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Phytoplankton in coastal waters of the English Channel :

Spectral Fluorometric characterization and comparison with Cytometry

Kiel, june 2014

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Introduction

Our general research topic :

To better understand :

1/ Phytoplankton physiology variability

in a high hydrodynamic system

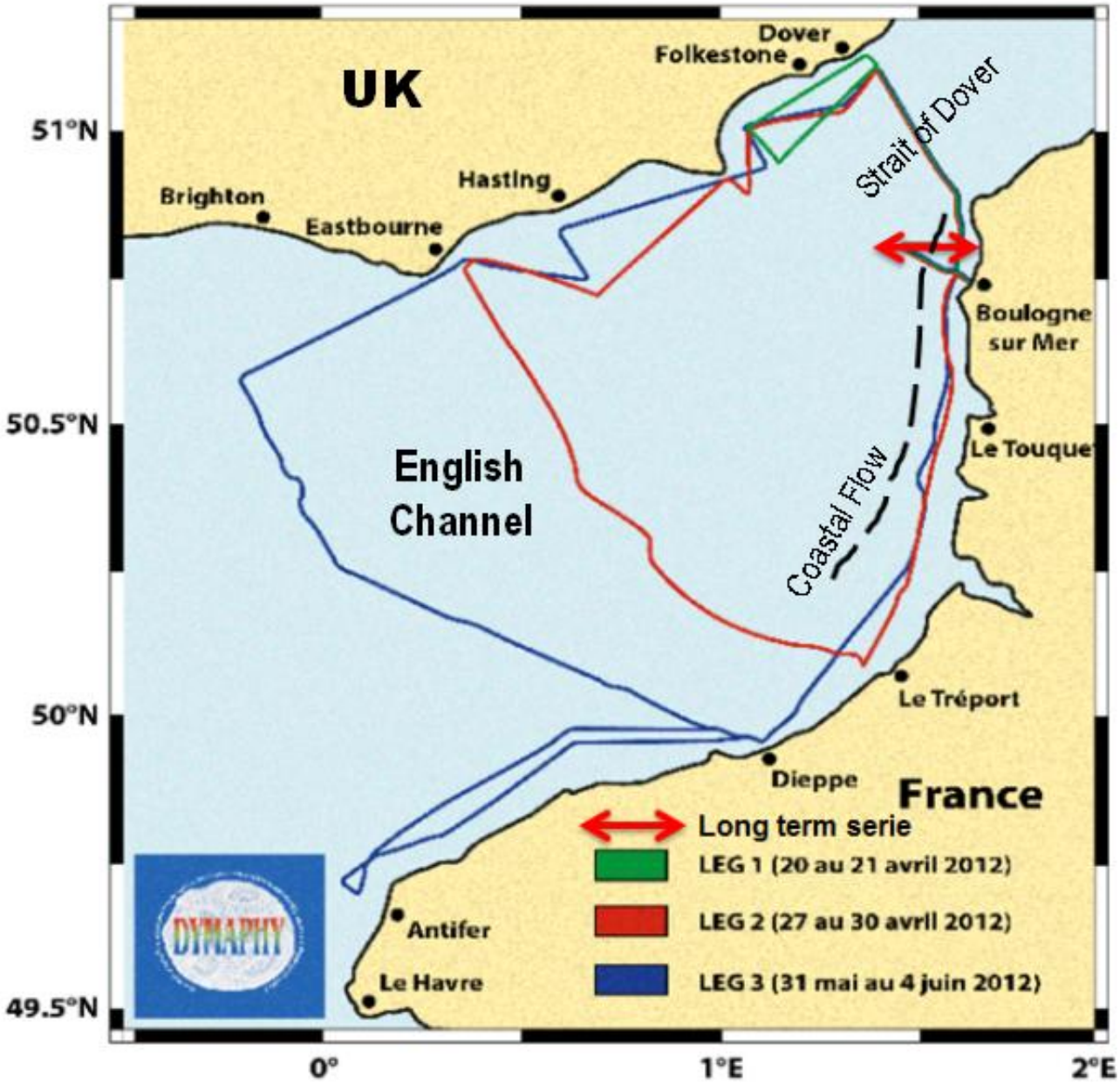
2/ Biodiversity & phytoplankton group distribution, shift

- At seasonal and long-term time scale

- Across environmental gradients at micro-scale

- In the water column

Introduction



Introduction

Specific goal in Dymaphy project :

- to characterize a local invasive species, Phaeocystis, with an alternative technique

- to study the possibility to monitor phytoplankton with spectral fluorometry at seasonal … scale

- to compare predictions of algae group composition by spectral fluorometry to those from cytometry

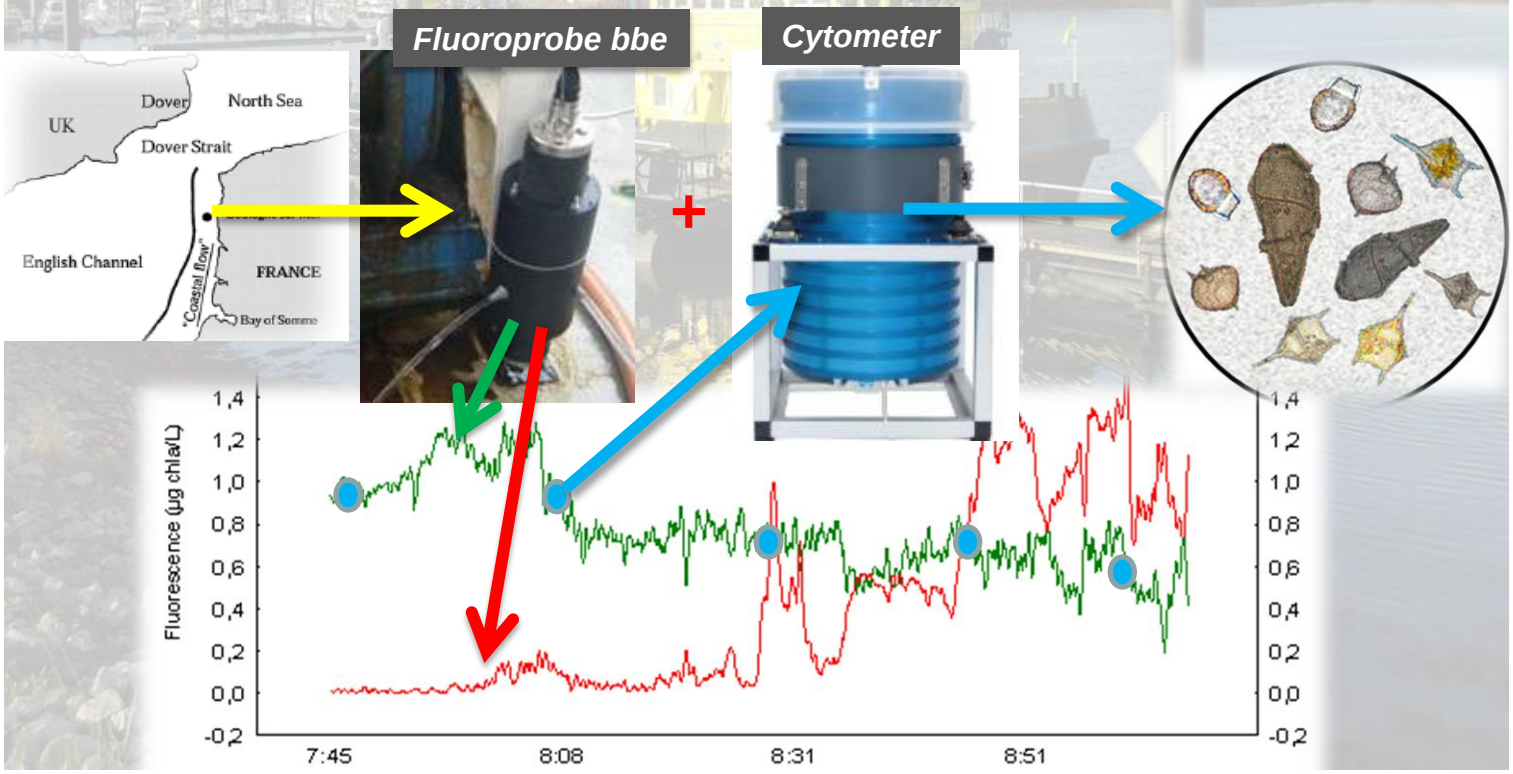
- in order to use a coupled approach for phytoplankton monitoring in coastal ecosystems !

NB : not to assess the performances of the used technology

More precisely :

The ultimate goal could be for phytoplancton monitoring :

- to conduct automatic and **coupled sampling**
- with spectral fluorescence at high frequency (±1,5 sec) → *global info on biomass by spectral groups*
- and with cytometer at medium frequency → accurate determination of phytoplancton classes or species



Plan

1/ Materials : Fluorometer and Cytometer

2/ Fingerprints, Classification tests on cultures and Haptophyte characterization

3/ Time / space variabilility :

- high scale : season, English Channel

- small scale : across environmental gradients

4/ Comparative study of Spectral Fluo. and Cytometry on natural samples :

-The Zeeland case Study

-The English Channel case study

5/ Conclusion & prospects

1
Materials

1/ Materials: Spectral fluorometry

➔ We use mainly the **FluoroProbe n° 16-16** (a submersible instrument), recently the **FLP n° 22-15**, and the **AOA** from the IFREMER Ferry Box or AEAP (north France fresh water agency)

➔ **For a detailed** description of the technique, see :

- *Beutler et al., Photosynth. Res., 2002 …*
- *MacIntyre et al., in « Chla fluorescence in aquatic sciences: methods and application »; Suggett et al. Eds, Springer, 2010*

➔ **The 4 basic spectral classes are not interesting in all systems, as the English Channnel** :

- ✓ ~~*« green » algae (Chlorophyta)*~~
- ✓ *« blue-green »algae (Cyanobacteria with phycocyanin)*
- ✓ *« brown » algae (Chromophyta, Dinophyta)*
- ✓ *« mixed » (Chryptophyta & and other algae with phycoerithrin)*

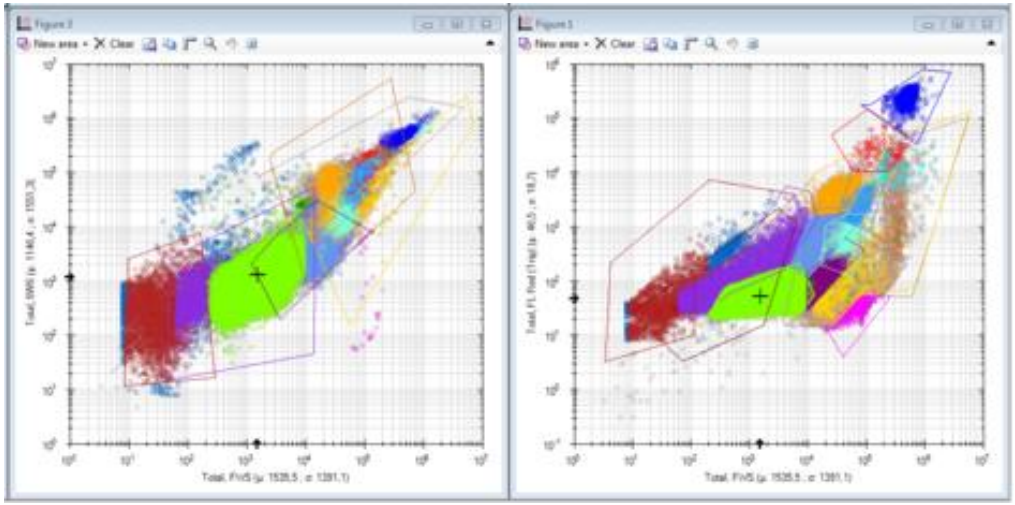
Replace with a local interesting algae group ?

1
Materials

Cytometer

➔ We use the The portable CytoSense **benchtop flow cytometer**, designed for use in the lab, on a shipboard, or anywhere else :

⇒ Data provided in this work come from the CEFAS CytoSense (Creach) and the ULCO CytoSense (Artigas, Bonato Thyssen), with CytoClus software



2
Fingerprints, Classification, Haptophyte

2/ Fingerprints, classification tests and Haptophyte characterization:

➔ **Measurements of spectral fluorescence have been realized on more than 40 pure cultures**

⇒ ***In order to test :***

- i/ the good agreemment between classification group by Fluoroprobe and the theoretical group

- ii/ and to characterize a new group : the Haptophytes as Phaeocystis globosa

2
Fingerprints, Classification, Haptophyte

➔**The most significant results was :**

JOURNAL OF PLANKTON RESEARCH | VOLUME 34 | NUMBER 2 | PAGES 136–151 | 2012

Spectral fluorometric characterization of Haptophyte dynamics using the FluoroProbe: an application in the eastern English Channel for monitoring *Phaeocystis globosa*

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- The new fingerprint was realized from a natural pop dominated by Phaeocystis (99%), single cell. & colony

- Many classification tests for pure cultures and mixtures…

➔**Phaeocystis can be well characterized, but some interactions exist when green fingerprint is activate :**

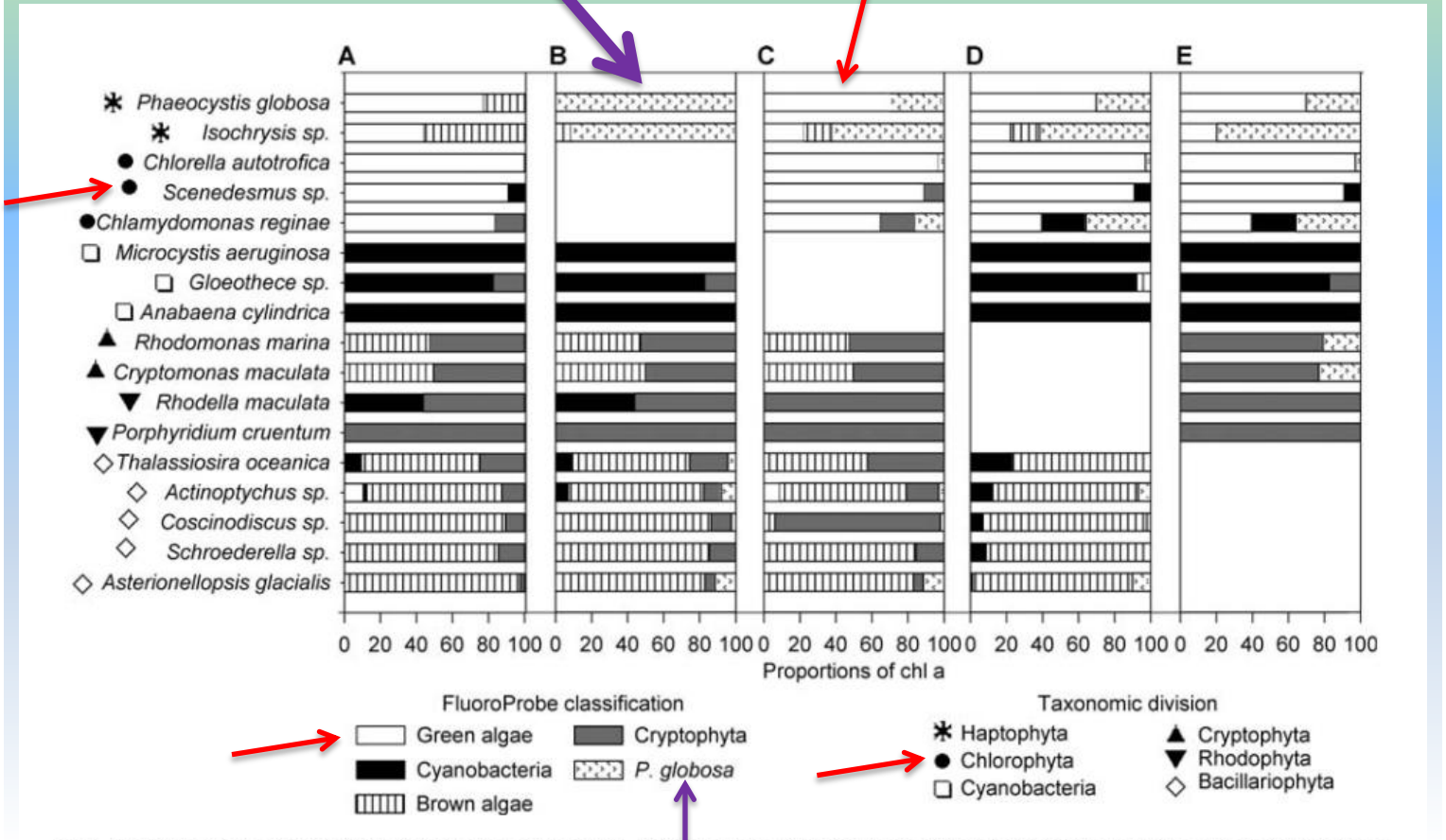


Fig. 2. FluoroProbe classification of 17 phytoplankton pure cultures using either the four original fingerprints or three original fingerprints + *Phaeocystis globosa*'s fingerprint. Original fingerprints (Cyanobacteria + brown algae + green algae + Cryptophyta) (**A**). Fingerprints of Cyanobacteria + brown algae + Cryptophyta + *Phaeocystis globosa* (**B**). Fingerprints of green algae + brown algae + Cryptophyta + *Phaeocystis globosa* (**C**). Fingerprints of Cynaobacteria + green algae + brown algae + *Phaeocystis globosa* (**D**). Fingerprints of Cynaobacteria + green algae + Cryptophyta + *Phaeocystis globosa* (**E**). Colours correspond to the FluoroProbe classification, whereas symbols situated in front of the species names correspond to the taxonomic division of species.

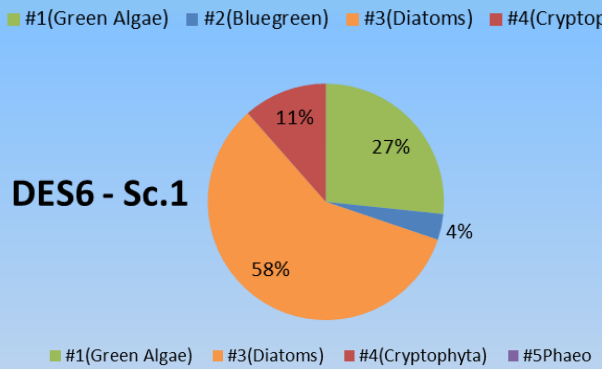
From Houliez et al., JPR 2012

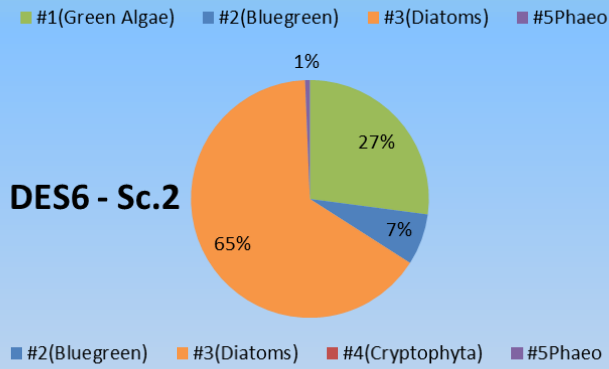
2
Fingerprints, Classification, Haptophyte

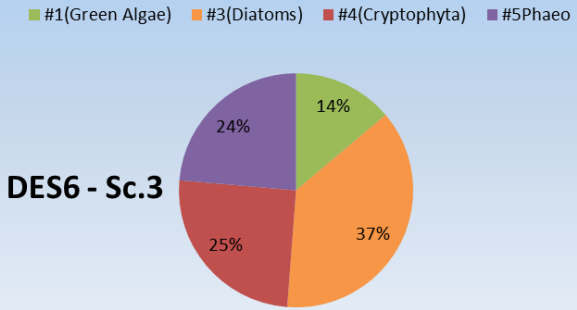
➔ **<u>Watch Out « 1 »</u> : Complex Interactions on natural unknown populations can occur :**

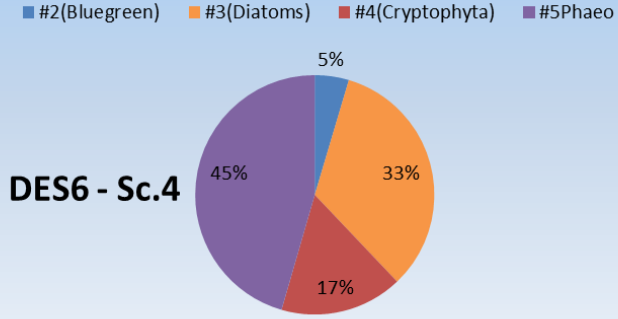
⇒ ***ex. of tests using 4 fingerprint scenarii (Sc)***

Sc1	Green	Bluegreen	Brown	Cryptophyta
Sc2	Green	Bluegreen	Brown	Phaeo
Sc3	Green	Brown	Cryptophyta	Phaeo
Sc4	Bluegreen	Brown	Cryptophyta	Phaeo









⇒ ***According to the scenarii of fingerprint used, Phaeocystis can be detect while there is no Phaeocystis from cytometer data !***

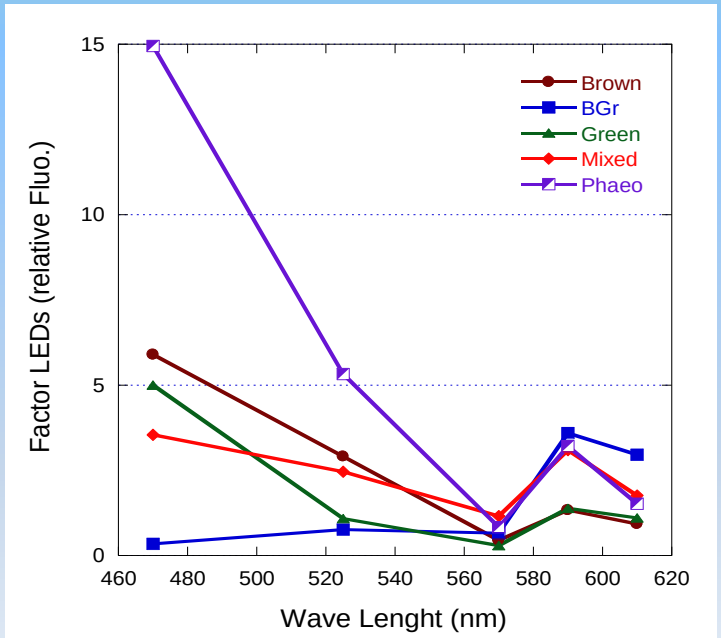
2
Fingerprints, Classification, Haptophyte

➔ Watch Out « 2 » : effect of the fingerprints origin :

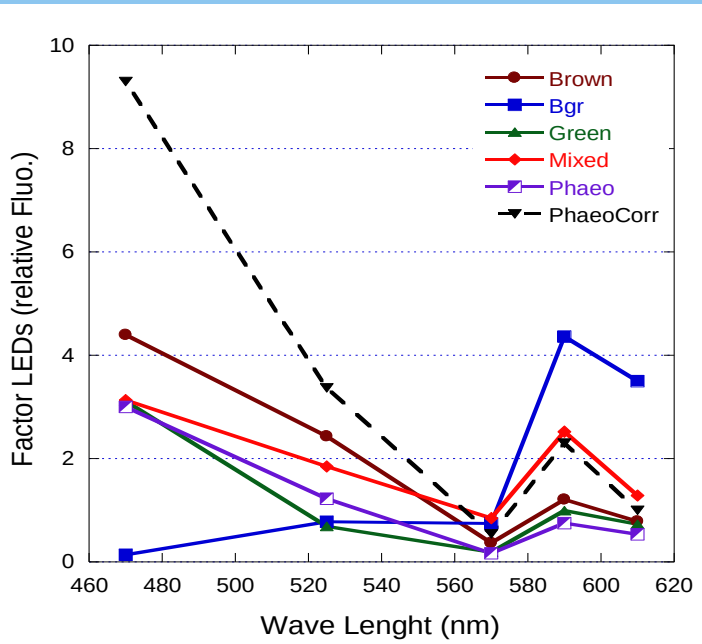
⇒ *Comparison between phaeocystis fingerprints for*

A natural population dominated by Phaeocystis (99%) :

Fingerprint used <2012, with 16-16 FLP



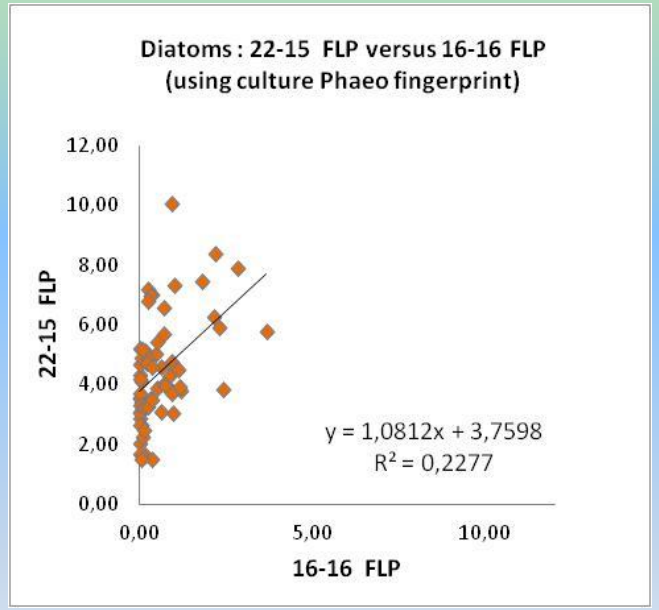
A culture, after a new calibration: raw (--) and corrected(--) fingerprints (16-16 FLP, > 2012)

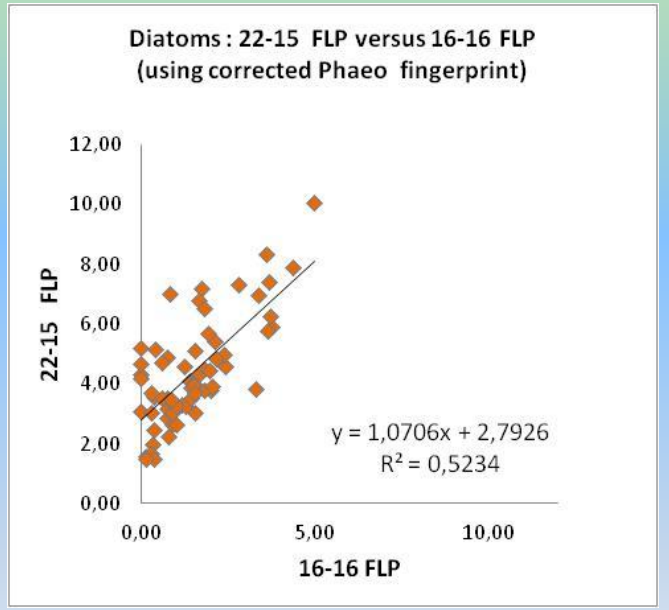


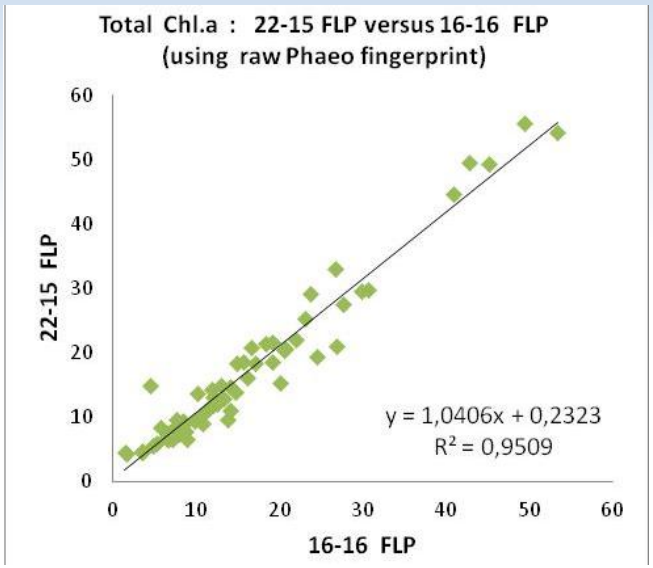
⇒ *Impact on the determination of Haptophytes and diatoms in natural population ?*

2
Fingerprints, Classification, Haptophyte

⇒Yes… according to comparisons between 2 FluoroProbes (16-16 and 22-15 FLP) using 2 different Phaeocystis fingerprints (2014 spring data set) :



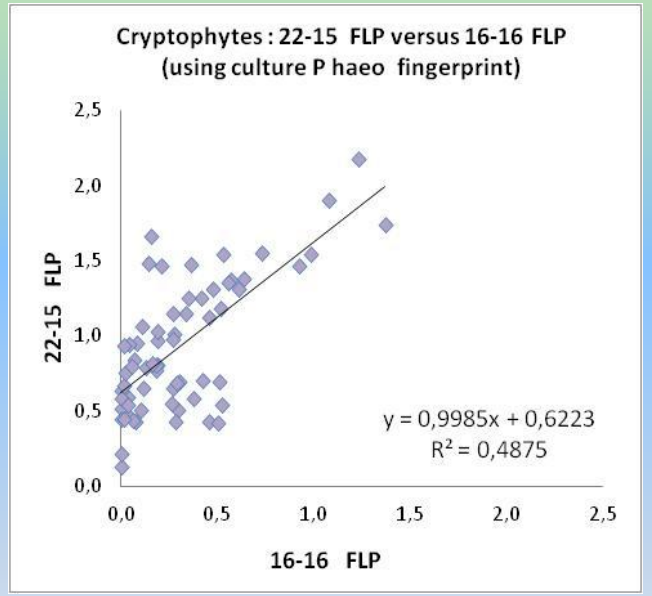


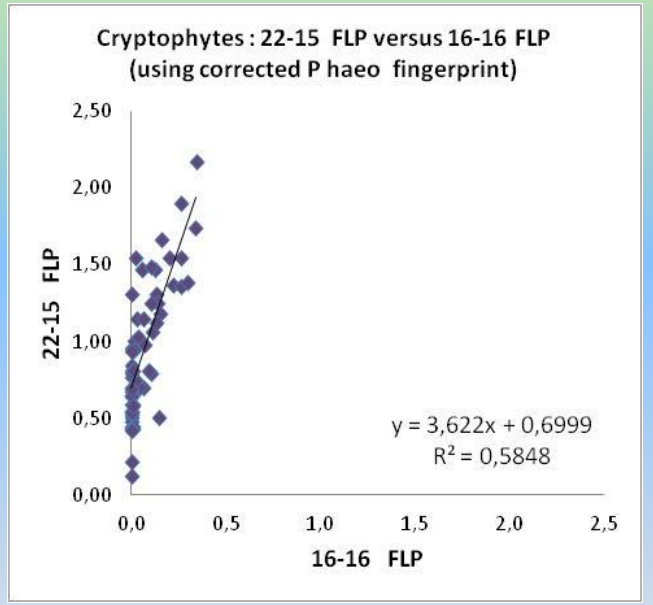


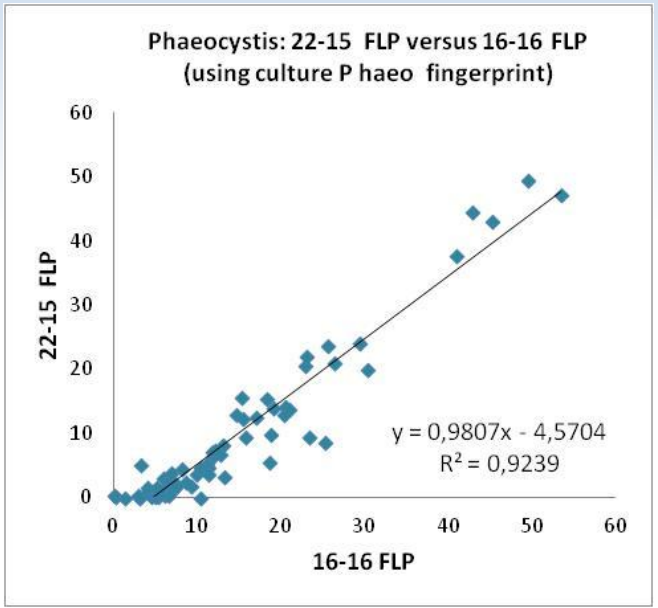
Total Chla. show a good relationship between the 2 FLP, using culture (fig) or corrected Phaeo fingerprints

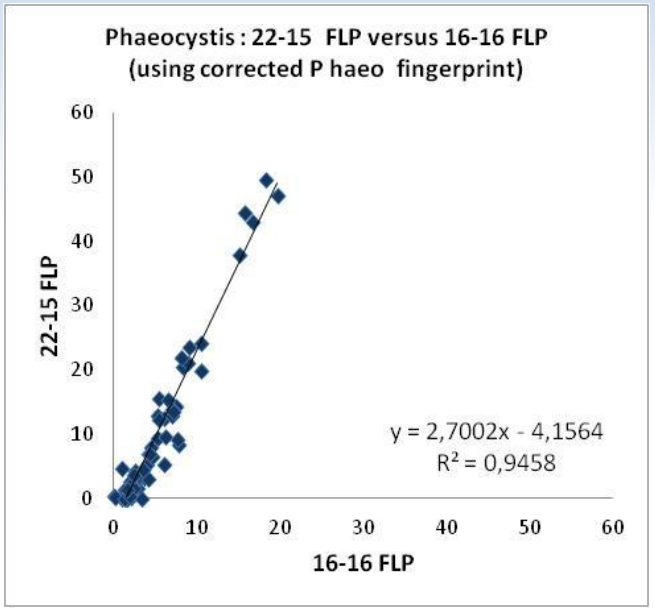
2
Fingerprints, Classification, Haptophyte

⇒ *Idem for the other phytoplankton groups when a « bad » fingerprint is used :*







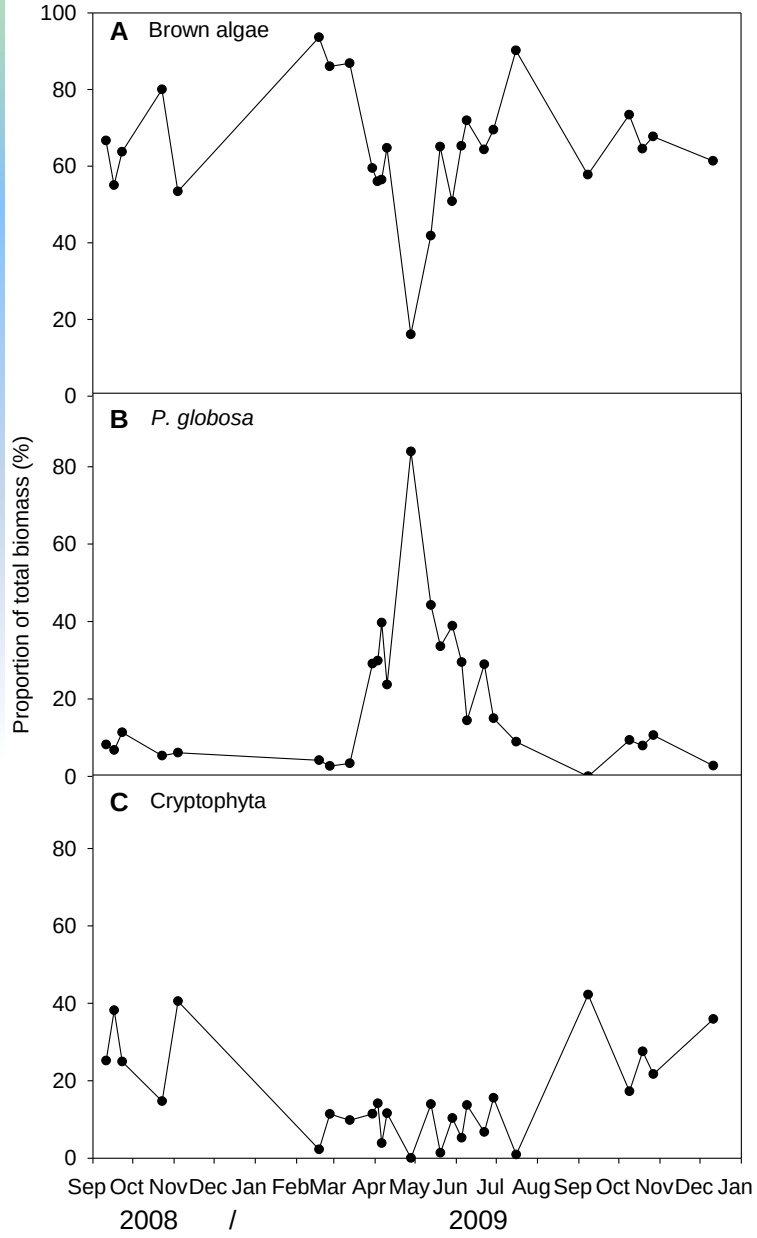


3
Tme / Space variability

3/ Time / space variability :

➔**Phaeocystis, diatom and cryptophyte variability has been well characterized during the annual cycle :**

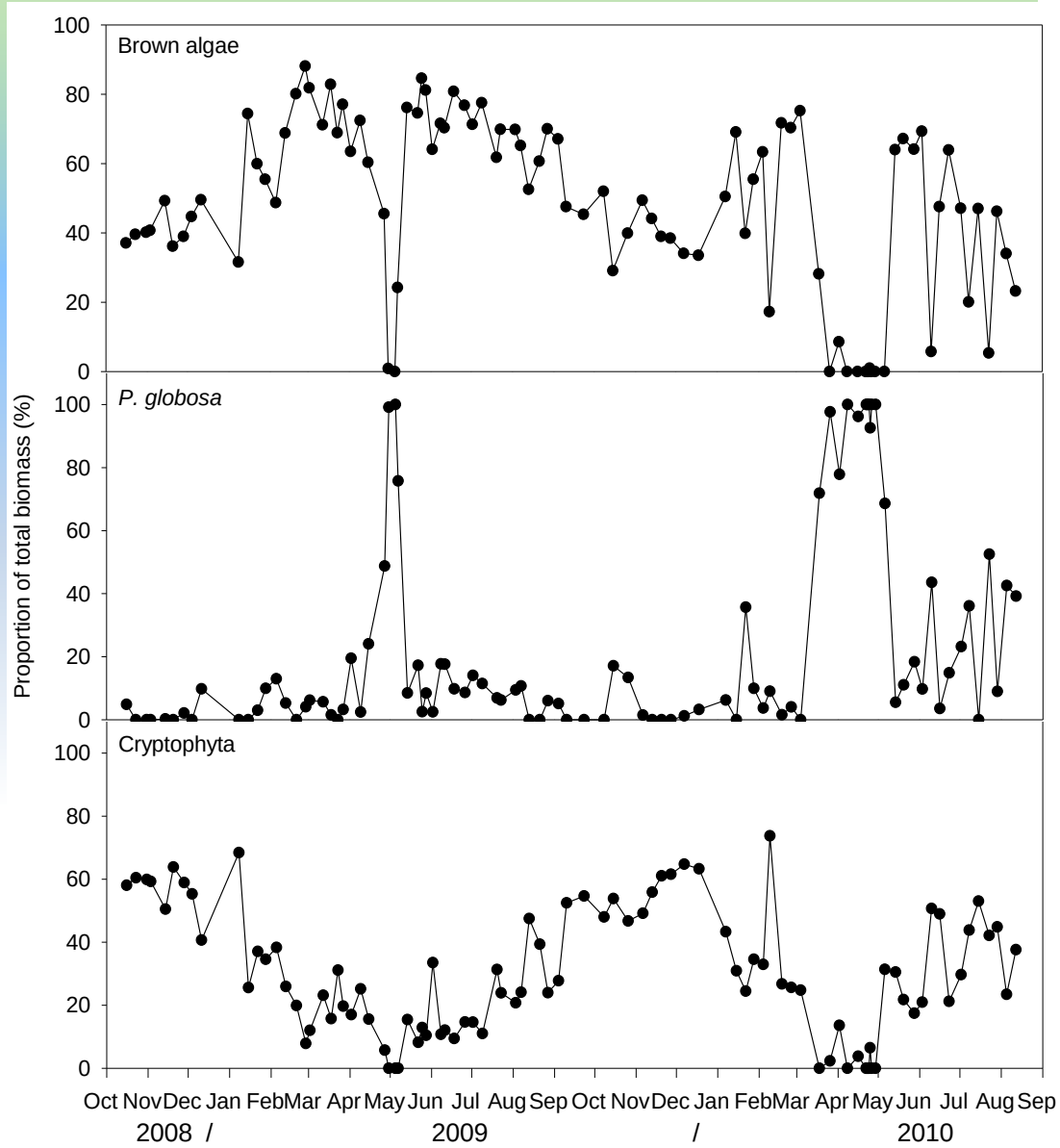
⇒ *EX.1: Phytoplankton groups in coastal waters of the Strait of Dover (station R1)*



From Houliez et al., Marine Biology 2013

3
Tme / Space variability

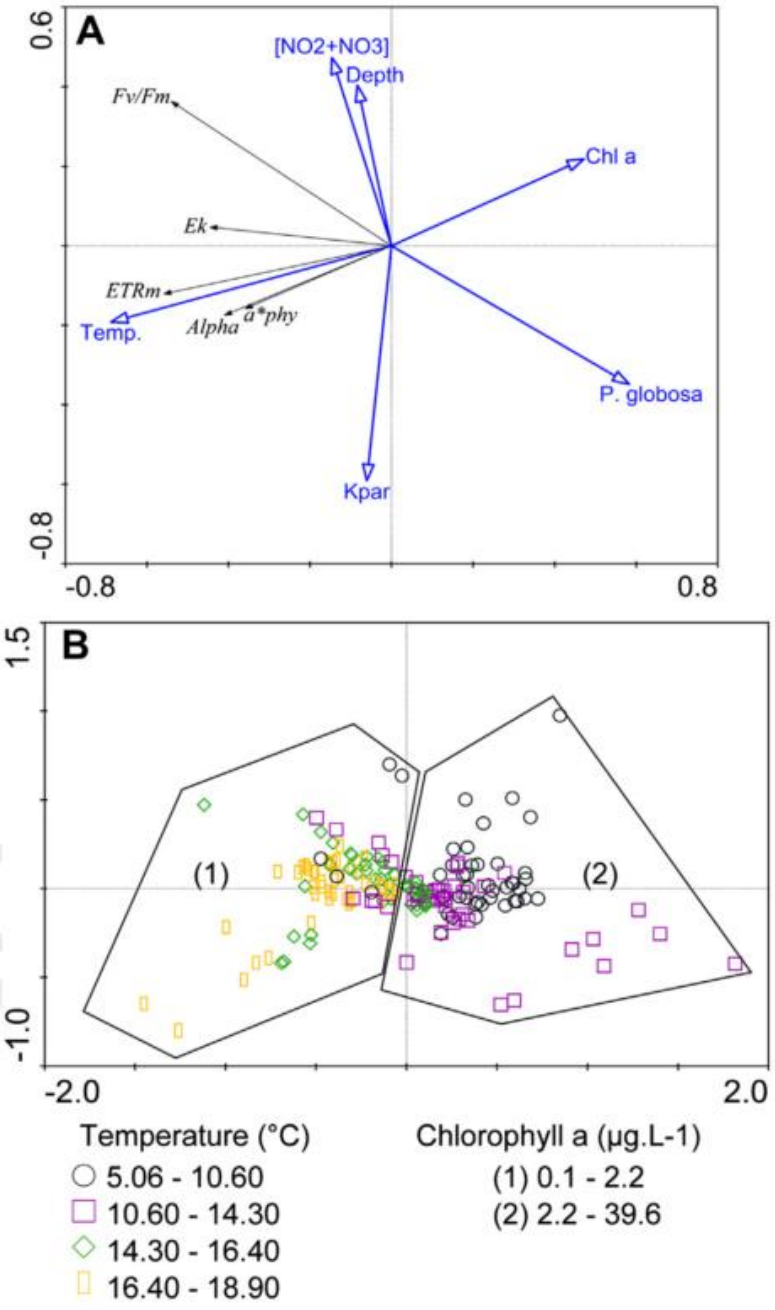
⇒ *EX.2: Phytoplankton groups in coastal waters of Wimereux, weekly sampling during > 2 years*



From Houliez et al., Journal of Marine Systems, 2014

3
Tme / Space variability

⇒ ***FP*** *data allow to characterize physiological state and controlling factors of Phaeocystis (FLP 16-16 < 2012) during the spring bloom*



From Houliez et al., ECSS, 2013

Fig. 10. Redundancy analysis (RDA). A) Ordination biplot showing the photosynthetic parameters in relation to environmental and biological variables. Eigenvalues on the first and second axis are respectively 0.225 and 0.050. The cumulative variance of the

3
Tme / Space variability

⇒ *and algae* ***photosynthetic properties*** *by period of the bloom or dominant group in the English Channel*

Average ± standard deviation of photosynthetic parameters as a function of dominant taxa and phytoplankton blooms. α: maximal light utilization efficiency (µmol e^- mg chl a^{-1} s^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$), ETR_m: maximum electron transport rate (µmol e^- mg chl a^{-1} s^{-1}), E_k: light saturation coefficient (µmol photons m^{-2} s^{-1}) and F_v/F_m maximum photosynthetic yield

	α	ETR_m	E_k	F_v/F_m
Brown algae dominated (> 70%)	0.0035 ± 0.0021	0.80 ± 0.43	231.83 ± 47.99	0.55 ± 0.07
P. globosa dominated (> 70%)	0.0027 ± 0.0027	0.73 ± 0.85	277.18 ± 86.19	0.49 ± 0.09
Cryptophyta dominated (> 50%)	0.0047 ± 0.0003	0.94 ± 0.62	202.74 ± 55.92	0.52 ± 0.06
1st Brown algae 2009	0.0040 ± 0.0023	0.84 ± 0.45	209.34 ± 23.49	0.55 ± 0.07
P. globosa bloom 2009	0.0017 ± 0.0010	0.38 ± 0.26	229.70 ± 31.36	0.44 ± 0.09
2nd Brown algae 2009	0.0030 ± 0.0017	0.76 ± 0.40	262.06 ± 51.09	0.54 ± 0.06
Brown algae bloom 2010	0.0041 ± 0.0030	0.79 ± 0.53	193.90 ± 27.18	0.60 ± 0.04
P. globosa bloom 2010	0.0030 ± 0.0033	0.87 ± 1.05	305.10 ± 98.58	0.50 ± 0.08

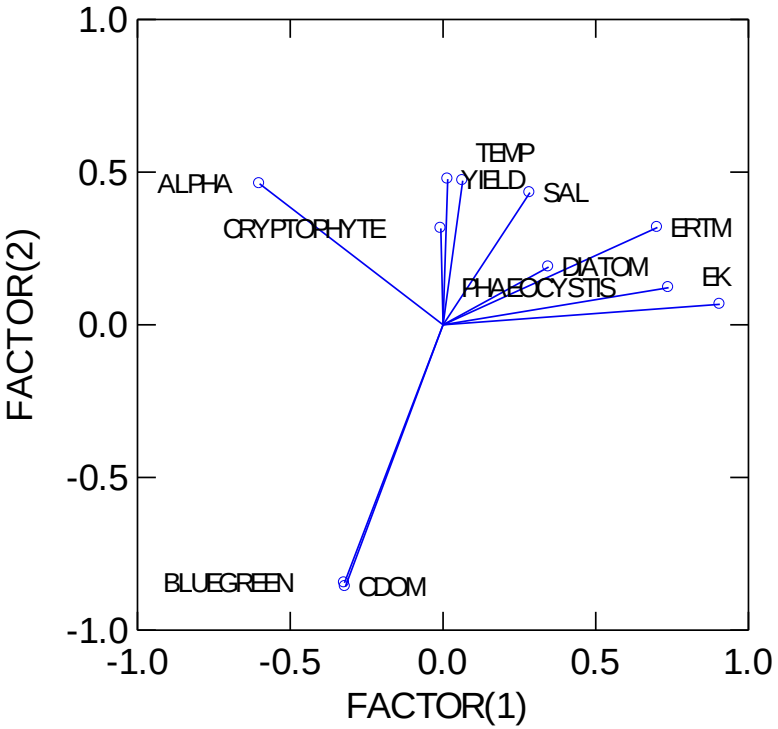
From Houliez PhD, 2012

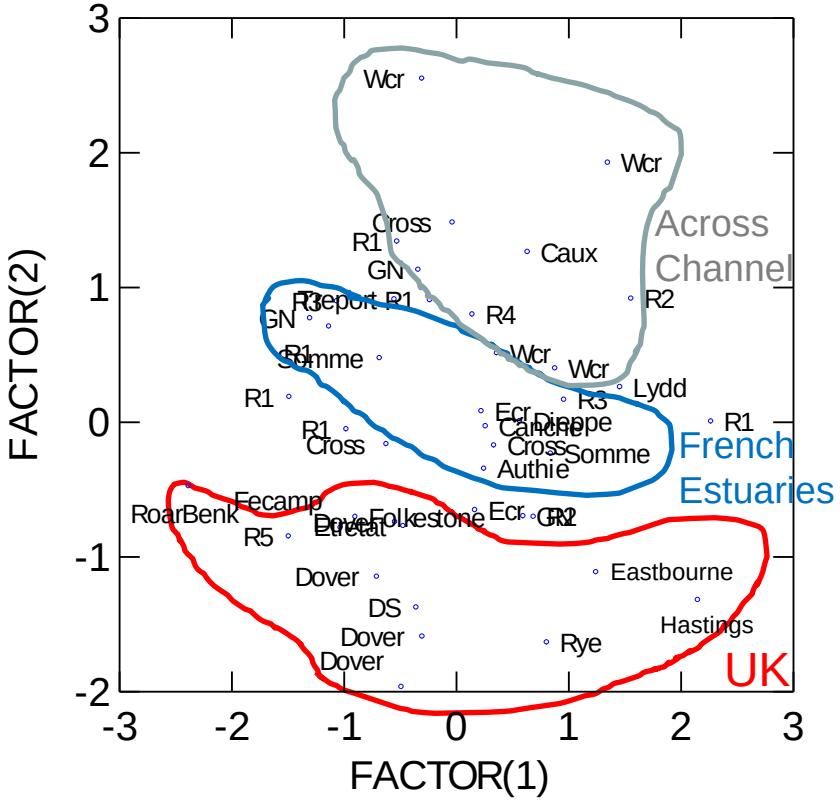
3
Space / Time variability

➔**Water masses of the English Channel can be well characterized by spectral groups :**

☞ **at high scale : differences between UK, French estuaries and transect across the Channel:**

ACP on spectral groups and physiological parameters (+ 70 discreet sampling stations): 55% var. expl.

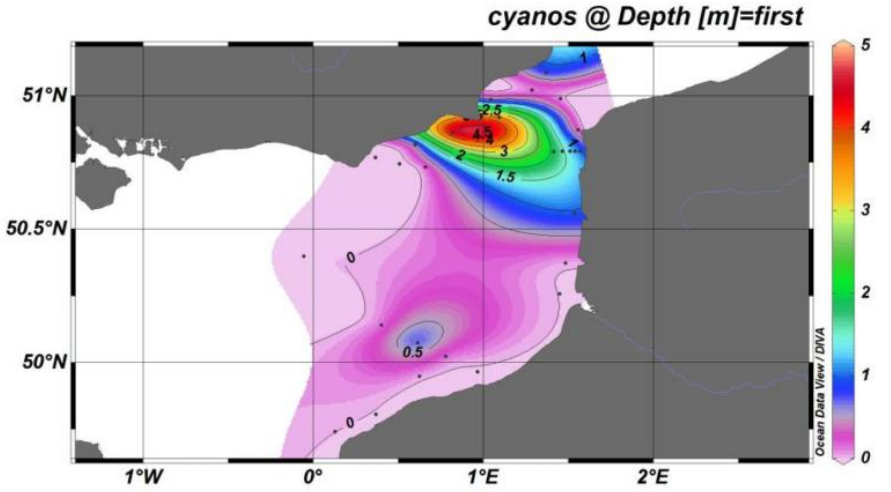


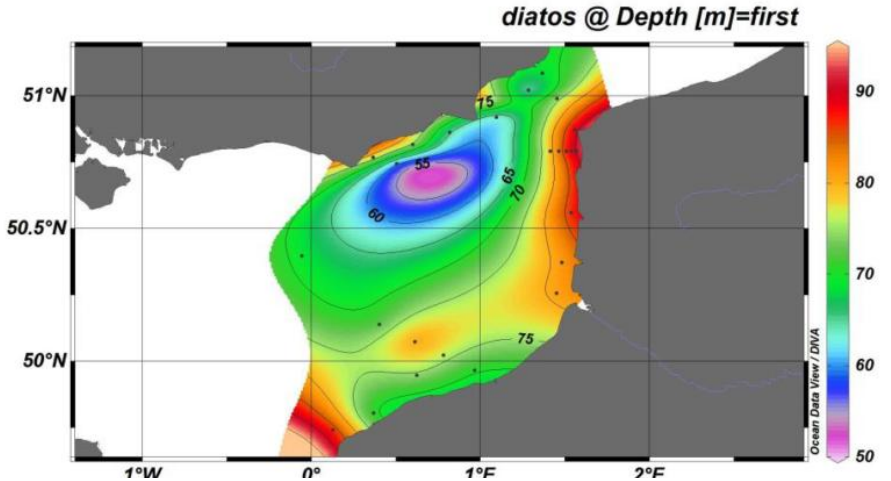


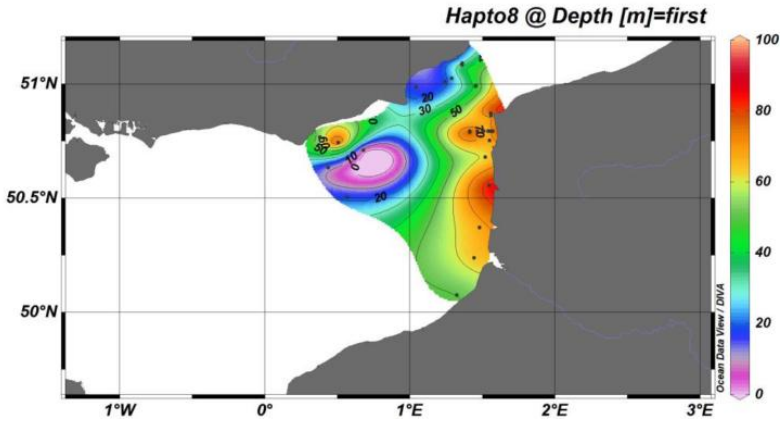
3
Space / Time variability

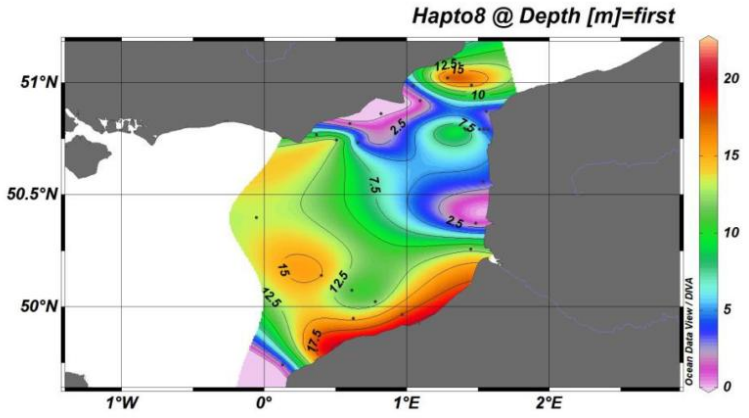
⇒in agreement with **Chemotaxonomic methods** (from Pigments HPLC analysis) in the English *Channel*

(Chemtax analysis: L. Lampert, Ifremer Nantes)









3
Space / Time variability

☞ at small scale with high frequency measurement along an inshore-offshore transect :

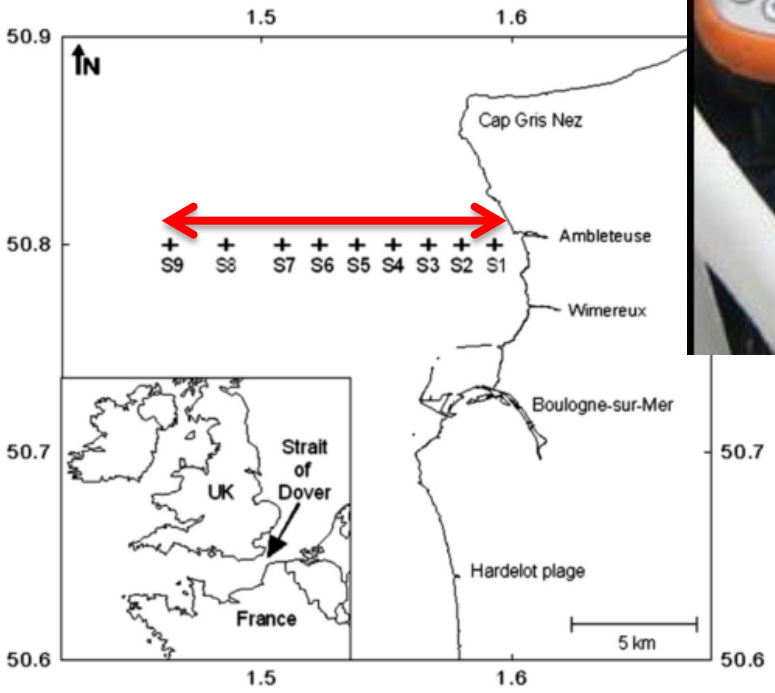
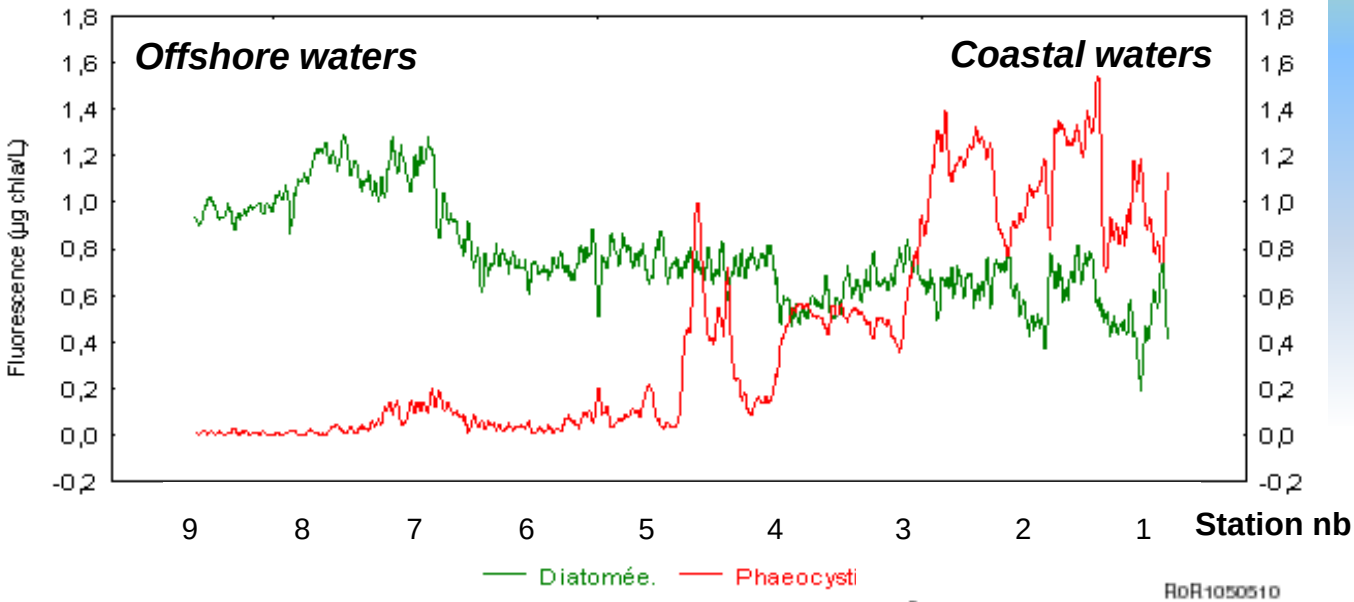


Fig. 1. Map of the Strait of Dover with enlarged area representing the location of sampling stations. Crosses indicate sampling stations.

⇒ frequency measurement of 1,5 sec with the « natural » fingerprint for Phaeocystis

3
Space / Time variability

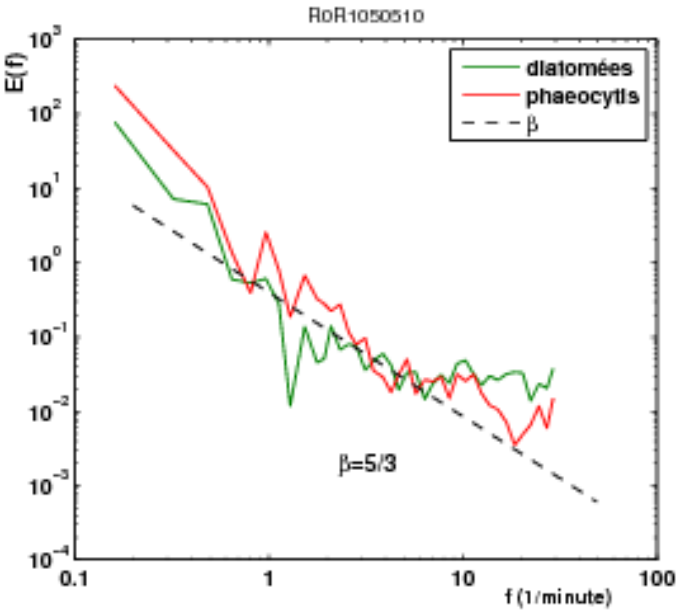
⇒ **Shift in dominant phytoplankton groups** can be clearly identified between coastal and offshore waters :



⇒ ***Spectral analyses show for the smallest scales :***

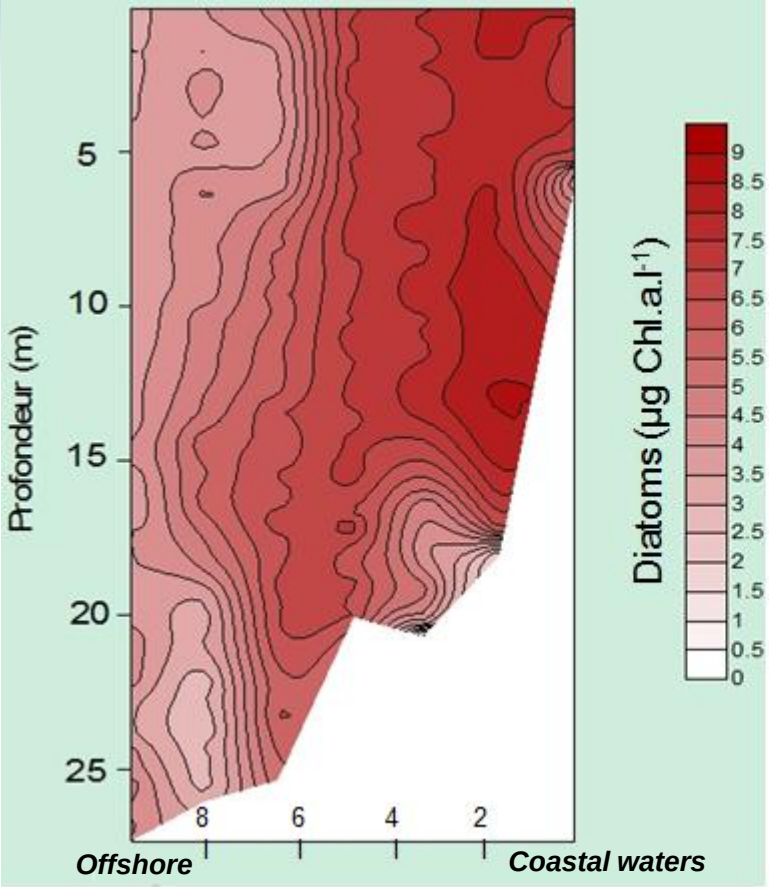
- ***different slopes from the theoretical value of 5/3***
- ***and different slope between Phaeocystis and « Brown » algae :***

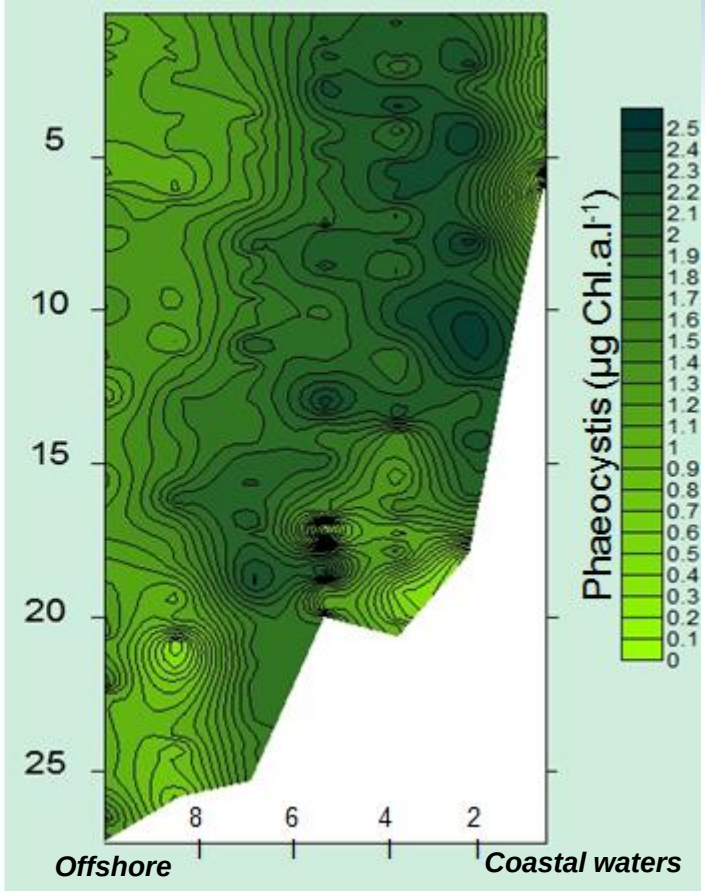
due to patchiness and turbulence !



3
Space / Time variability

⇒ Or in the **water column** : vertical profiles of FP along a coastal / offshore gradient in spring 2009



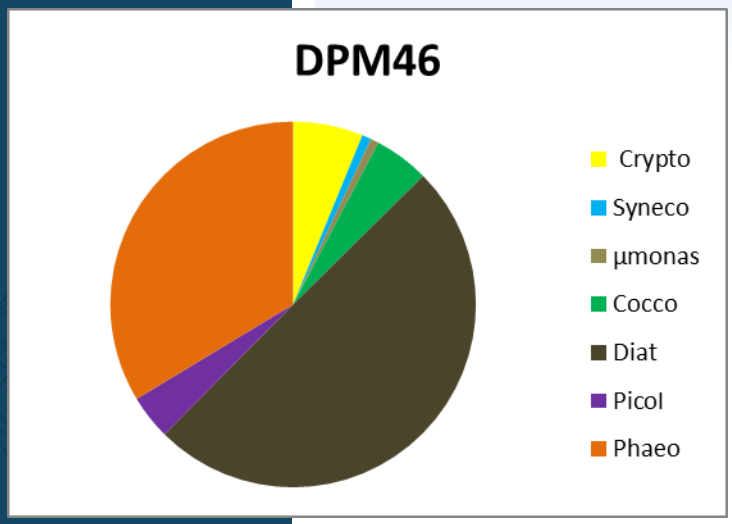


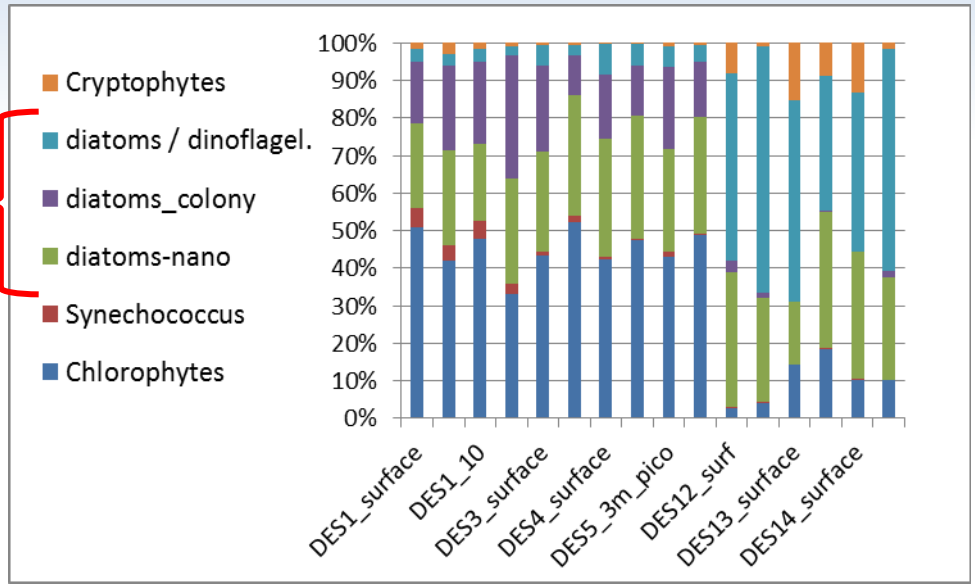
4/ Comparative study of Spectral Fluo. and Cytometry on natural samples

- ***> 95 stations have been sampled during the Dymaphy joint sampling campaigns (Channel + Zeeland Sea)***

⇒In order to compare FP spectral groups in equivalent Chla with the cytometry data, red fluorescence signal is used and added for different species according to spectral fluorescence groups

⇒Because, cytometry is more accurate and can divide groups as :





4
Spectral Fluo. / Cytometry

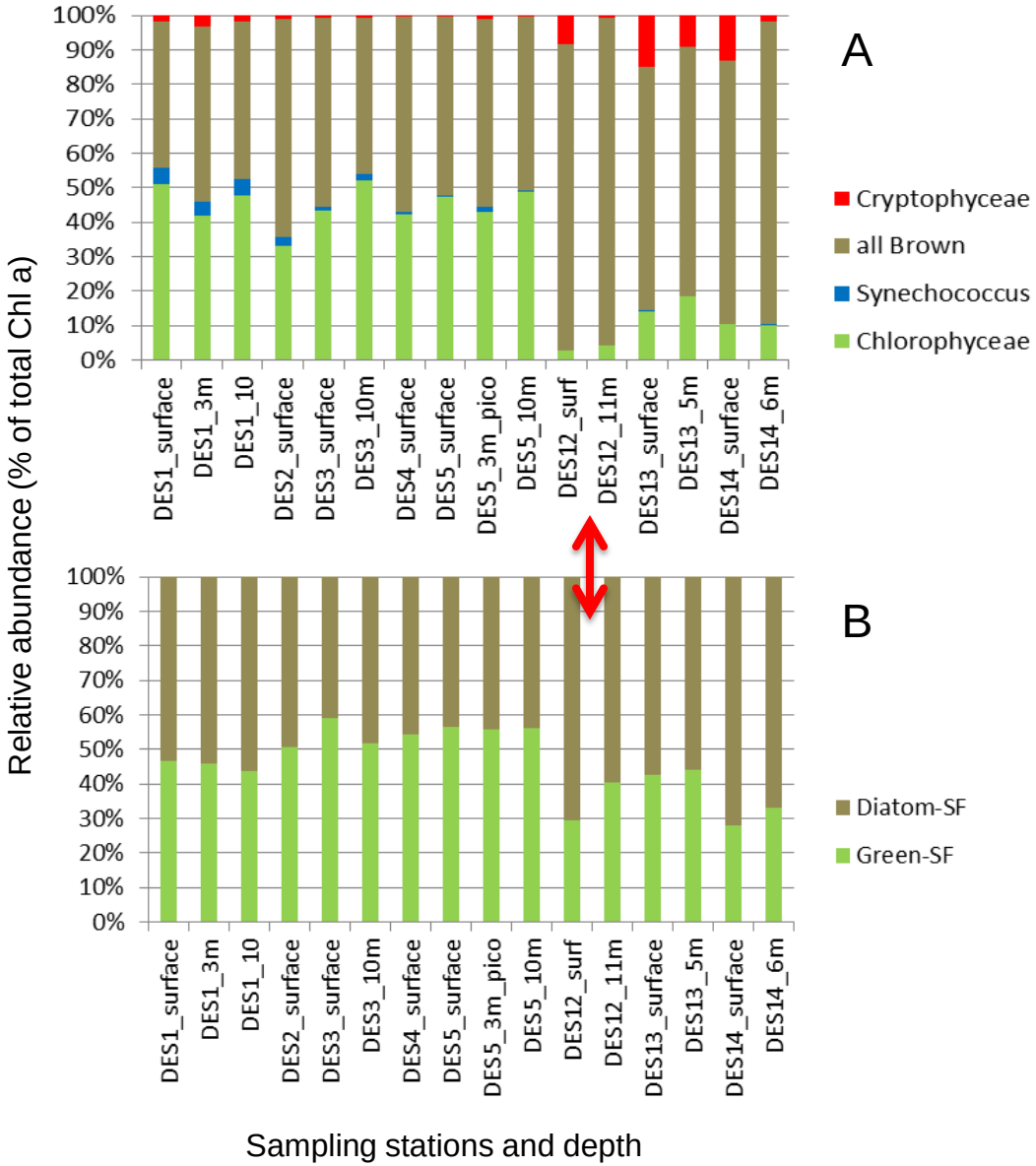
➔1/ The Zeeland case study :

→ ***Materials : 3 Cytometers, Ferry-Box with AOA, FluoroProbe, PhytoPam and env. param.***

→ ***3 sampling area : Westerchelde, Osterchelde and Dreischor***

4
Spectral Fluo. / Cytometry

→ *Relative abundance of 4 phytoplankton groups for cytometry (A; Cefas) and of 2 gps for spectral fluorescence (B) :*

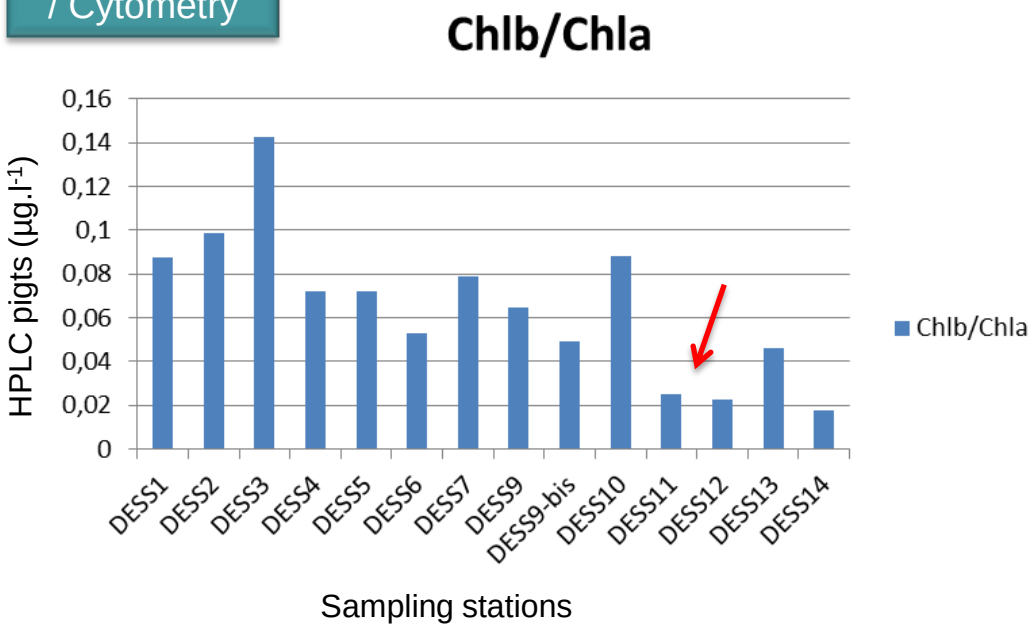


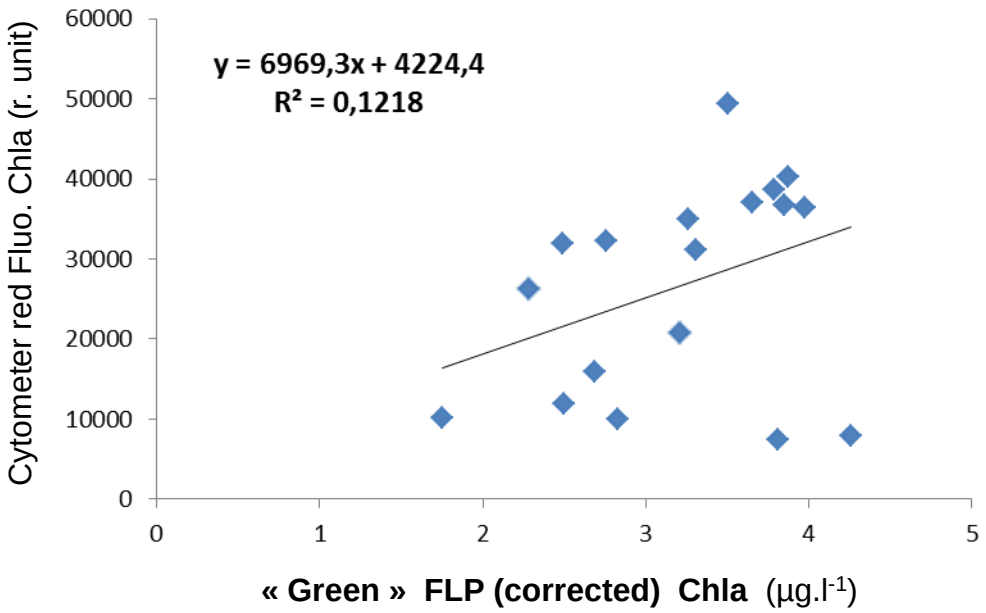
→ ***Only 2 groups are classified by the Fluoroprobe : « Green » & « Brown » algae***

⇒ *usual « bias » when relative abundance are < or ±10%*

→ ***Similar variations of the relative abundance are found except for stations "DES" 12 at 0 & 11 m***

4
Spectral Fluo. / Cytometry





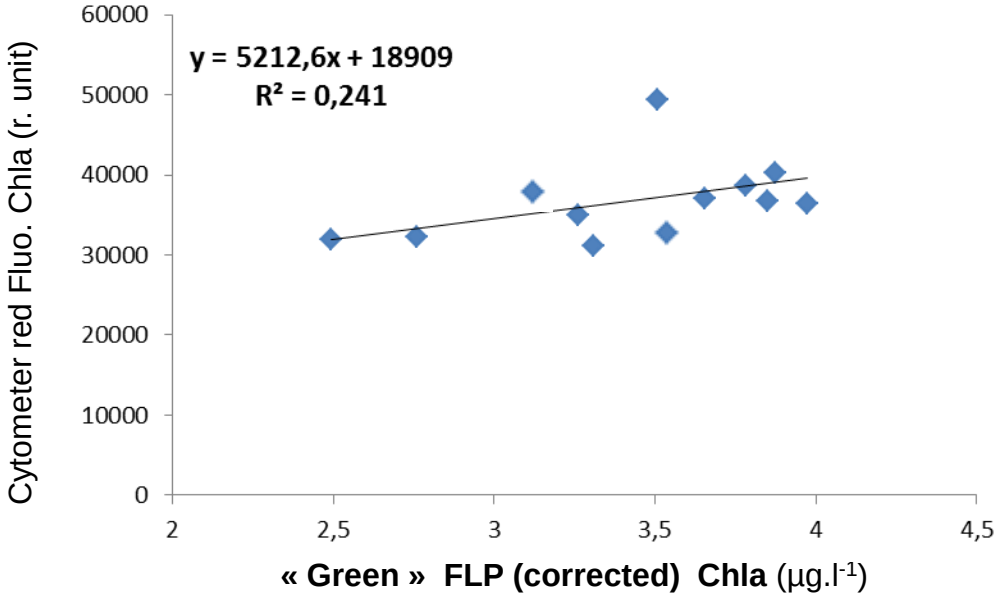
→Due to an overestimation of « green » algae by spectral fluorescence ?

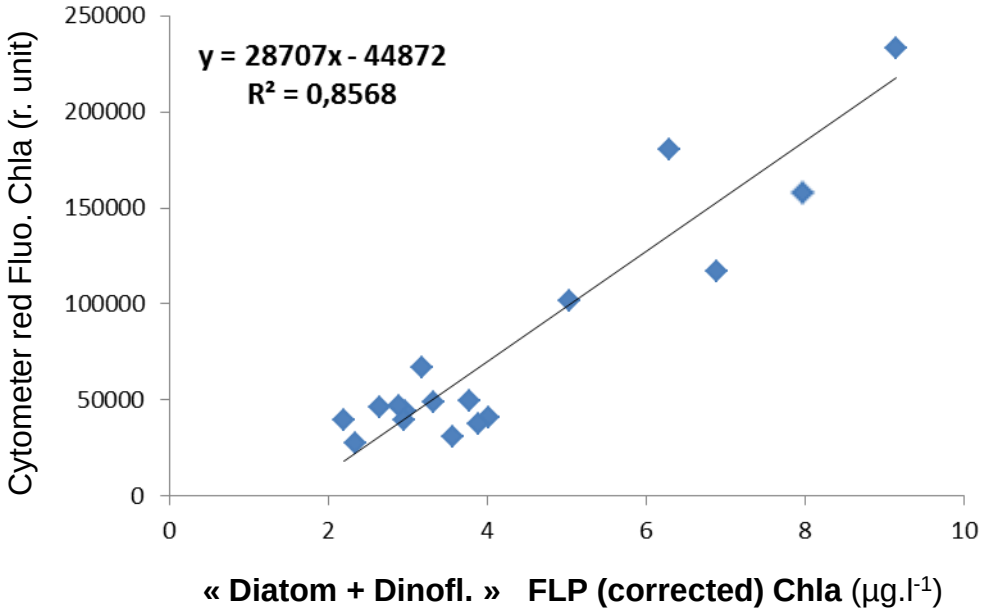
⇒ yes: Chl b / Chl a ratio (an index of green algae) from HPLC are lower for Dreischor stations, as cytometer data

→This could explain that no correlation was found with all data between cytometer fluorescence and "green" Chla from spectral fluorometer

→but…

4
Spectral Fluo.
/ Cytometry





→Removal Dreischor data, the relationship is better but stay no significant for « Green » algae (r = 0,490)

⇒ There was probably a mis-classification by spectral fluo. at Dreischor stations : local diff. species ?

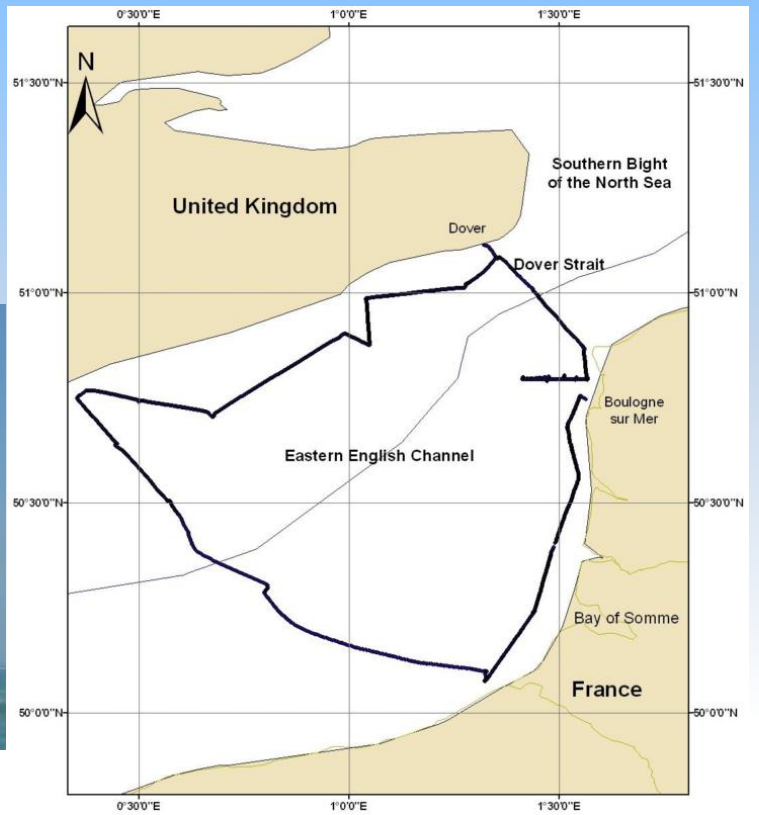
→However a significant correlation (r = 0,925) was found for Diatoms + Dinoflagellates considering all stations !

4
Spectral Fluo. / Cytometry

➔2/ The Eastern English Channel case study :

→ ***Materials : 2 or 3 cytometers, Ferry-Box with AOA , FluoroProbe, PhytoPam & env. par.***

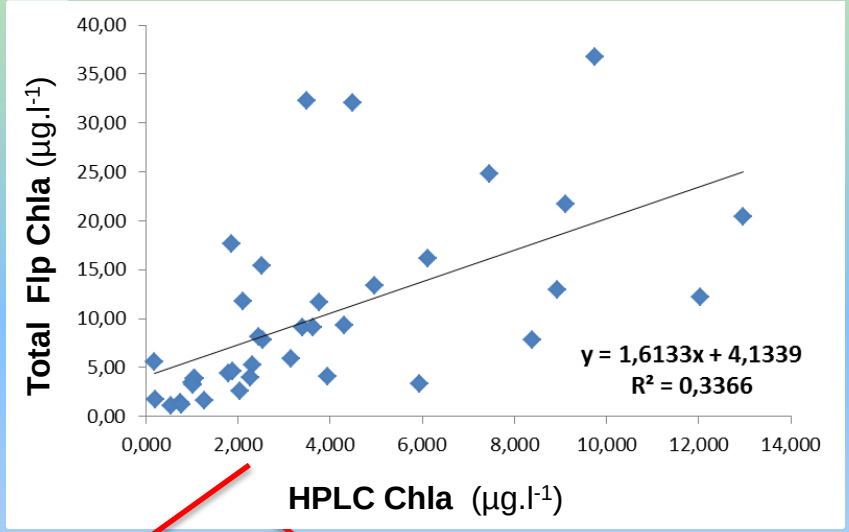
→ ***Sampling in 3 parts due to bad meteorological conditions***



→ 1/ Relationship between Chla predicted by Fluoroprobe and HPLC total chl.a for n= 36 from surface waters :

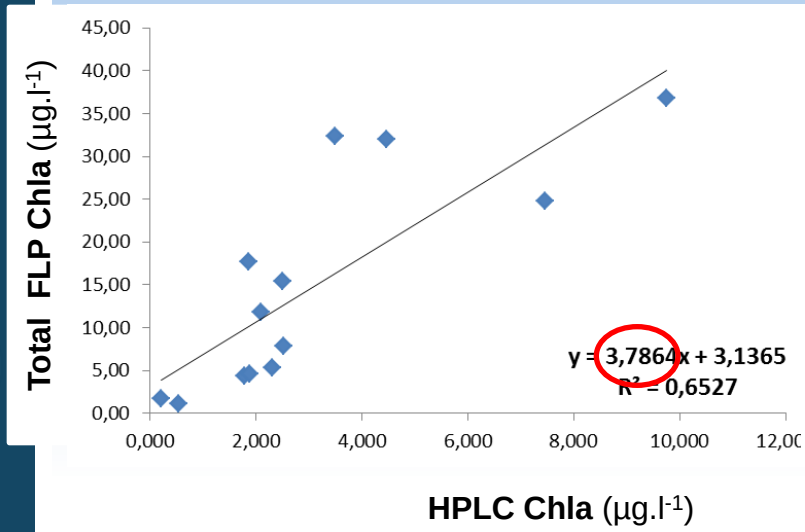
⇒ No good agreement between the 2 techniques !

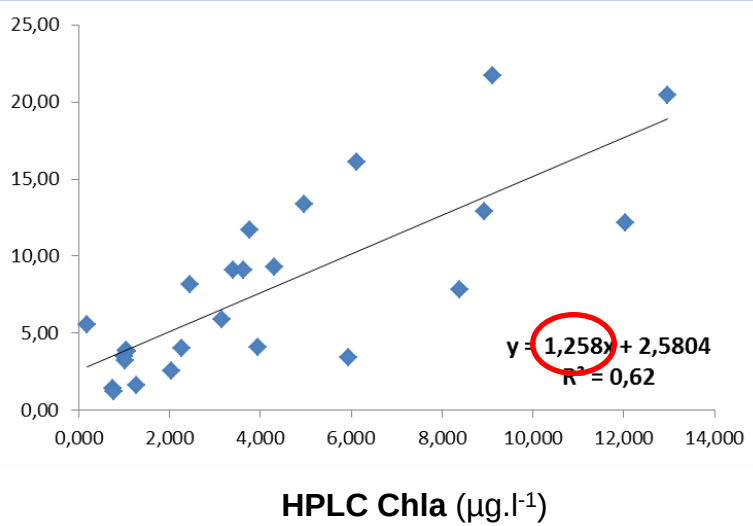
⇒ Relationship significant but with different slope when data are divided by sampling period:



Late april: great overestimation by FP

Mid-may :





→ ***This different relationships can be explained by a shift of dominant phytoplankton groups :***

☞ ***From the cytometer data set:***

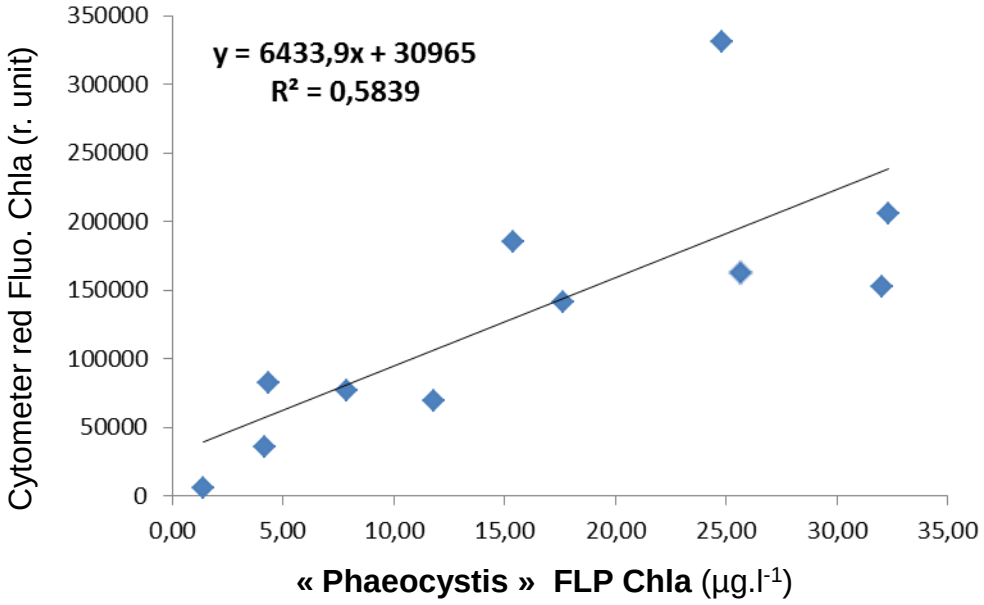
⇒ *Phaeocystis is dominant in april*

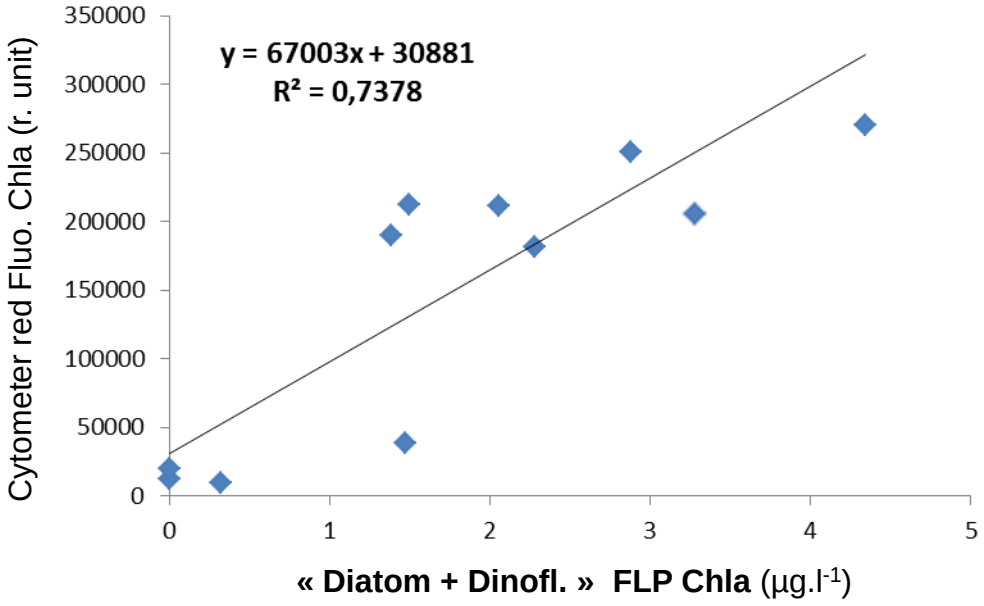
⇒ *While diatoms dominate in may*

→ ***So, in phaeocystis bloom, Chl a could be overestimated by the spectral fluorometer that have been recalibrated with a culture fingerprint in 2012 !***

→ ***Some mis-classification of diatom by Flp can also occur in this case***

→ 2/ Spectral Fluorescence / cytometer relationships





late april :

→<u>Significant correlations are found</u> :

⇒ for Phaeocystis between cytometer data and spectral fluo. (r = 0,764)

⇒and for « Brown » algae between cytometer data and spectral fluo. (r = 0,858)

⇒<u>Pb</u>. : no good cytometer data in mid-may to test relatioships !!

5
Conclusion

5/ Conclusion and prospects :

☞ **Spectral Fluorescence is a good tool** for characterization of phytoplankton global variation pattern, in time at a particular sampling station or across small environmental gradients !

☞ **However for monitoring in space at great scale :**

- *spectral fluo. must be regularly compared with discret samples for Chl.a and taxonomy check*
- *knowledge of dominant species in a phy. group is necessary to optimize characterization by a specific fingerprint*
- *New Fingerprints must be taken on freshly isoleted species from the sea where measurements will be realized :*

5 Conclusion

☞ ***This is a limiting factor in order to conduct sampling at great scale as the French sailor Bernard Stam during the last Vandée Globe Challenge !***

- missing group must not been always disabled : sensitivity analyses with different scenarii of fingerprint are recommanded

-Intercalibration of total Chl a with HPLC is highly recommended

☞**<u>Spectral fluo is on agreement with cytometer data</u>**

- for dominant phytoplankton groups

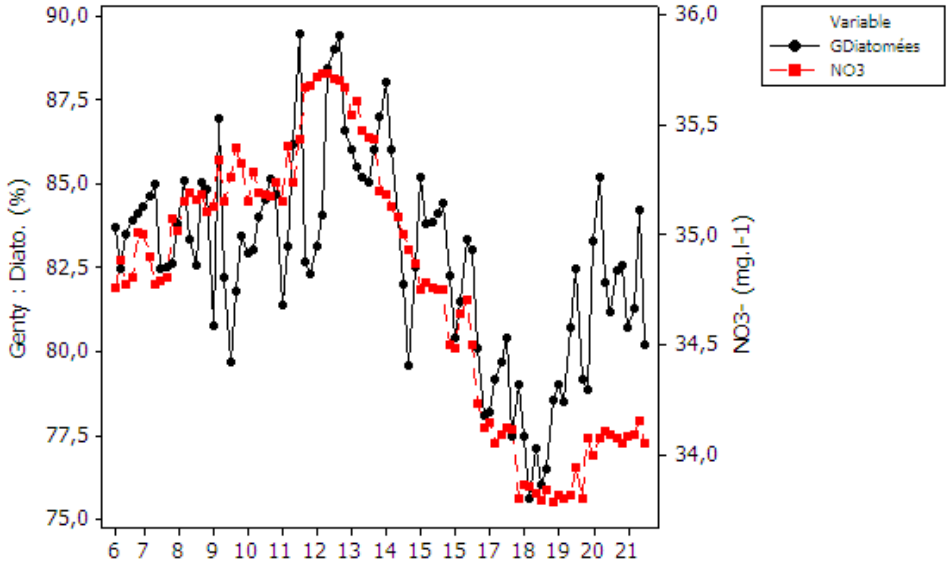
- according to the time / space scale of the sampling

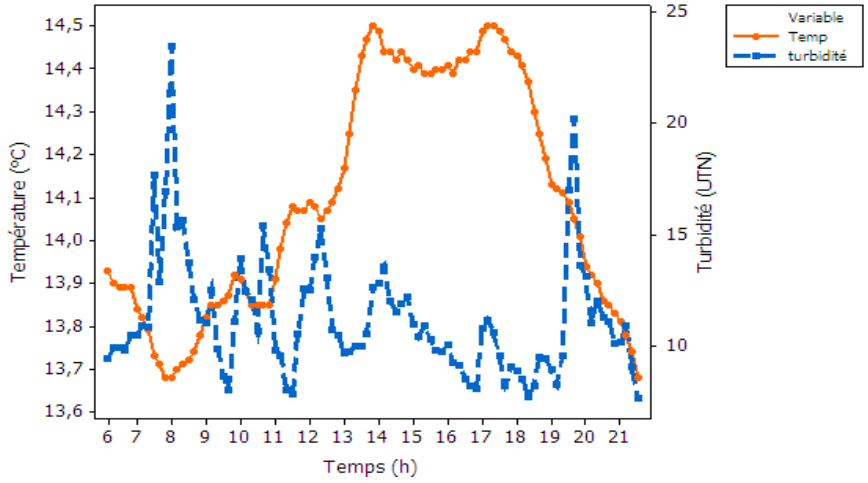
- minor cytometry groups must be grouped with corresponding major spectral groups cautiously !

5
Conclusion

☞ **Prospects : By analogy between fresh and sea water results:**

With high frequency AOA data coming from the north french water agency, we have found **strong correlations** between spectral group biomass and/or physiology (GP or Fv/Fm) and environmental parameters :





5 Conclusion

⇒ **Report of the stepwise multiple linear regression** results (with 3800 AOA data) relating the total, the 3 spectral group biomass and the Genty Parameters (GP as Fv/Fm) to environmental factors ; classified from the highest to the lowest (1 to 4) according to % of variance explained

☞at montly scale :

AOA	Genty Par.	PO4	NH4	NO3	Conduct.	Temp.	turbid.	Irrad.	Pluie	% Var.
Chla totale	1			3		2	4			56*
Diato	3	2		1				4		46,4*
Chloro		1		3		2	4			25,5*
Crypto	4				3	2	1			17,9
GP - Chla T.	-			2	4	1	3			38,5*
GP - Diato	-	4				2	3	1		50,6*
GP - Chloro	-	2	4	1			3			37,7*
GP - crypto	-			2	4	1	3			38,5*

1
2
3
4

*Significatif

5 Conclusion

☞at daily scale :

⇒ Controlling environmental parameters can be different according to the spectral groups and the studied scale in fresh water for high frequency measurements :

what ‘s happening in a high hydrodynamic marine system ?

1
2
3
4

Diatomées (AOA)

Dates	G. P.	PO4	NH4	NO3	Cond	T°C	tur.	Irrad.	Pluie	% Var.
15/avr				4		1	2	3		96,3
16/avr				2		1	3	4		92,8
17/avr				4		2	3	1		79,1
21/avr				2	4		3	1		67,3
22/avr				3	4	1	2			70,4
23/avr			3	4		1	2			91,5
24/avr		4				1	3	2		85,3
25/avr					4	1	3	2		79,9
26/avr				4		2	3	1		64,2
27/avr		2		1		4	3			81,6
28/avr				2	4		3	1		48,2
29/avr						1	3	2		74
30/avr				2		1	3	4		55,1
01/mai		3		2		4	1			48,7
02/mai				3		1		2		83,3
03/mai				3	4		2	1		70,6
04/mai		3	1				2	4		65,9
05/mai					4	1	2	3		64,2
06/mai			1	3	4			2		63,7
07/mai				2		4	3	1		69,1
08/mai	3		1	4		2				63,5
09/mai		2		4		3	1			36,7

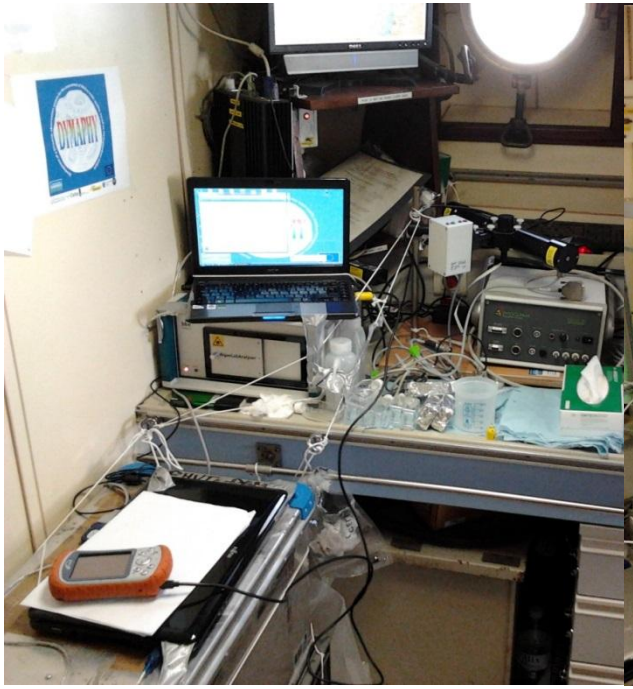
Chlorophycées (AOA)

Dates	G. P.	PO4	NH4	NO3	Cond	T°C	tur.	Irrad.	Pluie	% Var.
15/avr	1	4					3	2		52
16/avr	3			1		2		4		72,3
17/avr	3	4		1			2			61,2
21/avr	1		3	2			4			73,6
22/avr		4	3			1	2			82,1
23/avr				4		1	2	3		77,2
24/avr		3		4		1	2			75,9
25/avr	3	1				4	2			68,8
26/avr	3	4		1			2			69,2
27/avr	2	3		4		1				79,5
28/avr	2	1		4			3			52,9
29/avr			1			2	3	4		51,7
30/avr		2	4			1	3			72,4
01/mai		2	1			3		4		70,1
02/mai			2		4	1	3			39,4
03/mai	3	4	2			1				72
04/mai	4	2	3			1				66,7
05/mai	1		3		4		2			58,6
06/mai		4		3	2	1				64,2
07/mai	2	3				1	4			69,9
08/mai	3			2		1	4			63,1
09/mai	2					1	3	4		72,8

2 Mers Seas Zeeën
INTERREG IV A
FRANCE - ENGLAND - VLAANDEREN - NEDERLAND

DYMAPHY

Thanks for your attention !



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