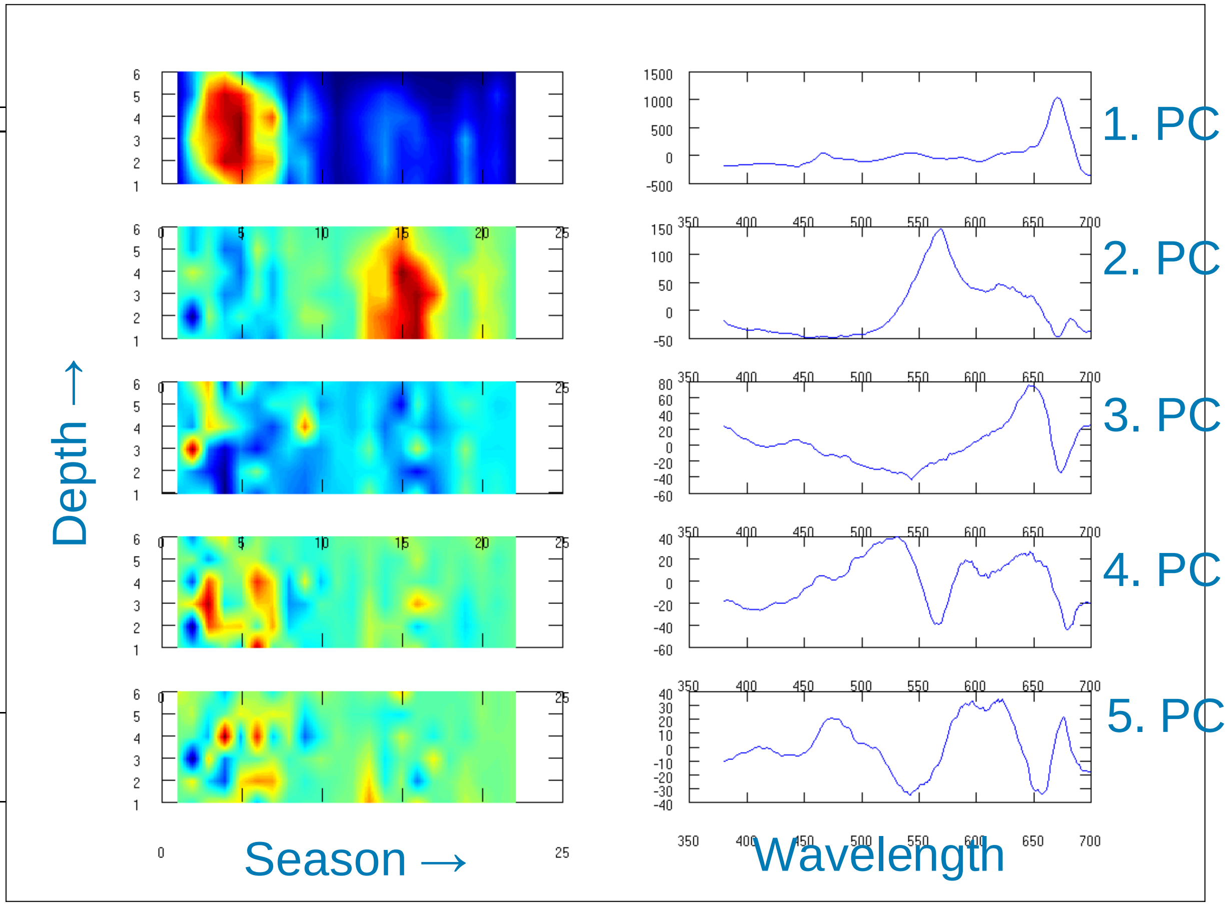

An overview of spectral *in vivo* fluorescence methods for phytoplankton taxonomy

What next?

- ❑ Calibration / validation (HPLC, FlowCAM, FCM, EO)
- ❑ Collection of validation data (intelligence)
- ❑ Instrumentation (preferably spectral)
- ❑ New algorithms (multivariate)
- ❑ Use of data