INTERREG IVA 2 Mers Seas Zeeën Crossborder Cooperation Programme 2007-2013
Part-financed by the European Regional Development Fund (ERDF)

Combination of high frequency devices to characterize the phytoplankton community and the physico-chemical supporting parameters in the Eastern English Channel and the Dutch estuaries.

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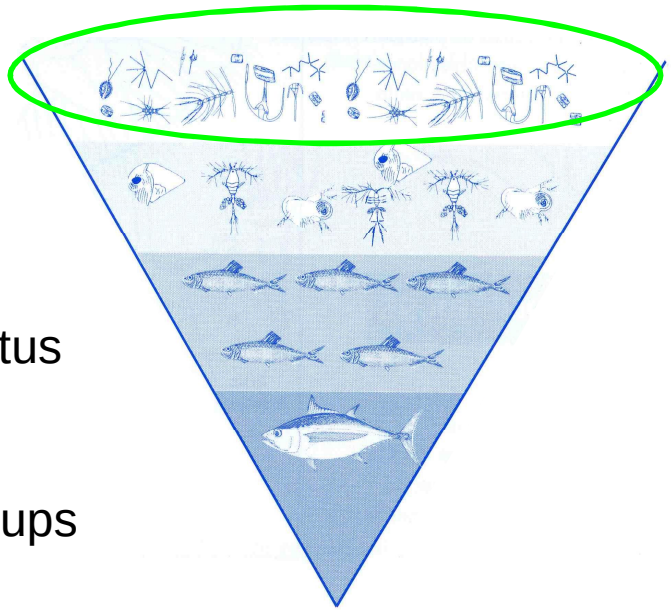
The DYMAPHY project

Development of a **DY**namic observation system for the assessment of **MA**rine water quality, based on **PHY**toplankton analysis

Main objectives

* Development of standard operational procedures to monitor phytoplankton community structure *in situ* and in real-time.
* Better assessment of the quality of marine waters in the “2 Seas” Region (English Channel – North Sea).



Focus on **phytoplankton** as it represents the basis of all food chains in the sea and reflects the environmental status and water quality with consequences in socio-economic issues and human health.
Species composition and relative abundance of algal groups are fundamental determinants of aquatic ecosystem structure and function.

Research Context

- Marine biodiversity as a key for goods and ecosystem services
- Fondamental knowledge (patrimony)
- Regime shifts in phytoplankton communities (natural vs anthropogenic)
- Phenology of phytoplankton blooms
- Occurrence of Harmful Algal Blooms
- Global change

Montoring Context

- The OSPAR strategies (1992) (Common Procedure – Eutrophication strategy)
- The Water Framework Directive (2000/60/EC)
- The Marine Strategy Framework Directives (2008/56/EC)

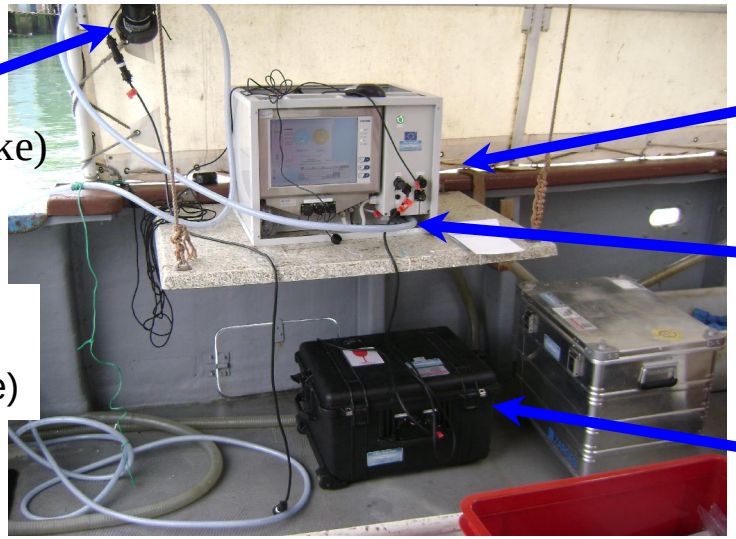
⇒ Reliable, cost-effective, (near) real-time information need for scientifics, regional managers and policy makers

DYMAPHY - Actions 1 & 3

Test and implementation of automated systems for water quality assessment

The Pocket Ferry Box on the RV “ Sepia II ” (CNRS/INSU)

Pump
(Water Intake)



The Pocket Ferry Box
(PFB, *4H-JENA*©)
on the RV « Sepia II »

Possibility to add a data Sonde on the water output (complementary parameters)

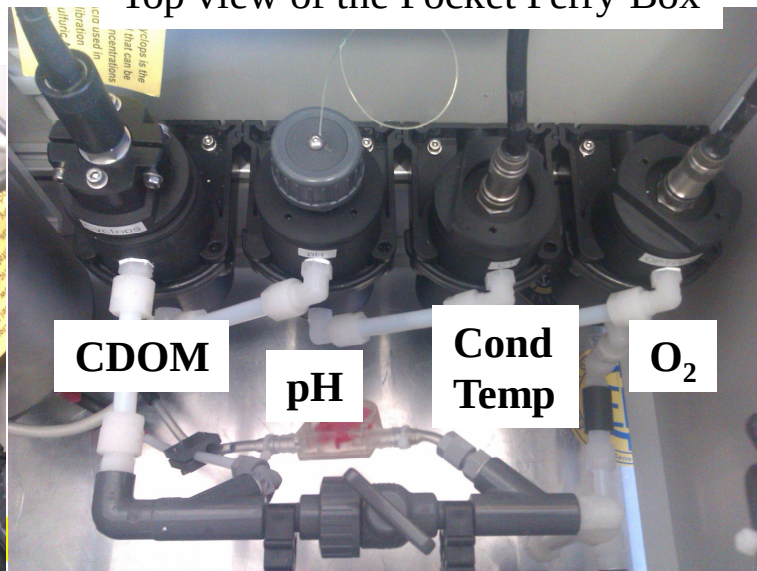
Battery

All « out of water » system
=> Easy access (maintenance)

Top view of the Pocket Ferry Box

Algae Online Analyzer
(AOA, *bbe*©)

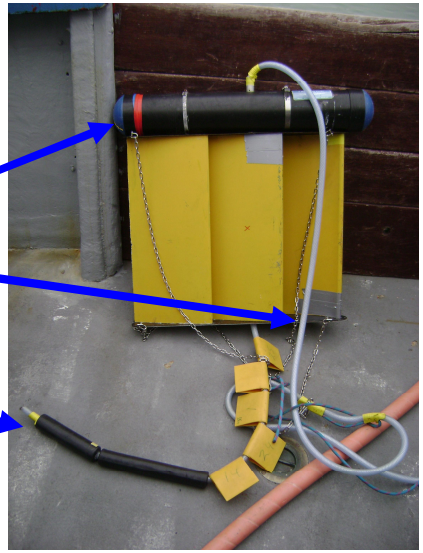
Top view of the Pocket Ferry Box



INTERREG IVA 2 Mers Seas Zeeën Crossborder Cooperation Programme 2007-2013
Part-financed by the European Regional Development Fund (ERDF)

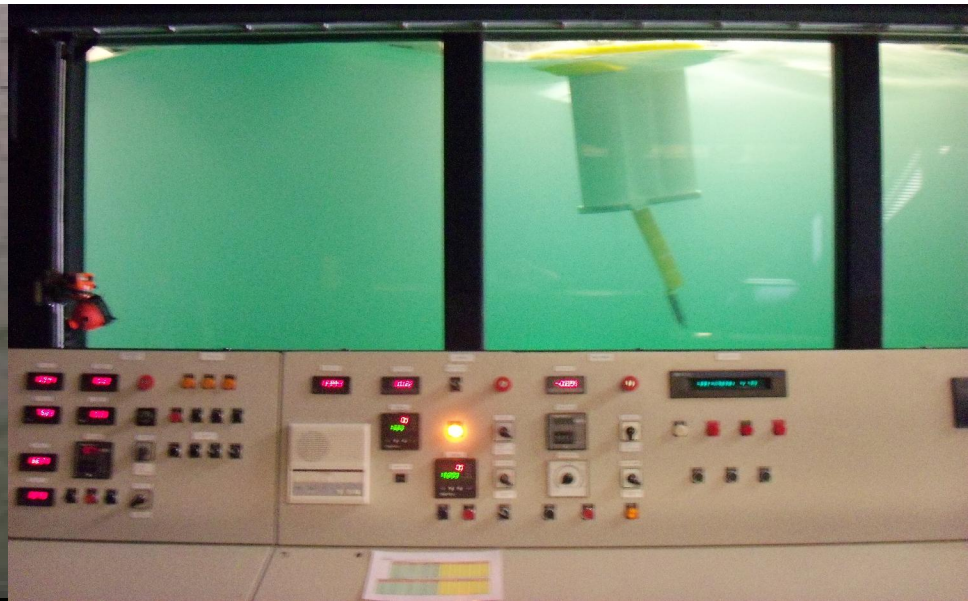
Development of the underwater sampling unit

(The DymaPhin = Dymaphy + Dolphin)



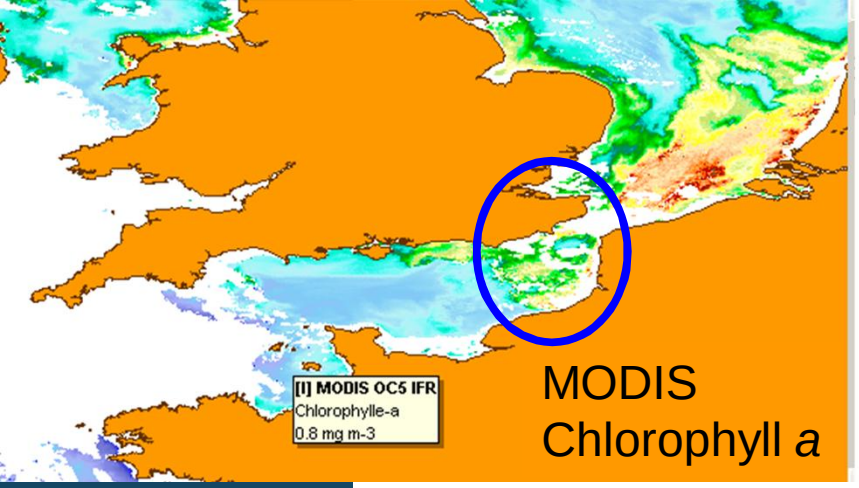
In situ test with the RV “Sepia II”

Test in the wave and current basin (Ifremer)



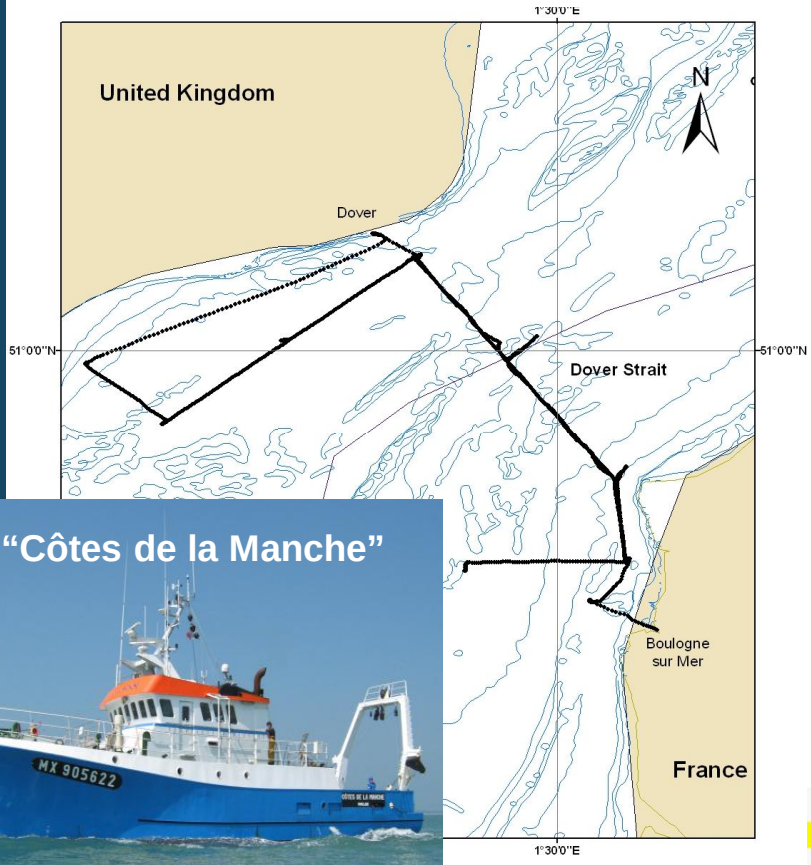
The Dymaphy campaign on the RV “Côtes de la Manche” in the English Channel

Towards a synoptic *in situ* view thanks to HFM

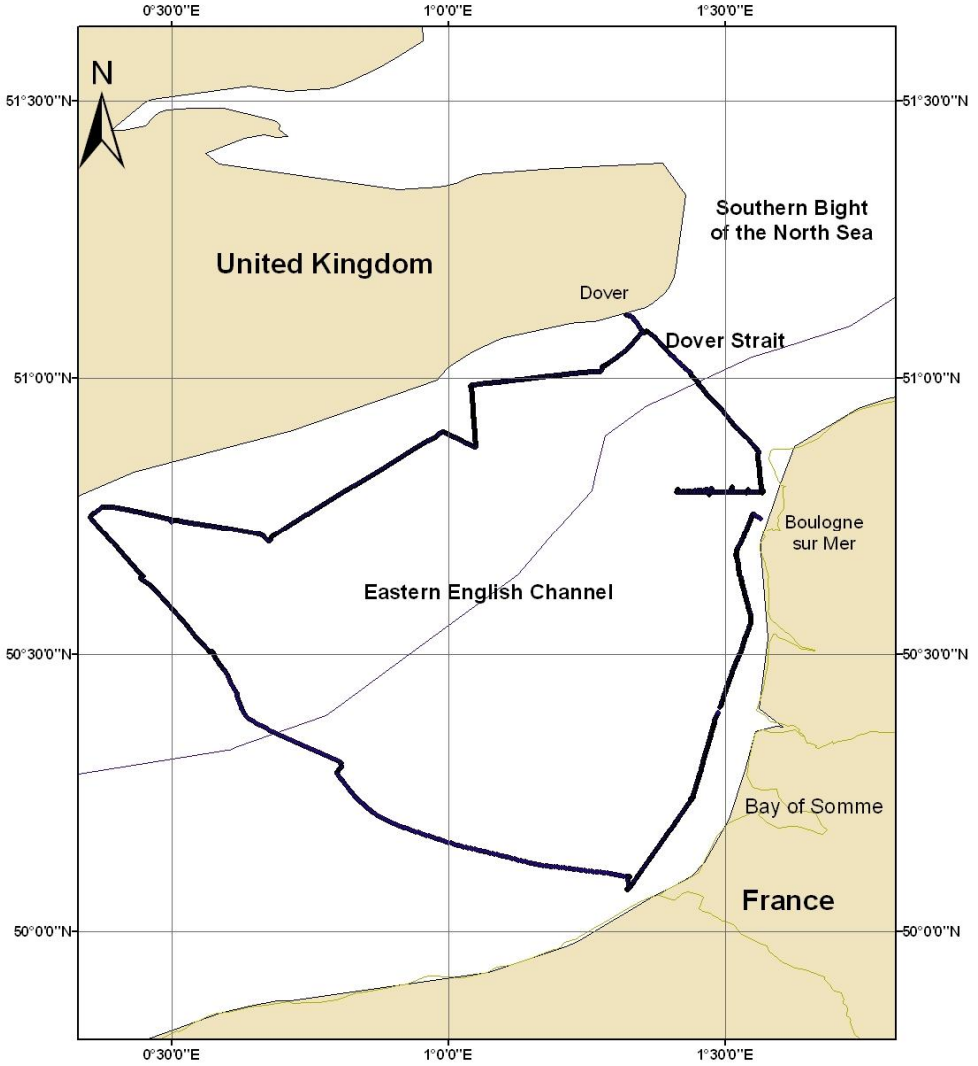


MODIS Chlorophyll *a*

Leg 1 – April 20-21, 2012

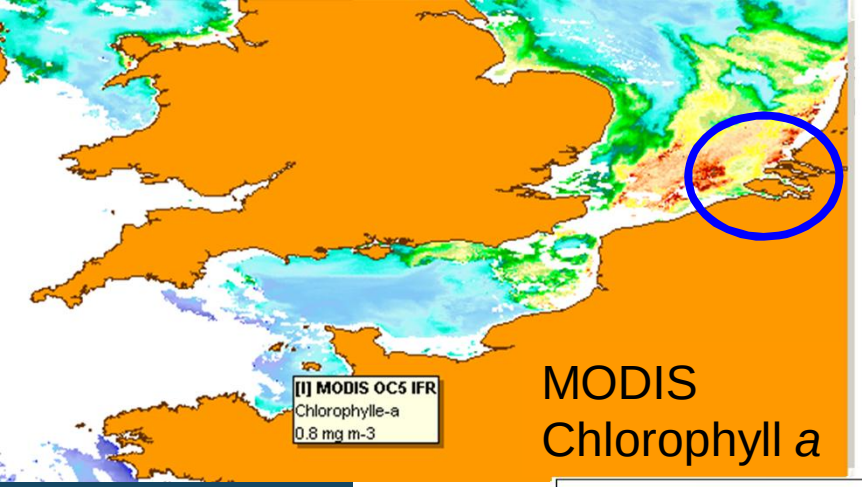


Leg 2 – April 27-29, 2012



The Dymaphy campaign on the RV “Argus” (RWS, NL) in the Dutch Estuaries

Towards a synoptic *in situ* view thanks to HFM



MODIS Chlorophyll *a*

September 10 – 14, 2012

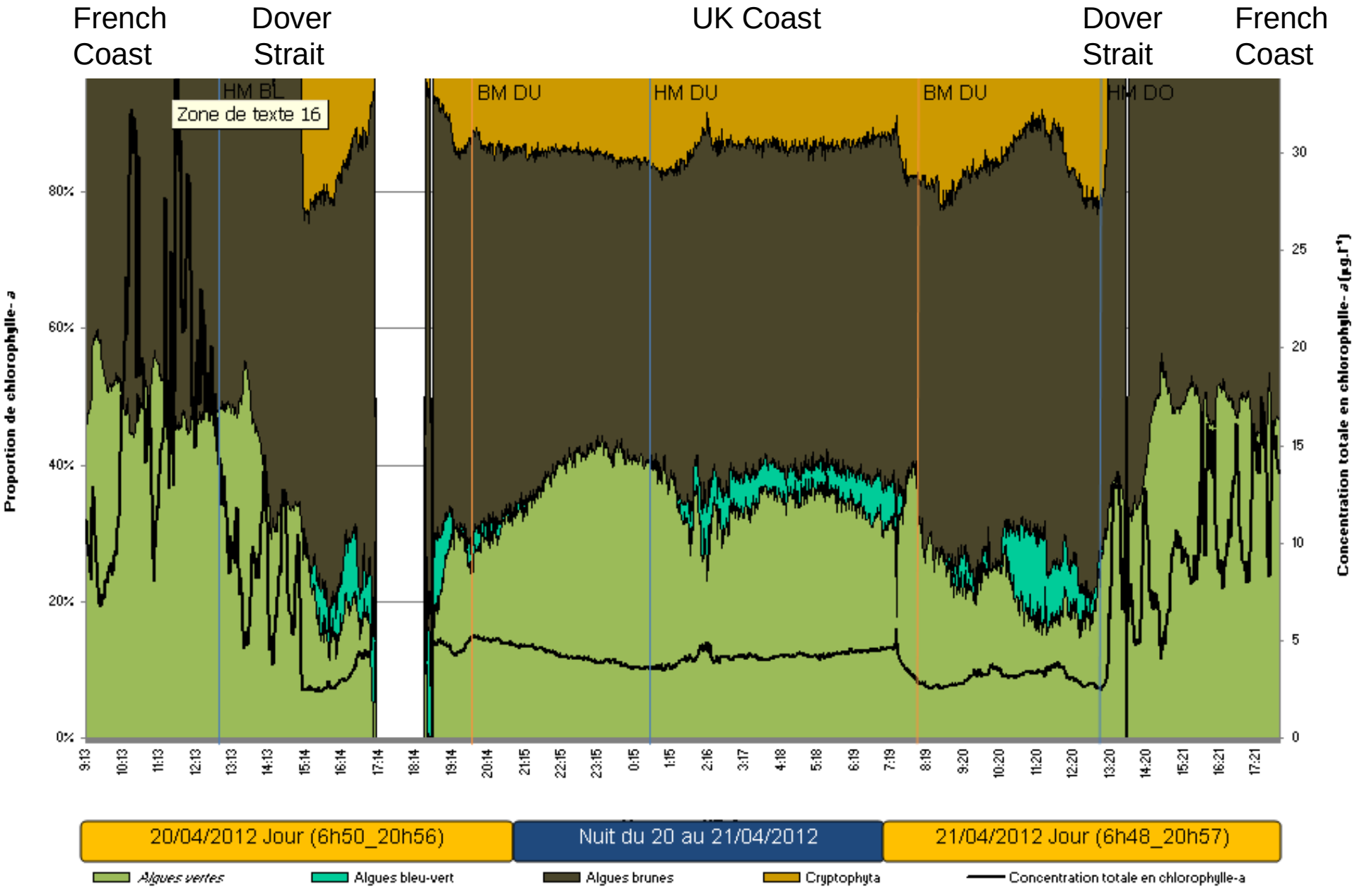
Locatienr.	Locatienaam	Omschrijving	X	Y	Watersysteem
1	VLISSBGRISSVH	Vlissingen Boei SSVH	28280	381900	Westerschelde
2	BORSSELE	Borssele	37415	383800	Westerschelde
3	TERNZBD0	Ternneuzen Boei 20	46290	374200	Westerschelde
4	HOEDKKKBI4	Hoedekenskerke Boei 4	53000	382800	Westerschelde
5	HANSWGL	Hanswaert Geul	59530	383900	Westerschelde
6	ZUIDERGAT	Zuidergat	60900	381195	Westerschelde
7	LDDSGT	Lodijkse Gat	67830	390230	Oosterschelde
8	Z-01	Zandmeer	49750	395917	Oosterschelde
9	Z13PE	Zijpe	65700	407000	Oosterschelde
10	OUWERKERK-MP16	Ouwerkerk	58430	403725	Oosterschelde
11	ZIERZDVL	Zierikzee De Val	51420	405600	Oosterschelde
12	WISSKKE	Wissenkerke	39540	402730	Oosterschelde
13	WALCRN2	Walcheren 2 km	17834	377180	Noordzee
14	VLAKTE VD RAAN	Vlakte van de Raan	60750	392714	Noordzee
15	WIELGN	Wielingen	138530	382049	Noordzee

Rijkswaterstaat
Ministerie van Verkeer en Waterstaat

Leg 1: 20-21/4/2012

Map of *in vivo* fluorescence intensity along the ship track

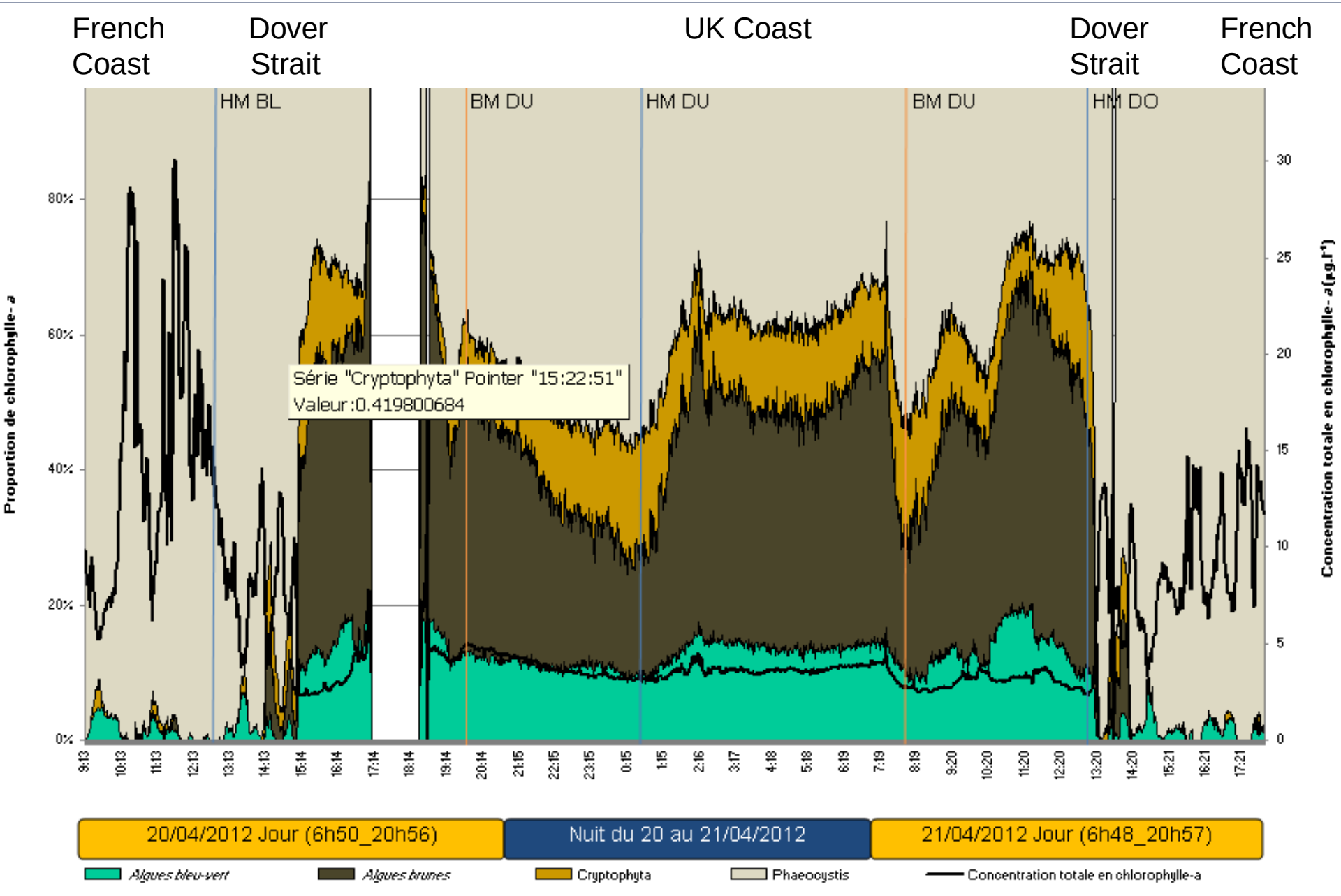
Original Fingerprints: Green / Blue Green / Crypto / Brown



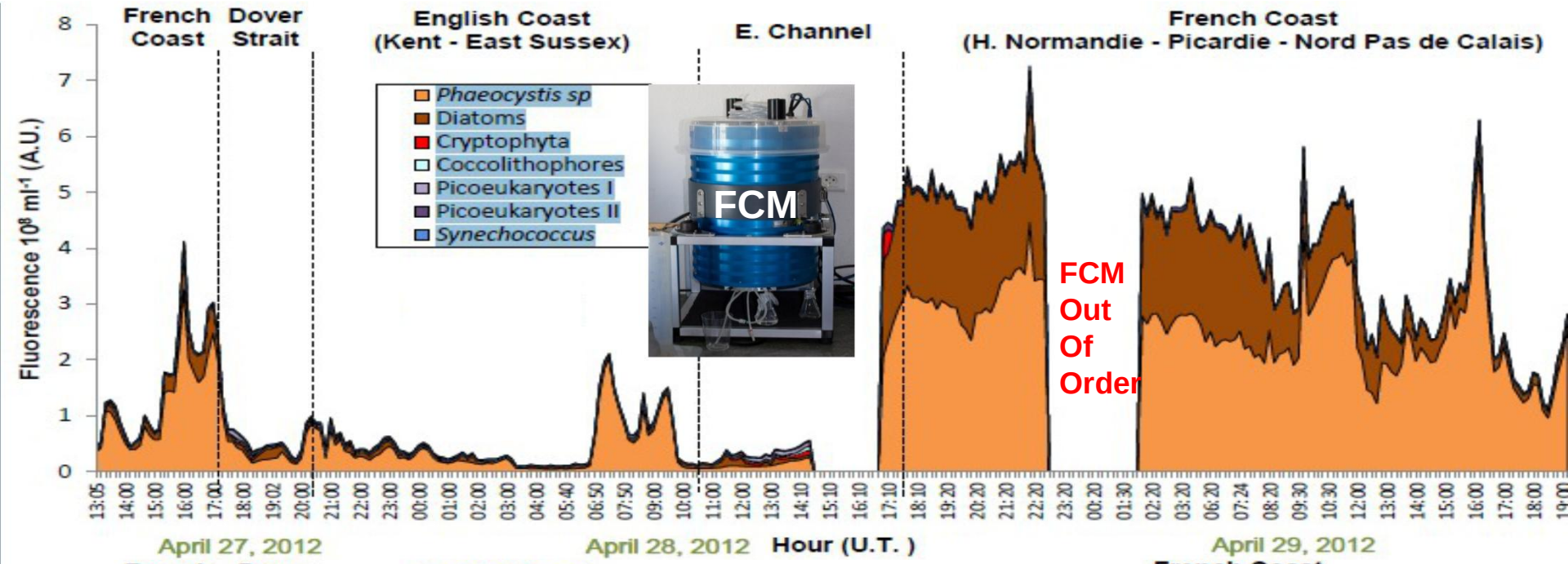
Leg 1: 20-21/4/2012

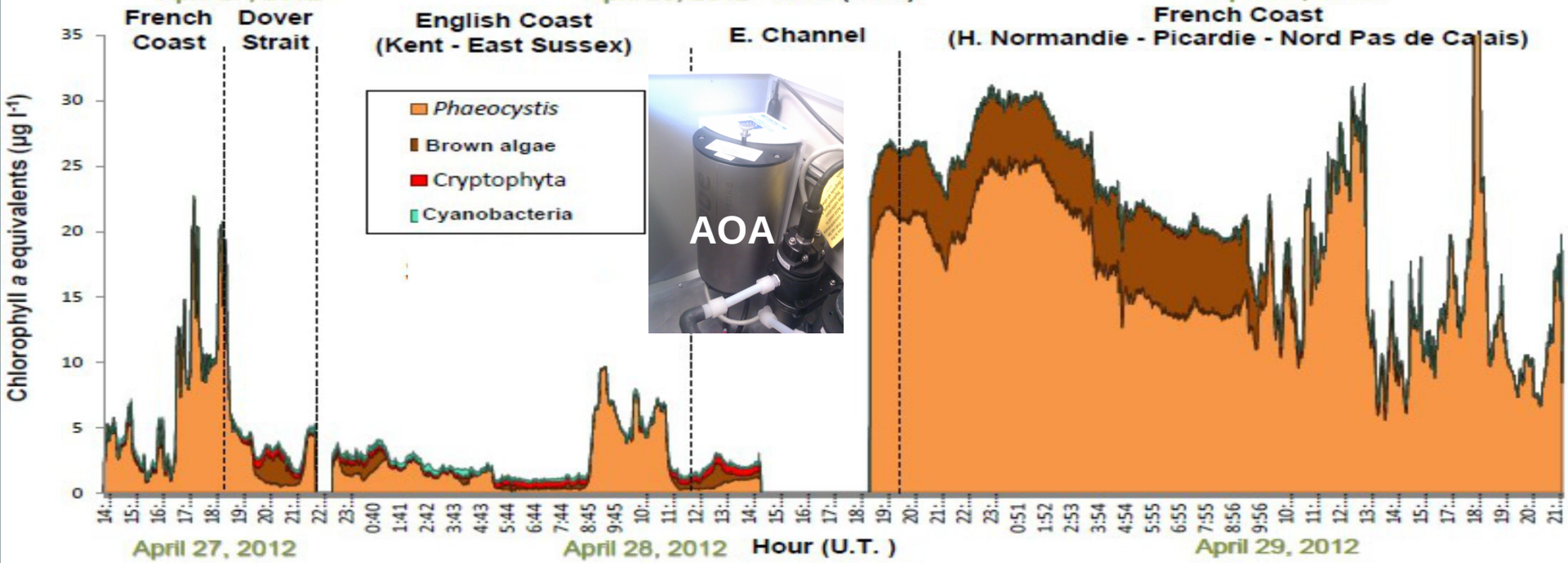
Map of *in vivo* fluorescence intensity along the ship track

Fingerprints: *Phaeocystis* / Blue Green / Brown / Cryptophyceae



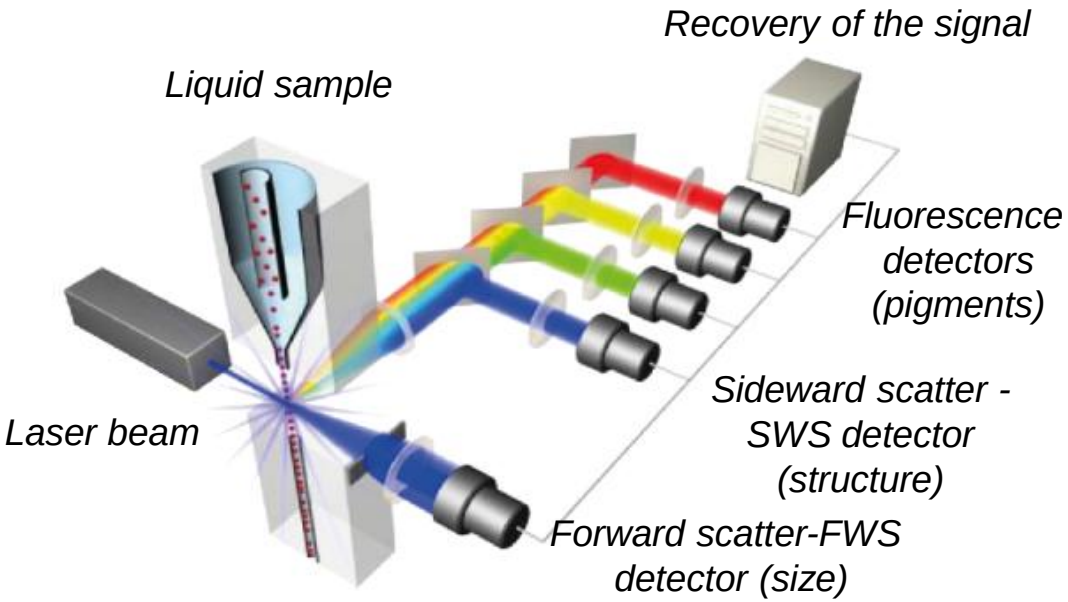
Leg 2: 27-29/4/2012 Flow Cytometry & Algae Online Analyser



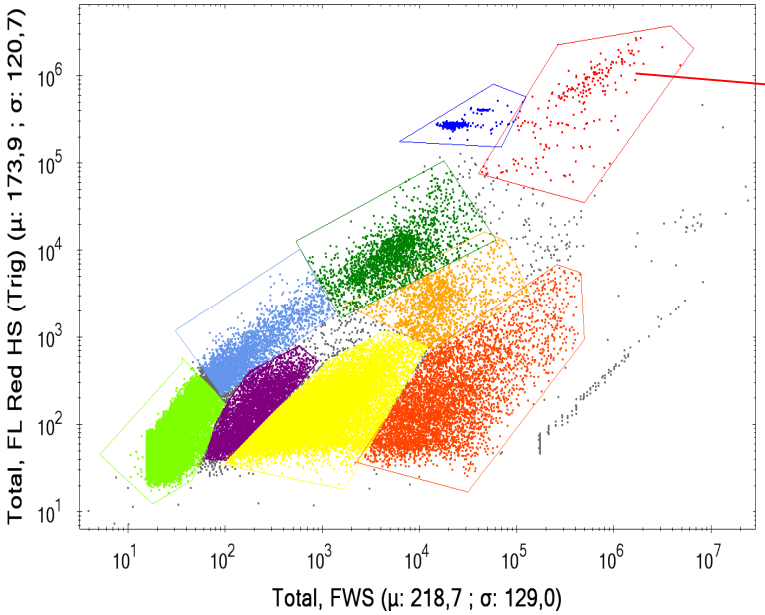


Flow Cytometry

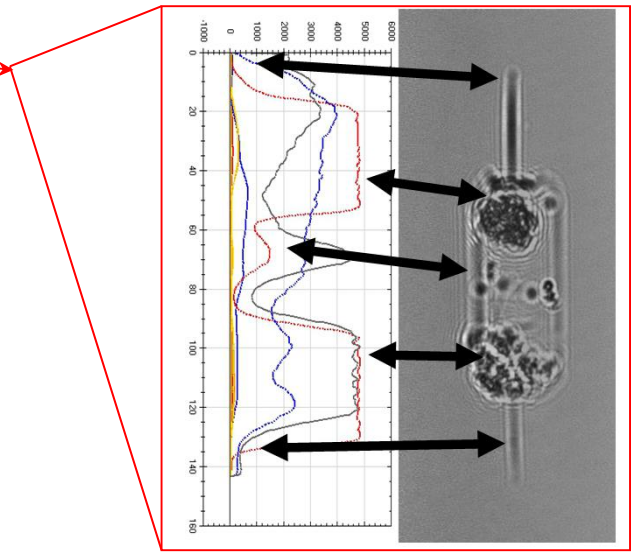
Single cell analysis
1 to 800 µm



Functional diagramme of the Flow Cytometer (CytoSense, CytoBuoy©)



Cytogramme (each cluster correspond to a phytoplankton group, each dot to a cell)



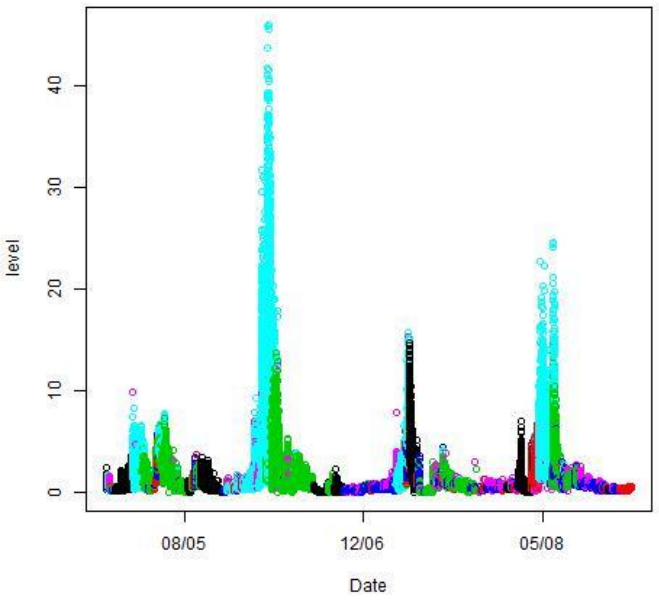
Optical profile and picture of one phytoplankton cell

INTERREG IVA 2 Mers Seas Zeeën Crossborder Cooperation Programme 2007-2013
Part-financed by the European Regional Development Fund (ERDF)

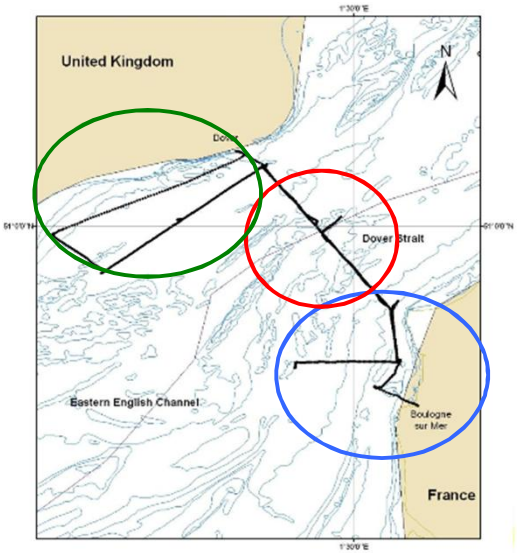
Improvement of HF data analysis

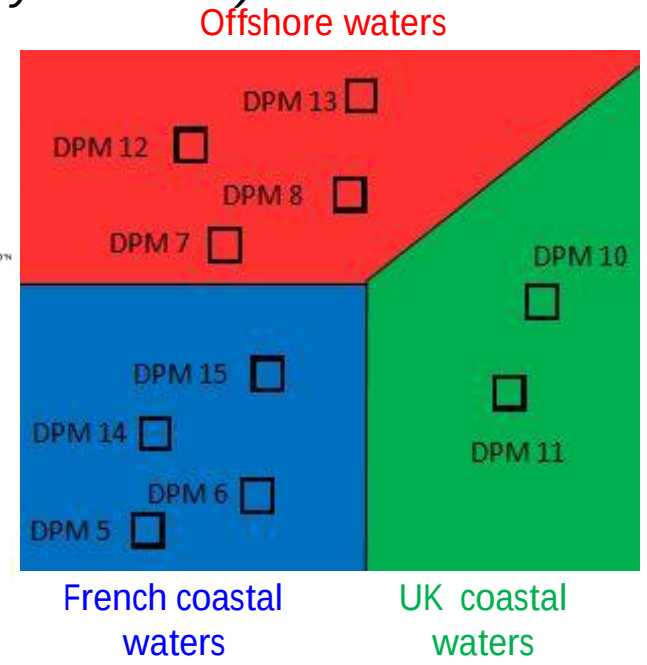
*See Poster: Detection of contrasted physico-chemical and biological environmental status using **unsupervised classification tools**.*
Rousseeuw Kévin, Lefebvre Alain, Caillault Emilie, Hamad Denis

A) Different clusters *i.e.* ecological status *(MAREL buoy data)*



B) Different clusters *i.e.* ecological regions *(Pocket Ferry Box data)*





Next step: system combining
K-means classifier (without *a priori* knowledge) with one Hidden Markov Model

This PhD work is funded by IFREMER and the Artois-Picardie Water Agency.

The IFREMER's Department REM and its unit Research & Technological Development have operational control for servicing the MAREL system.

Conclusions

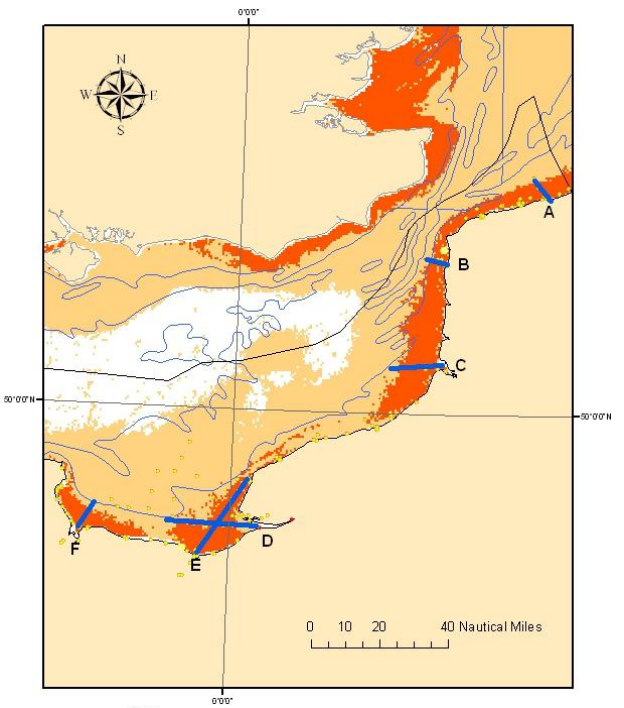
- HFM useful, otherwise essential, for monitoring of phytoplankton community composition and supporting physico-chemical parameters (WFD, MSFD, OSPAR,…).

- Useful to define, to adapt and to optimize the Sampling Strategy during a scientific cruise (to track the bloom and to target the collection of discrete calibration samples)

- Useful as an Early Warning System (HAB, direct and indirect effects of Eutrophication,…)

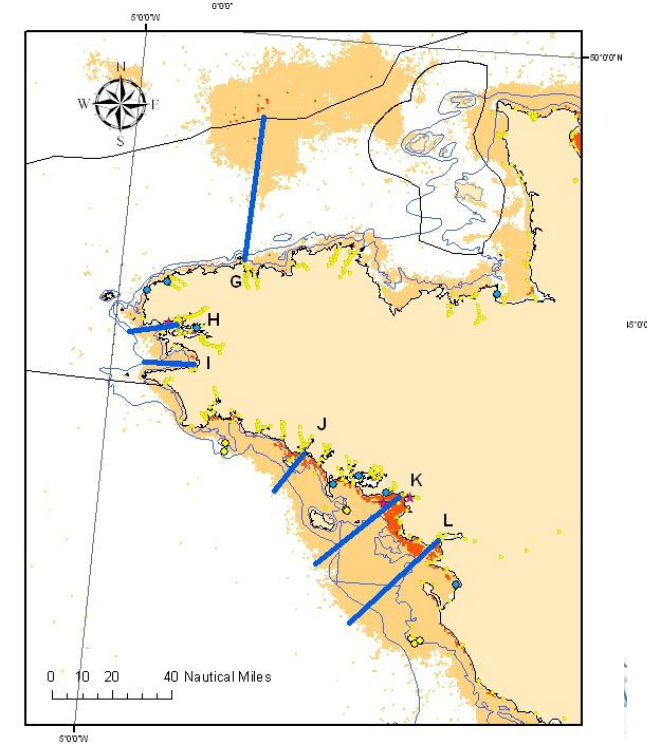
- Different objectives from research to management purposes => Need to couple systems (PFB + flow cytometry + PhytoPam, satellite imagery …)

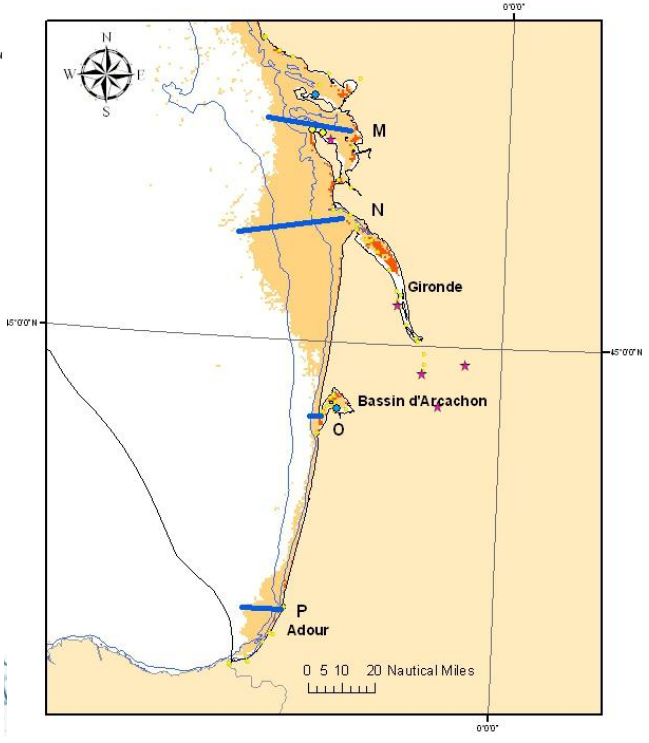
- Need to optimize HF data analysis methodologies (classification, modelling)

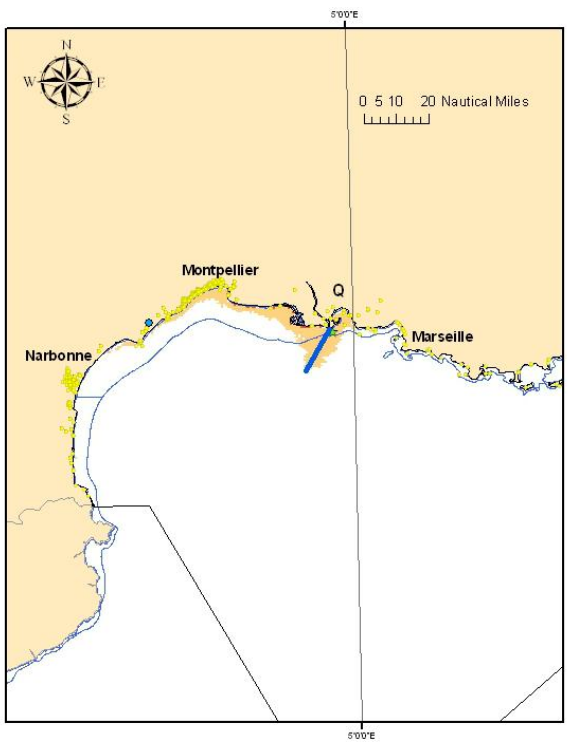


Short term perspective:

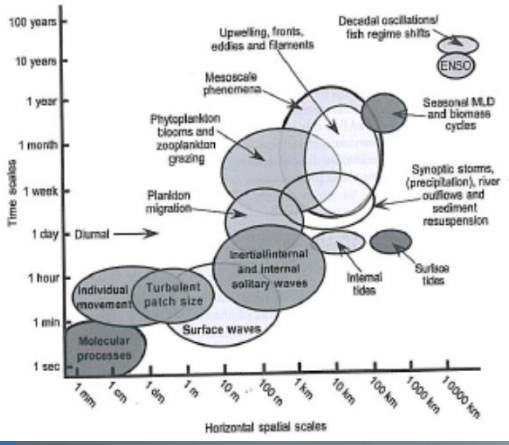
The Monitoring Programme of the **MSFD**:
Proposal for a scenario using HFM to cover the needs for the Descriptors Biodiversity, Invasive Species, Trophic Networks, Eutrophication for the 4 French marine sub-regions.

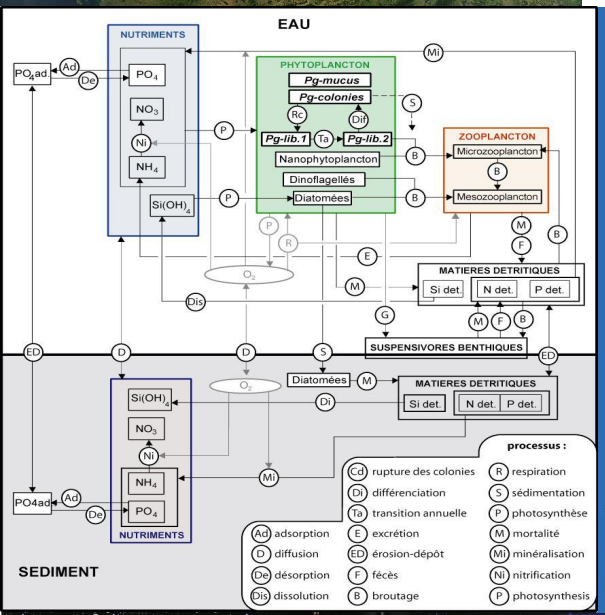


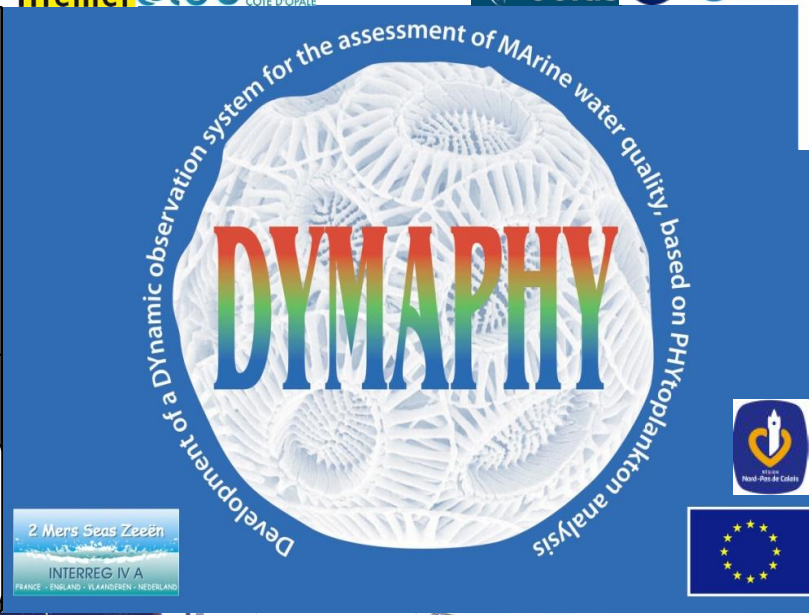


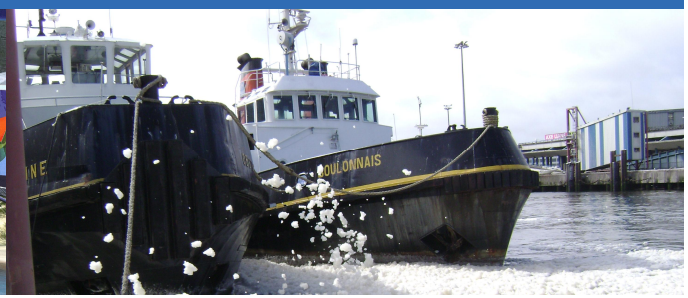


Thanks for your attention!









http://www.dymaphy.eu

Expected outcomes

Improve our ability to implement an ecosystem based approach to the management of human activities, to allow the sustainable development of the marine environment in the cross border area

Support the identification of new potential marine protected areas

Contribute to the definition of new international policy instruments.

Contribute to an integrated vision and consideration of the cross-border maritime dimension by sharing the results of the new monitoring strategies with stakeholders, environmental agencies and the public.

The Algae Online Analyzer (bbe)

- Fixed-wavelength spectral fluorometer
- LED centered at 470, 525, 570, 590 and 610 nm (+ CDOM 370 nm)
- Emission measured at 680 nm.
- Built-in data-analysis and reporting
- Specification of the library of spectral fluorescence (algal fingerprints) => Characterization of the phytoplankton community as spectral groups (as a preliminary taxonomic determination)
- Original fingerprints described in AOA parlance as Green, BlueGreen, Diatoms, Cryptophyceae

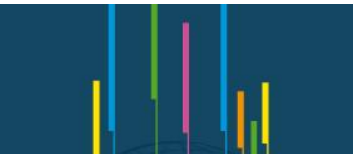
Main principle

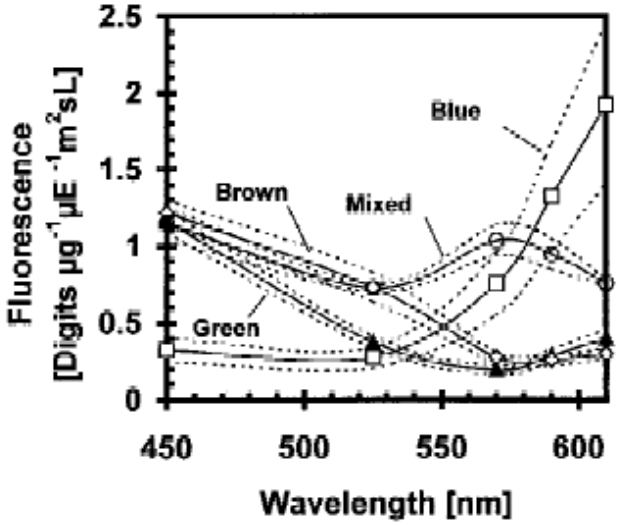
Shape of the spectral fluo signature

=> taxa discriminbation

Fluo intensity and the group-specific fluo/chl ratio

=> total phytopl biomass (chla)





Mean fluorescence-excitation probabilities for 4 spectral algal groups (norm spectra) (Source : Beutler et al., 2002)

Sampling frequency: 1 min. continuous sampling mode / Spatial resolution approx. 0.1 nm

System coupled with a YSI 6600 water-quality probe connected with a flow-through system (for comparison / added parameters) and with a flow cytometer (sampling frequency: 10 min.)

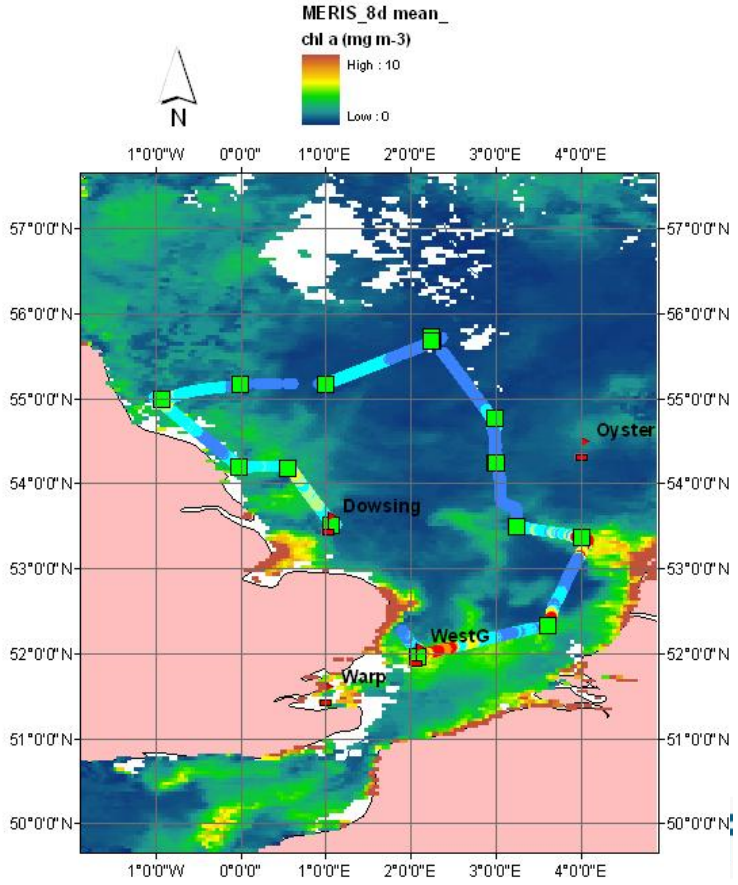
Discrete samples (water quality, phytoplancton, HPLC, spectro, fluo, flow cytometry)

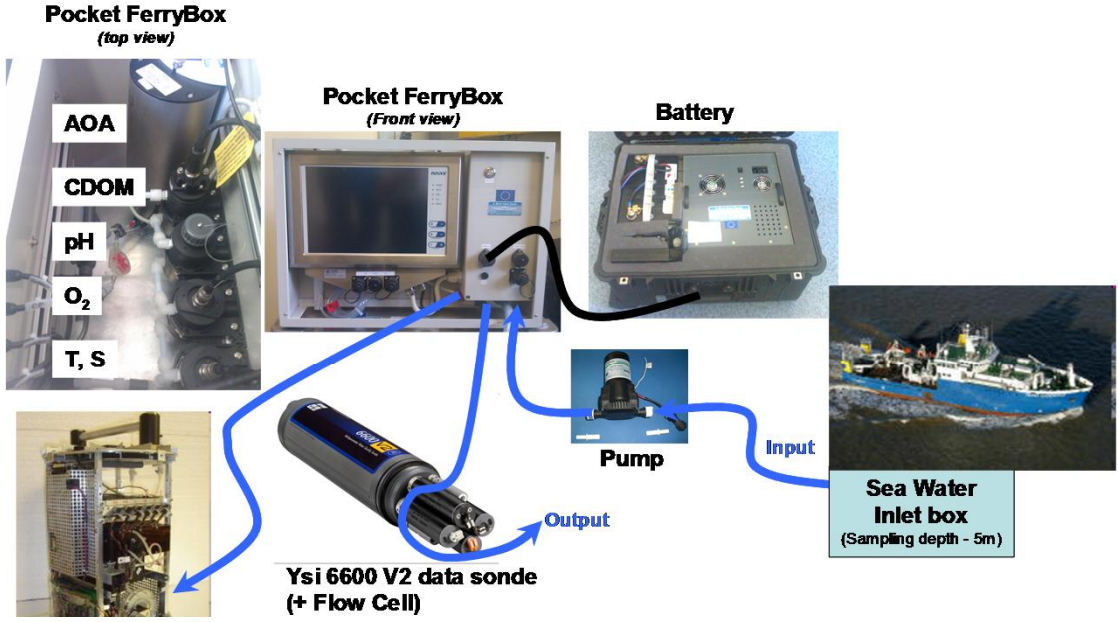
The Protool / Dymaphy campaign
7 – 13th May 2011

The **Pocket Ferry Box** (4H-JENA©) coupled with a multiple-fixed wavelength **spectral fluorometer** (AOA, bbe©), an **Ysi data Sonde** and a **Cytosense flow cytometer** (Cytobuoy ©) was implemented during the scientific cruise PROTOOL (+ PAM, Fastracka, PSI fluorometers, radiocarbon) on the RV « Cefas Endeavour ».

= > Test of the reliability of the system

=> Exchanges on « problems » or update needs => training sessions with 4H-JENA, BBE

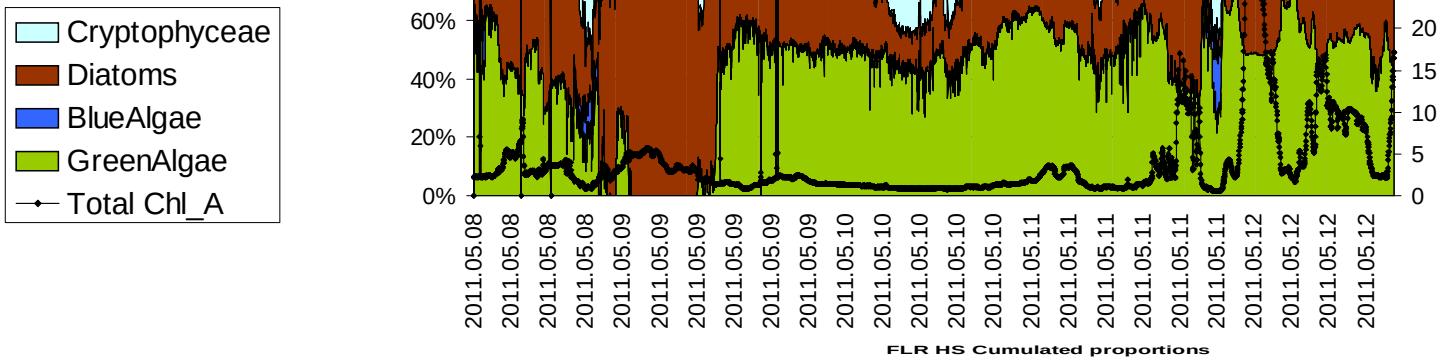




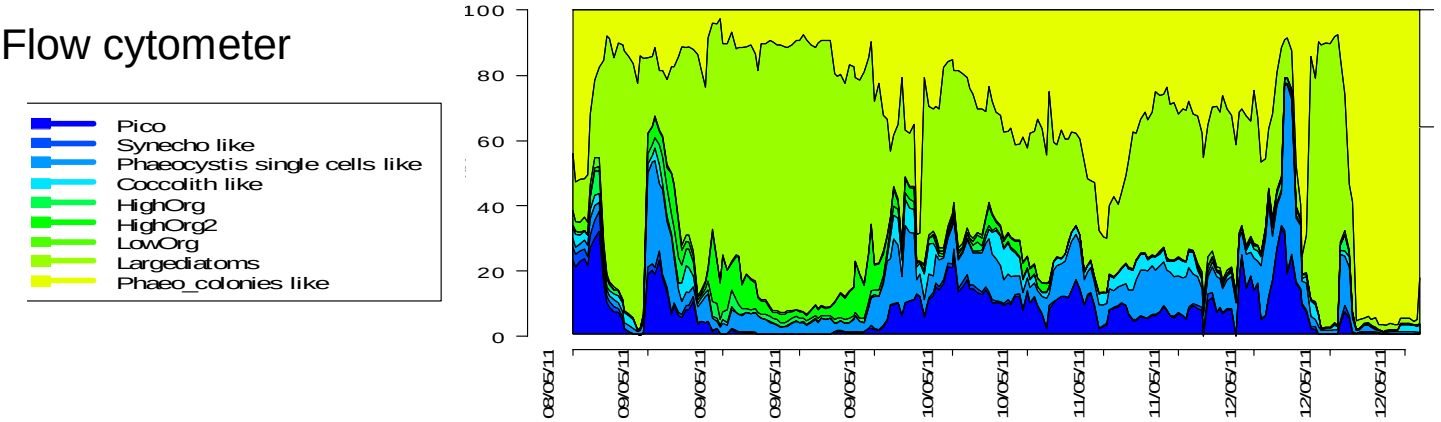
The Protool / Dymaphy campaign
7 – 13th May 2011

England East coast | Central North Sea | Dutch coastal Outer Thames

Algae Online Analyser



Flow cytometer



A total of 9 groups were distinguished by flow cytometry based on their optical properties.

We show **high spatial temporal changes in the phytoplankton community** which could hardly be detected by employing discrete sampling strategies. High frequency measurements (HFM) allows to identify precisely, in time and space, the **contrasted environment** sampled. Moreover within a given environment, HFM highlight **changes in the composition of the phytoplankton community.**

Main problem :
Before / after cruise control of the parameters of fit
⇒ Control of the fingerprints according to references (discrete samples)

Parameters of fit (2011-03-24)

Offsets LEDs — filtrated water — distilled water

Hold for Refit | Cancel

	1	2	3	4	5	UV
F	11.093	3.3564	3.4712	3.3331	9.3363	16.953
F0						—
FM						—

global corr. factor 1

Algae classes

fit	type	name	factor LEDs 1	2	3	4	5	UV	standard deviation of LEDs 1	2	3	4	5	UV
☑	A	Green Algae	1.313	0.249	0.417	0.308	2.417	2.793	0.1	0.1	0.1	0.1	0.1	0.1
☑	A	Bluegreen	0.955	1.041	2.217	1.431	0.237	1.259	0.1	0.1	0.1	0.1	0.1	0.1
☑	A	Diatoms	7.149	0.794	0.746	0.633	6.972	6.647	0.1	0.1	0.1	0.1	0.1	0.1
☑	A	Cryptophyta	4.115	1.578	1.056	1.202	3.129	3.149	0.1	0.1	0.1	0.1	0.1	0.1
☐	A	Phaeocystis	1	1	1	1	1	1	0.1	0.1	0.1	0.1	0.1	0.1
☐	A	Pseudonitzschia	4.49748	0.50878	0.48567	0.45056	4.50818	1.87941	0.1	0.1	0.1	0.1	0.1	0.1
☐	Y	#6	1	1	1	1	1	1	0.1	0.1	0.1	0.1	0.1	0.1
☑	Y	Yellow substances	3.165	0.298	0.13	0.201	4.574	15.185	0.1	0.1	0.1	0.1	0.1	0.1

Original Fingerprints

Implementation of new fingerprints : *Pseudonitzschia*, *Phaeocystis*, *Isochrysis*

Next steps (*ongoing analysis*)

- Verification of phytoplankton community composition with those from microscopy (Utermöhl 1958)

- Compare estimates of biomass with HPLC values and variations
  (effect of light intensity and nutrient availability on fluo quenching)

(general assumption = poor prediction of biomass by the AOA but good job in its characterization of overall trends in phytopl comm composition)

- Compare estimates of taxonomic structures with those from HPLC-derived marker pigment by ChemTax

- Relations of the highlighted patterns with physico-chemical supporting factors
  (nutrients, turbidity, …)

Recommendations

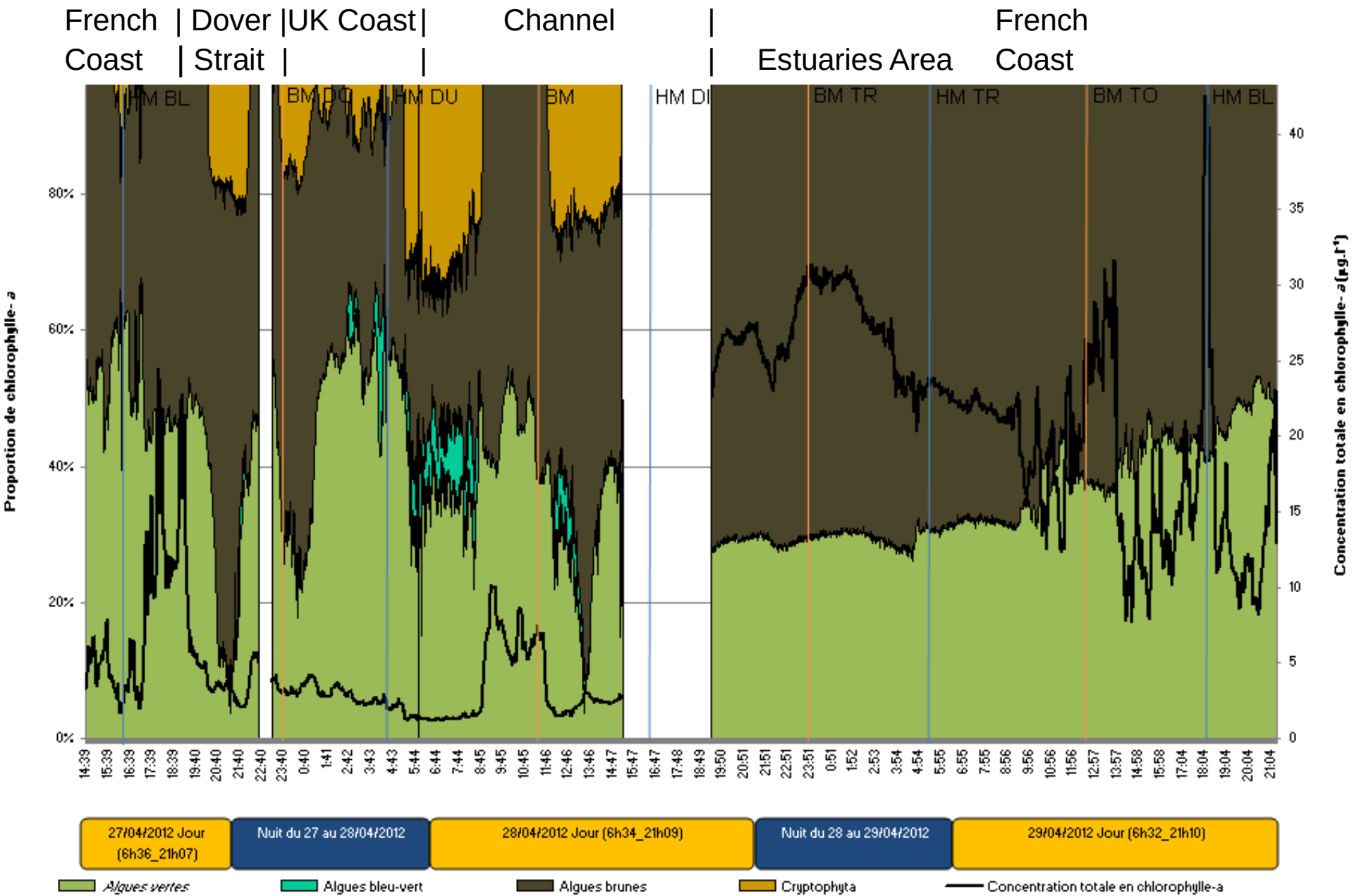
- Frequent calibration by discrete sampling collection

- AOA : Calibration/Fingerprint with mixture of species (not with a single sp.) representative of the ecoregion of interest to avoid mis-classification, then over-estimation of contribution

Leg 2: 27-29/4/2012

Map of *in vivo* fluorescence intensity along the ship track

Original Fingerprints



Leg 2: 27-29/4/2012

Map of *in vivo* fluorescence intensity along the ship track

Fingerprints Blue Green / Brown / Cryptophyceae / *Phaeocystis*