

Banyuls, septembre 2015



Mediterranean Institute
of Oceanography



PHYTOPLANKTON SOUS INFLUENCES: DYNAMIQUE À ÉCHELLES VARIABLES

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...et tous nos collaborateurs (MIO, LOG, LOV-OOV, Roscoff, I2M, SBR, etc.)**

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<https://precym.mio.univ-amu.fr/>



LE PHYTOPLANCTON : IMPORTANCE ET COMPLEXITÉ

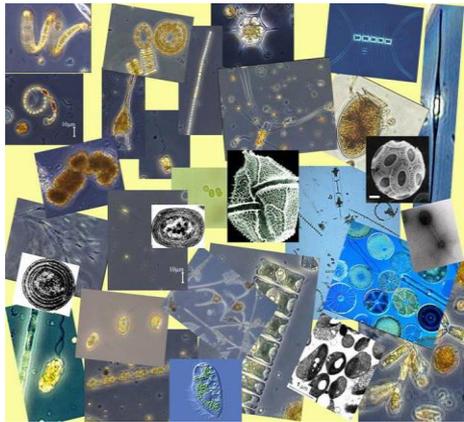


Importance :

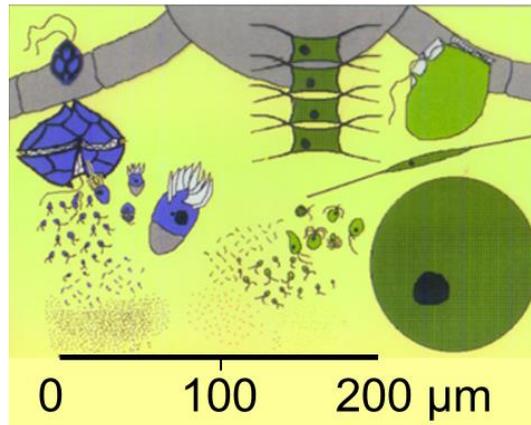
- Fonctionnement de l'Ecosystème de la planète
- Micro-organismes (unicellulaires)
- Dynamique rapide (jour)

Complexité:

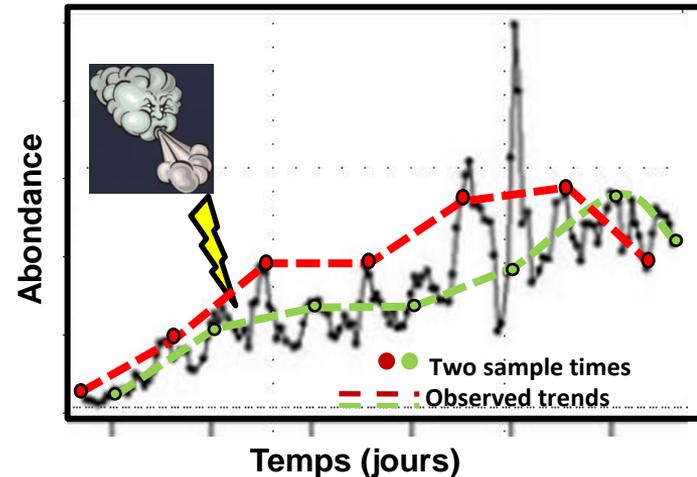
Diversité



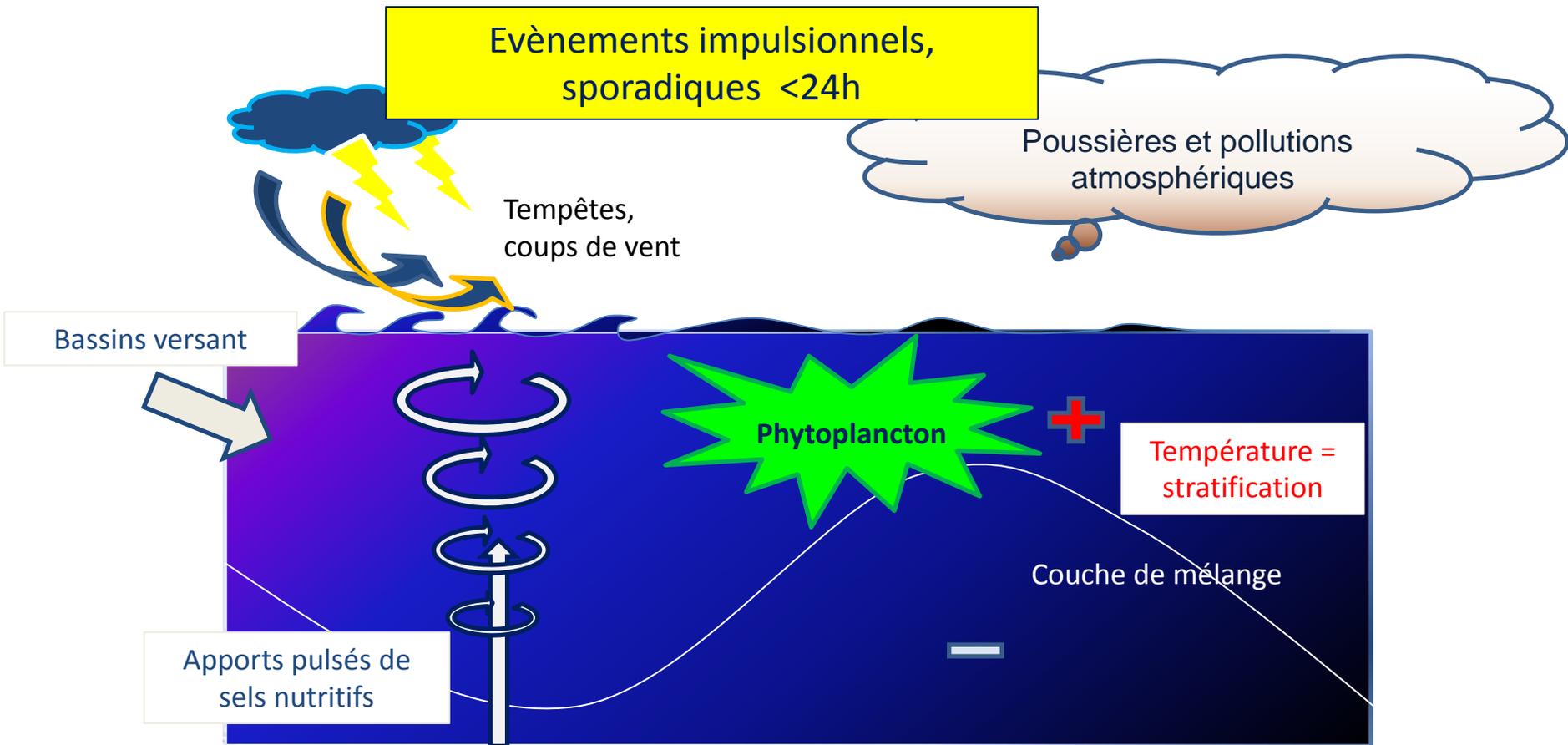
Grande gamme de tailles/abondances



Dynamique



DYNAMIQUE DU PHYTOPLANCTON : INFLUENCES DES VARIATIONS PHYSIQUES DU MILIEU



- Une variation de $\pm 10\%$ des coups de Mistral peuvent engendrer 54% de variabilité sur la production annuelle en mer Ligurienne (Lacroix & Nival, 1998)
- Des tempêtes hivernales en mer des Sargasses peuvent induire des pics de croissance qui sont à l'origine d'exportation de matière de l'ordre de 20% de l'exportation annuelle (Lomas *et al.* 2009).

AUTOMATED HIGH FREQUENCY IN-SITU (SUBMERSIBLE OR NOT) FLOW CYTOMETERS

➔ Fluorescence and light scatter intensities recorded for each particle (cell)

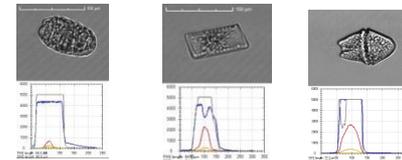
Imaging FlowCytobot
([IFCB](#))
McLane Research Laboratories



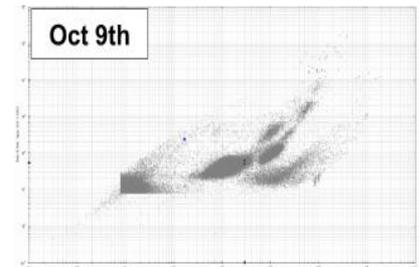
(Sosik et al.)

Images

Cytosense flow
cytometer
Cytobuoy b.v.

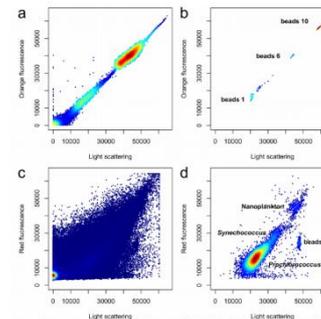


Optical pulses + Image in flow



(Thyssen et al.; Dugenne et al.,
Pommati et al.; Artigas et al., etc.)

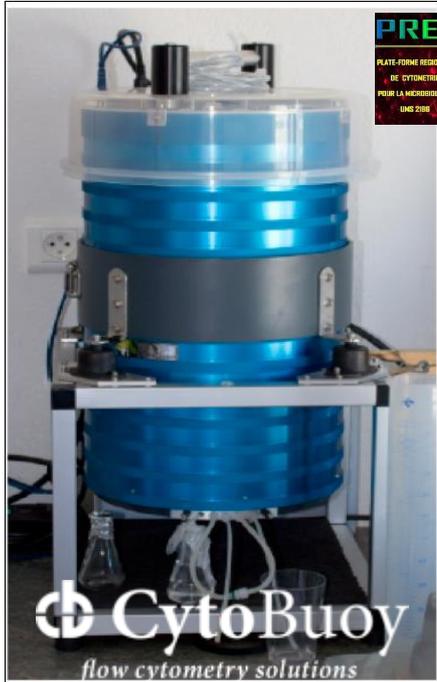
SeaFlow cytometer
School of Oceanography,
University of Washington



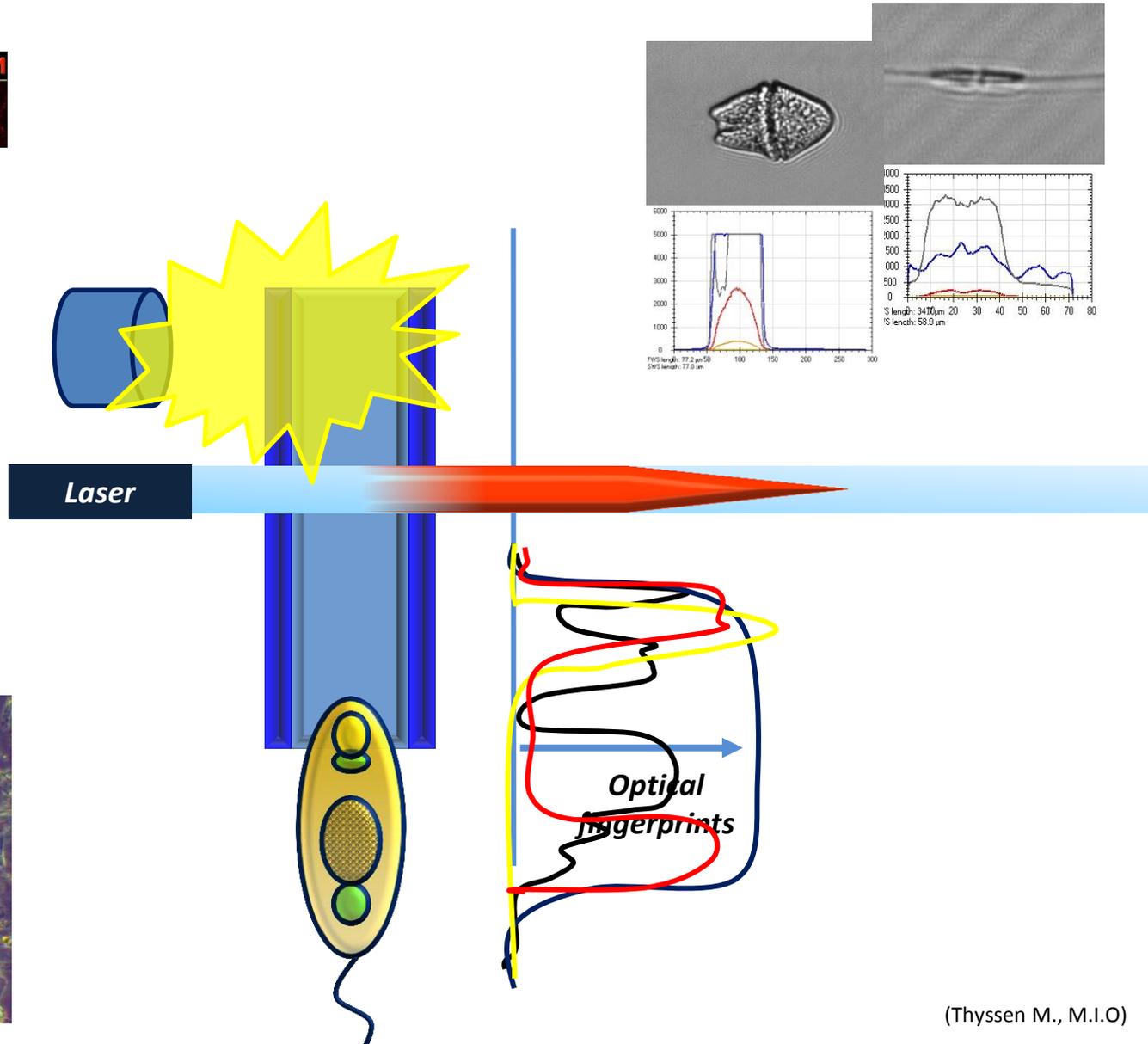
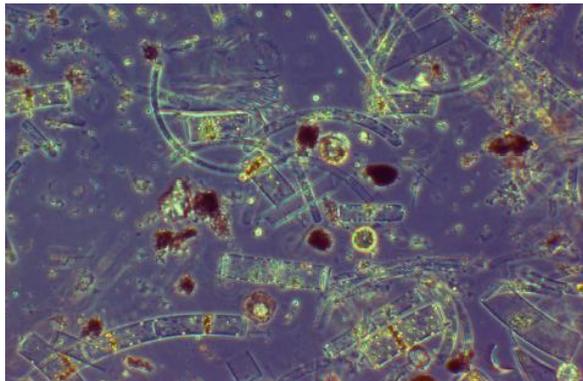
**Continuous, real-time
shipboard observations
of phytoplankton cells**

(Ribalet et al.)

CAPTEUR INNOVANT ÉPROUVÉ: CYTOMÈTRE EN FLUX AUTOMATISÉ CYTOSENSE (CYTOBUOY)

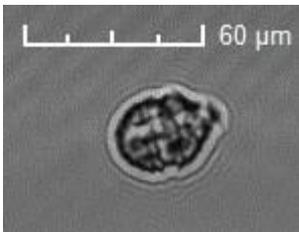


+

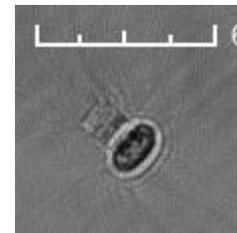
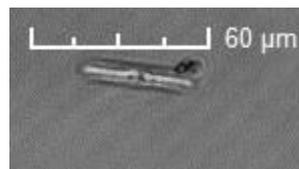
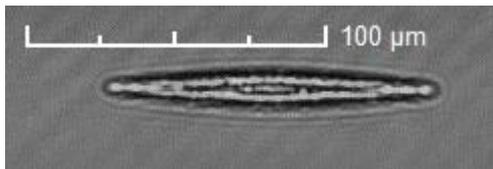


Exemples de photos prises par le cytomètre automatisé

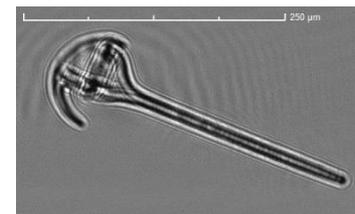
Scrippsiella



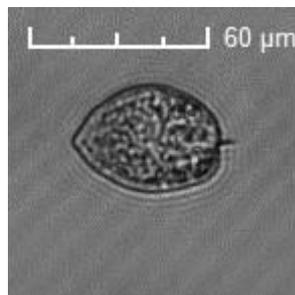
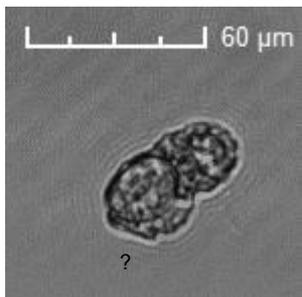
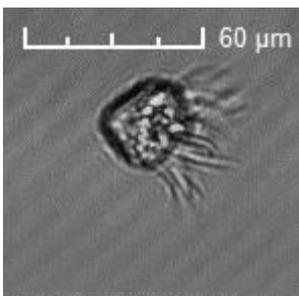
Diatoms



Chaetoceros
With an auxospore...

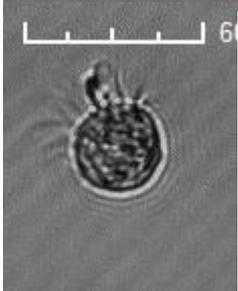
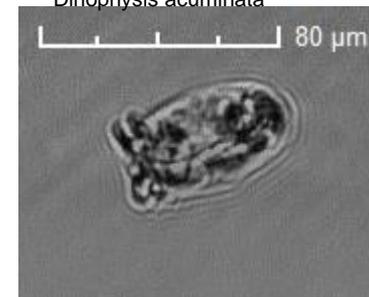


Ciliates

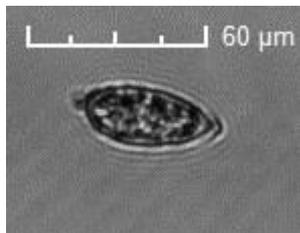
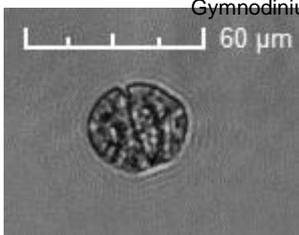


Prorocentrum micans

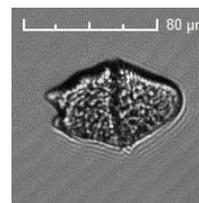
Dinophysis acuminata



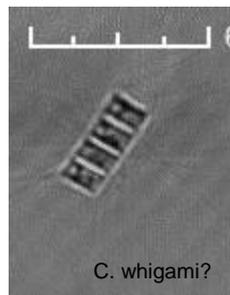
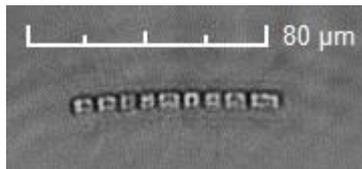
Gymnodinium?



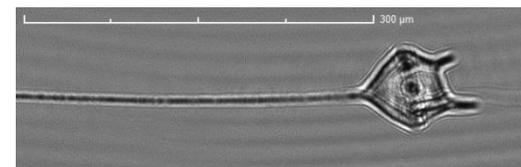
Prorocentrum ?



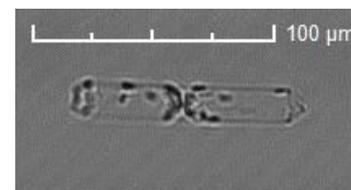
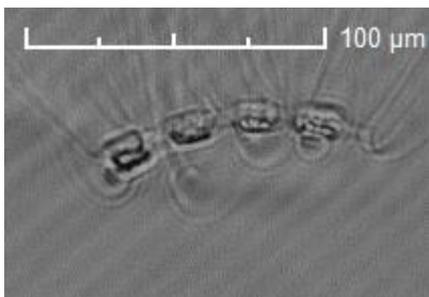
Chaetoceros spp.



C. whigami?

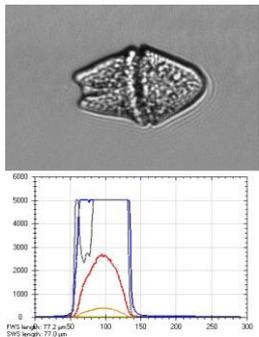


C. Curvisetus or pseudocurvisetus

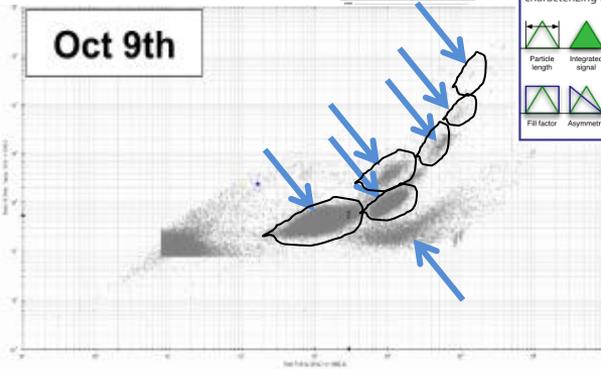


DE LA CELLULE À LA COMMUNAUTÉ PHYTOPLANCTONIQUE ET À SA DYNAMIQUE IN SITU

Cytométrie en flux automatisée avec prise d'images



Chlorophyll. Fluo. (au)



Forward scatter - Size (au)

PARAMETERIZING
The pulse profiles are parameterized by a set of eight parameters for each channel. These contain 2 absolute entities and 6 entities characterizing the profile shape.

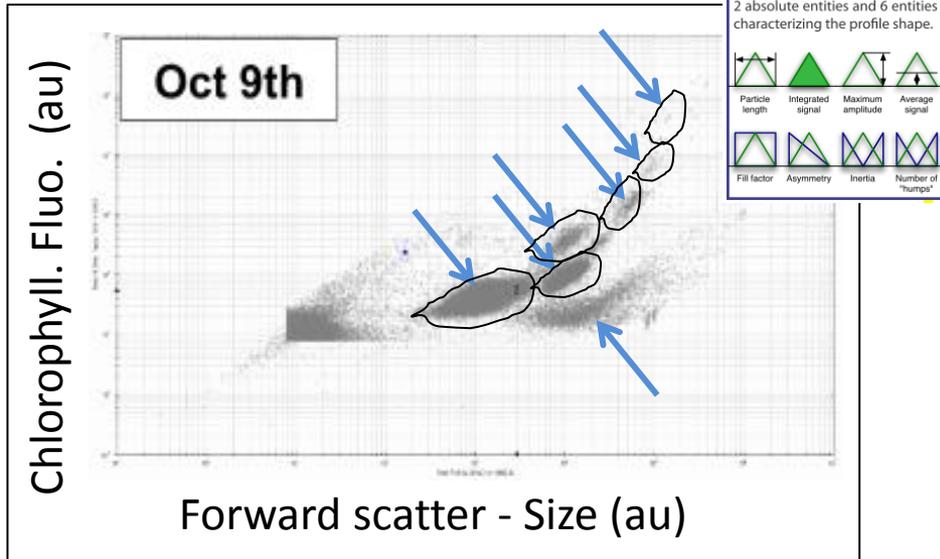
Particle length	Integrated signal	Maximum amplitude	Average signal
Fill factor	Asymmetry	Inertia	Number of "humps"

Analyse à l'échelle individuelle de la structure des communautés du pico- au microphytoplancton



ANALYSE DE DONNÉES POUR CHAQUE ÉCHANTILLON

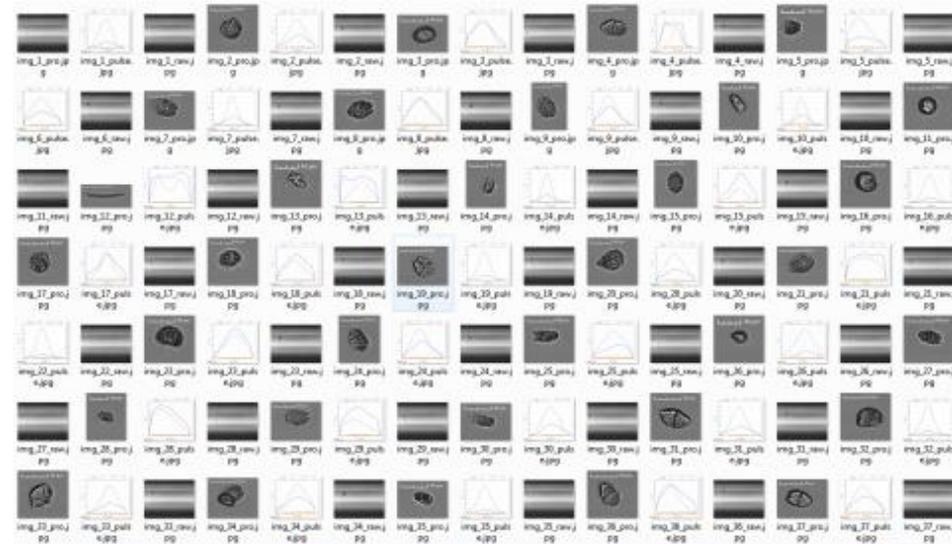
Groupes cytométriques (ataxonomique)



Abundances, average fluorescence and scatter intensities per cell:

→ Chla content, cell size, biomass

Images (taxonomique?)

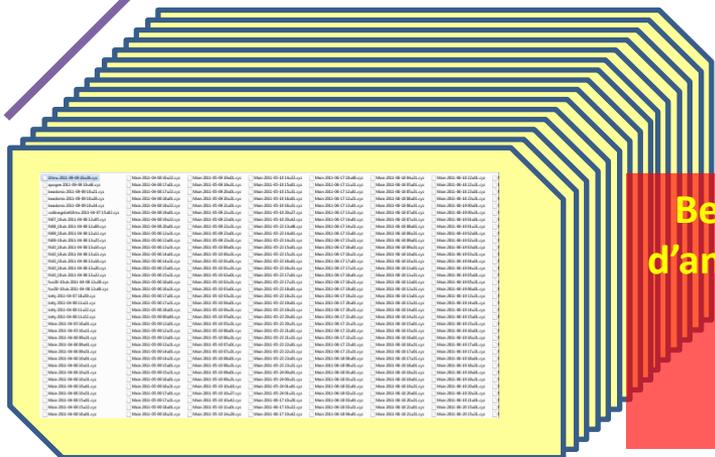


Pictures of the cells

ANALYSE DE DONNÉES POUR L'ENSEMBLE DES ÉCHANTILLONS

Strategie :
Haute fréquence
(plusieurs fois/h)

Time / space



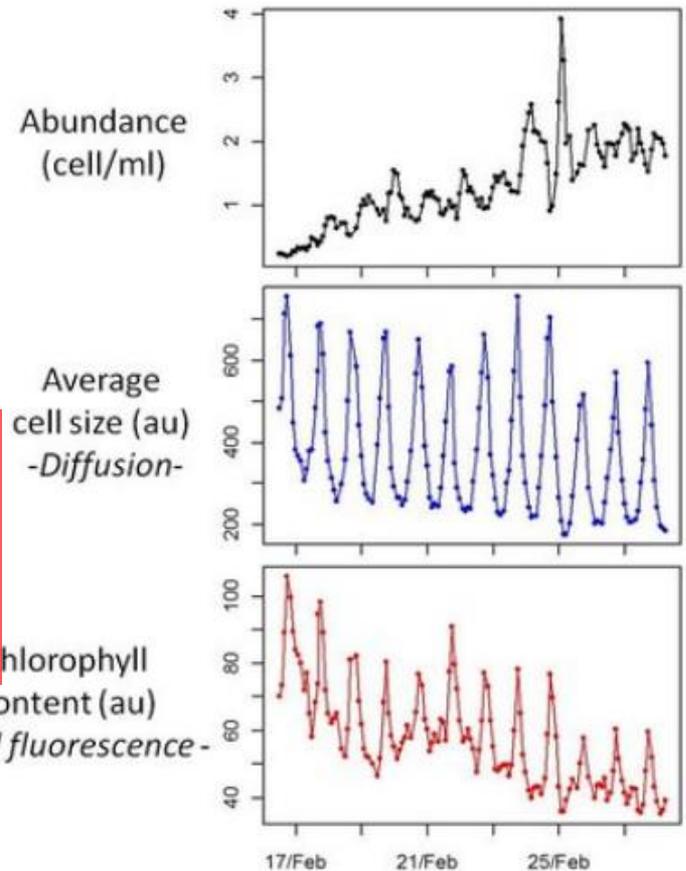
**Besoins de méthodes
d'analyses automatisées
(clustering)
+
Data mining**

**Many samples collected
(several per hour)**

(Cytometer programmed, remotely operated)

On a Buoy (EOL), on a Ferry (LEFE - Cel2sat),
a research vessel (DEWEX), or in a lab (EC2CO-MISE)

Dynamique des groupes



Project CNRS EC2CO-DRIL « Mise » (P.I. G. Grégori)

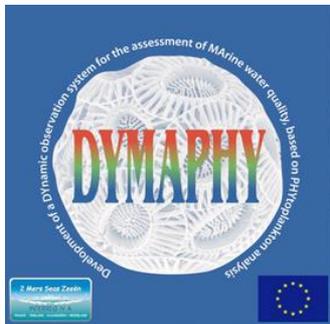
STANDARDISATION ET AUTOMATISATION DU TRAITEMENT DES DONNÉES

Association of traditional and such innovative approaches :

- Need for developing standardised procedures to measure phytoplankton diversity, abundance, and biomass
- and to perform better data analysis automation

DYMAPHY European Project (INTERREG IV A “2 Mers Seas Zeeën” project)

- DYMAPHY sets out to improve the assessment of the quality of marine waters in the 2 Seas (English Channel and Manche) area by using the phytoplankton as indicator. This INTERREG IV A “2 Mers Seas Zeeën” project



— DYMAPHY Partners:

- Université du Littoral Côte d'Opale (Dunkerque - Wimereux, France) **(PI: F. Artigas)**
- Cefas (Suffolk, England)
- Rijkswaterstaat (Zeeland, Netherlands)
- Ifremer (Boulogne sur Mer, France)
- Université Lille 1 (Villeneuve d'Ascq - Wimereux, France)
- CNRS (Lille - Wimereux, France)

NECESSITÉ DE METHODES AUTOMATISÉES DE TRAITEMENT DES DONNÉES (DÉTOURAGE DES GROUPES, COMPTAGE, STATS, TENDANCES...)

- A.Malkassian - Classification tools for flow cytometry phytoplankton monitoring (MIO)
- Guillaume Wacquet - Constrained Spectral Clustering Algorithm FCM application (ULCO/ LISIC-Team)
- P.A. Hebert - Clustering and identifying Phytoplankton groups by FCM signal analysis, with a new R-toolbox (ULCO/ LISIC-Team)
- K. Rousseeuw - Unsupervised Hidden Markov Model building for high frequency data (IFREMER-ULCO)
- P.A. Hebert - DYMAPHY's Results DataBase InputOutput Data, Structure, and Web Access (ULCO/ LISIC-Team)
- T. Rutten - Easy Clus software applications to in situ monitoring (Thomas Rutten Projects)

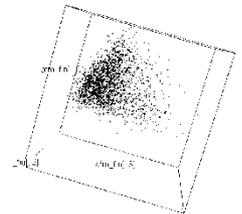
Methods:

● Clustering methods: Partitioning

-K-medoids, Fuzzy analysis [Kaufmann, 2005], Model Based clustering [Fraley ,2002].

● Visualization: MultiDimensional Scaling (MDS)

-Starting from the D_{global} matrix, we seek for a 3D display of the dataset (i.e. the coordinate of particles in a 3D space) [Kruskal, 1978].

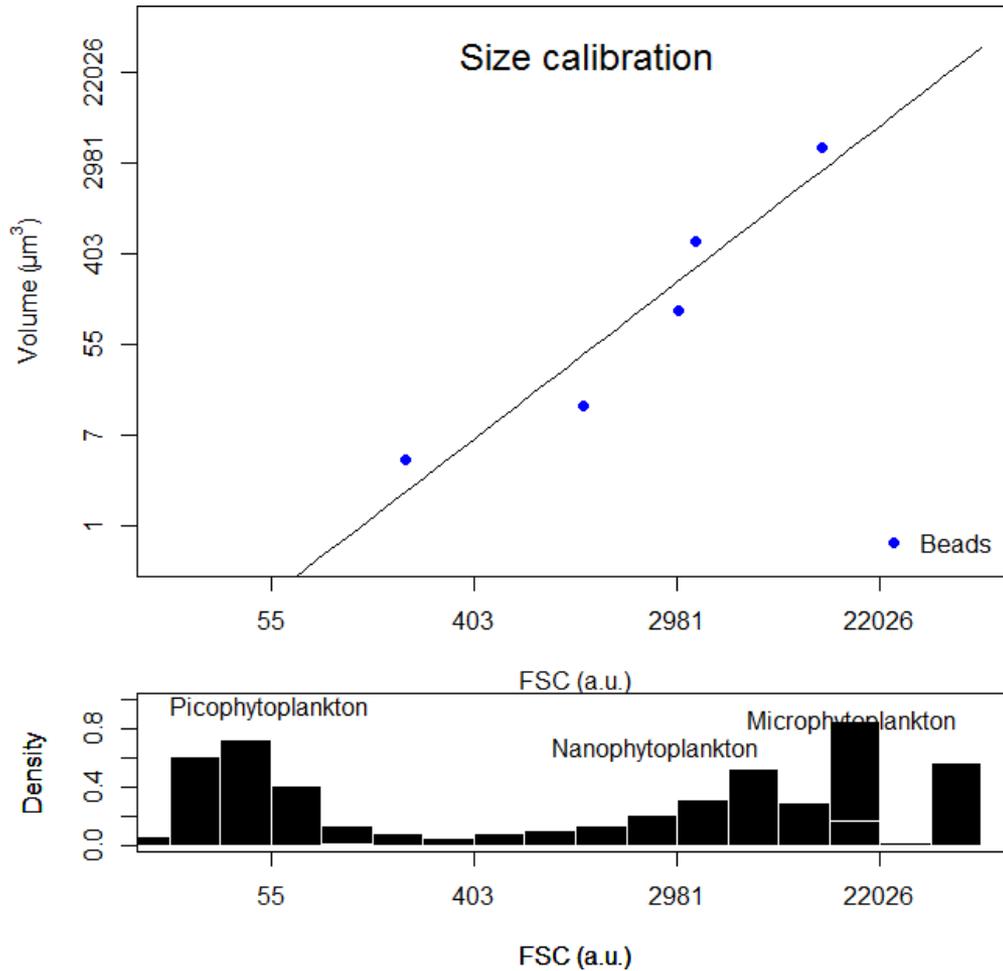


● Estimation of the optimal number of clusters

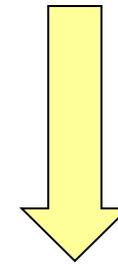
-The silhouette coefficient (SC) measures the amount of clustering structure that is discovered by the classification algorithm for K-medoids and Fuzzy analysis [Kaufmann, 2005]
-Bayesian Information Criterion (BIC) is used for Model Based clustering [Fraley ,2002].

● Unsupervised Hidden Markov Model

ESTIMATION DU BIOVOLUME PAR CYTOMÉTRIE EN FLUX

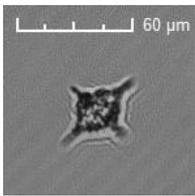


Forward Angle Light Scatter (FSC)
is a proxy of the size



Thanks to microbeads of various
diameters,
conversion of Forward Angle
Light Scatter (FSC)
in volume (μm^3)

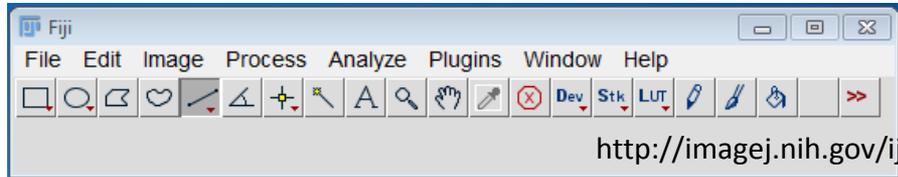
ESTIMATION DU BIOVOLUME À PARTIR DES IMAGES PRISES PAR CYTOMÉTRIE EN FLUX



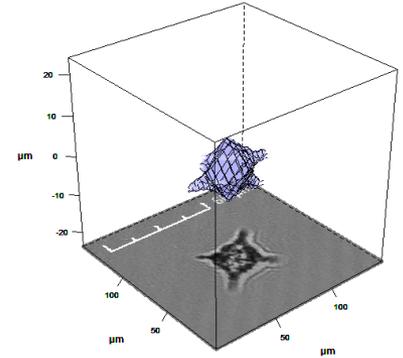
Picture



Fiji Software (ImageJ)

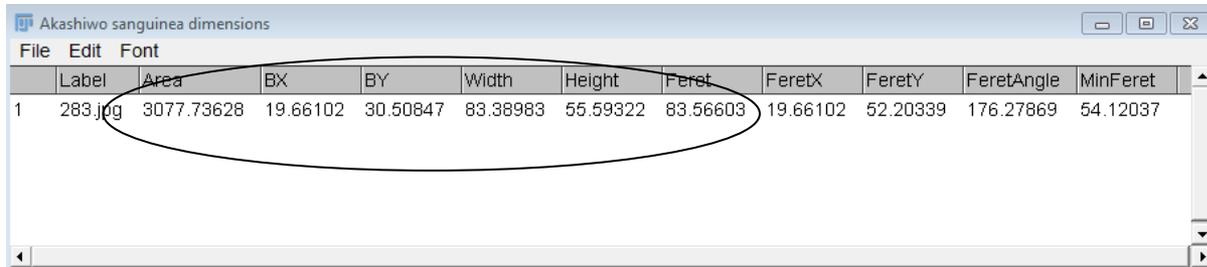


Object detection (edges)



Distance map
biovolume algorithm
(Moberg & Sosik)

Limnol. Oceanogr.: Methods 10,
2012, 278–288



Label	Area	BX	BY	Width	Height	Feret	FeretX	FeretY	FeretAngle	MinFeret	
1	283.jpg	3077.73628	19.66102	30.50847	83.38983	55.59322	83.56603	19.66102	52.20339	176.27869	54.12037

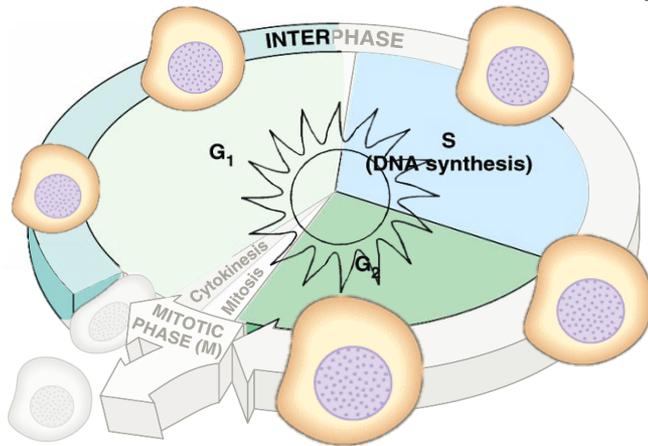
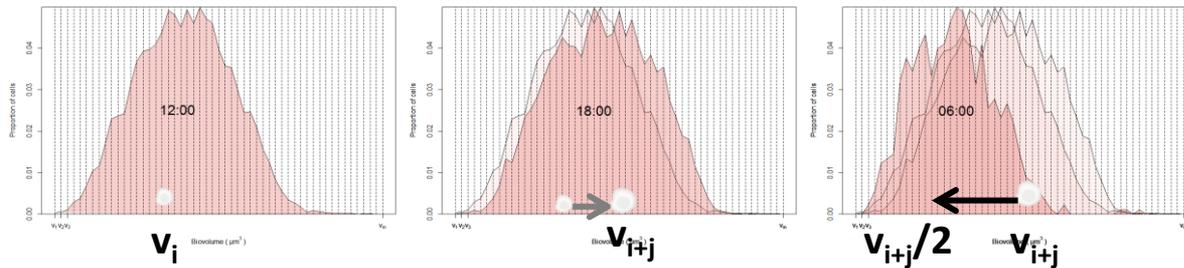
Object dimensions (→ ESD, etc.)



Estimation du biovolume

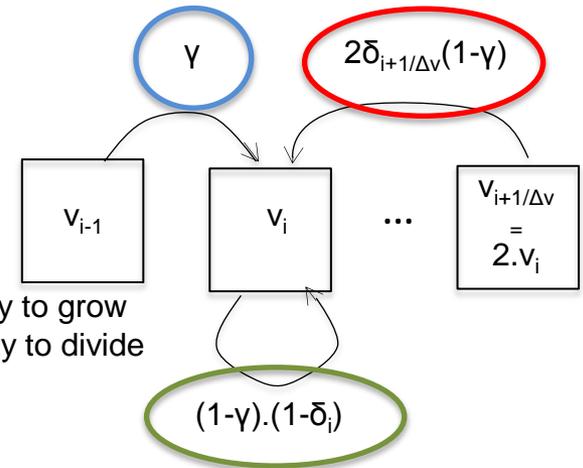
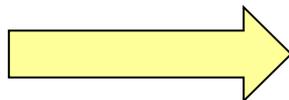
MODÈLE DE POPULATION STRUCTURÉE EN BIOVOLUME POUR CARACTÉRISER LA DYNAMIQUE DU PHYTOPLANCTON

Cytometric cluster biovolume distribution



Copyright © Pearson Education, Inc., publishing as Benjamin Cummings.

A matrix population model,
fit to hourly cell size distributions,
accurately estimates division rates
of natural populations



$\gamma(t)$: probability to grow
 $\delta_i(t)$: probability to divide

Phytoplankton biovolume distribution is
linked to the growth rates, even when
populations are asynchronous.

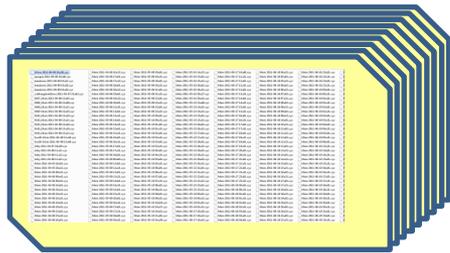
Modelisation

(Sosik et al., 2003)

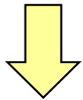
(Courtesy M. Dugenne)

DYNAMIQUE DU PHYTOPLANCTON SUR STATION FIXE

Automated flow cytometer on multi-instrumental platform



(hundreds of samples)



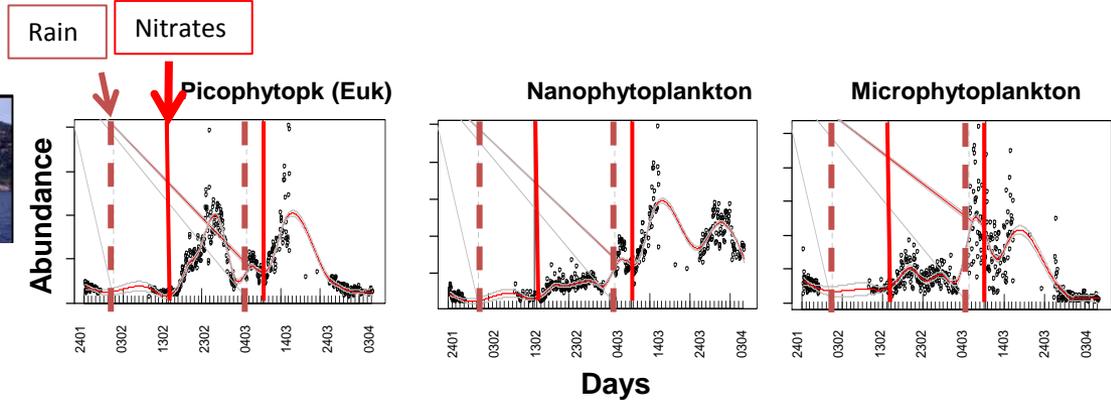
High frequency sampling remotely controlled (satellite, internet, Wifi)



Sampling frequency adaptation (sudden event)

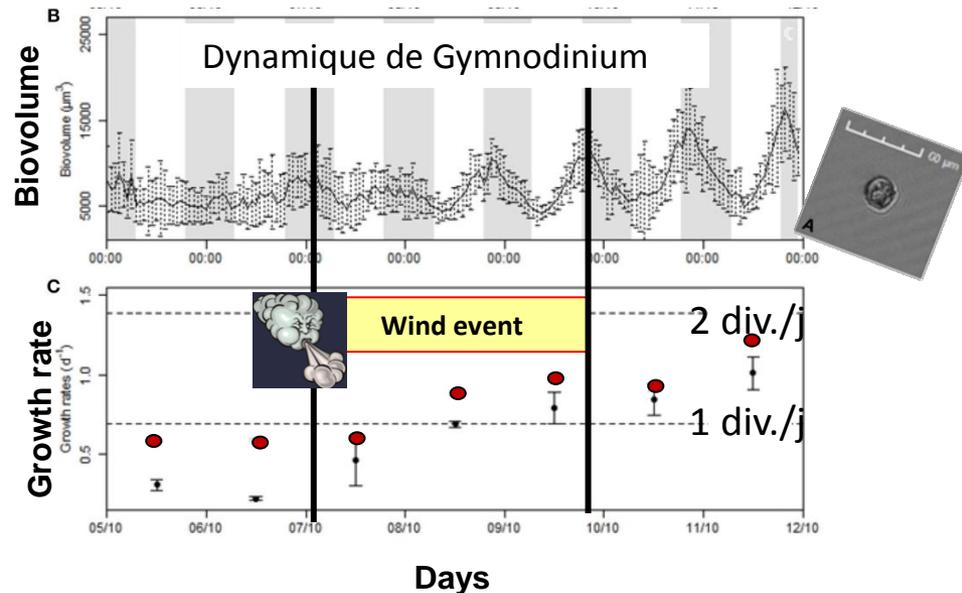


- EOL Buoy (NW Mediterranean) : M. Thyssen (this session)



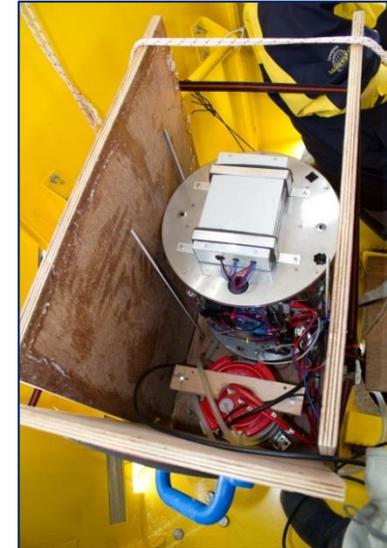
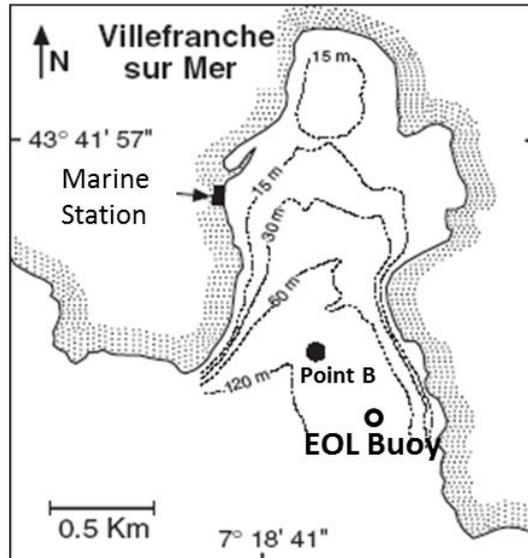
- Berre Lagoon (NW Mediterranean) : M. Dugenne

Session 71, Thursday, 3:15 PM



INFLUENCE D'ÉVÈNEMENTS IMPULSIONNELS SUR LA MISE EN PLACE DU BLOOM PRINTANIER

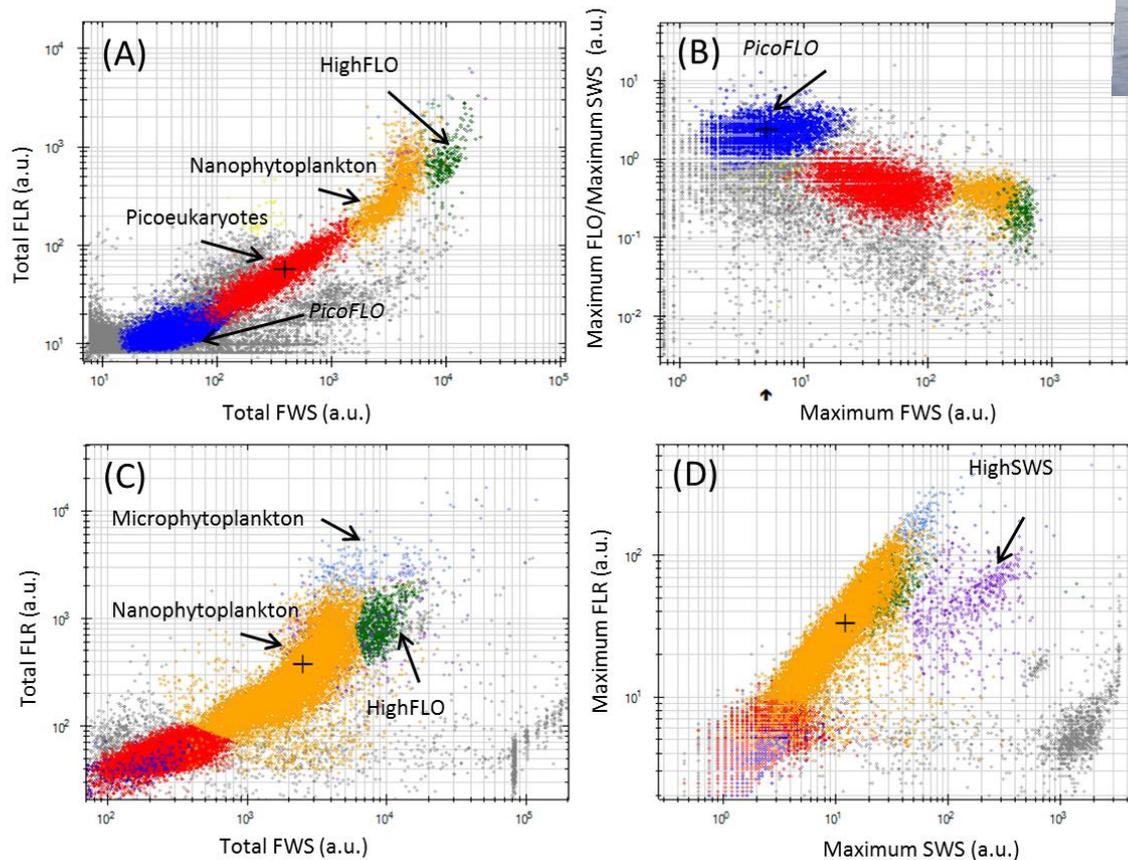
Baie de Villefranche, bouée EOL
25 janvier - 6 avril 2012



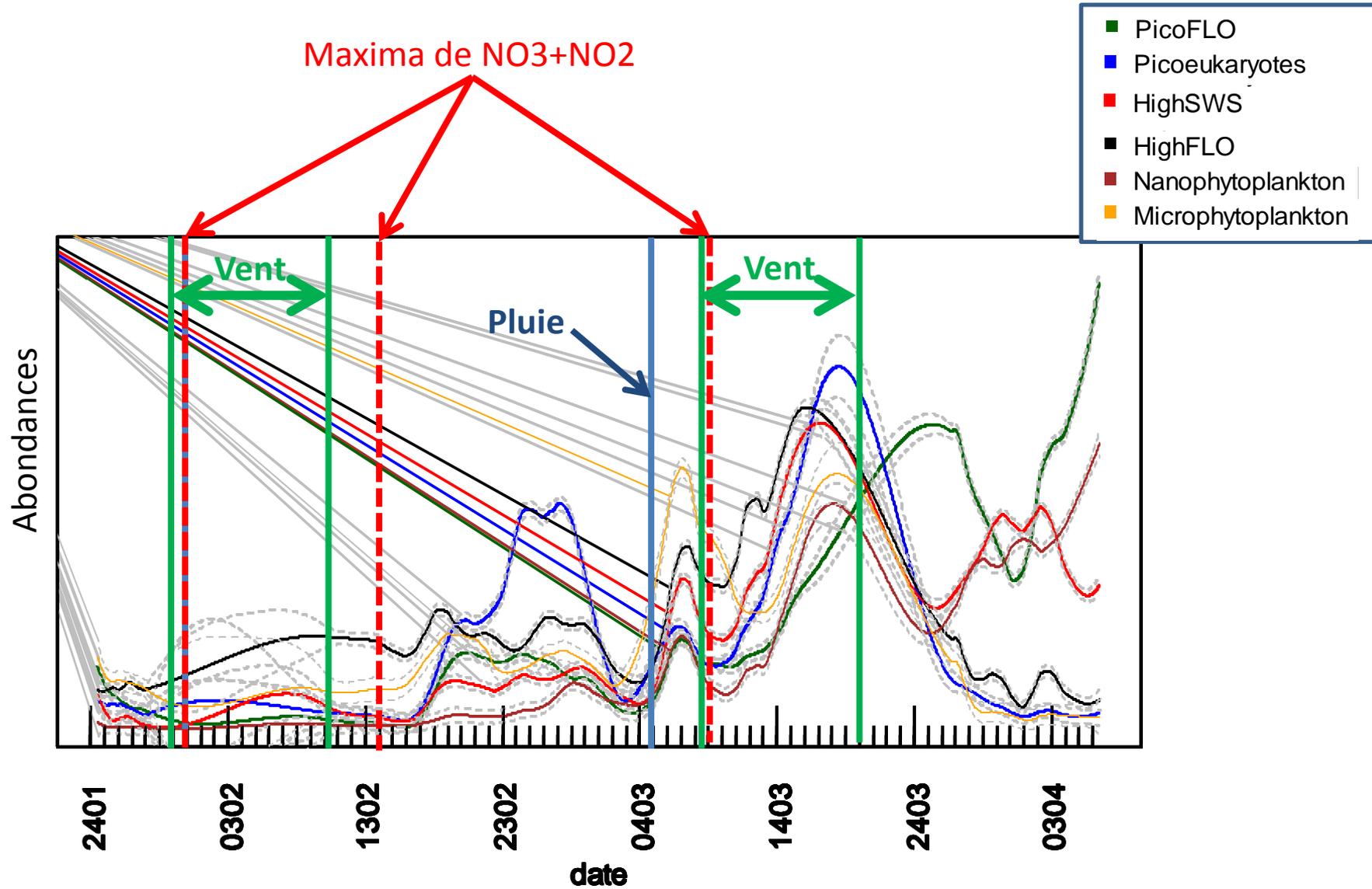
Thyssen M, Grégori GJ, Grisoni J, Pedrotti M, Mousseau L, Artigas LF, Marro S, Garcia N, Passafiume O and Denis MJ(2014). **Onset of the spring bloom in the northwestern Mediterranean Sea : influence of environmental pulse events on the in situ hourly-scale dynamics of the phytoplankton community structure.** *Front. Microbiol.* 5:387. doi:10.3389/fmicb.2014.00387

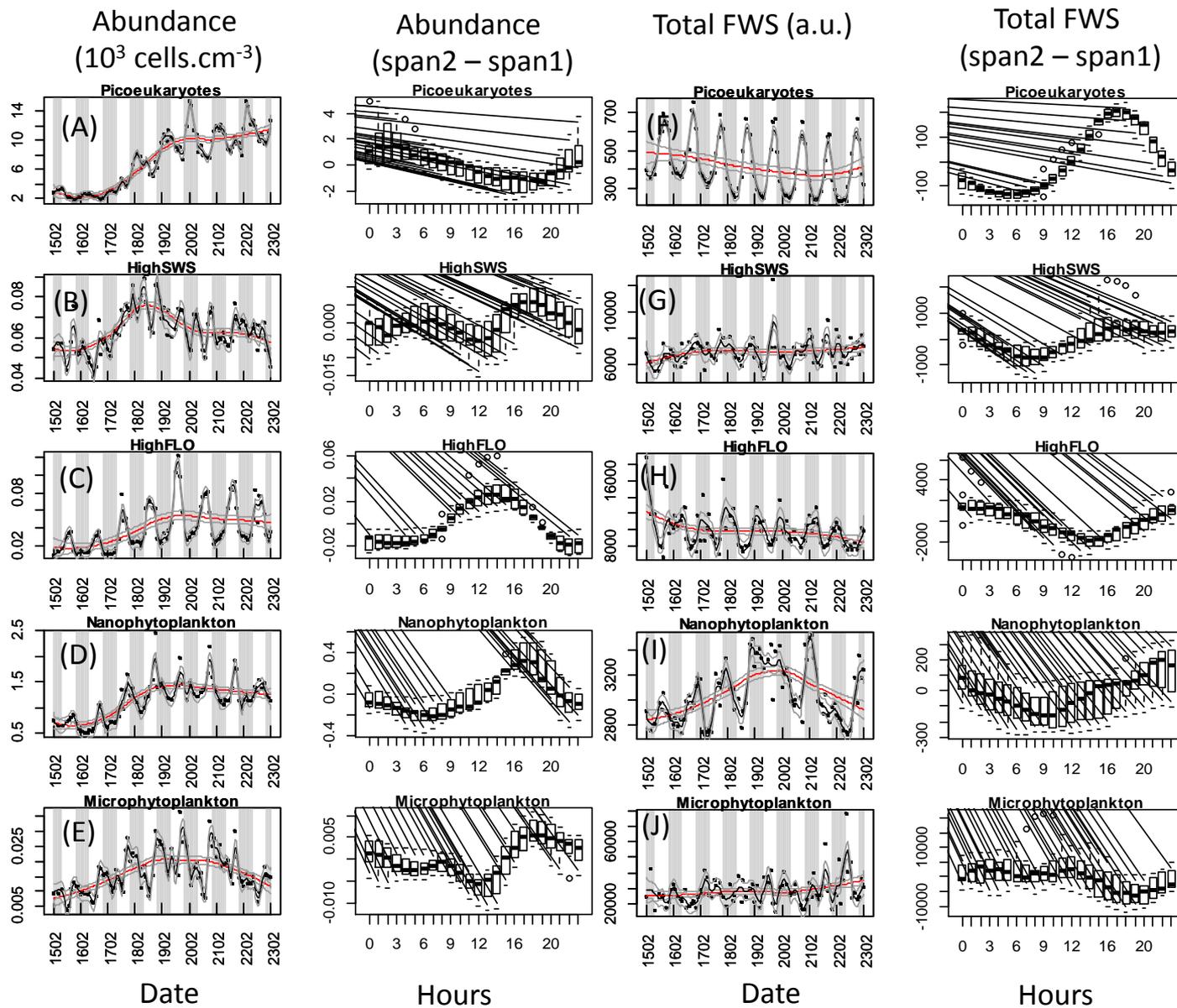
GROUPES FONCTIONNELS DÉFINIS PAR CYTOMÉTRIE EN FLUX

- Jusqu'à 6 groupes fonctionnels de phytoplancton mis en évidence.
- Echantillonnage toutes les 2 heures
- Contrôle à distance en direct par liaison wifi



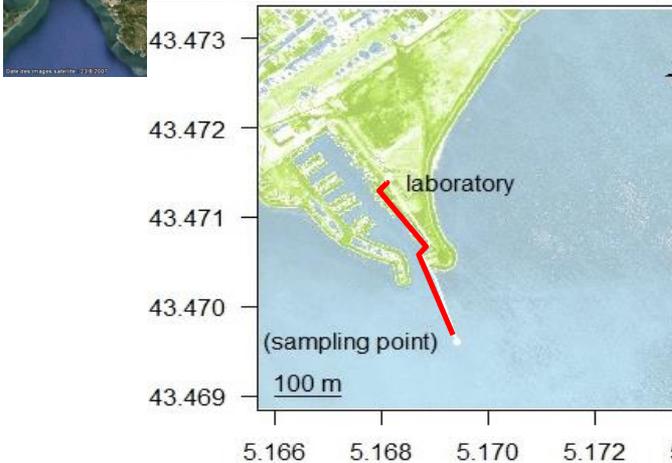
DYNAMIQUE DES GROUPES FONCTIONNELS





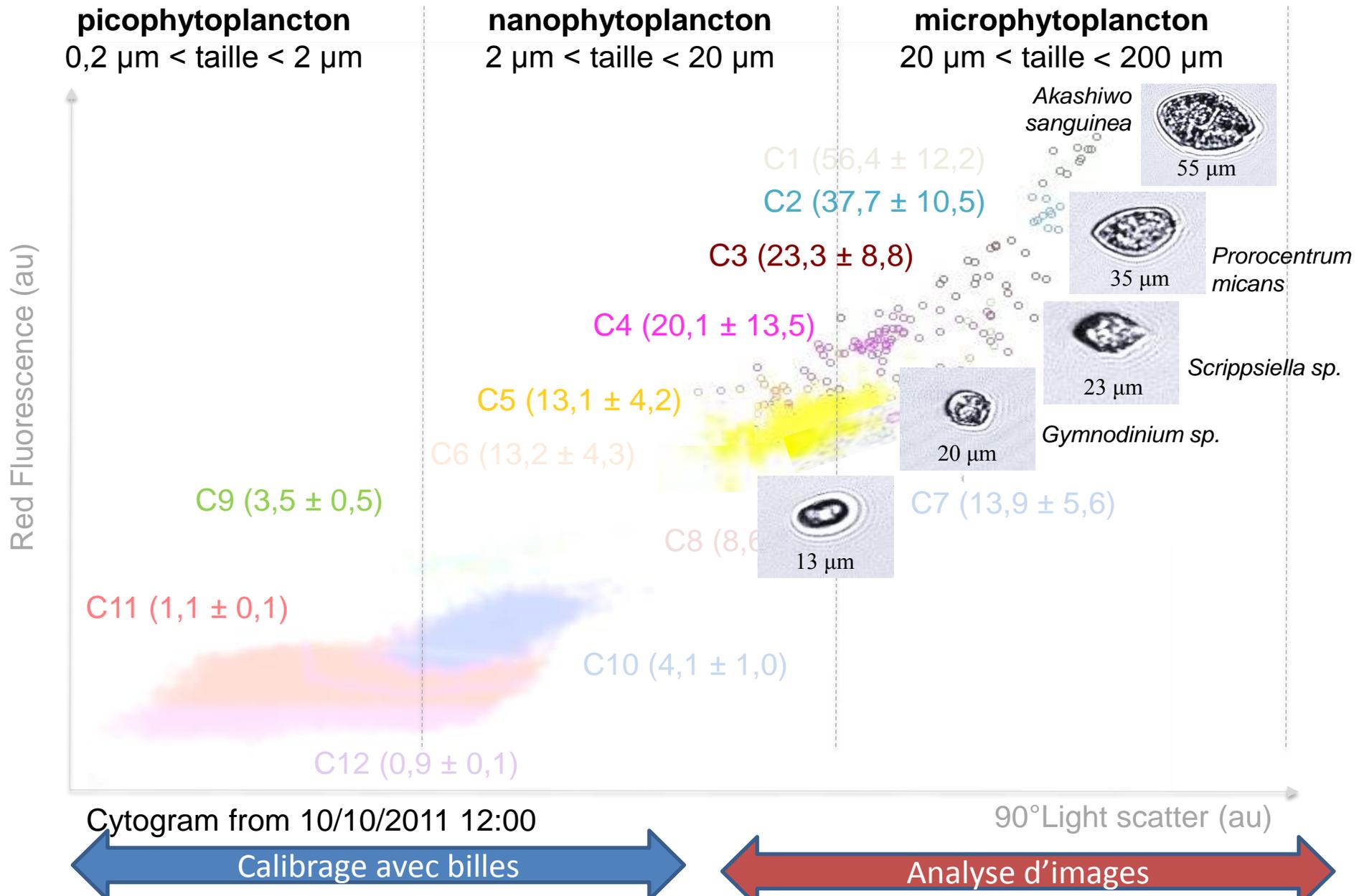
La variation journalière des abondances et de la diffusion est liée au cycle cellulaire et permet d'estimer une croissance nette in situ (Sozik et al. 2003, Ribalet et al., and Dugenne et al. 2014)

INFLUENCE D'ÉVÈNEMENTS IMPULSIONNELS SUR LA DYNAMIQUE D'ESPÈCES PATHOGÈNES

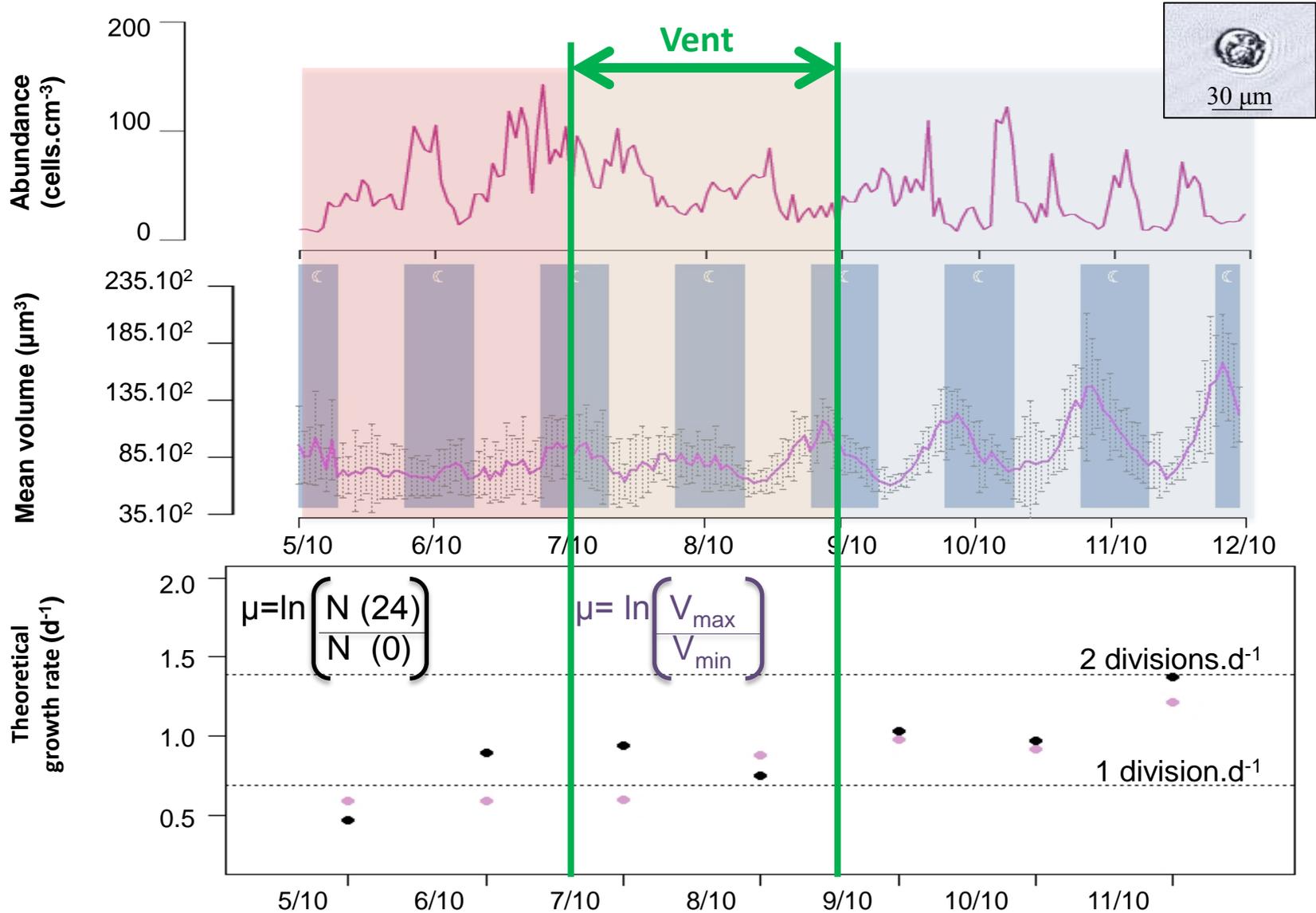


Dugenne M, Thyssen M, Nerini D, Mante C, Poggiale J, Garcia N, Garcia F and Grégori GJ (2014). **Consequence of a sudden wind event on the dynamics of a coastal phytoplankton community : an insight into specific population growth rates using a single cell high frequency approach.** *Front. Microbiol.* 5:485. doi : 10.3389/fmicb.2014.00485

GROUPES FONCTIONNELS DÉFINIS PAR CYTOMÉTRIE EN FLUX



Effet du vent sur la dynamique de *Gymnodinium*





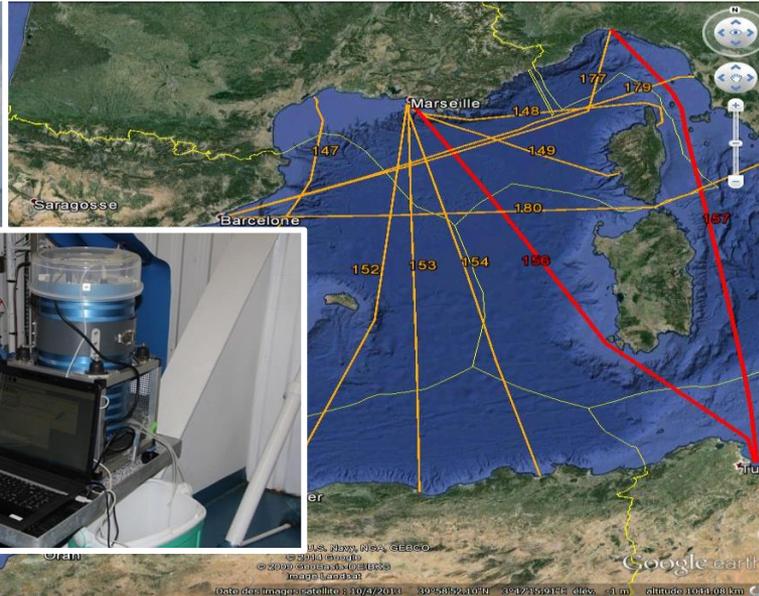
Prospective 2 : Continuous and high resolution observation of the NW Mediterranean Sea (A*MIDEX CHROME 350 k€)



Institut de athématiques de Marseille I2M



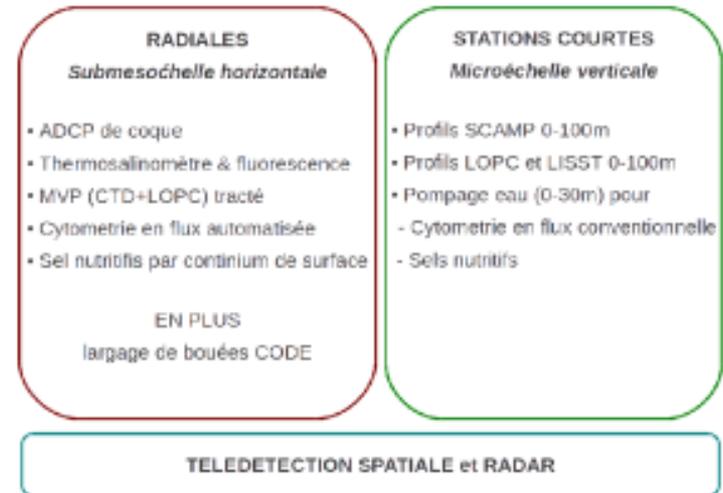
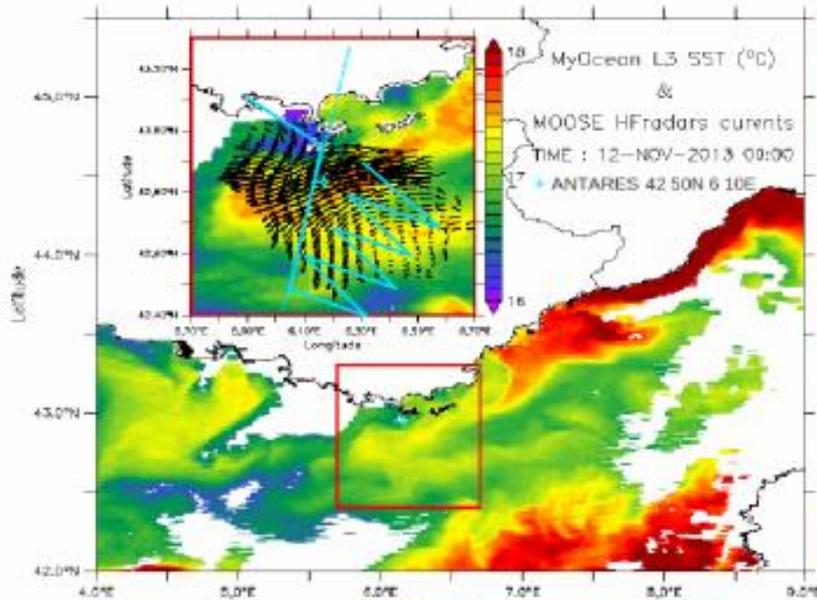
University of Bologna (SINCEM)



- Salinité
- Oxygene
- Fluorescence
- Turbidité
- CDOM
- PCO2 et pH
- Cytométrie automatisée

- Relation physique/chimie et diversité fonctionnelle du phytoplancton sur des critères de résolution espace/temps similaires
- Couplage avec PHYSAT (LOG) pour une extrapolation des groupes fonctionnels à l'échelle du bassin
- Océanographie opérationnelle et modèles biogéochimiques

Prospective 3 : Observing Submesoscale Coupling At High Resolution (OSCAHR, nov. 2015, Pis: A. Doglioli et G. Grégori)



- Relation physique/chimie et diversité fonctionnelle du phytoplancton sur une structure physique
- Couplage avec satellites et radar pour physique adaptative
- Océanographie opérationnelle et modèles biogéochimiques