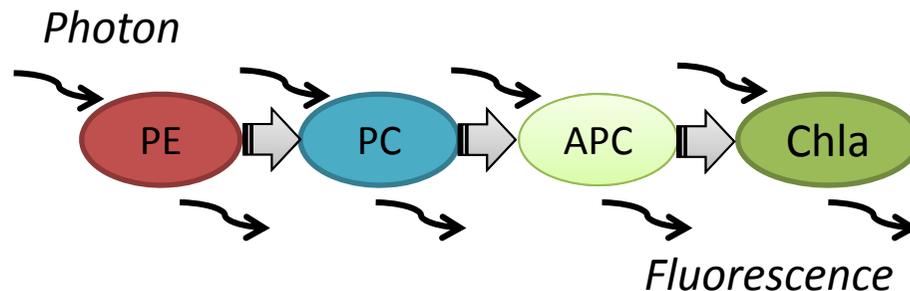


Phycoerythrin fluorescence in the Baltic Sea

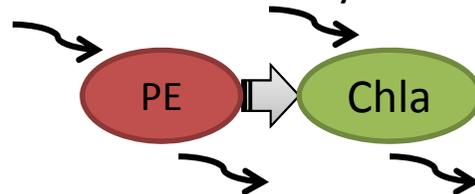
*Jukka Seppälä, Suvi Rytövuori,
Pasi Ylöstalo, Jani Ruohola, Petri Maunula, Seppo Kaitala
Finnish Environment Institute, SYKE
Jukka.seppala@ymparisto.fi*

Phycoerythrin, background

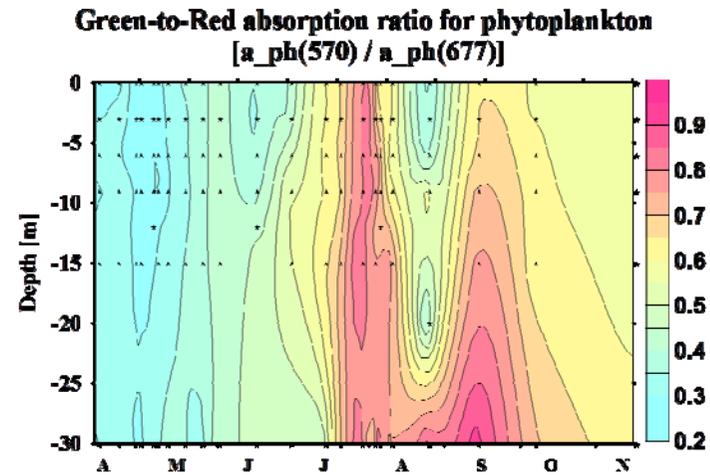
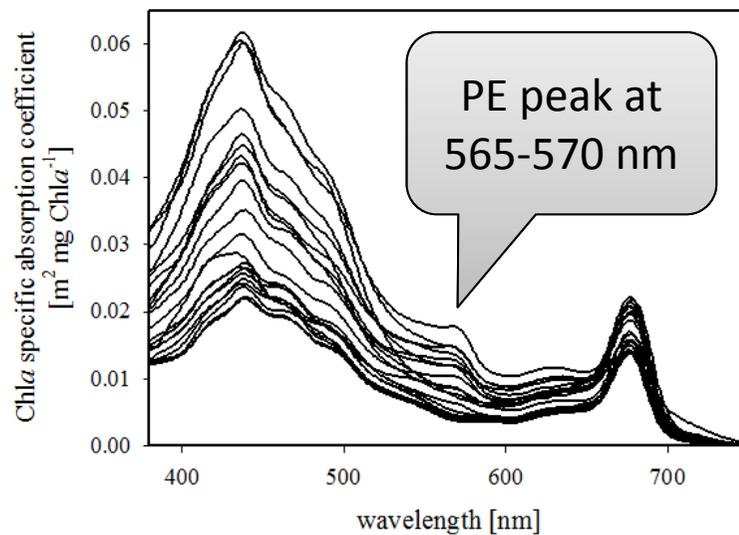
- Phycobiliproteins are major light harvesting pigments for cyanobacteria, red algae and cryptophytes but also found in few other species.
- Phycoerythrin (PE; “erythros” = red) is available in several forms, composed of several chromophores (PUB, PEB) with different absorption maxima. Their ratio varies (adaptation and acclimation) due to light climate - > differences between oceanic and coastal types.
- Depending on the chromophores PE has A_{\max} 475-580 nm and fluorescence emission maxima at 570-580 nm.
- Energy transfer in cyanobacteria



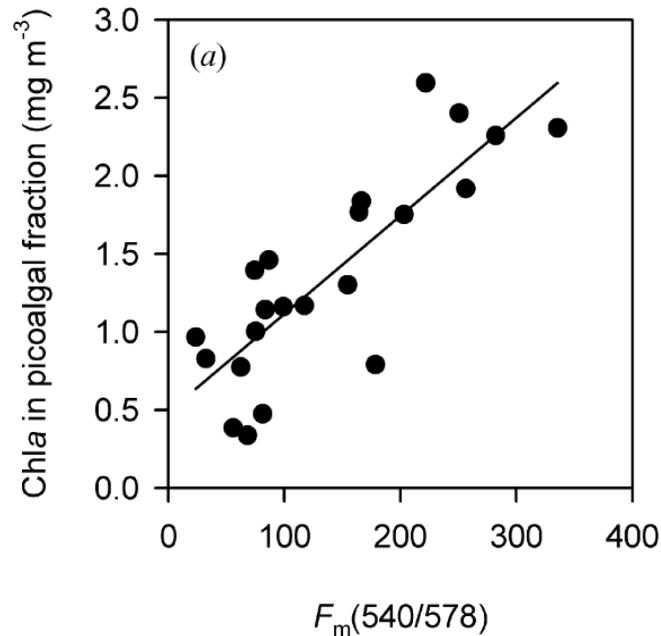
- Energy transfer in eucaryote



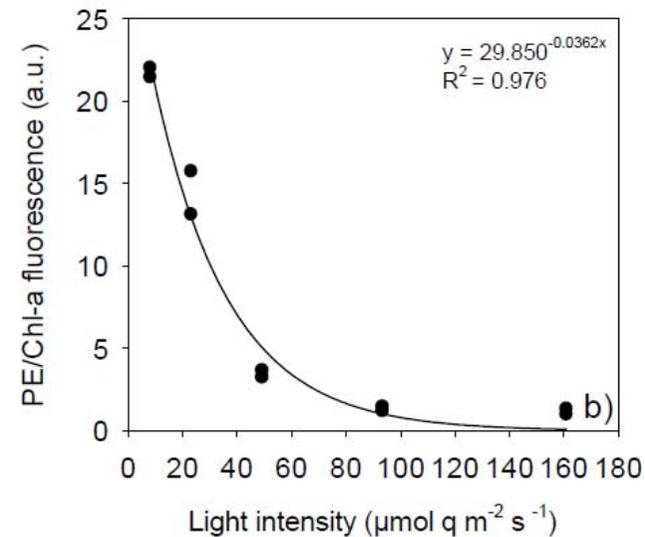
- Early studies on PE fluorescence and absorption since 1990's.
- Seasonality, high values in summer
- Vertical variability, high values in deeper layers (matching the green light that penetrate deepest)



- Early studies on PE fluorescence and absorption since 1990's.
- Seasonality, high values in summer
- Vertical variability, high values in deeper layers (matching the green light that penetrate deepest)
- Relationship to picoalgae fraction (cells <2µm)
- For cyanobacteria, PE regulated by light availability (low light -> high PE)



Seppälä, Ylöstalo, Kuosa 2005



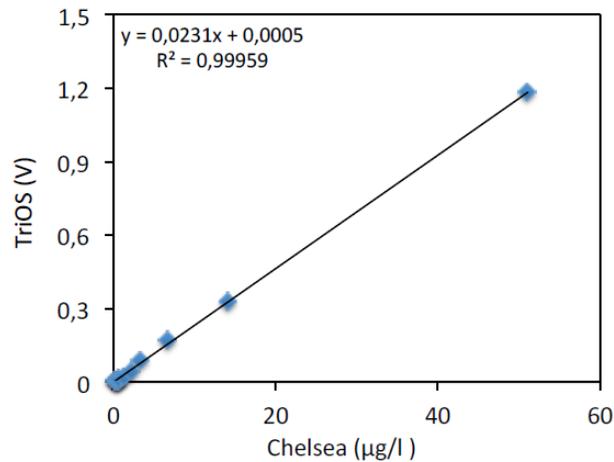
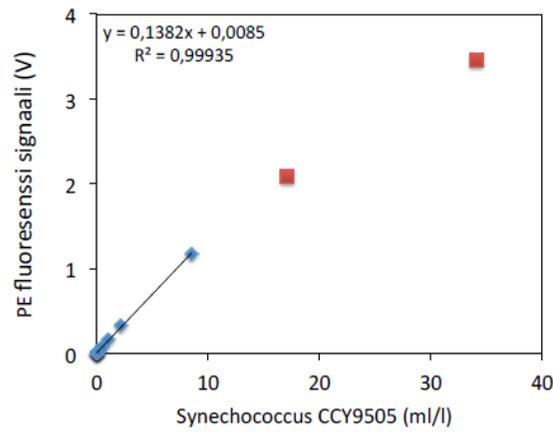
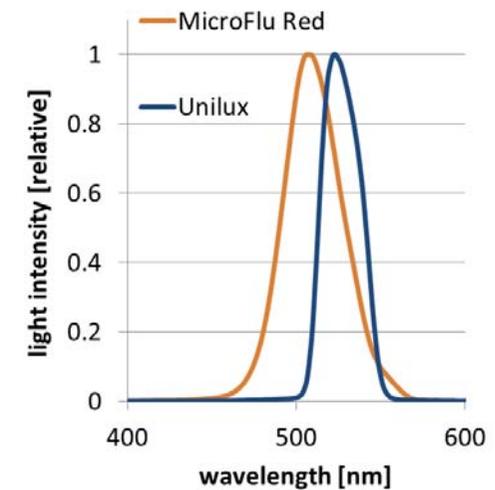
Niiranen 2008

Research questions

- 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 and cell count measurements
- Does phycoerythrin fluorescence provide added value in monitoring the state of the sea and its biodiversity

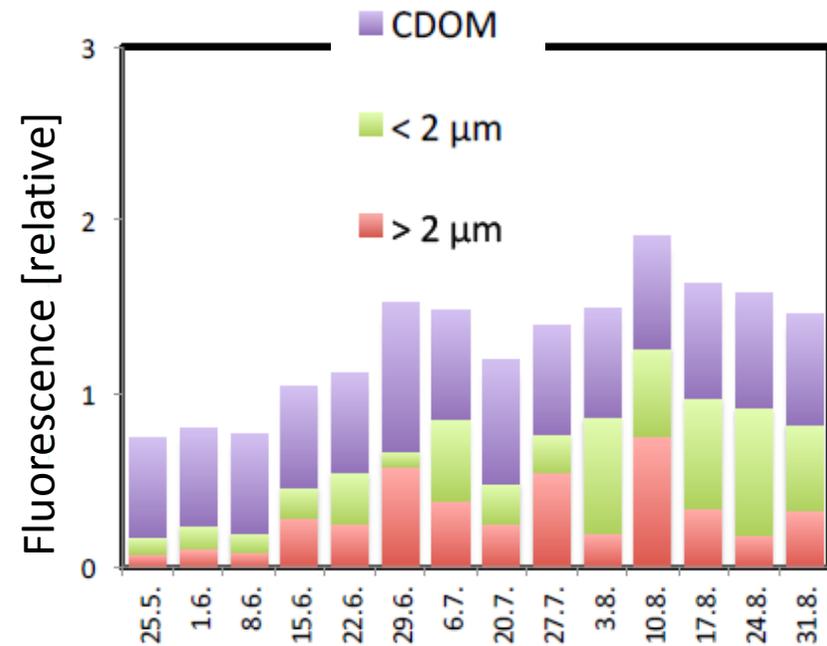
	Exc. (nm)	Em. (nm)	Filament cyanob.	Picocyanob. green red	Other cyanob	Crypto- monads	Mesodinium Dinophysis
Phyco- erythrin	490- 575	570- 580	-/+	- +	-/+	+/-	+
Phycoeryth- rocyanin	570- 595	625- 635	+/-				
Phyco- cyanin	615- 640	635- 645	+	+ +	+	+/-	
Allophyco- cyanin	620- 655	660- 675	+	+ +	+		

- Two commercial instruments tested: MicroFlu Red (Trios GmbH) and Unilux (Chelsea Technologies Group Ltd, UK)
- Intercalibrated: MicroFlu; 5 V = Unilux; 200 µg/L
- No interference from non-PE species
- No interference from flow-through cuvette
- Linear for 30-50 % of full scale

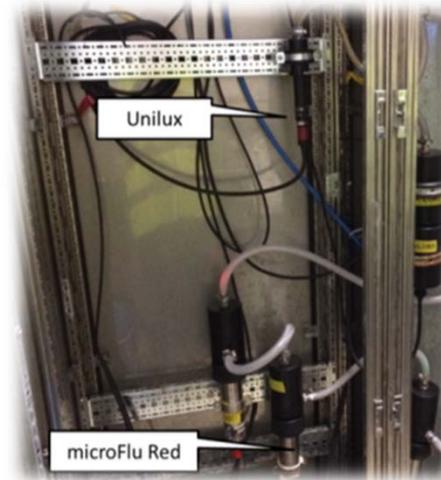


- Readings influenced by dissolved organic matter, "blank" vs. CDOM (or salinity) relationship to be established
- Field measurements close to LOQ and lower part of linear range
- Samples measured from different size-fractions, using spectral fluorometry and by simulation the fluoroprobe wavebands, indicate >30% of PE signal is due to CDOM fraction.

	Unilux [$\mu\text{g/L}$]	MicroFlu Red [V]
LOD: Milli Q		0.0052
LOQ: Milli Q		0.0073
LOD: 6 PSU	0.35	0.022
LOQ: 6 PSU	0.50	0.025
Linear range	0-100	0-2
Range 2016	0-3	0-0.1



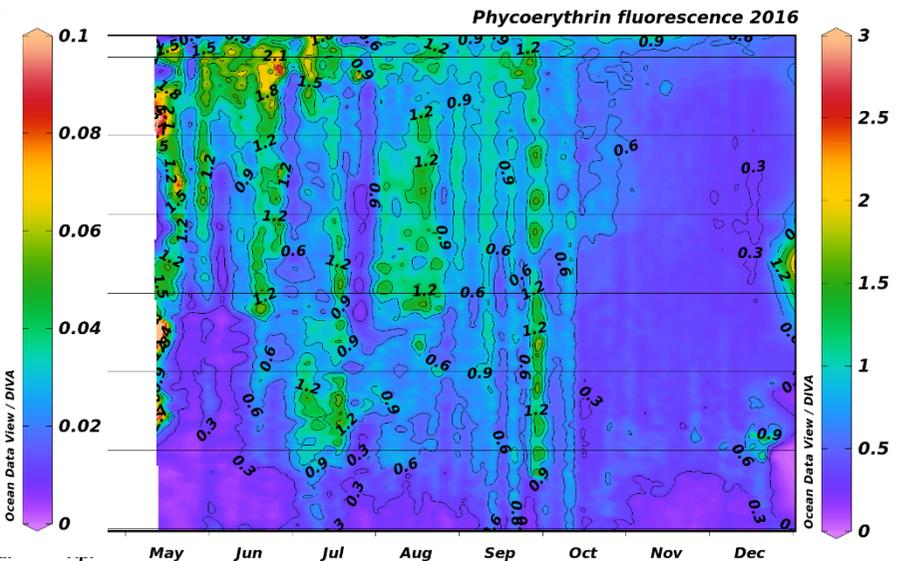
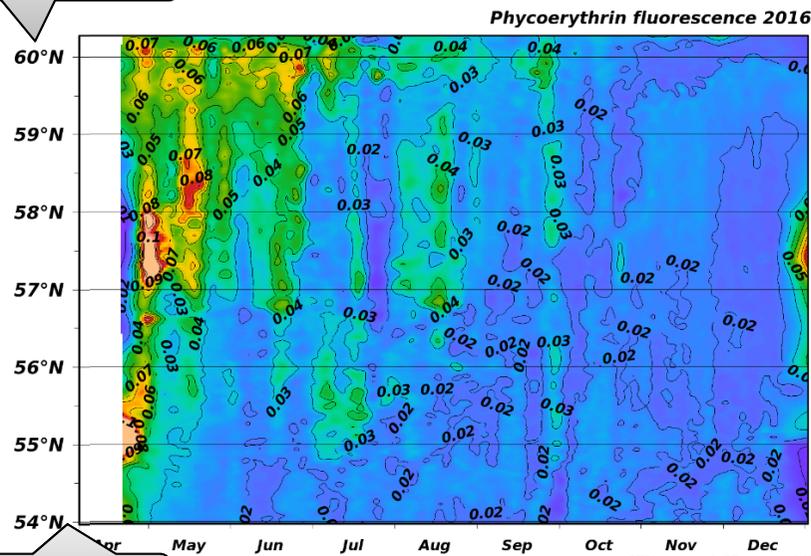
- Instruments onboard Finnmaid in 2016
- Weekly sampling at three stations for additional data
- Clear seasonality and spatial structure



MicroFlu Red; LOQ 0.025

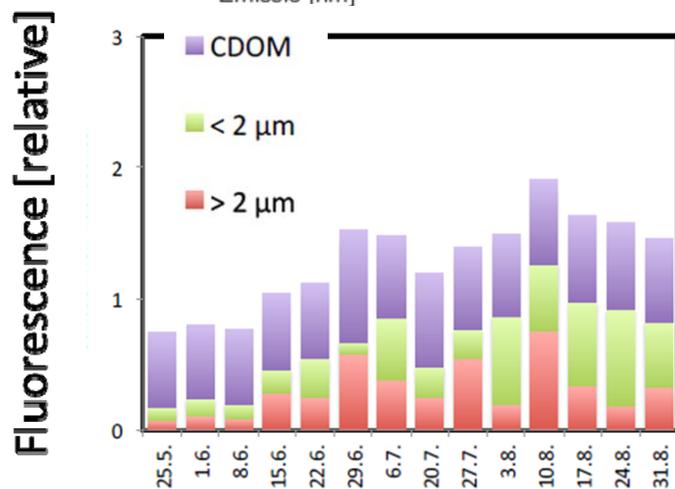
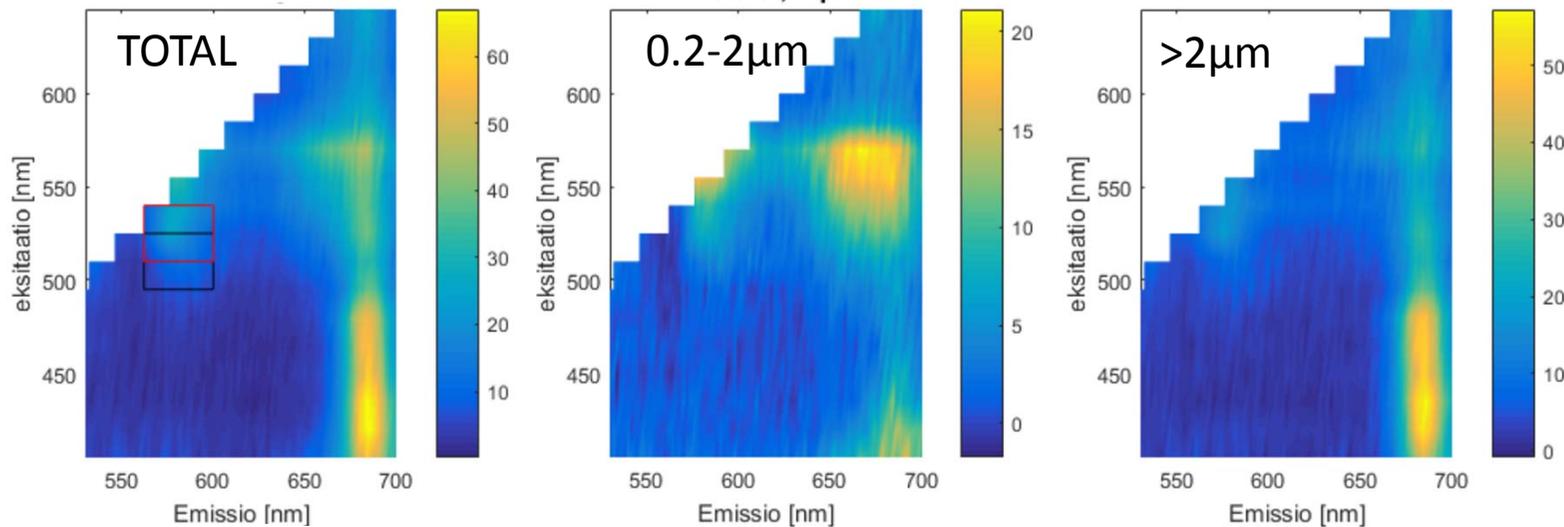
Unilux; LOQ 0.5

Helsinki



Travemunde

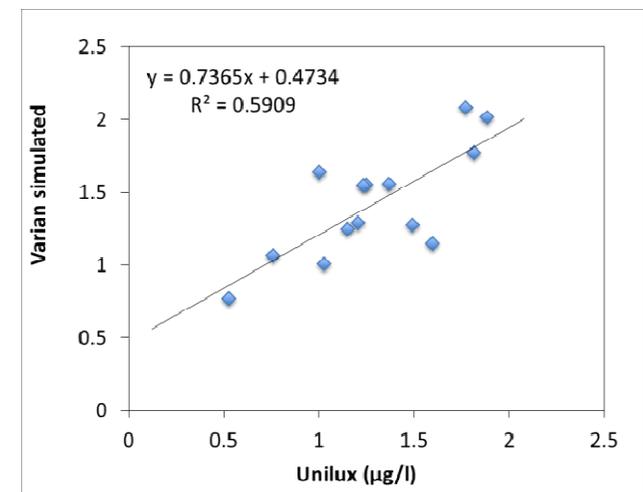
- Discrete samples EEM fluorescence analysed in lab (Varian Cary Eclipse). MicroFlu Red and Unilux results simulated from EEM data. -> not perfect match, time diff. between analyses?



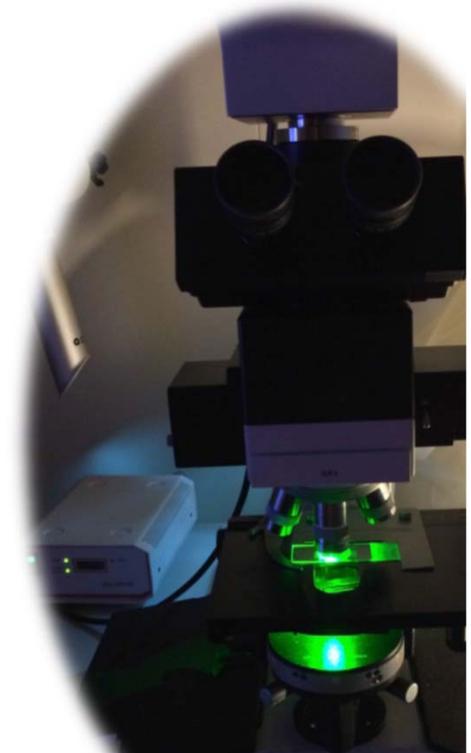
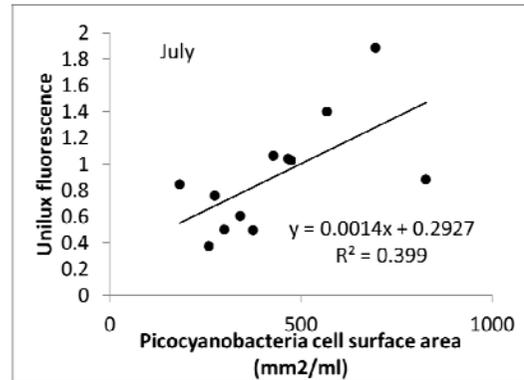
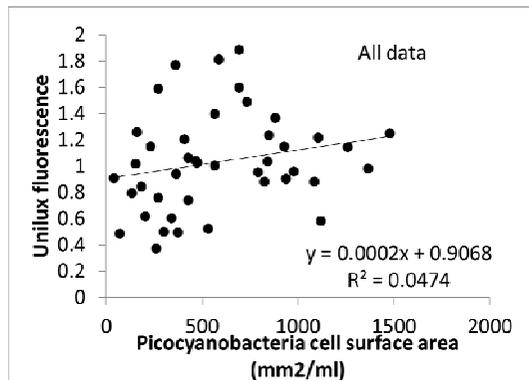
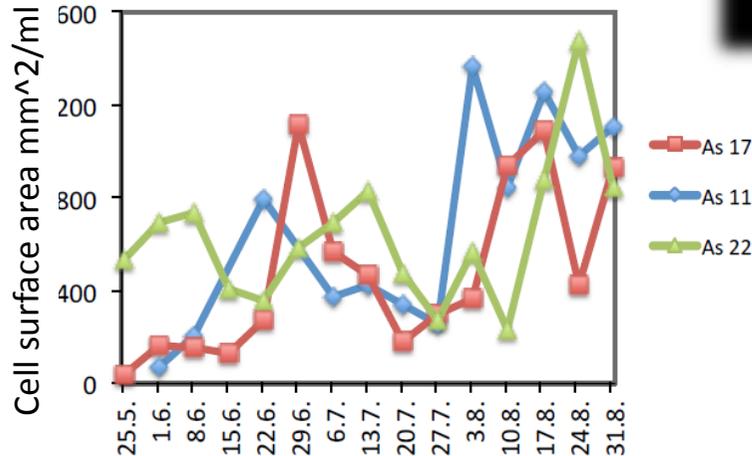
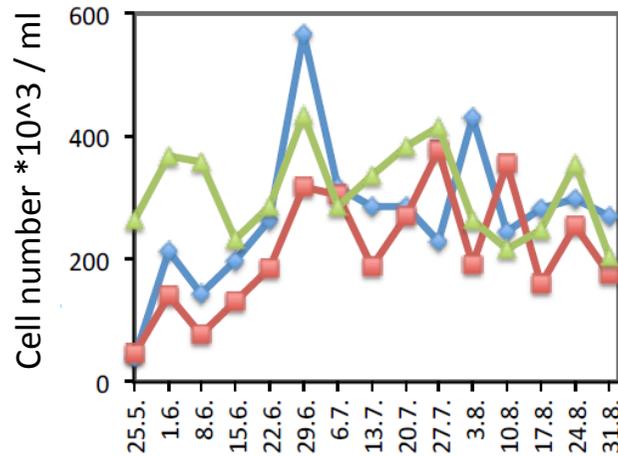
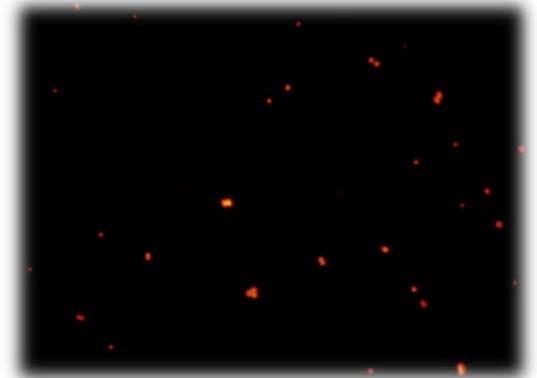
Northernmost station

Early summer:
PE in large species

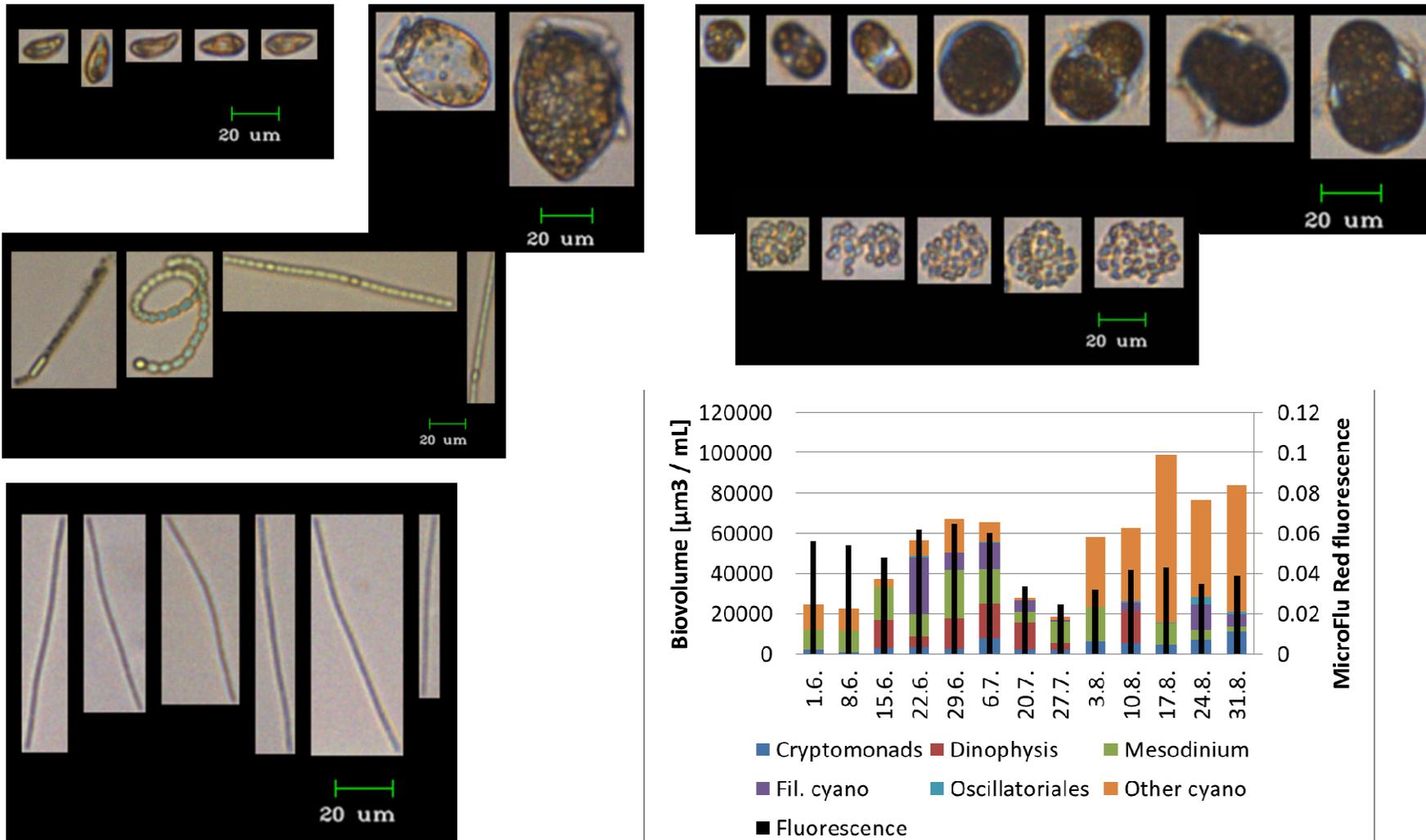
Late summer:
PE in small species



- Counting picocyanobacteria that contain PE, image analysis software to decide between PC and PE containing cells, measure cell abundance and dimensions for each cell (especially surface area).
- No overall correlation with fluorescence, in June relationship evident

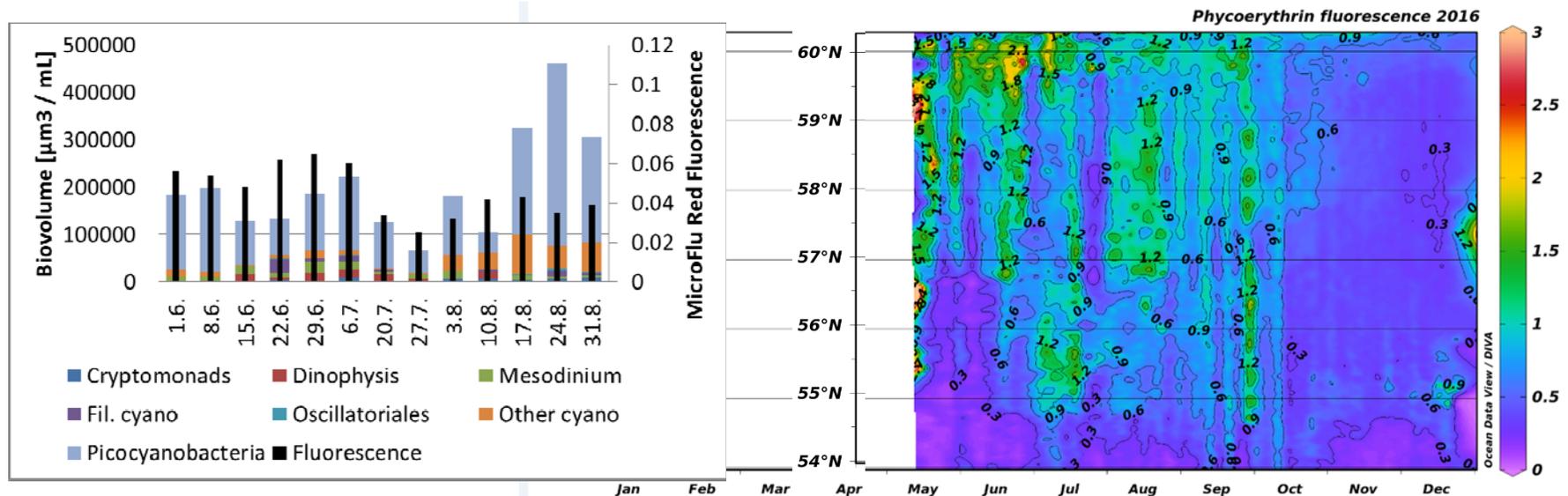


- Imaging PE containing large cells triggered with 532 nm laser and 575 nm emission. Image analysis software to measure dimensions for each cell, manual sorting for abundance. (Also fluorescence per cell available for further analysis)



- Multiple sources of PE fluorescence
 - Early summer with Mesodinium, noted e.g. by characteristic behaviour during filtration
 - Late summer with picocyanobacteria 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 events, which need to be evaluated also by other more specific methods.



For future

- Variability in species specific PE content (in natural conditions)
- Light/nitrogen control of PE (constrains, thresholds, rates)
- Changes in the PE fluorescence yield (Cell physiology, pigment connectivity)
- Technical developments (sensitivity, specificity)
- Analytical methods (extraction, flowcytometry)

Message home:

Picocyanobacteria, thus phycoerythrin, has huge importance for global BGC cycle!

We need combination of methods to understand their abundance and responses.

Thanks!

