SYKE

FinMARI Finnish Marine Research Infrastructure

JERICO next

JOINT EUROPEAN RESEARCH INFRASTRUCTURE NETWORK FOR COASTAL OBSERVATORIES

# Online measurements for phytoplankton biology

## Lessons from Algaline and JericoNEXT

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# Outline

**Phycoerythrin fluorescence** in the Baltic Sea, ***Alg@line ferrybox*** since 2016 ->

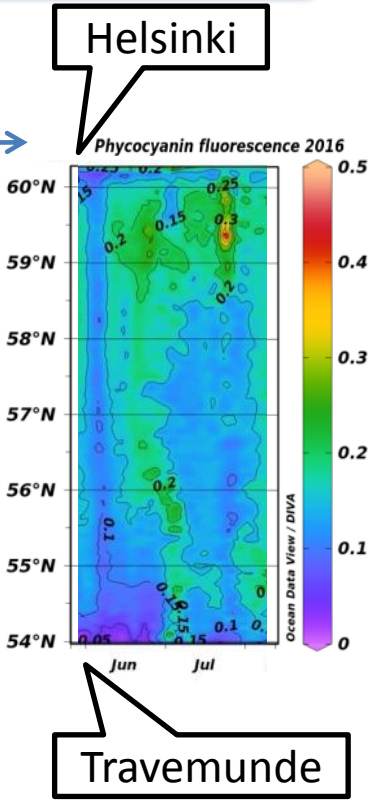
- In the Baltic Sea, how does the phycoerythrin fluorescence vary in time and space, when recorded using up-to-date commercial fluorometers
- How is this variability related to other optical measurements, phytoplankton community structure and cell count measurements
- Does phycoerythrin fluorescence provide added value in monitoring the state of the sea and it's biodiversity

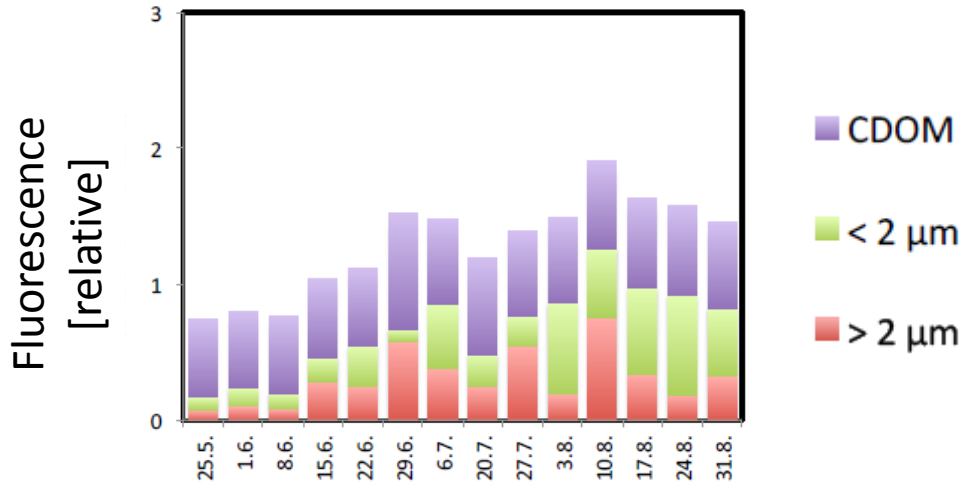
Multi-instrument studies at ***Utö Atmospheric and Marine Research Station***, 2016-18

- Usability of **backscattering meter BB3**
- How does **spectral absorption photometer OSCAR** compare with traditional filter pad and CDOM absorption measurements
- What is the sensitivity, stability, usability… of **Multiexciter (ME) spectrofluorometer** in online measurements
- What is the functionality of **FRRF** in continuous measurements
- First trials of **Imaging FlowCytobot** to analyse phytoplankton community structure

# PE fluorescence

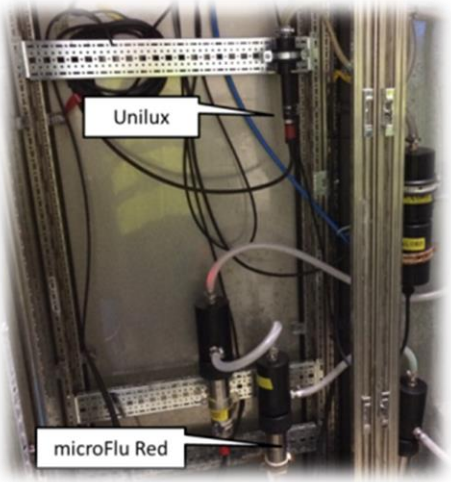
- Phycobiliproteins are major light harvesting pigments for cyanobacteria, red algae, cryptophytes, also found in few other species.
- In Alg@line ferries, phycocyanin fluorescence (fil. cyanobacteria) since 2005, phycoerythrin (picocyanobacteria, cryptohytes, Mesodinium, Dinophysis) since 2016
- Two commercial Phycoerythrin (PE) instruments tested in 2016: MicroFlu Red (Trios) and Unilux (Chelsea Technologies Group)
- PE field measurements close to LOQ and lower part of linear range
- PE readings influenced by dissolved organic matter; >30% of PE signal is due to CDOM fraction.




# PE fluorescence, Field data

- Instruments onboard Finnmaid in 2016
- Weekly sampling at three stations for additional data
- Clear seasonality and spatial structure

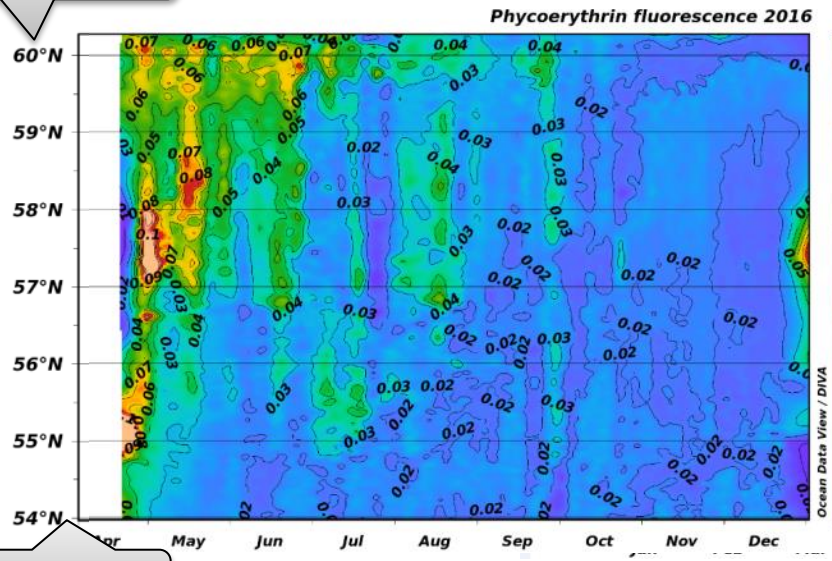


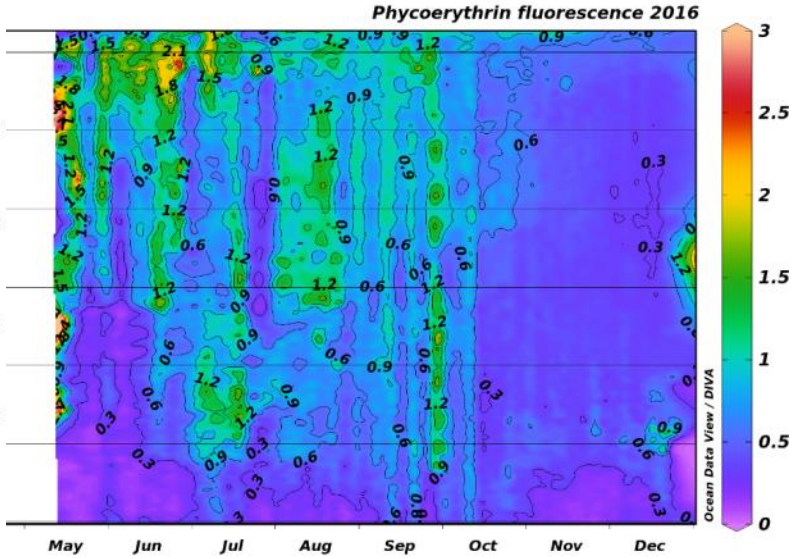


MicroFlu Red; LOQ 0.025

Unilux; LOQ 0.5

Helsinki

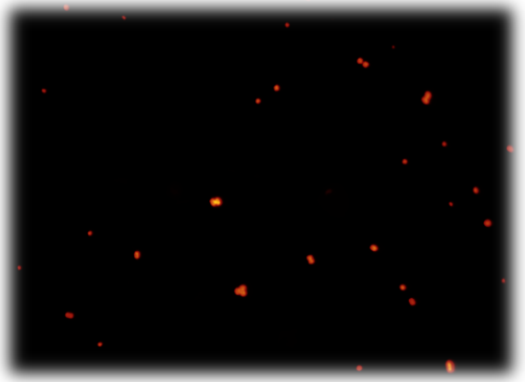


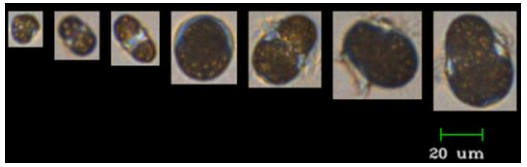



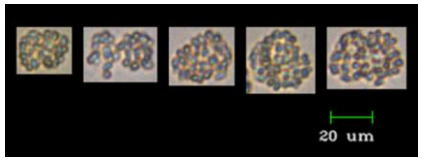

Travemunde

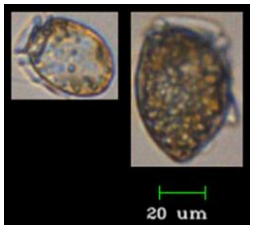
# PE fluorescence validation, cell counts

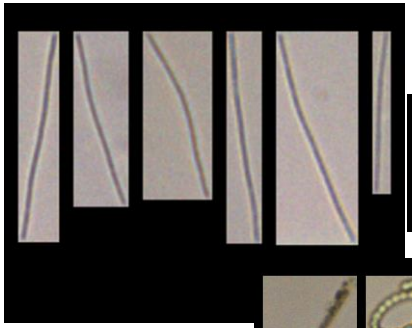
- Epifluorescence microscopy: Counting picocyanobacteria that contain PE, image analysis software to decide between PC and PE contaning cells, measure cell abundance and dimensions for each cell (especially surface area).
- FlowCAM: Imaging PE containing large cells triggered with 532 nm laser and 575 nm emission. Image analysis software to measure dimensions for each cell, manual sorting for abundance. (Also fluorescence per cell available for further analysis)
- correlation between cell counts and fluorescence not straightforward

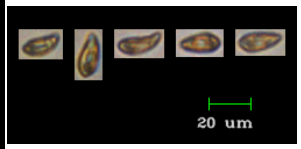


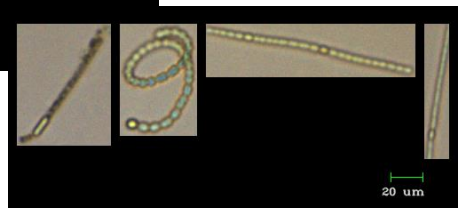


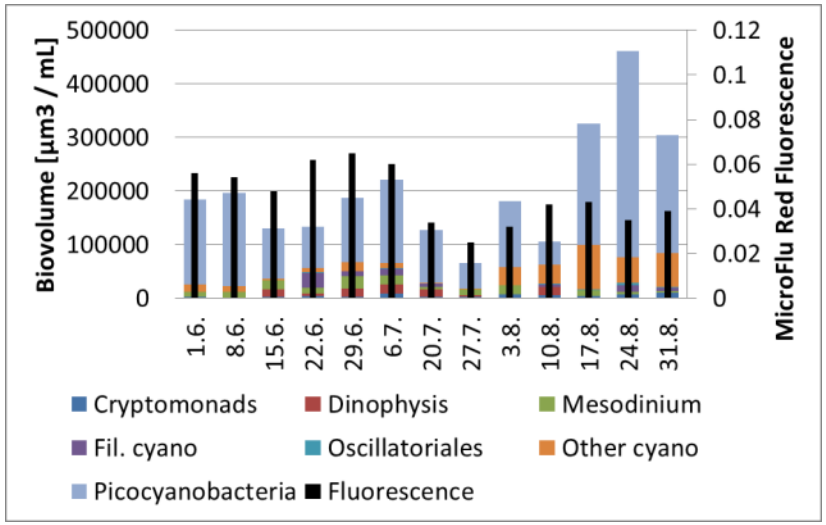



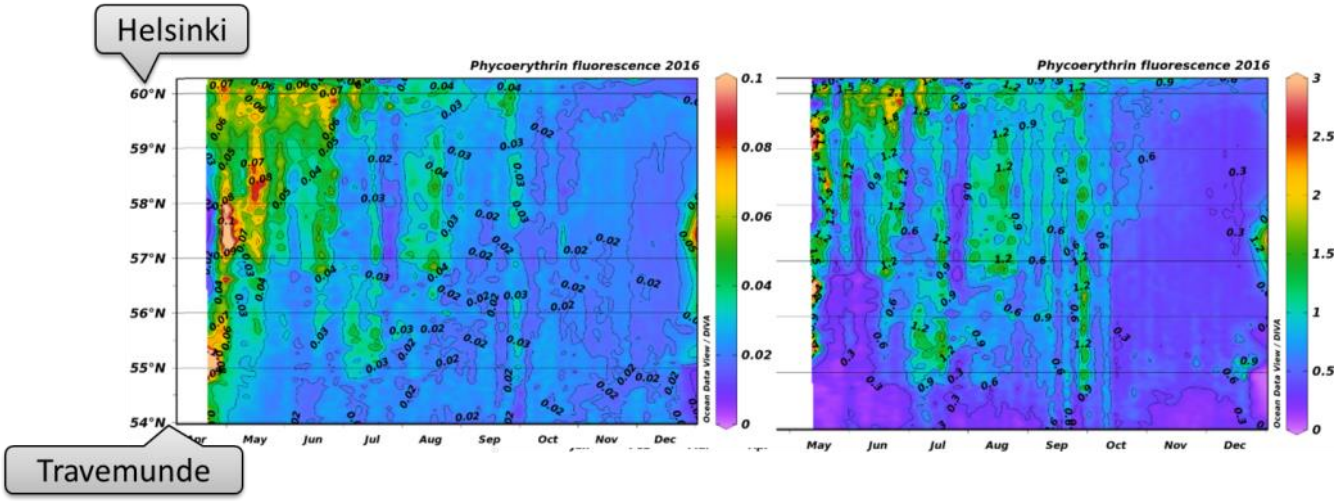


# PE fluorescence, summary

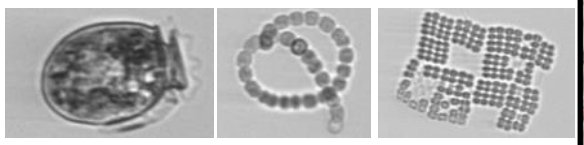
- Multiple sources of PE fluorescence
- Early summer with Mesodinium, noted also by other methods
- Late summer with picocyano and coccoid cyanos
- Occasional PE increases due to upwelling (populations staying normally in deeper, and rich of PE, are taken to surface)
- Light acclimation and nitrogen availability largely affect the PE content of species, making cell volume vs. fluorescence comparisons rather complex
- PE fluorescence can be used to track spatiotemporal phytoplankton events, which need to be validated by various lab methods.
- Validation not an easy task: Biomass ≠ pigment conc. ≠ fluorescence
- 2018 new sensors are/will be installed in additional ferries, Utö station, and in Utö profiling buoy



# Utö studies during the JericoNEXT field campaign: spring 2017-spring 2018

- Data analysis has been just started, some snaphots available

Imaging FlowcytoBot – phytoplankton imaging





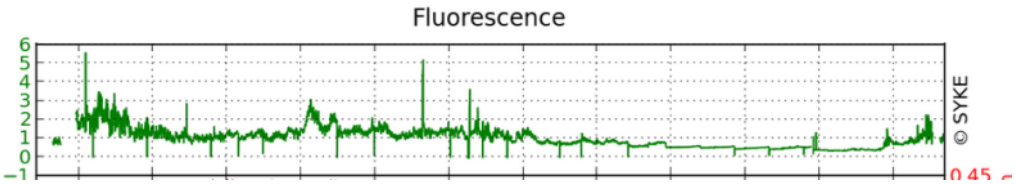
Backscattering meter – for ocean color cal/val

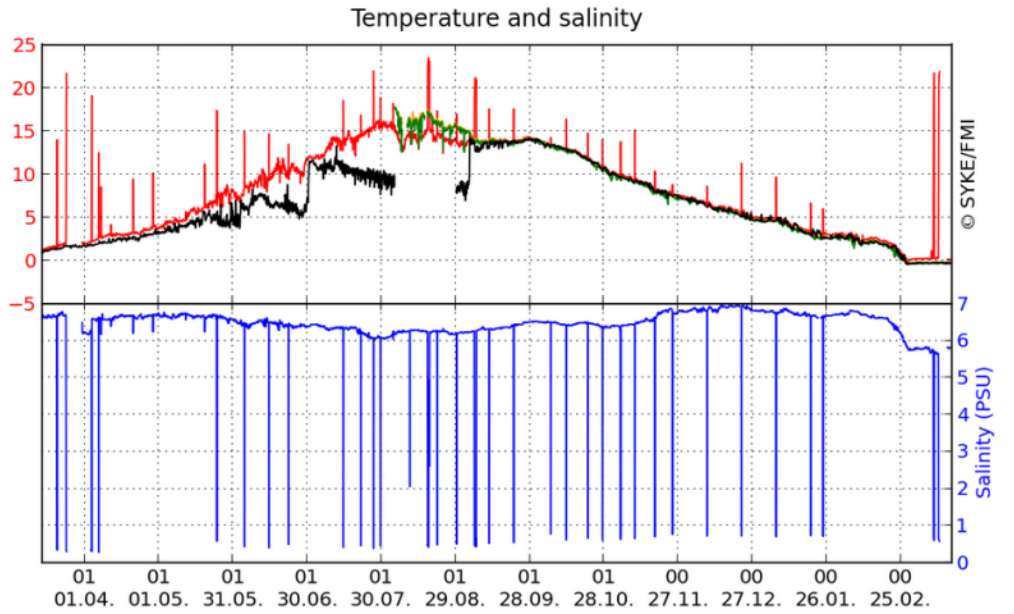
Spectral fluorometry – phytoplankton pigments groups

Fast repetition rate fluorometry – primary productivity

Spectral absorption – in-water optical components

# Utö studies




http://swell.fmi.fi/Uto/latest.html

www.finmari-infrastructure.fi/field-stations/uto-fmi

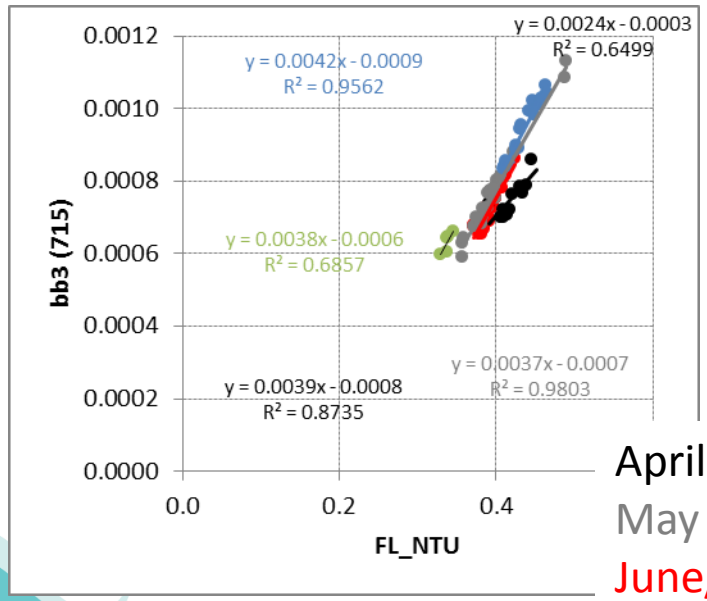
Contact: Lauri Laakso, FMI

# Utö studies

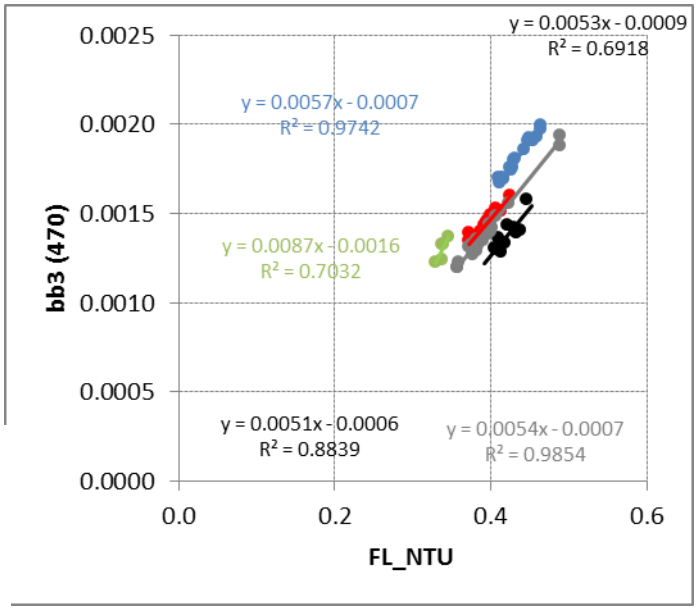
Backscattering, important parameter for Ocean Colour.

Testing the Wetlabs BB3 in flow-through system.

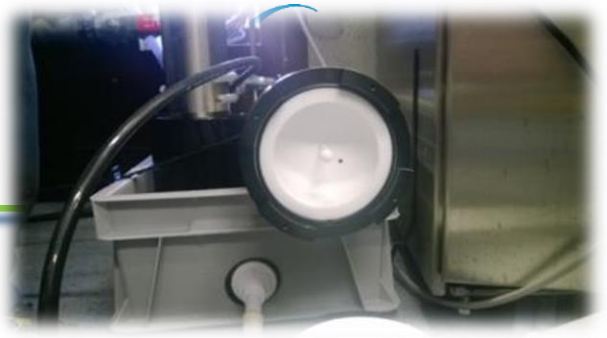
- Seasonality in the scattering-turbidity slope; change in particulate materials or instrument drift?
- BB3 needs frequent HQ cleaning




April 2017
May 2017
June/July 2017
August 2017
Sept 2017

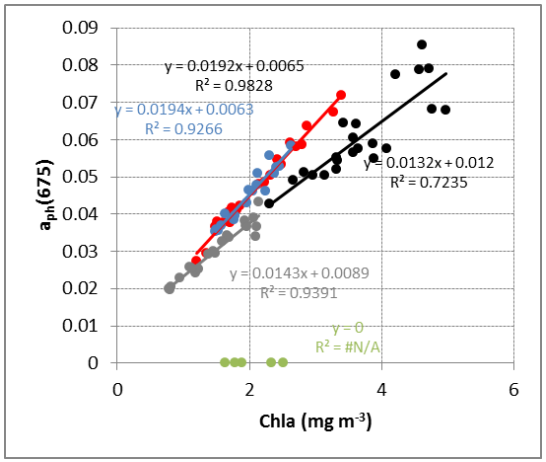


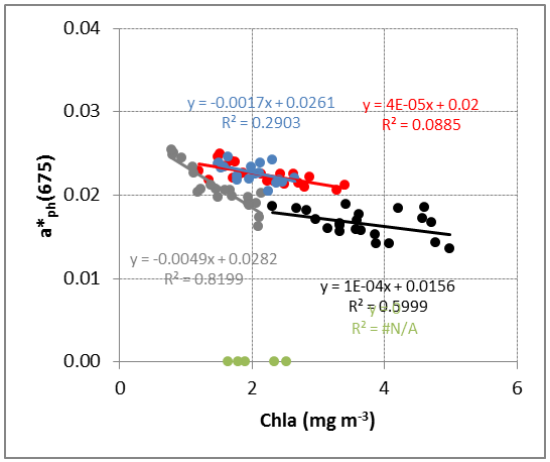
# Utö studies



OSCAR, integrating cavity spectrophotometer,
Sensitive measurements due to a long optical path length,
eliminates errors introduced by light scattering by particles.
→ *Spectral absorption, Chla, TSM, phytoplankton community composition*

- OSCAR data still no QC.
- Reference measurements: Spectral light absorption using filter pad absorption, CDOM absorption: Good range of Chla concentrations and package effect (biomass, size)
- OSCAR needs frequent HQ cleaning, getting final data is not easy
- Good potential for Chla estimation

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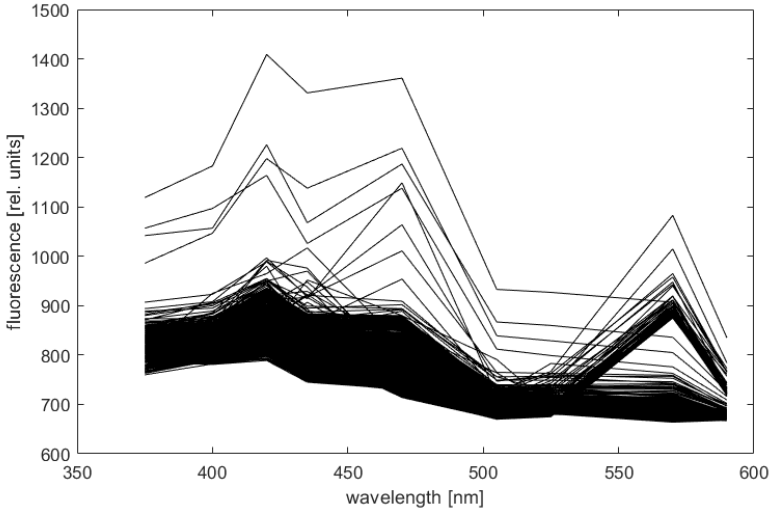
# Utö studies

Multiexciter spectrofluorometer
9 wavebands to *discriminate different phytoplankton groups* (green algae, brown algae, cyanobacteria …).

- Reference measurements: Chla fluorescence, Phycocyanin fluorescence (30 sec interval, for almost one year), spectrofluorometry (during campaigns, n≈85). Also comparison with community composition .
- Very robust instrument, some hardware issues but not critical
- Calibration pending (i.e. stability not checked)
- Multivariate spectral analysis to detect shifts in phytoplankton communities
- ME data still no QC.

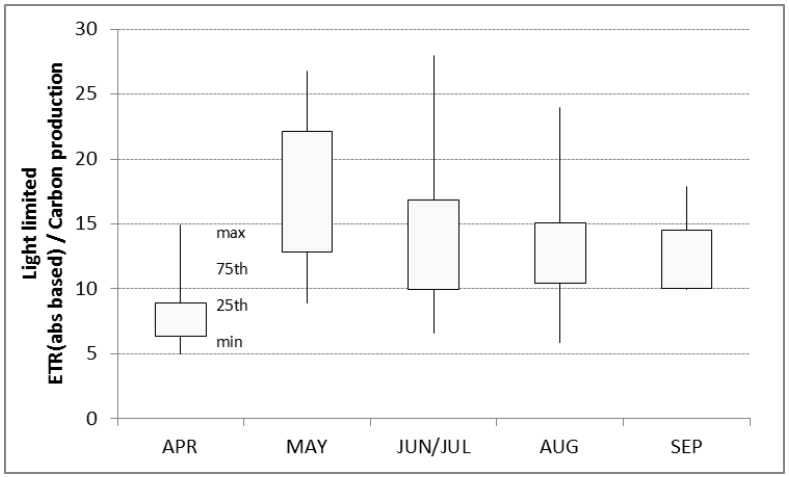
Spectral fluorescence variations during 10-day period in 2017.
No QC done yet.



# Utö studies

Fast Repetition Rate Fluorometry, FRRF
Fluorescence induction measurements result in several parameters describing the state of *photophysiology of the phytoplankton cells and which can be used to estimate primary productivity*

- FRRF used continuously since April 2017, several breaks due to issues with internal pumps and flashdisk. During campaigns comparison with 14-C.
- Two methods for comparison: P-I curves & FLC and simultaneous FRRF/14-C incubation for 1 hour (during campaigns, n≈85).
- Preliminary data available, needs furher analysis + QC
- Key study issue, why does the conversion factor between C-fixation and electron transport rate vary



# Utö studies

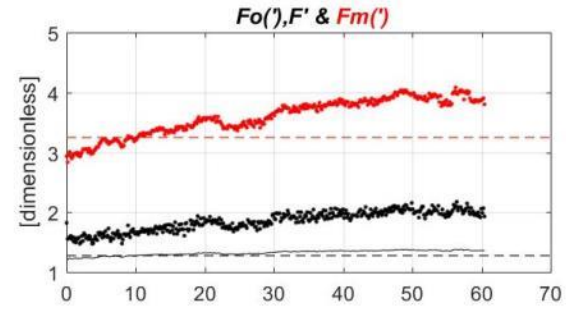
**SAMPLE DATA**
Sample No: **79**
Date: **4.4.2017 13:00:00**
Chla (tot/<2µm) = **3.4** / **0.5** mg $m^{-3}$

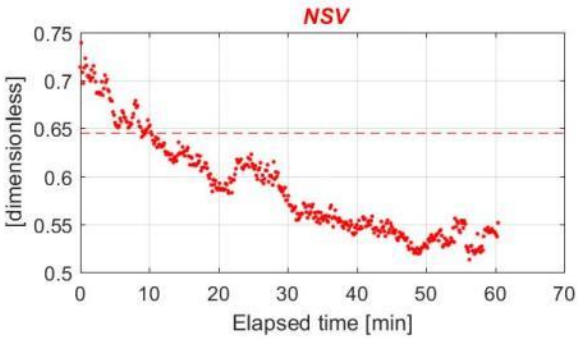
**FRRf derived parameters**
Actinic irradiance = **48** µmol photons $m^{-2}$ $s^{-1}$
Fv/Fm = **0.61**
Fq'/Fv' = **0.65 - 0.88**
$\sigma$ = **4.9** $nm^2$ $quanta^{-1}$
$\sigma'$ = **4.08 - 5.48** $nm^2$ $quanta^{-1}$
RCII = **5.09e-09** (mol RCII) $m^{-3}$
$1/n_{PSII}$ = **752** mol Chla (mol RCII) $^{-1}$
$a_{LHII}$ = **0.00723** $m^2$(mg Chla)$^{-1}$

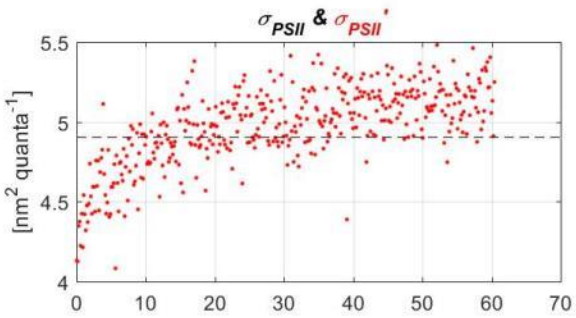
**Electron transfer rates (ETR) vs $C^{14}$ based production**
Abs. based ETR ≈ **891** mol $e^-$ (mol Chla)$^{-1}$ $h^{-1}$
Sigma based ETR ≈ **815** mol $e^-$ (mol Chla)$^{-1}$ $h^{-1}$

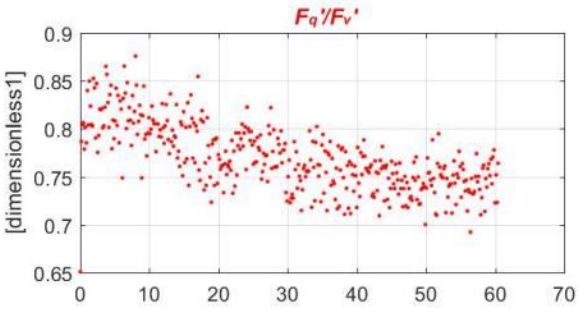
$C^{14}$ based production ≈ **140** mol C (mol Chla)$^{-1}$ $h^{-1}$

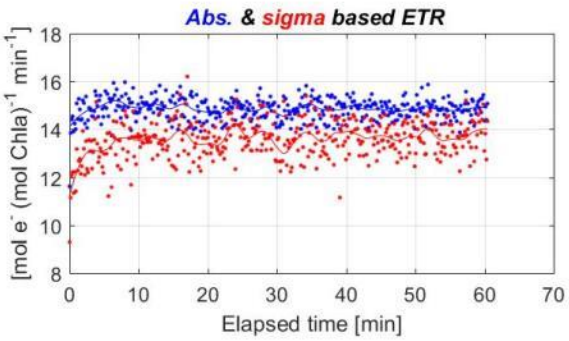
CF for abs. based ETR = **6.39** mol $e^-$ (mol C)$^{-1}$
CF for sigma based ETR = **5.84** mol $e^-$ (mol C)$^{-1}$



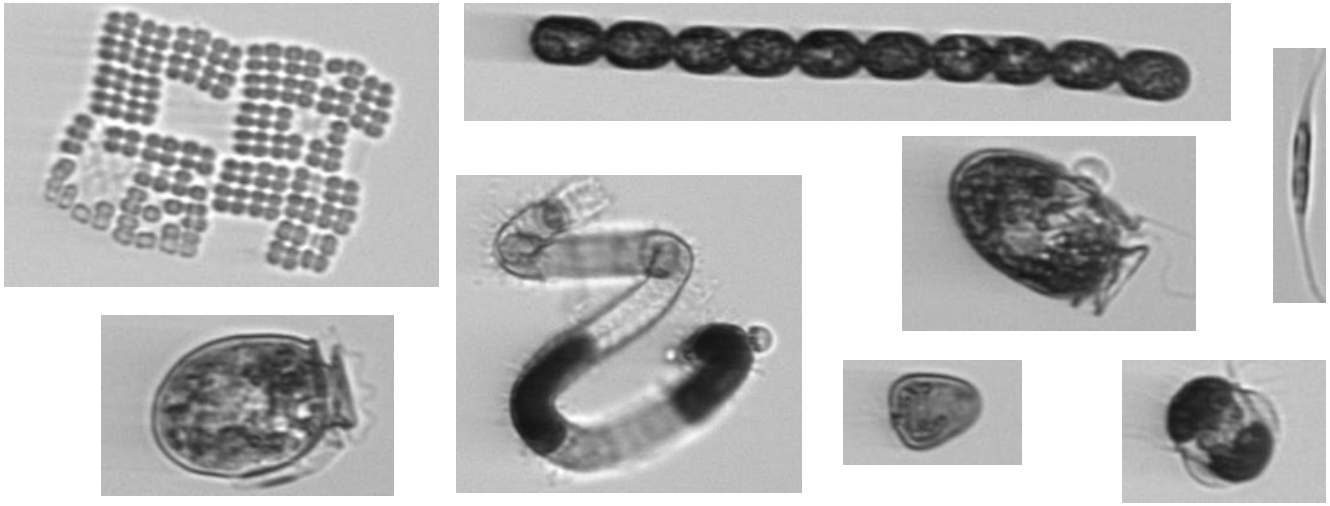



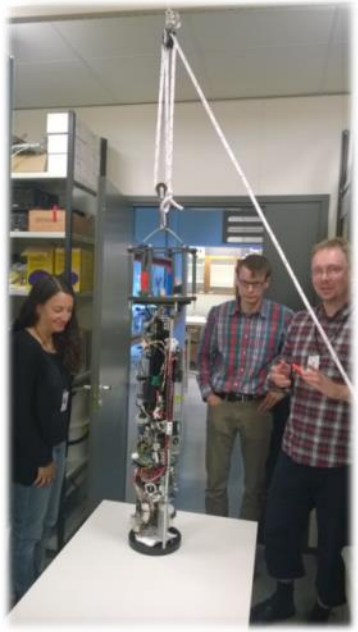



# Imaging FlowCytobot

- Imaging FlowCytobot (IFCB) is an in-situ automated submersible imaging flow cytometer that generates images of particles in-flow taken from the aquatic environment.
- Phytoplankton cells recognized by fluorescence
- High resolution (1 µm) images of suspended particles in the size range <10 to 100 µm
- Samples at a rate of 15ml of water per hour, 30,000 high resolution images per hour
- Algorithm to classify images
- primed, tested and run in the SYKE lab in winter 16/17
- deployed at Utö 03/2017, connected to flow through system inside research hut. Samples every 20 min and transfers raw images to Helsinki in real time
- Automated cleaning cycle, but due to clogging issues (due to large cells) this is now supplemented by remotely operated one





# Summary

Several techniques/instruments tested for the first time in Baltic. Slightly hectic and huge amounts of new types of data.

Within JericoNEXT, SYKE aims to analyse the usability of emerging technologies in the Baltic Sea conditions

- phycoeryhtrin fluorescence
- spectral fluorescence
- variable fluorescence
- backscattering
- spectral absorption.

Additional national PhD project started, using Imaging FlowCytobot to detect climate change relevant shifts in phytoplankton community composition.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 654410.