MULTIWAVELENGTH FLUORESCENCE STUDIES OF PHYTOPLANKTON IN THE NORTHERN BENGUELA UPWELLING SYSTEM

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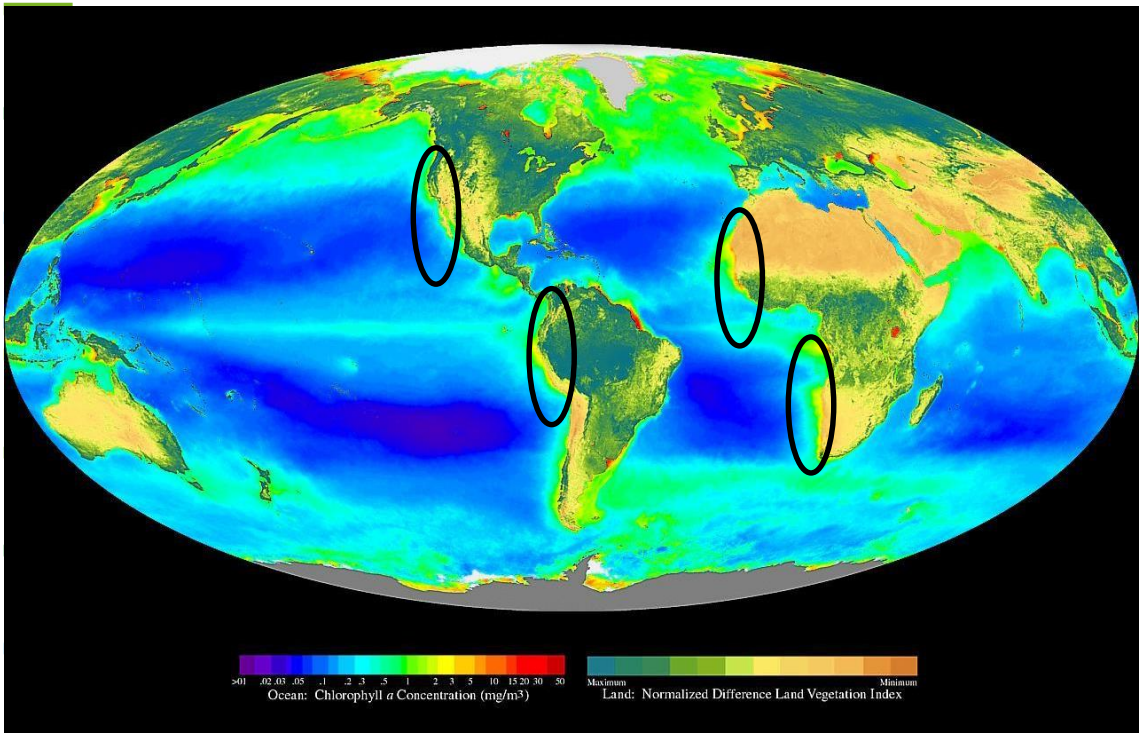
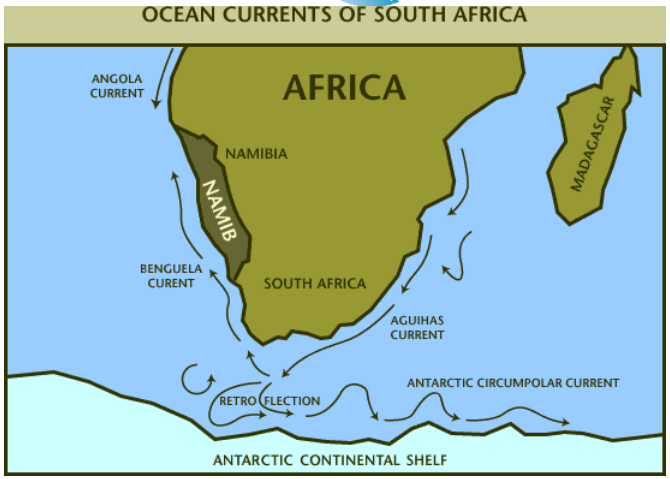
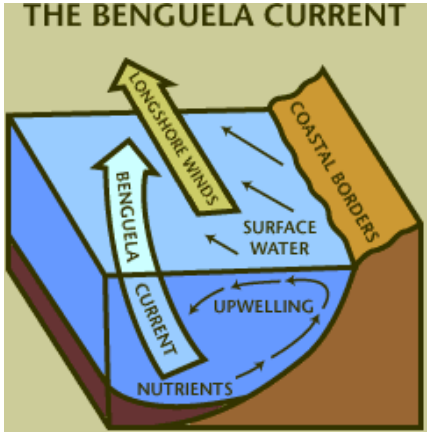
Northern Benguela upwelling system



NASA, SeaWiFS project





www.pbs.org/edens/namib/earth2.htm

Table I: Comparison of various productivity parameters of the four major eastern boundary upwelling systems (after Hutchings 1992)

Parameter	Benguela	Humboldt	California	Canary
Phytoplankton productivity (gC m^{-2} day^{-1})	1–5	3–10	0.1–1.4	1–3
Zooplankton biomass (mℓ 1 000 m^{-3})	200–250	250–1 500	100–600	270–1 060
Maximum fish yield (tons × 10^6)	3	14	1	1–2
Total fish biomass (tons × 10^6)	11	20–30	3–5	3–5

Boyer & Hampton (2001) South African Journal of Marine Science, 23:1, 5-35

Northern Benguela upwelling system

Phytoplankton abundance, taxonomy and productivity not yet well described.

- Some attempts for monitoring program in 1970s
- Since 1999/2000 regular monthly monitoring program along central coast off Namibia (23°S) including chl-a and phytoplankton abundance, + additional transects less frequently

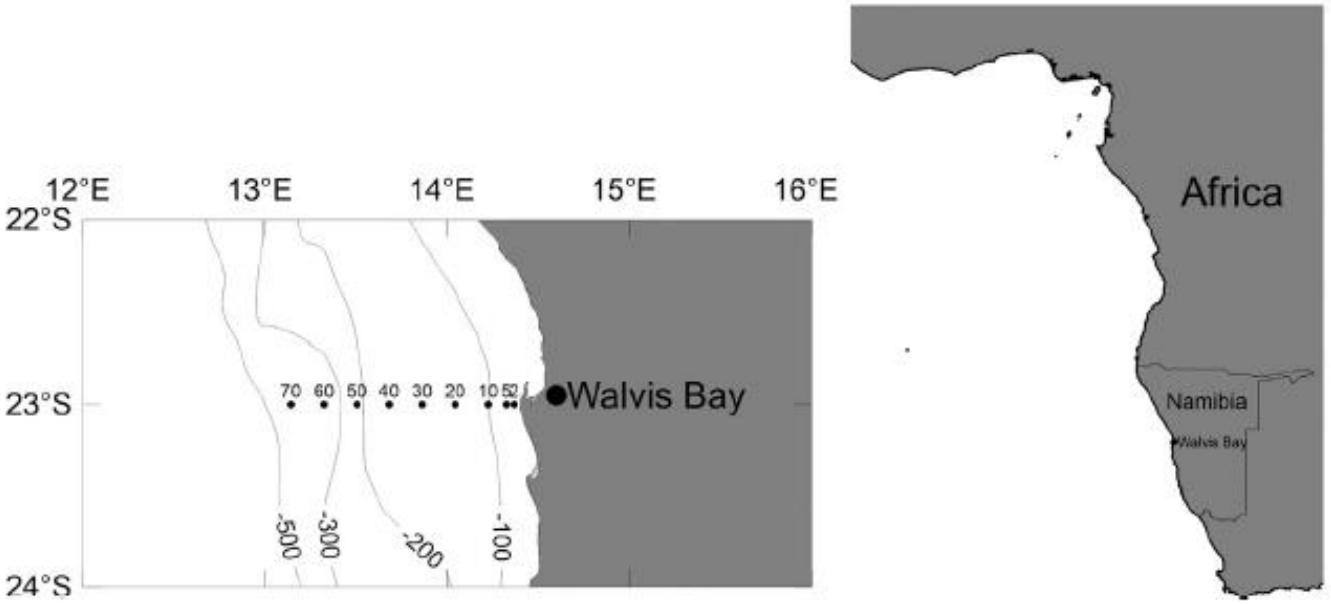


Fig. 1. Map of study area and 23°S transect off the coast of Namibia.

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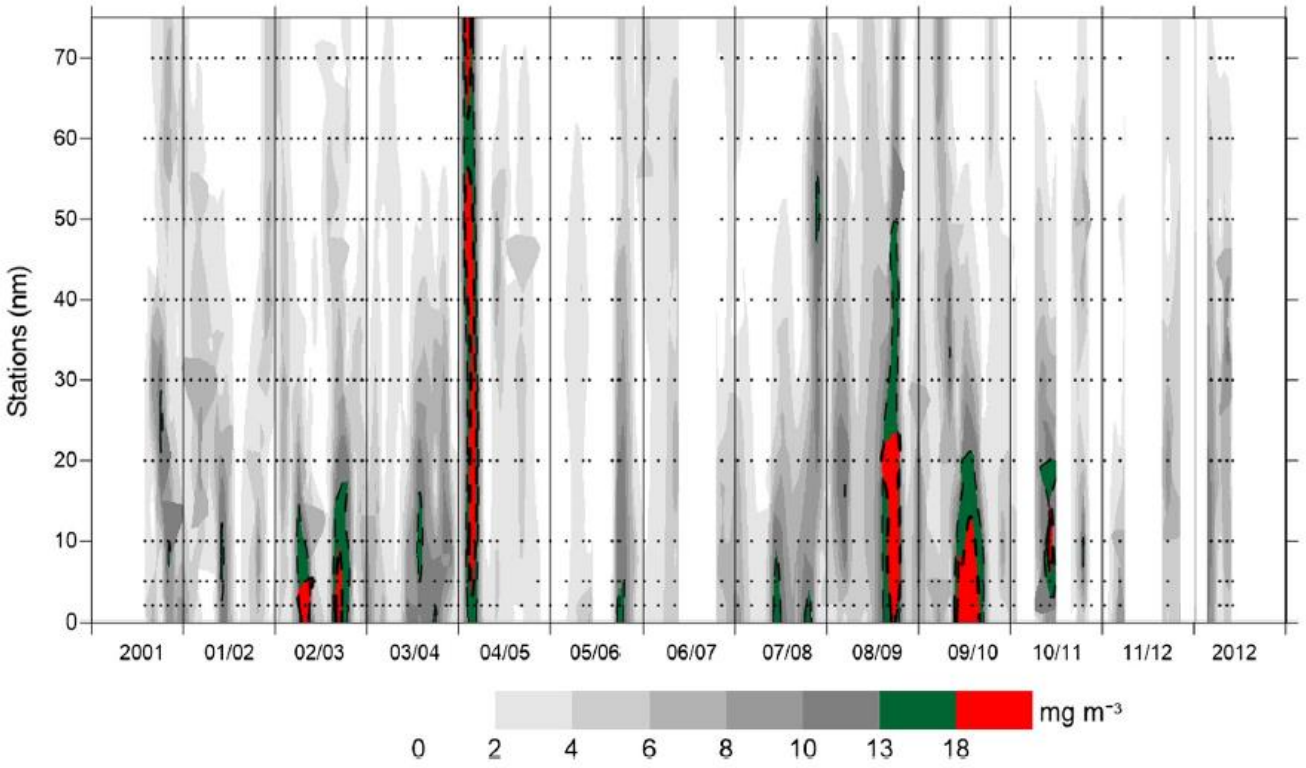

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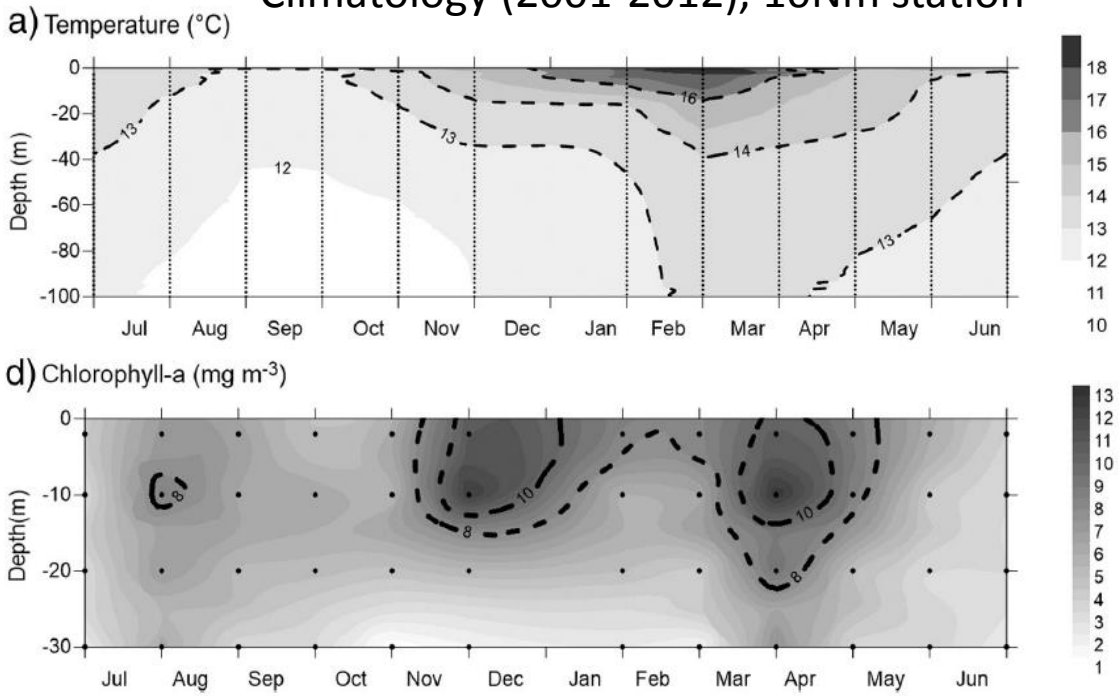
Northern Benguela upwelling system

- Phytoplankton biomass show high seasonal, interannual and spatial variability.
- Seasonal patterns are driven by different forcing mechanisms including wind, temperature, mixing, thermocline, stratification and nutrient availability.
- Challenge for traditional monitoring -> testing new technologies

Climatology (2001-2012), 10Nm station



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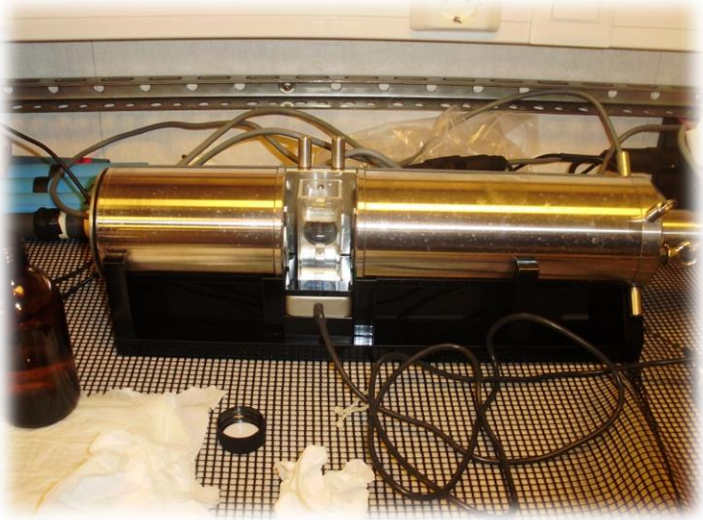
Background

- 2011, Ministry of Fisheries and Marine Resources of Namibia decided to commission a new fisheries and marine research vessel from the STX Finland shipyard in Rauma.
- 2012, delivery of new research vessel R/V Mirabilis
- 2012-2015, project MARINAM (Marine research capacity development in Namibia): with a subtask for data collection and equipment training
- 2014, Purchase of multiwavelength fluorometer FluoroProbe (bbe Moldaenke) for phytoplankton studies
- 2014-15, Two cruises with aim to define best practices for instrument use and collect data supporting site specific calibration

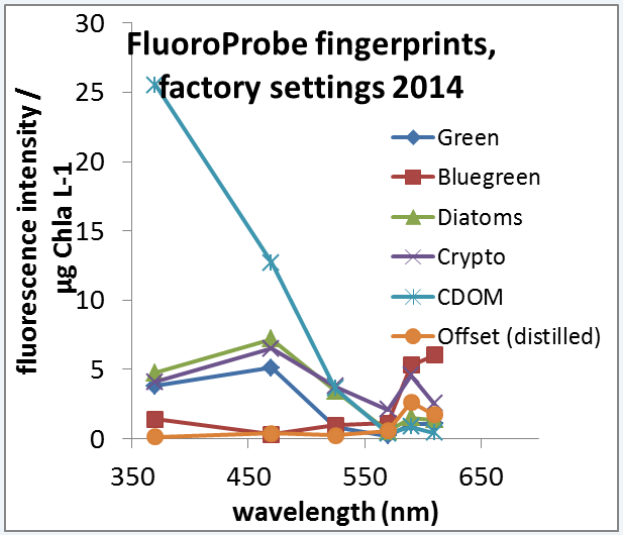


Multiwavelength fluorometry

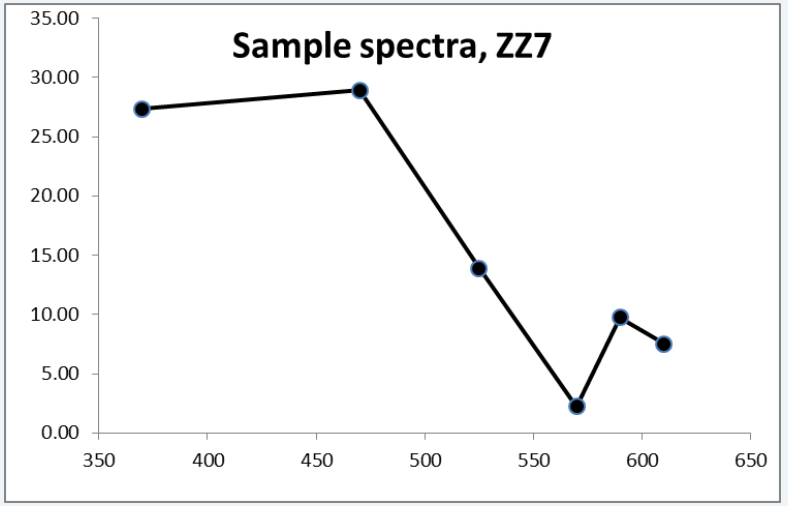
- Taxonomically different phytoplankton groups have different accessory pigments (chlorophylls, carotenoids, phycobilins), and thus differences in fluorescence spectra (fingerprints).
- These fingerprints are determined using phytoplankton cultures representing each taxonomic group (factory calibration).
- Typically, for given study area, 3-5 pigment groups can be identified
  - Green algae, brown algae (Diatoms + Dinoflagellates), Cryptophytes, Bluegreens with different colors
- Coloured dissolved organic matter (CDOM) need to be included as separate group.
- FluoroProbe has 6 wavelengths, thus mathematically it is possible to solve max. 6 compounds.
- Key problem is that obtained fingerprints are not stable, but vary between species and physiological conditions. Thus factory-build (or any) fingerprints are not fully representative for diverse phytoplankton communities we have in nature.

Example of FluoroProbe results

Fingerprints (k_i)

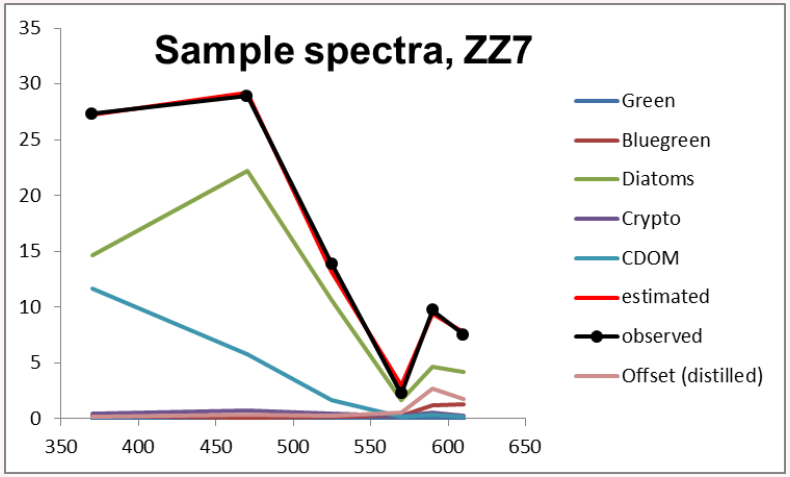


Spectra measured (SFS)



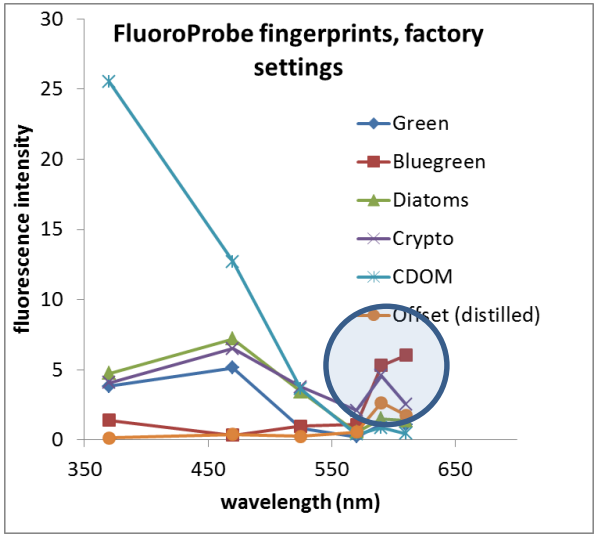
$$SFS(\lambda) = \sum_{i=1}^{n} c_i k_i(\lambda)$$

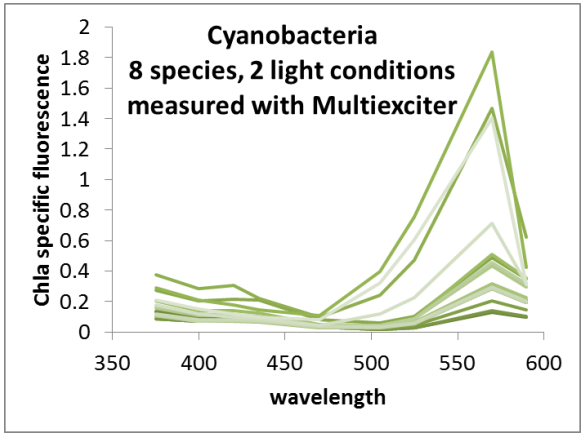
	µg /L
Green	0.00
Bluegreen	0.22
Diatoms	3.08
Crypto	0.12
CDOM	0.46

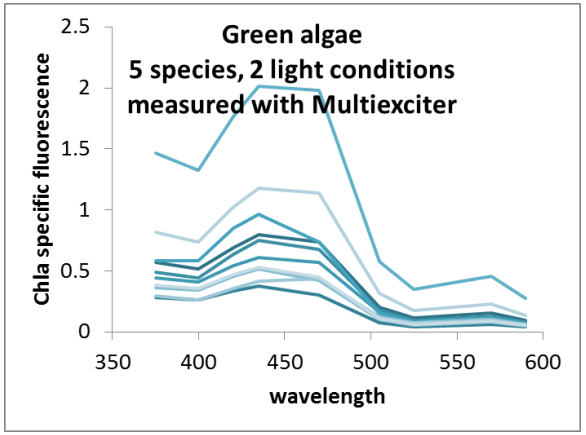


Known issues

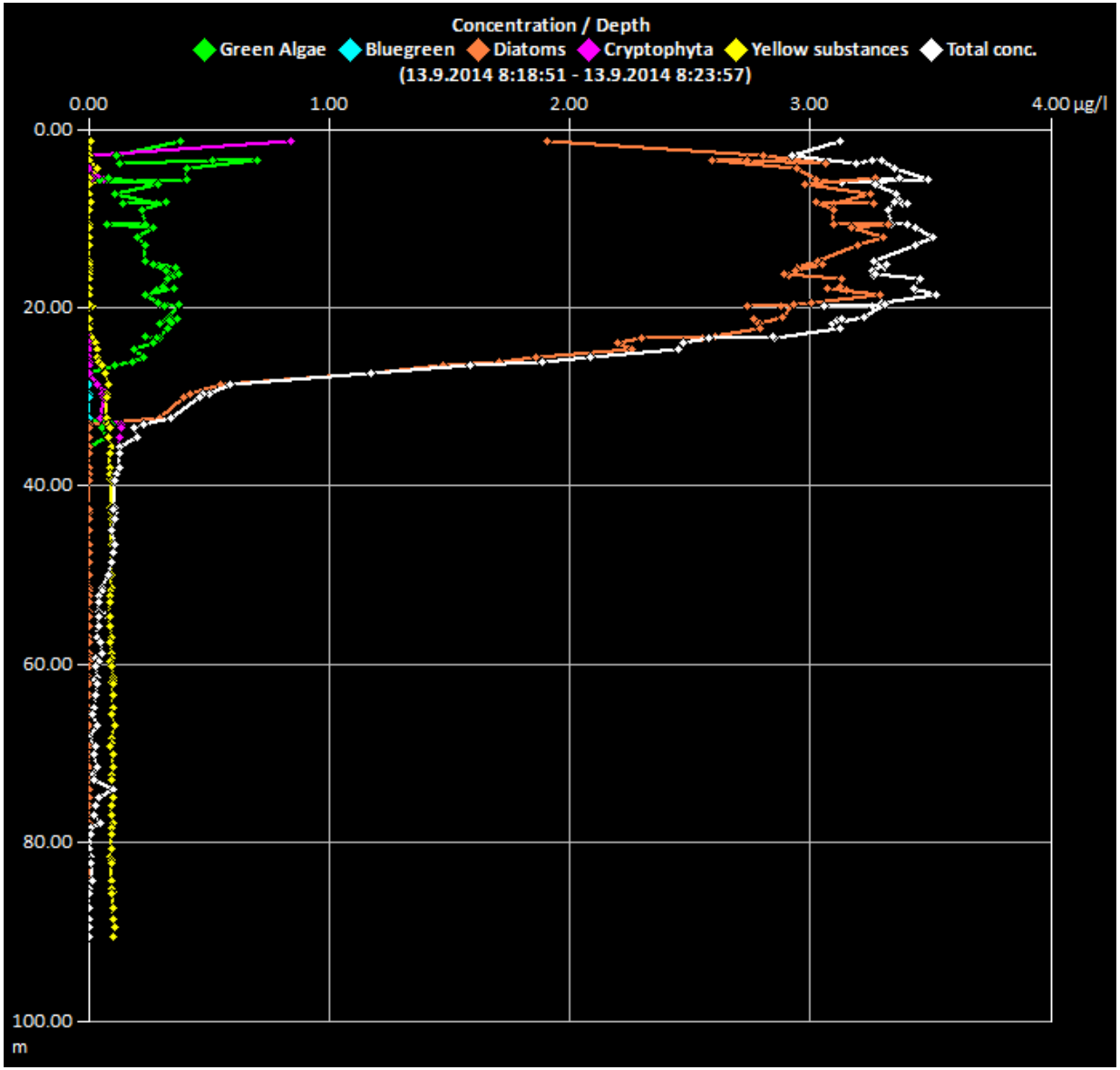
- Wrong fingerprints included (e.g. cyanobacteria in our case)
- Correct fingerprints not available (e.g. oceanic Synechococcus cyanobacteria).
- Some fingerprints too identical (e.g. diatoms vs. dinoflagellates).
- Chlorophyll concentration estimation relies on fingerprint spectra, while in reality chlorophyll-specific fluorescence is highly variable.







Example of FluoroProbe results



- Fluorescence profile to assist sampling
- Change in spectra indicate change in community structure
- Taxonomic information and concentrations need to be treated with care

Study aims

CHLA

- Test various algorithms for predicting Chla content using FluoroProbe data

PHYTOPLANKTON COMMUNITY STRUCTURE

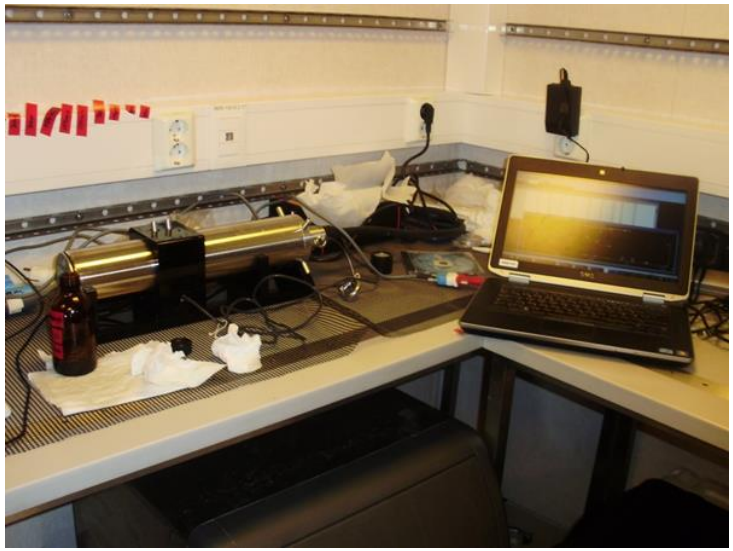
- FluoroProbe results vs. cell counts by microscopy/FlowCAM and vs. HPLC
- Analyse fluorescence characteristics of different size fractions (<2µm and <10µm) -> Do they show a specific spectral shape, which may be used as a spectral group in the FluoroProbe software (e.g. picocyanobacteria)
- statistical multivariate analyses of FluoroProbe data ->which are the major spectral components in the study area.

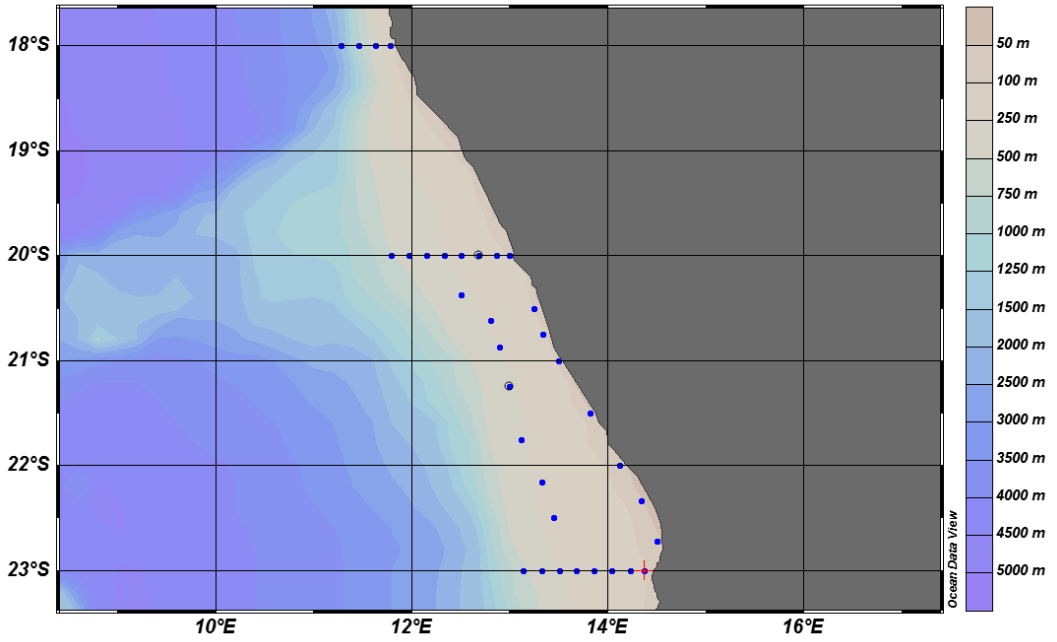
CDOM

- CDOM as measured with FluoroProbe vs. traditional CDOM analyses

Data collection

- Fluoroprobe profiles
- Chlorophyll samples at 0, 10, 20, 30,(+) m
- FlowCAM, microscopy, HPLC samples
- Size-fractions: <2µm, <10µm, total
  - Fluoroprobe in lab-mode
  - Chlorophyll a
- Size-fraction: <0.2µm (CDOM)
  - Fluoroprobe in lab-mode
  - Absorption and fluorescence analysis
- 2014: 34 stations
- 2015: 20 stations





Results

Chla estimation with FluoroProbe

The default Chla concentration is estimated as:

$SFS = C_g*S_g + C_{bg}*S_{bg} + C_d*S_d + C_c*S_c + C_y*S_y + offset + E$

$[Chla] = C_g + C_{bg} + C_d + C_c$

where

SFS = sample spectra at 6 wavelengths

[Chla]= total chla concentration of sample

C=concentration of algal class (µg Chla L-1)

S=spectral signal at 6 wavelengths

g=green algae

bg=blue green algae

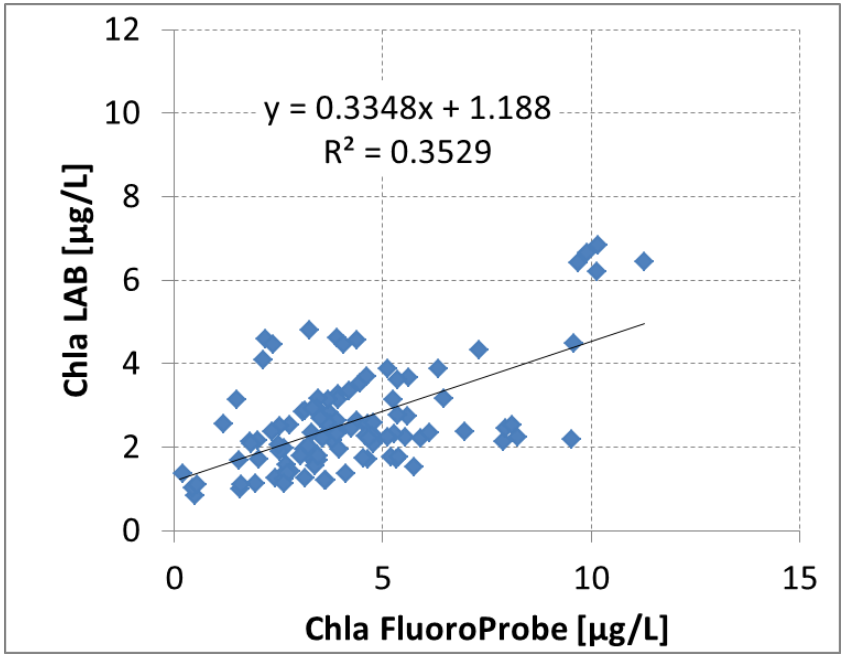
d=diatoms

c=cryptomonads

y=yellow substances

offset = offset due to distilled water

E=error spectra at 6 wavelengths to be minimized



Results

Chla estimation with FluoroProbe

Alternative 1. Regression between Chla and 470 nm fluorescence

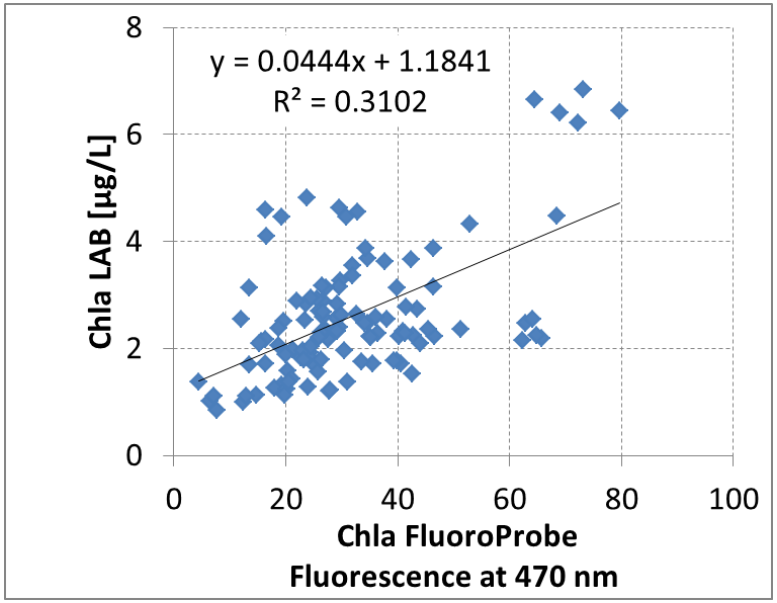
Alternative 2. Multiple regression

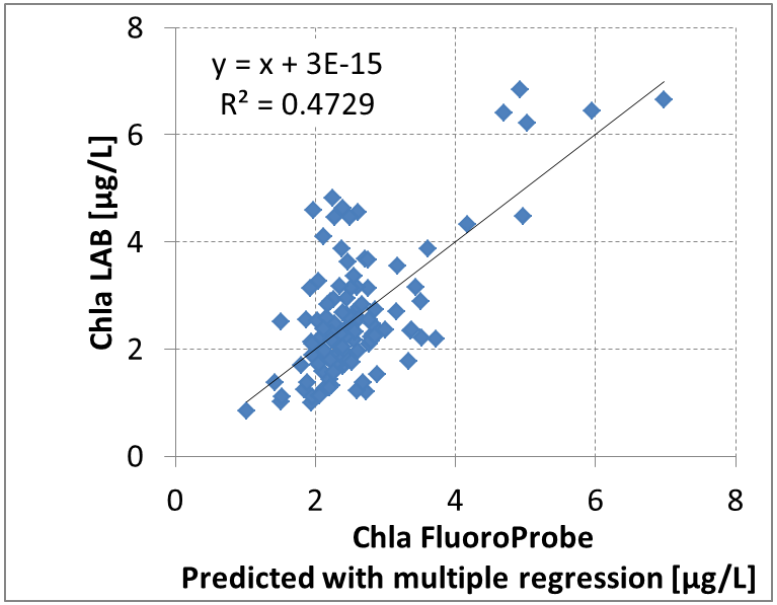
$[Chla] = b_1*S_{370} + b_2*S_{470} + b_3*S_{525} + b_4*S_{570} + b_5*S_{590} + b_6*S_{610} + b_7$

where

[Chla]= total chla concentration of sample

$B_{1\text{-}7}$ = regression coeffiecients

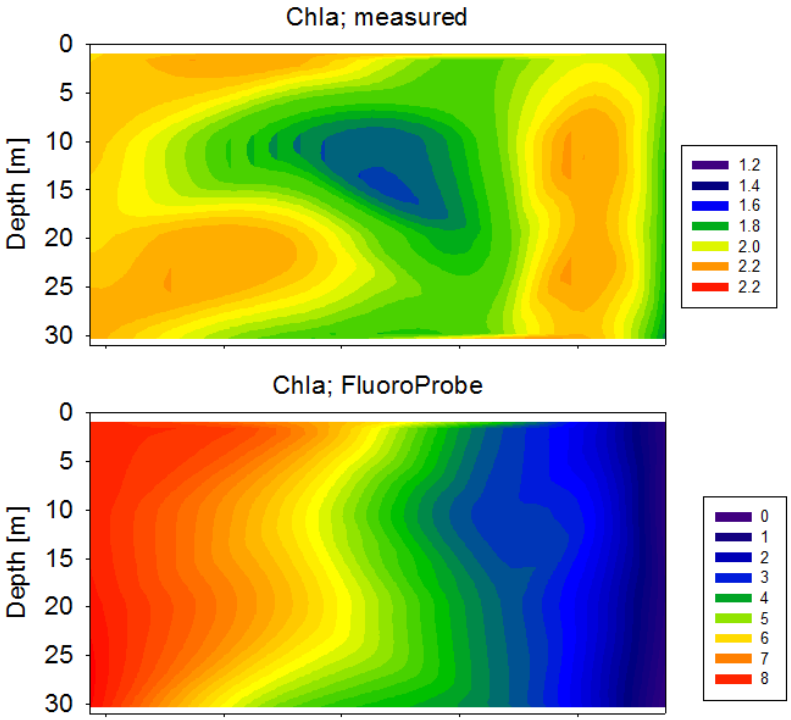


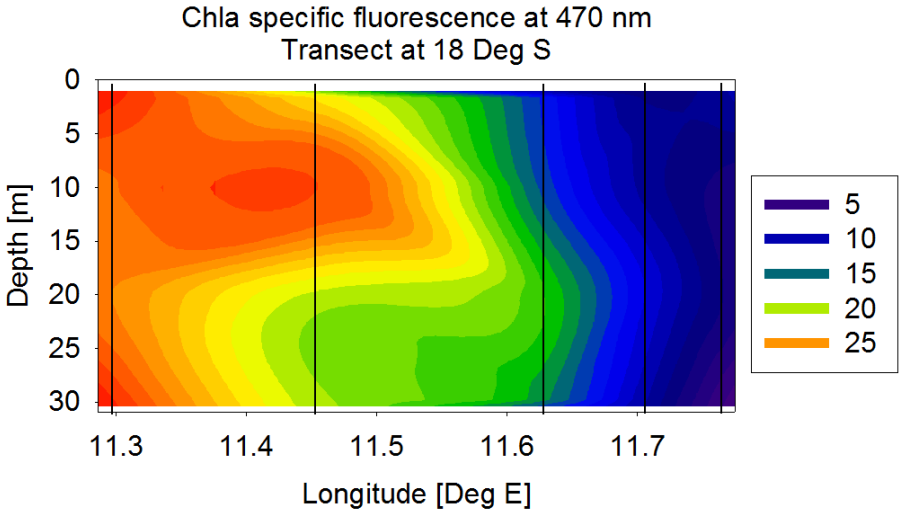


Results

Chla estimation with FluoroProbe

Alternative 3. Whatever regression model used, spatial/temporal variations in chla fluorescence vs. concentration need to be included (include light/depth/time in regression or use autocorrelation!)





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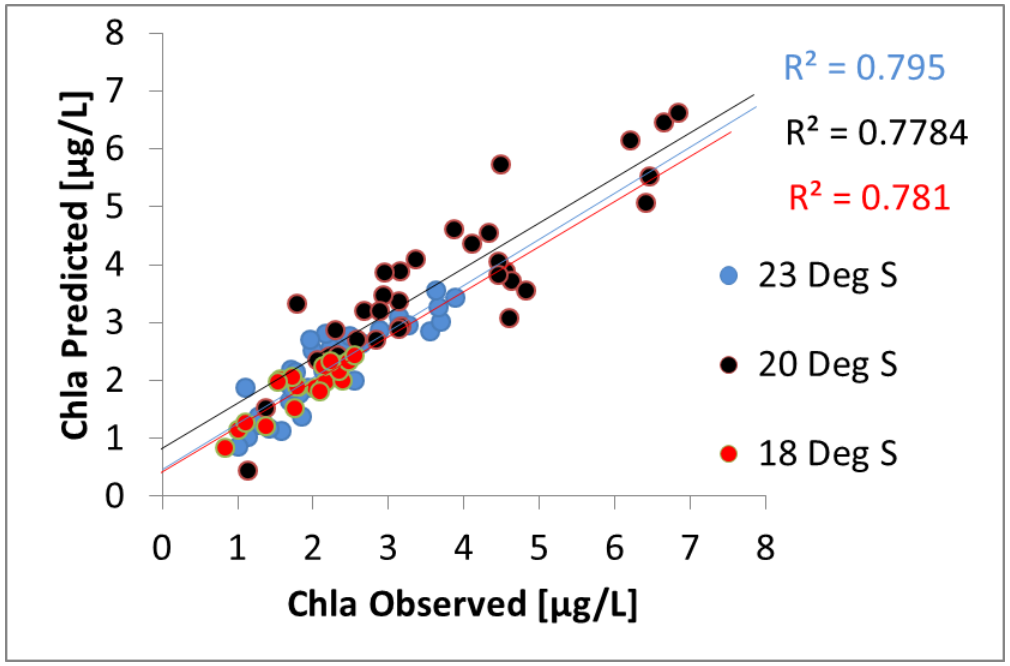
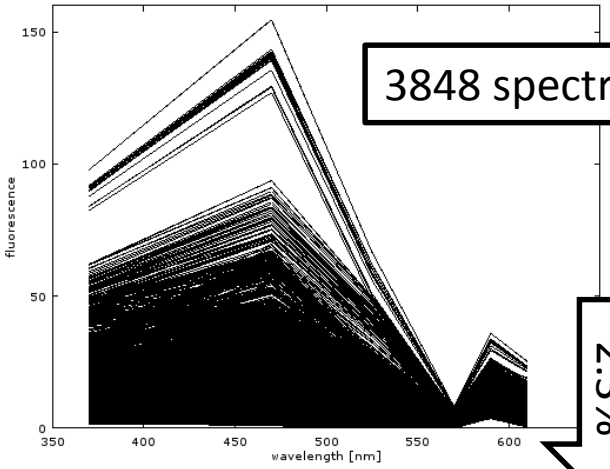


Predicted Chla calculated using multiple regression including fluorescence data, depth, time of day, distance from shore.
Separate regression for each transect
Need to be studied further (nonlinearities, residuals)

Results

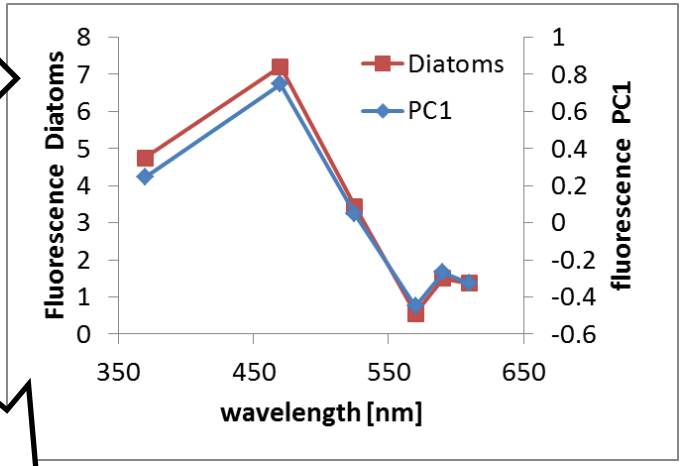
Taxonomic information with FluoroProbe

Principal components analysis to find out major spectral components.
Three components (PC1, PC2 and PC3) contribute 99.9 % of the total spectral variability.

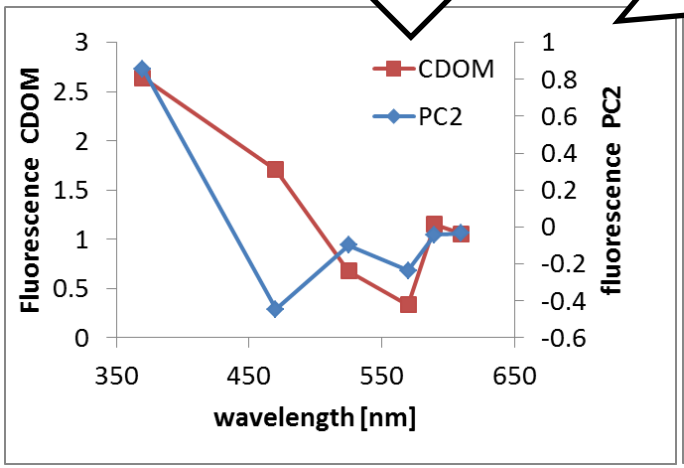


3848 spectra

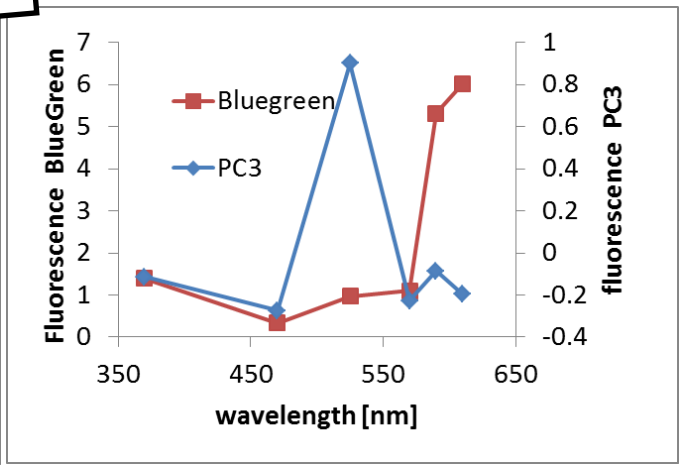
97.1%



2.3%



0.5%



Results

Taxonomic information with FluoroProbe

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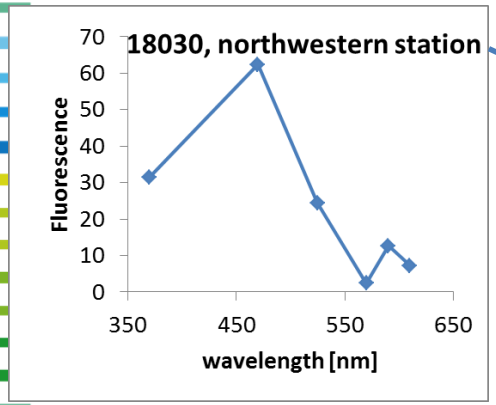
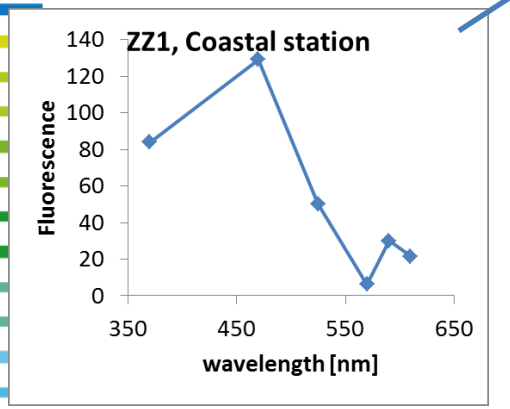
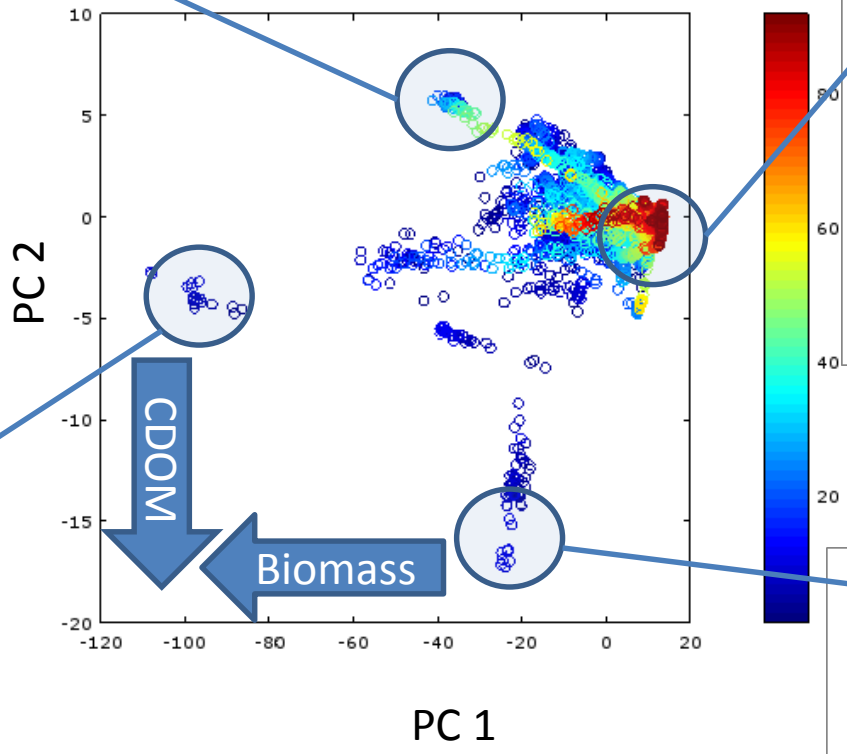
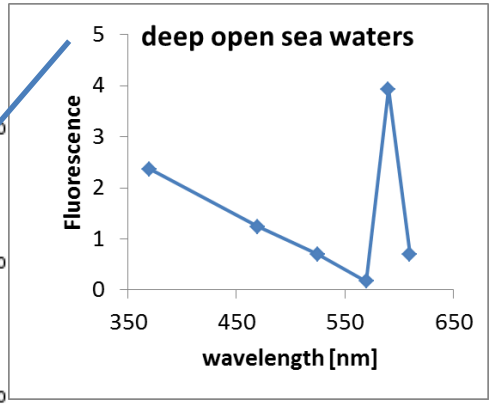
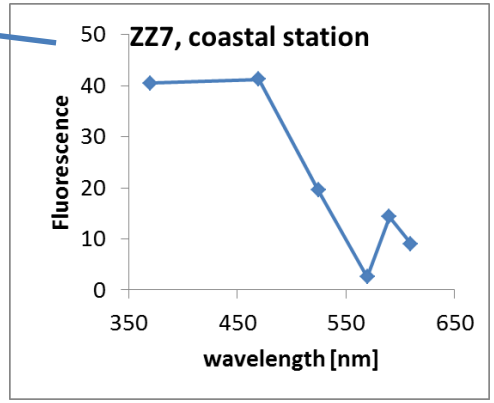








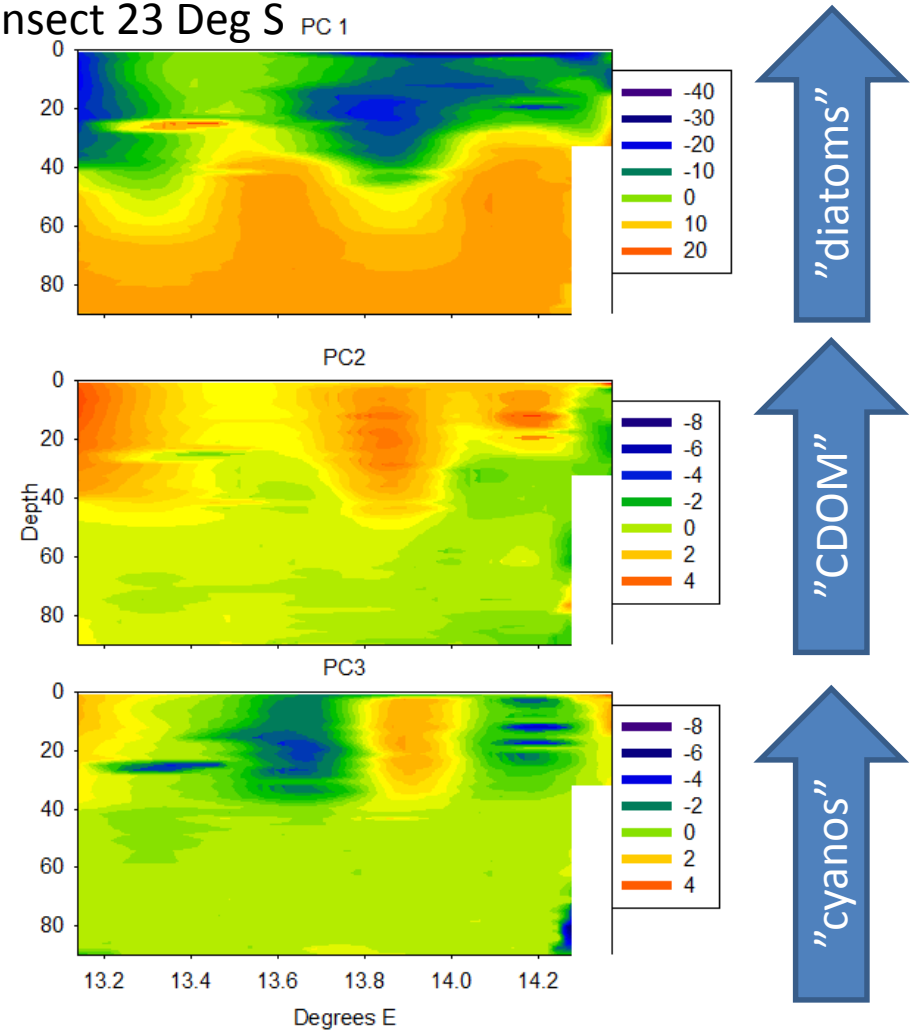


Results

Taxonomic information with FluoroProbe

Principal components analysis to find out major spectral components.

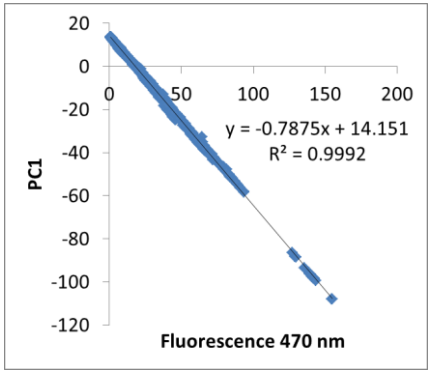
Transect 23 Deg S

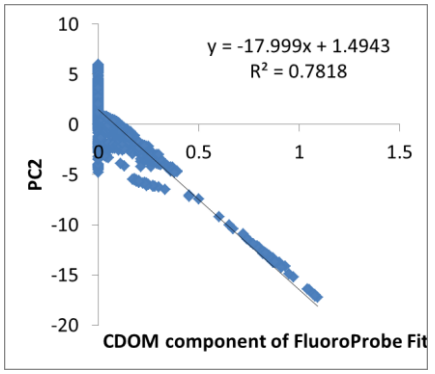


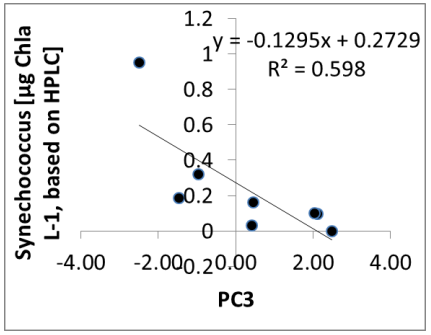
"diatoms"

"CDOM"

"cyanos"



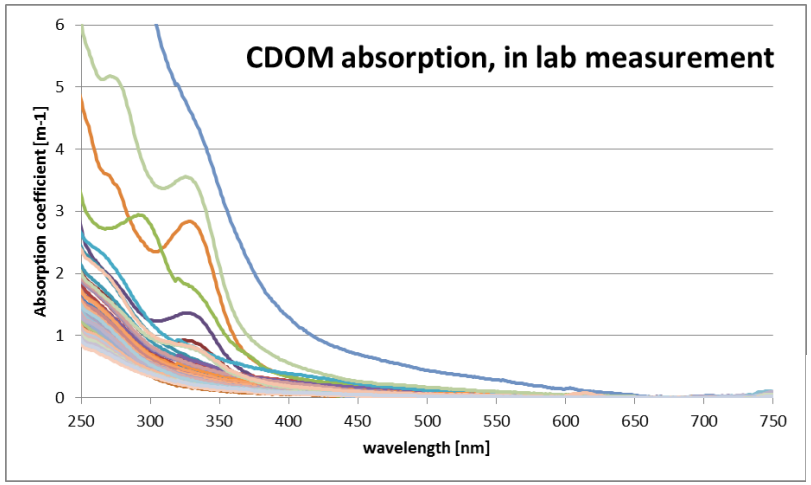




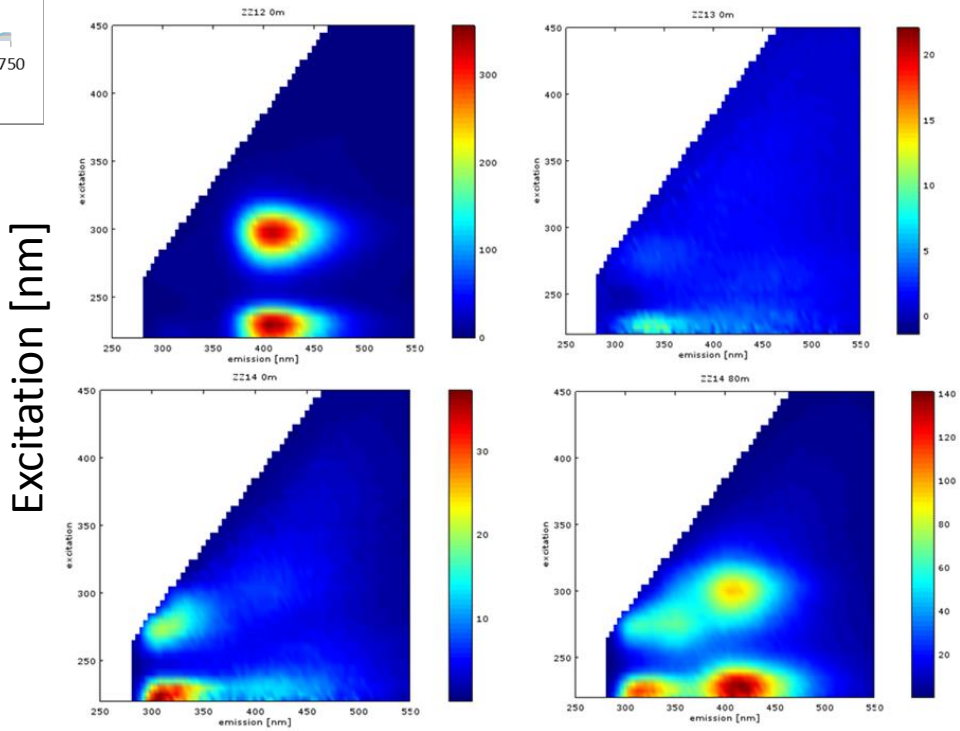
HPLC data extracted from Barlow, Louw, et al

Results

CDOM as measured with FluoroProbe vs. traditional CDOM analyses



Range a(440) 0.013-0.770 m^{-1}



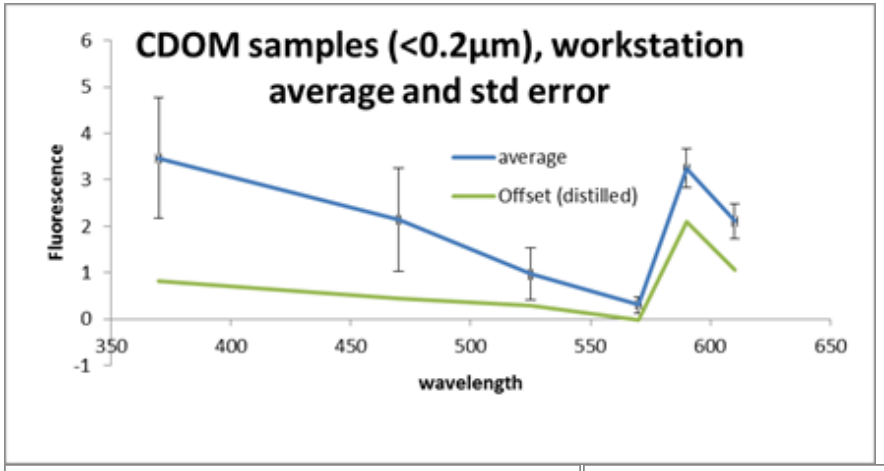
Excitation emission fluorescence spectra for stations ZZ12 (0m), ZZ13 (0m), ZZ14 (0m) and ZZ14 (80m); showing the huge variability in fluorescence compounds.

Excitation [nm]

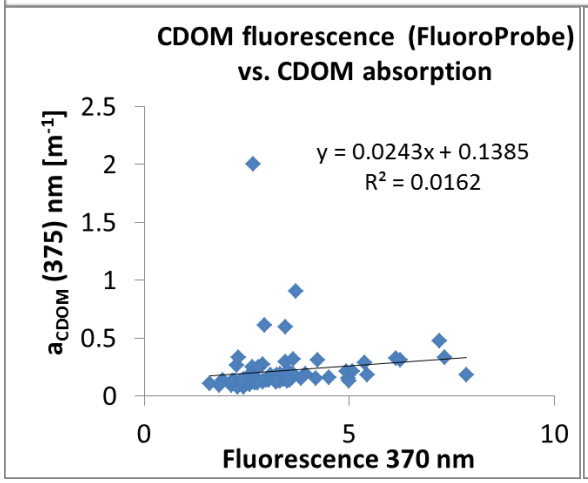
Emission [nm]

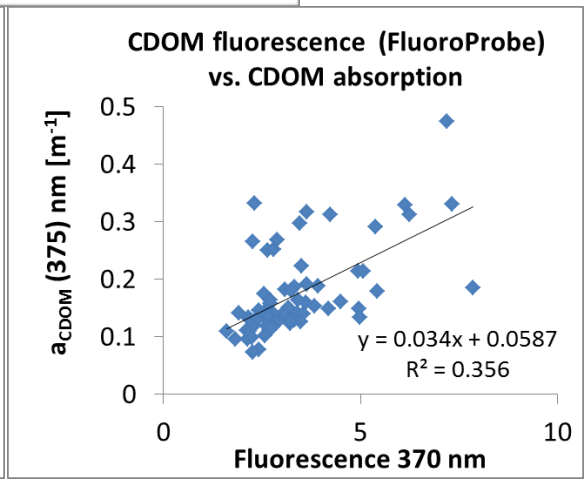
Results

CDOM as measured with FluoroProbe vs. traditional CDOM analyses



Fluorescence spectra of CDOM (<0.2µm) as measured with FluoroProbe





Left: relationship between CDOM fluorescence (measured with FluoroProbe using <0.2µm samples) and CDOM absorption.

Right: the same, but outliers with high absorption removed

Conclusions and perspectives

Field data

- Multiwavelength fluorescence is a tool to observe variability in phytoplankton distribution, information for Chla or taxonomy need to be treated carefully
- Taxonomic variability (Optical one!!) in Benguela upweling system was very moderate during study.
- Current data does not allow diatom/dinoflagellate distinction, need to wait for FlowCAM HPLC data.
- Cyanobacteria distribution need to be studied more carefully (samples for microscopy, HPLC). FluoroProbe need to be checked with appropriate cultures.
- Chlorophyll levels can be predicted, but not using factory build or simple regression algorithms. No single solution available, need to build up a decision tree type of approach?
- CDOM levels at the low range of the FluoroProbes detection capability.

Issues with calibration:

- There is no optical characterization of instrument (no traceability).
- Instrument send to service -> new factory settings -> not possible to compare spectral readings before/after service (unless one trust that the ”factory cultures” have constant fluorescence spectra, which they cannot)
- Need to bring calibration issue up in Jerico NEXT and start discussions with manufacturers (WP2/WP3).

Ferrybox part of the story …

Thank you

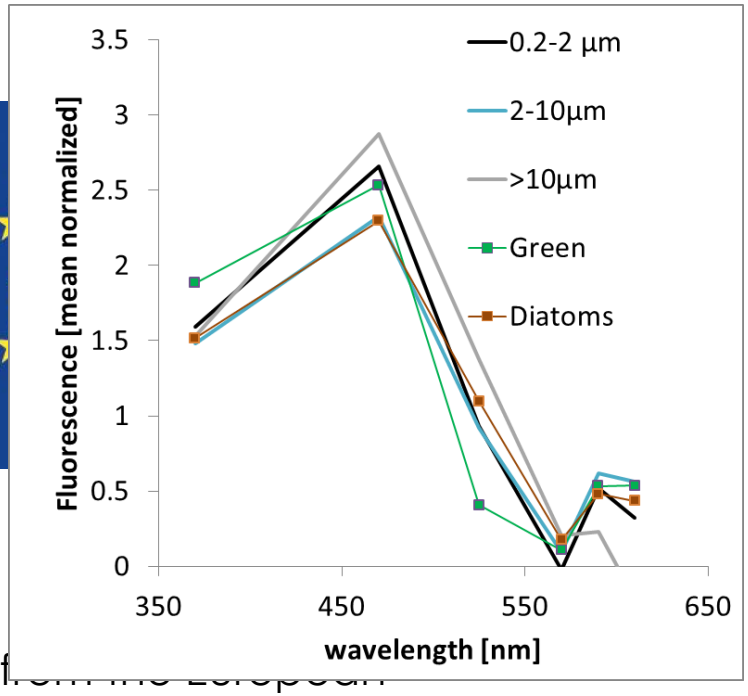
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 654410.

Results

Taxonomic information with FluoroProbe

In the Benguela upwelling system diatoms and dinoflagellates dominate (hard to resolve spectrally), while amount of picocyanobacteria is unknown (but 4-74% of Chla was found in <2µm fraction!).

Between size-classes hardly any consistent difference in spectra (n=71-74).



Very high correlation between wavelengths!

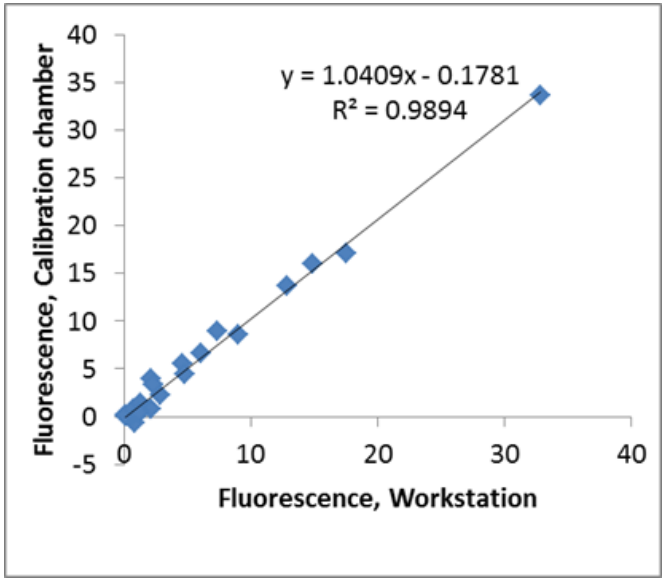
This project has received funding from the European ... programme

	LED 6 [370 nm]	LED 8 [470 nm]	LED 3 [525 nm]	LED 4 [570 nm]	LED 7 [590 nm]	LED 5 [610 nm]
LED 6 [370 nm]	1					
LED 8 [470 nm]	0.97	1				
LED 3 [525 nm]	0.98	0.98	1			
LED 4 [570 nm]	0.95	0.94	0.97	1		
LED 7 [590 nm]	0.99	0.97	0.99	0.97	1	
LED 5 [610 nm]	0.99	0.96	0.98	0.96	0.99	1

Results

Comparing data collected in calibration chamber and in workstation

Aim: to allow quantitative comparison of data collected in different operational modes



Measurements were linearly related and showed a slope close to 1 and intercept close to 0.