Etude des successions saisonnières des communautés phytoplanctoniques marines : la série à long terme SOMLIT-Astan.

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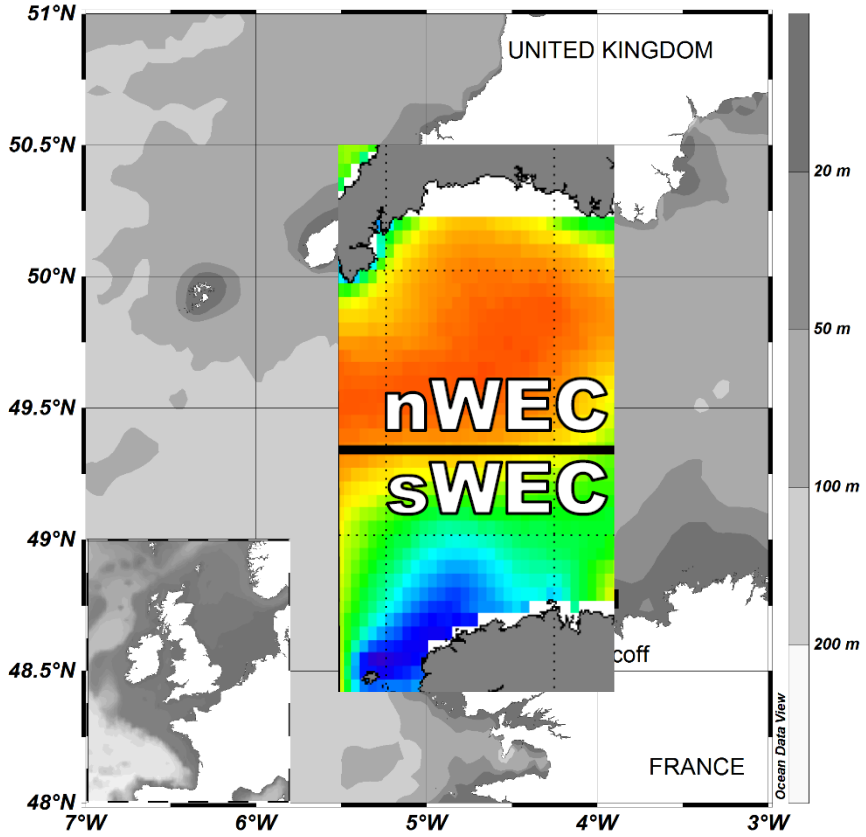
and ECOMAP Team, Chemistry Team and Observation Facility

Roscoff,
10 Octobre 2019

SOMLIT sampling stations



somlit since january 2000

Service d'Observation en Milieu Littoral depuis 1997

- 2 samples/month
- High tide – Neap tide
- 15 hydrological parameters

- **SOMLIT-Estacade** - littoral, 10m depth (high tide)
  - anthropogenic influence
- **SOMLIT-Astan** - 2,5 miles, 60m depth (high tide)
  - permanently mixed water column
  - continental influence limited / typical high seas of the WEC

Phytoplankton diversity

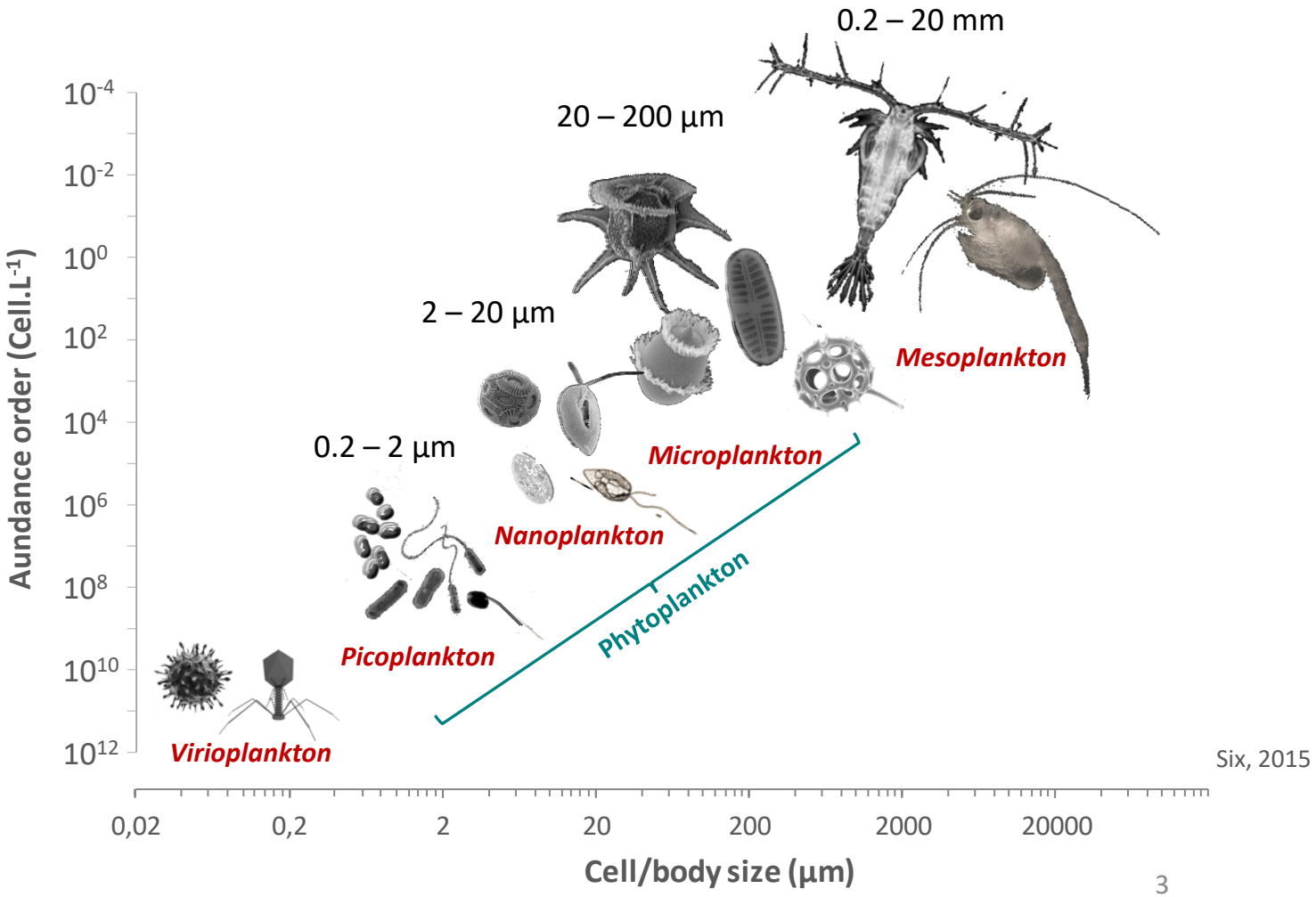
Phytoplankton is the plant fraction of the plankton.

Unicellular organisms
Size and shape diversity
Taxonomic diversity

From few tens to several million cells/Liter

Basis of the marin food web

Sensitive to various environmental changes, from natural or anthropogenic sources.



Six, 2015

SOMLIT-Astan phytoplankton time-series

Since 2000

Different samples …

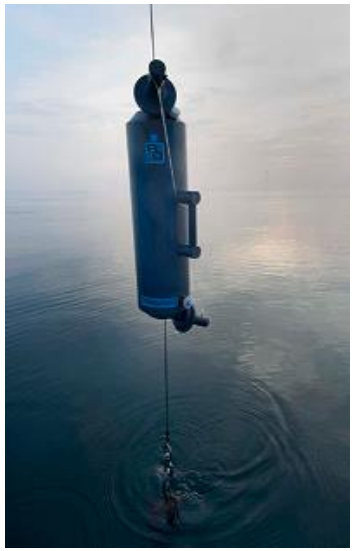
- sub-surface – Niskin and net > 20µm

… for different methods because of microbial biodiversity

- **Pico-nano-phytoplankton** → **Flow cytometry**

- **Microphytoplankton** → **Optical microscopy**
  List of species
  Taxa counts

- **Genetic microbial diversity** → **Metabarcoding**
  DNA filters (-80°C)
  # 2000 to2006 - fraction [0,2 - 3µm]
  # from 2007 - fractions [0,2 – 3µm] et [>3µm]



Niskin -1m

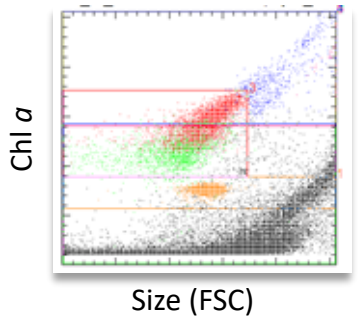
Trait de filet 20µm
Sub-surface, 3 minutes

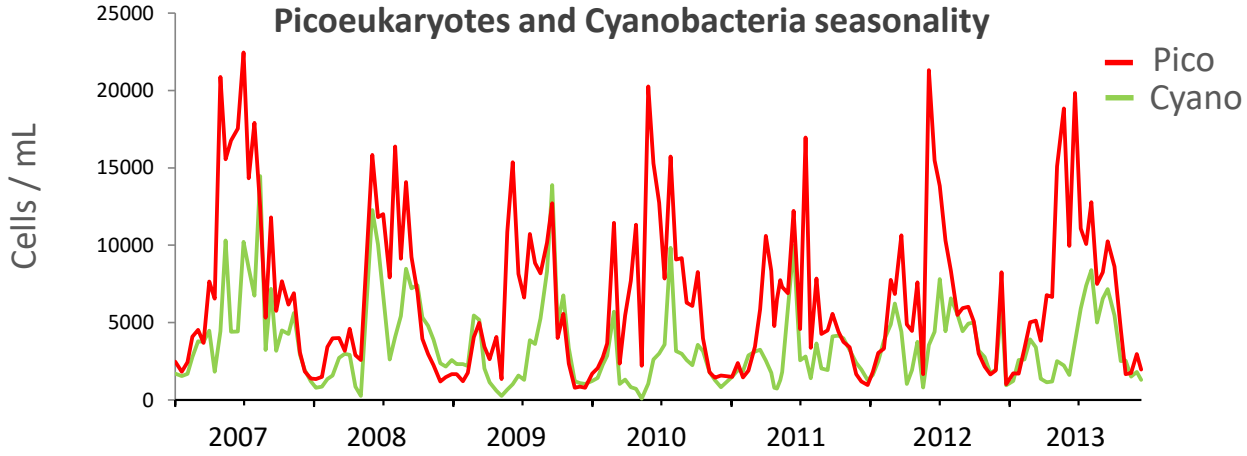
SOMLIT-Astan phytoplankton time-series

Flow cytometry < 20µm - 7 fonctionnal groups / 336 données per year

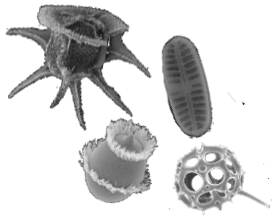
Picoplankton

Nanoplankton

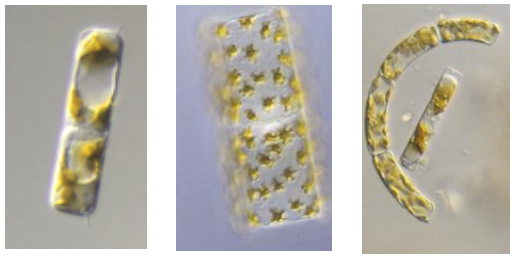




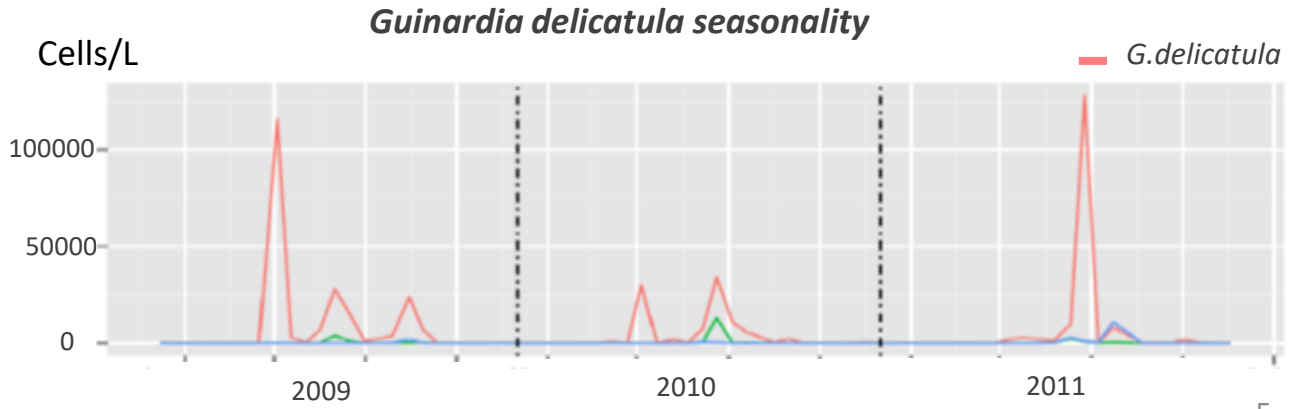
Optical microscopy > 10µm - 3 to 5h per sample / 230 taxa



Microplankton

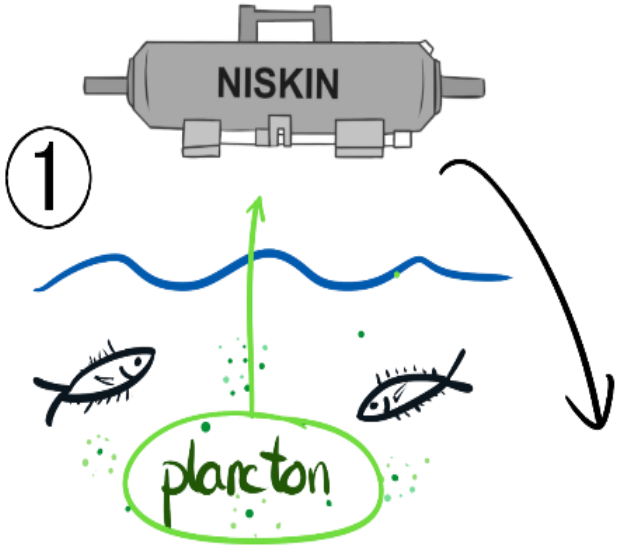


G.delicatula *G.flaccida* *G.striata*

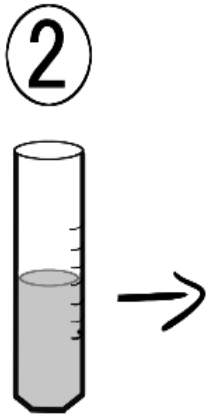


SOMLIT-Astan phytoplankton time-series

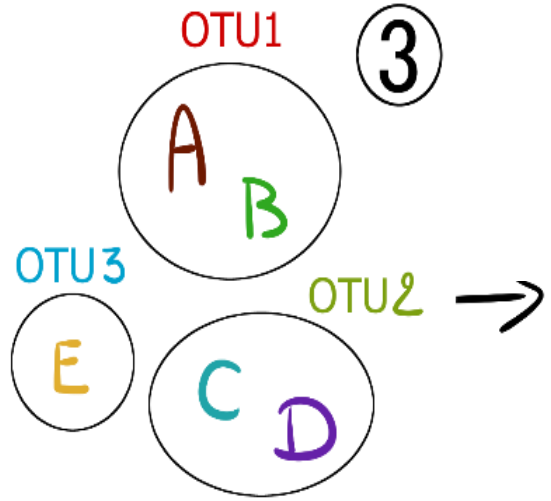
Metabarcoding > 0,2 µm -



Sampling
5 liters
2 fractions



DNA extraction
Amplification
V4 18S
High-throughput sequencing



OTUs
Taxonomic units
Genetic similarities

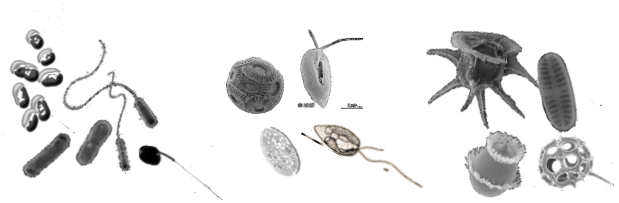
4

	Date 1	Date 2	Date 3	Date n
Esp / OTU 1	12	20	10	n1
Esp / OTU 2	5	5	2	n2
...				
Esp / OTU 23000	5	5	2	n2

OTUs assigned to known taxa (reference base)

Illustration J.Courboulès

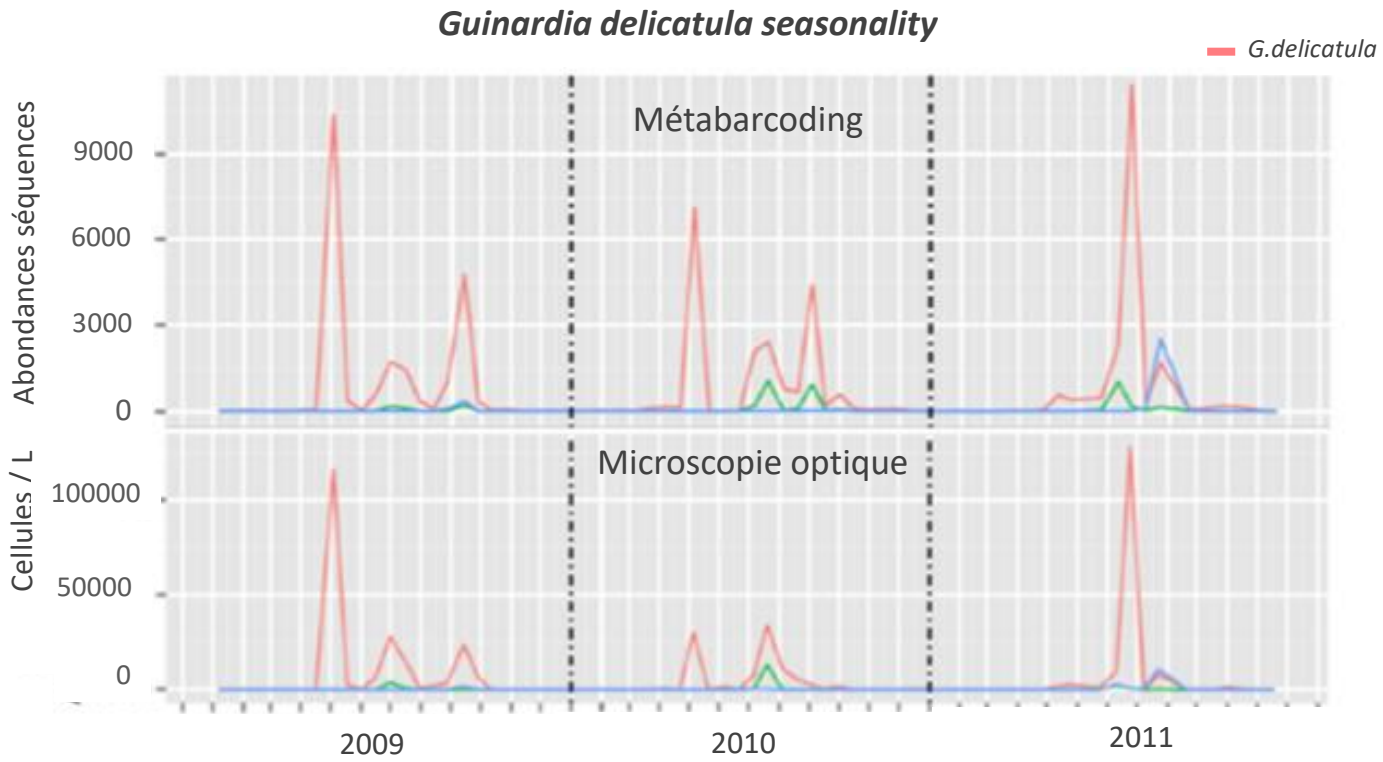
SOMLIT-Astan phytoplankton time-series



Picoplankton Nanoplankton Microplankton

	Date 1	Date 2	Date 3	Date n
Esp / OTU 1	12	20	10	n1
Esp / OTU 2	5	5	2	n2

...

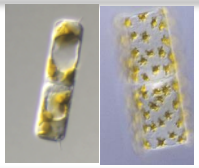


Seasonality of OTUs as with classical methods
biodiversity and successions

SOMLIT-Astan TIME-series

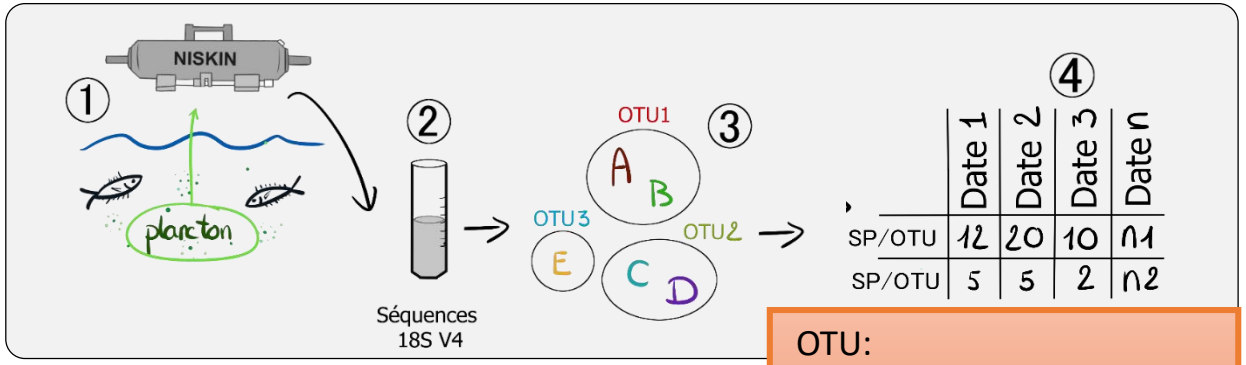
❖ Phytoplankton diversity (>10µm) – 2000 to 2017

- **Morphological taxa counts (optical microscopy)**



phytobs

❖ Genetic diversity – metabarcodes (> 0,2µm) – 2009 to 2016



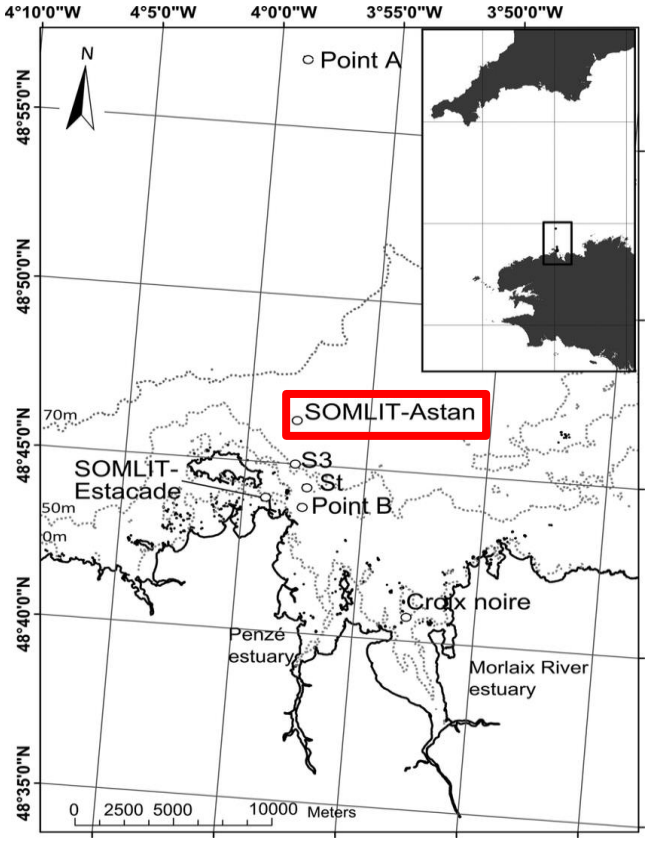
OTU:
Operational taxonomic unit

- Metabarcode data
- V4 Illumina sequencing of the nuclear 18S rDNA

❖ Physicochemical parameters

somlit
Service d'Observation en Milieu Littoral depuis 1997

Sampling twice a month 2.5 miles north-est of Ile de Batz

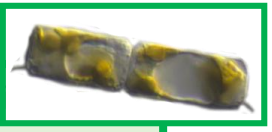


OBJECTIVE 1

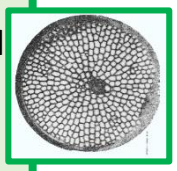
Description of the seasonal/interannual patterns of the pelagic eukaryotic microbes

- ❖ Alpha & Beta diversity
  - ✓ **NMDS/PCA**
  - ✓ **Shannon Index**

Spring bloom dom diatoms: ***Guinardia delicatula***



Genetic data dominated by small diatoms: ***Minidiscus trioculatus***



- ➢ Verify the reliability of genetic data for the whole time-series: **compare** with morphological data
- ➢ **Describe** seasonal patterns, detect trends

OBJECTIVE 2

Impact of hydrology and climate on successions

- ❖ Multivariate analyses (MVA)
  - ✓ **Redundancy analyses (RDA)**
  - ✓ **Correlation**

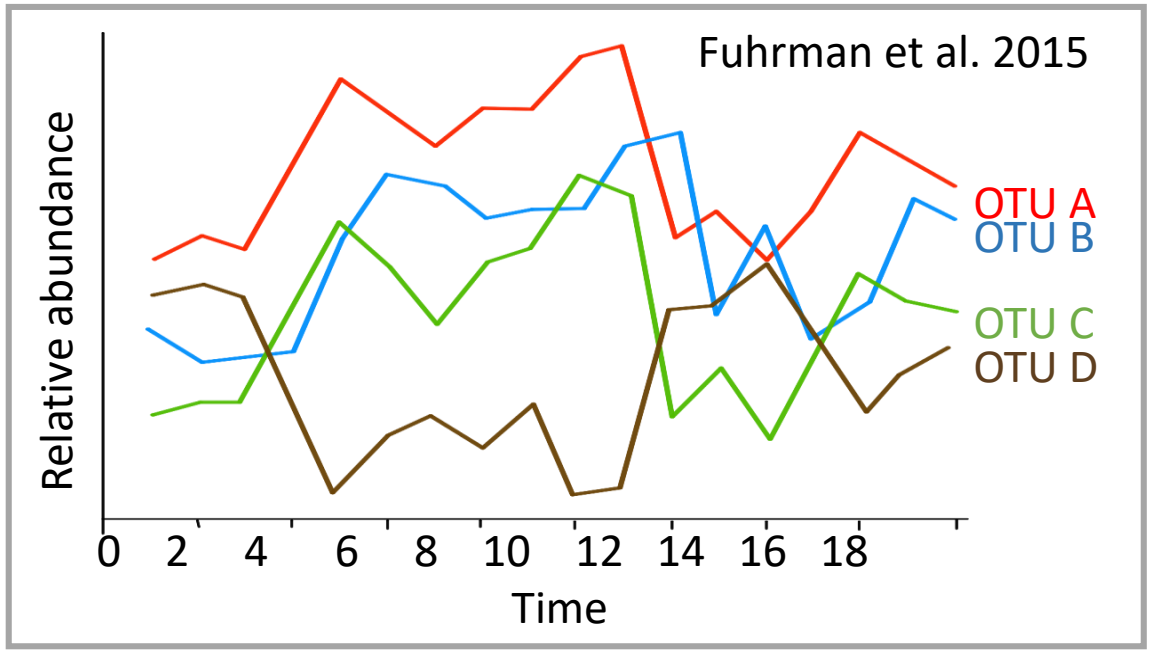
SOMLIT

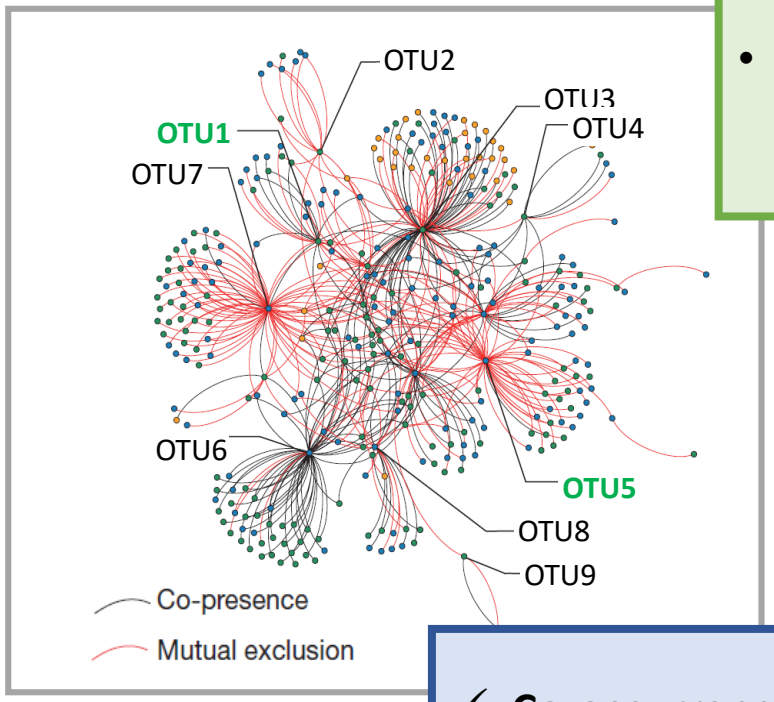
Climatic and hydrological data (Service National d'Observation)

- ➢ Investigate the **influence** of the environment on seasonal succession
- ➢ Describe seasonal pattern associated to hydrology anomailes

OBJECTIVE 3

Investigate biotic interactions





- **Positive interactions**: mutualism and commensalism
- **Negative interactions**: competition, antagonism and amensalism)

✓ **Co-occurrence/co-exclusion analyses**
✓ **Local similarity analyses (LSA)**

➢ Reconstruct **interaction networks**
➢ Identify **keystone** species
(Confirm using litterature and available experimental data)

Data management

❖ Microscopic counts

	> 20 µm
Number of taxa distinguished	187
Samples	272
Species	146

❖ Metabarcoding

	Removing Metazoan and bad samples	0.2-3 µm	> 3 µm
Number of clean reads	26.176.859	13.581.047	12.595.812
Samples	352	188	164
OTUs	28.373	22.713	22.814

❖ Hydrological variables

Environmental parameters selected:
Temperature, PH, Salinity, NO3, NO2, PO4, SIOH4, NH4, PAR.

http://somlit.epoc.u-bordeaux1.fr/fr/

OTUs table

- SWARM: fast clustering method for amplicon-based studies (https://github.com/frederic-mahe/swarm → pipeline)
- PR2 for taxonomy assignation (18S rRNA sequences - 170 000)

PROTIST RIBOSOMAL REFERENCE DATABASE

The Protist Ribosomal Reference database (PR²): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy

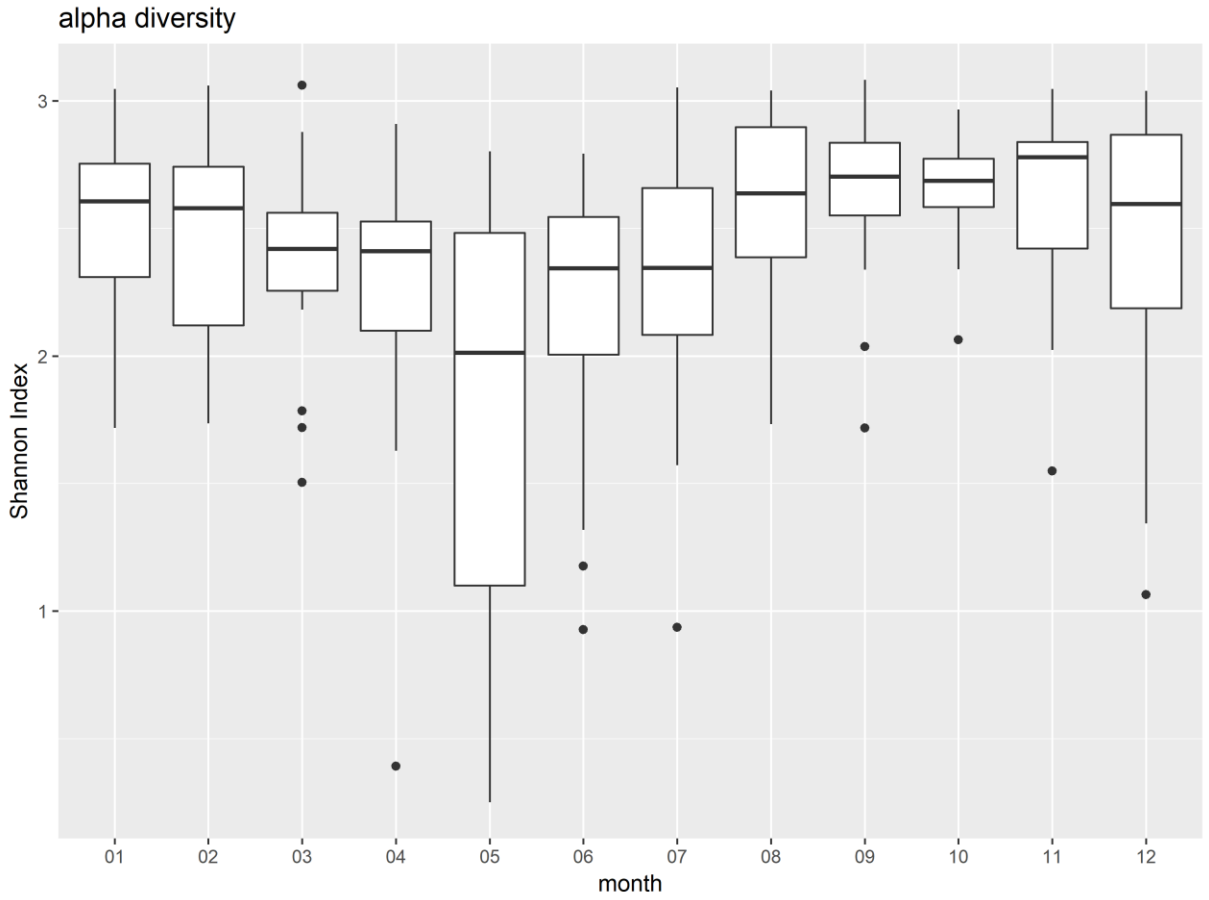
Laure Guillou[1,2,*], Dipankar Bachar[3,4], Stéphane Audic[1,2], David Bass[5], Cédric Berney[5], Lucie Bittner[1,2], Christophe Boutte[1,2], Gaétan Burgaud[6], Colomban de Vargas[1,2], Johan Decelle[1,2], Javier del Campo[7], John R. Dolan[8], Micah Dunthorn[9], Bente Edvardsen[10], Maria Holzmann[11], Wiebe H.C.F. Kooistra[12], Enrique Lara[13], Noan Le Bescot[1,2], Ramiro Logares[7], Frédéric Mahé[1,2], Ramon Massana[7], Marina Montresor[12], Raphael Morard[1,2], Fabrice Not[1,2], Jan Pawlowski[11], Ian Probert[14,15], Anne-Laure Sauvadet[1,2], Raffaele Siano[16], Thorsten Stoeck[9], Daniel Vaulot[1,2], Pascal Zimmermann[17] and Richard Christen[3,4,*]

[1]CNRS, UMR 7144, Adaptation et Diversité en Milieu Marin, 29682 Roscoff, France, [2]UPMC Université Paris 06, UMR 7144, Station Biologique de Roscoff, 29682 Roscoff, France, [3]CNRS, UMR 7138, Systématique

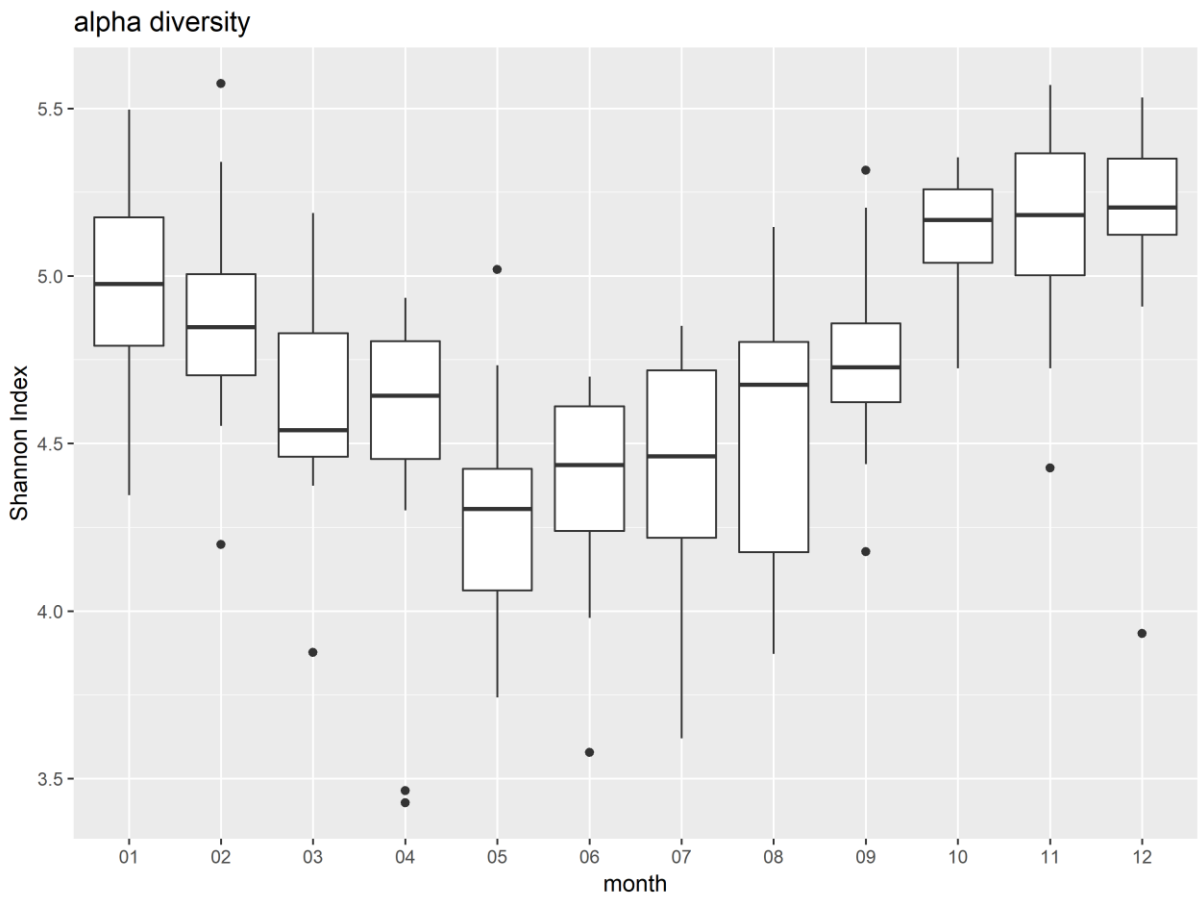
https://figshare.com/articles/PR2_rRNA_gene_database/3803709

Shannon Diversity Index

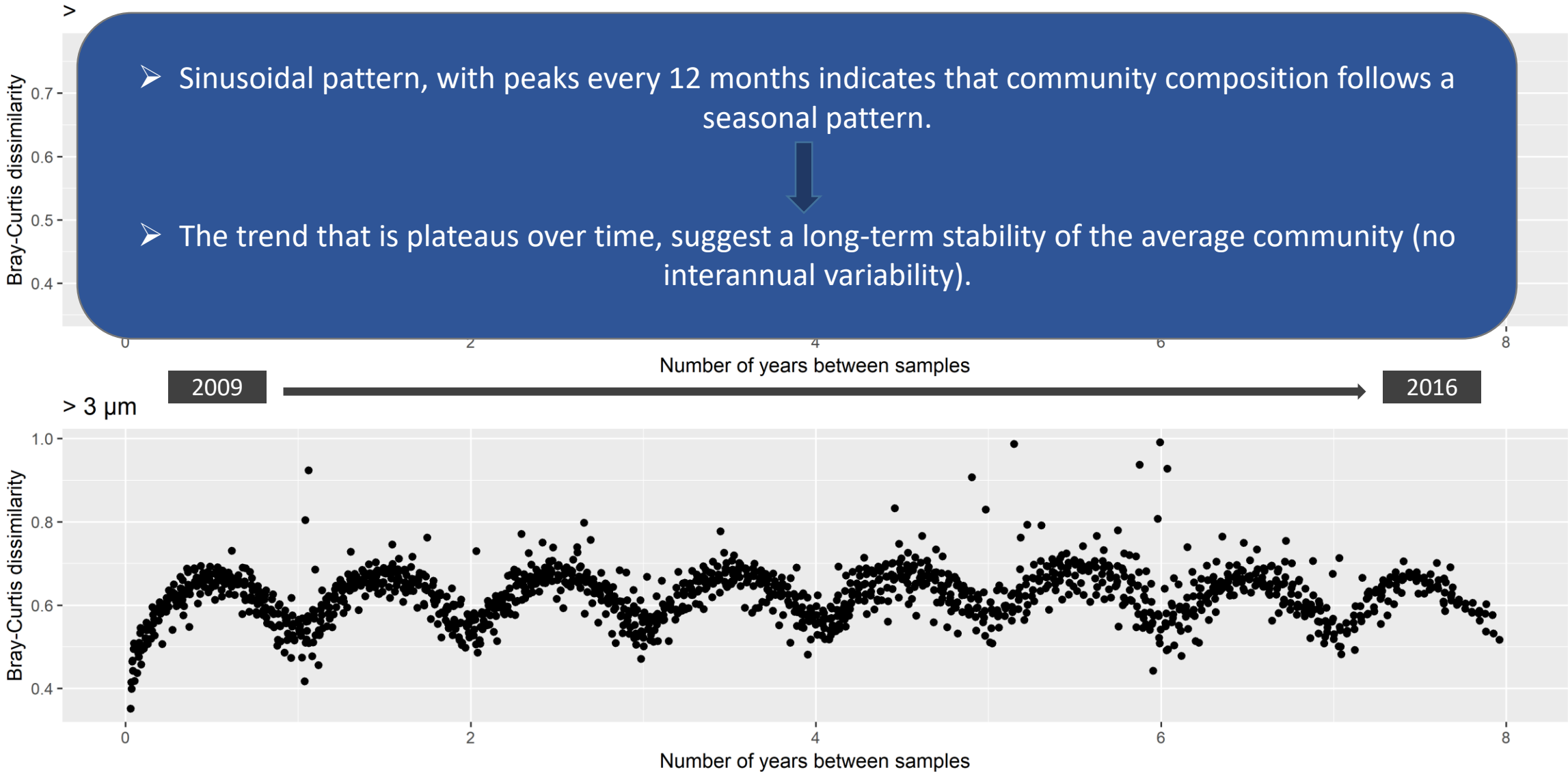
Morphologic data



MetaB data



Bray-Curtis dissimilarity



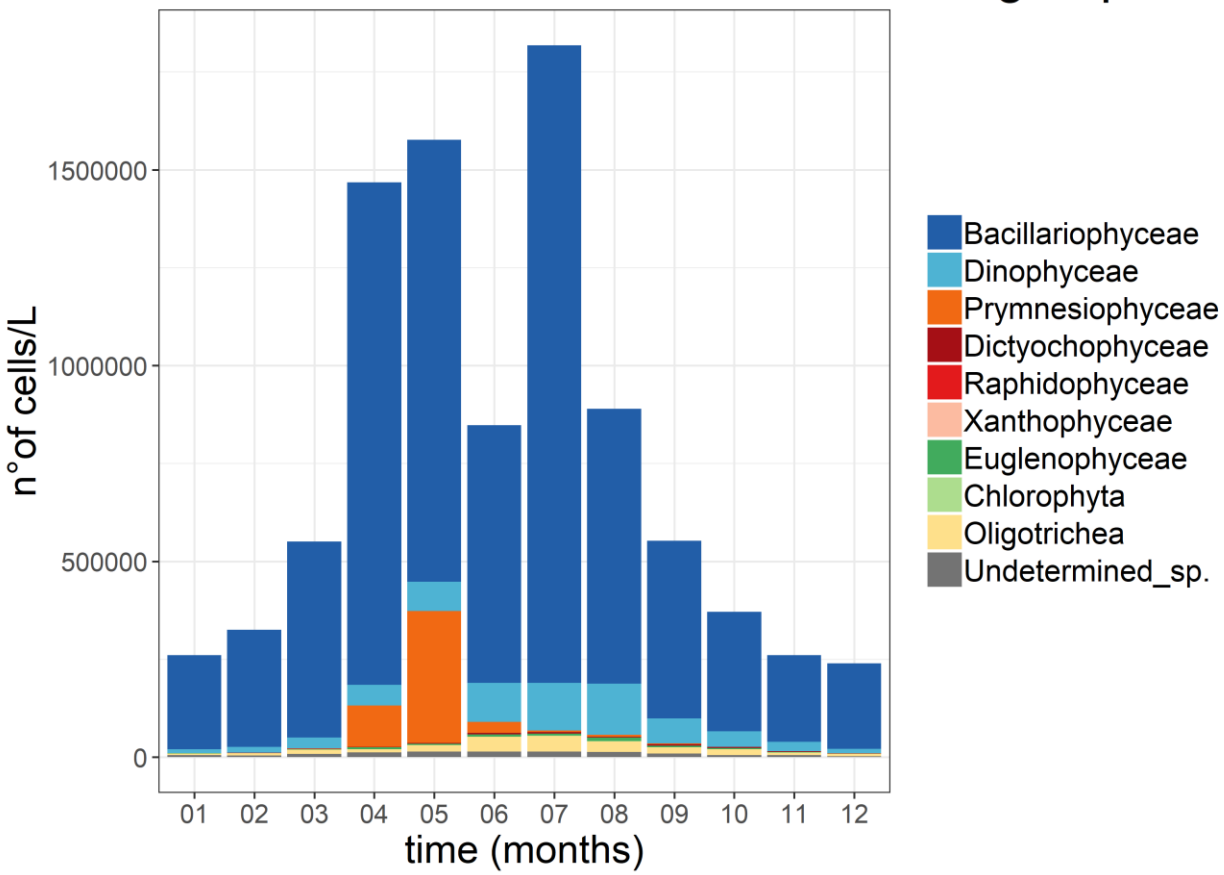
- Sinusoidal pattern, with peaks every 12 months indicates that community composition follows a seasonal pattern.

- The trend that is plateaus over time, suggest a long-term stability of the average community (no interannual variability).

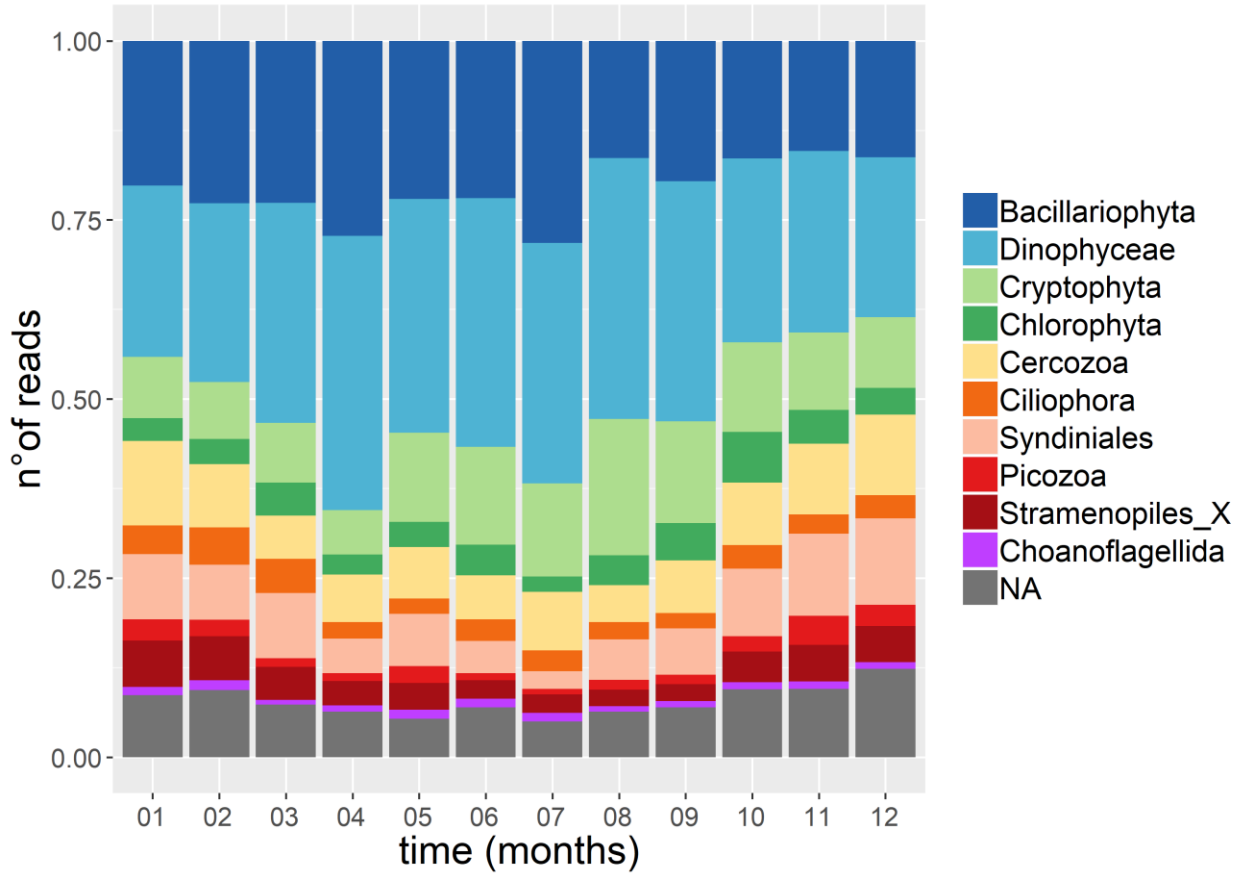
Absolute and relative abundance

- Different size fraction (3-20µm for metaB and >20 µm for morphologic)
- Different taxonomic resolution

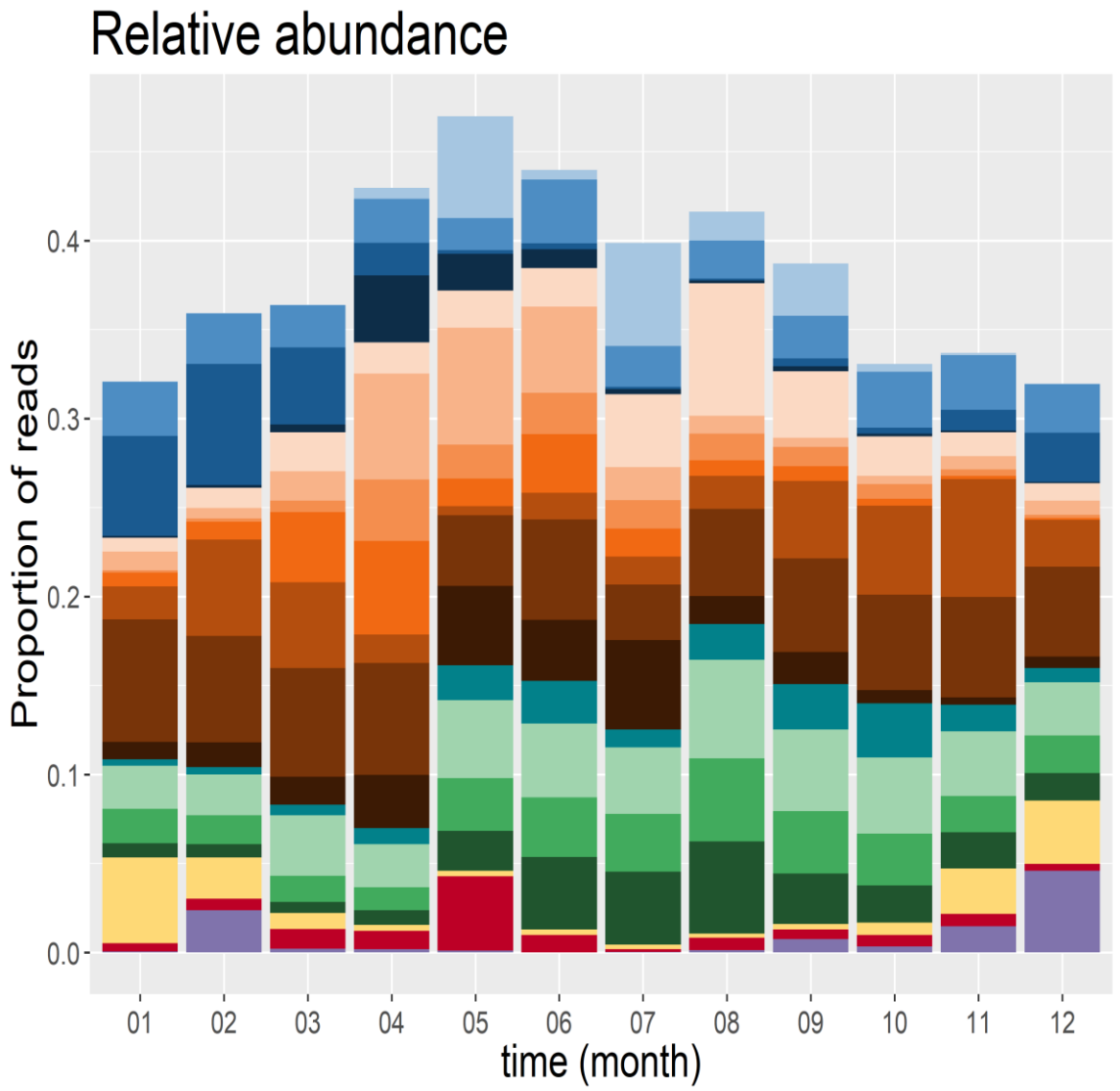
Morphologic data



MetaB data



Most abundant OTUs

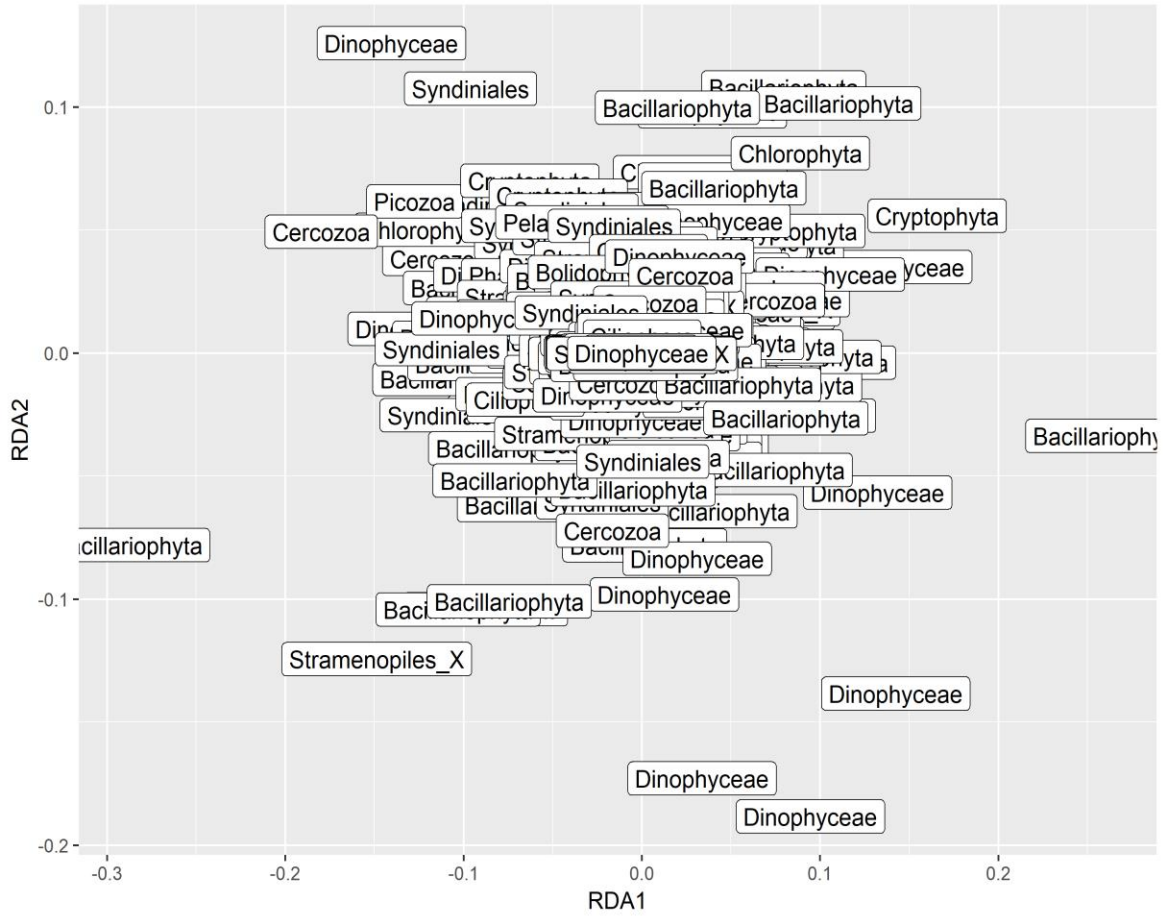


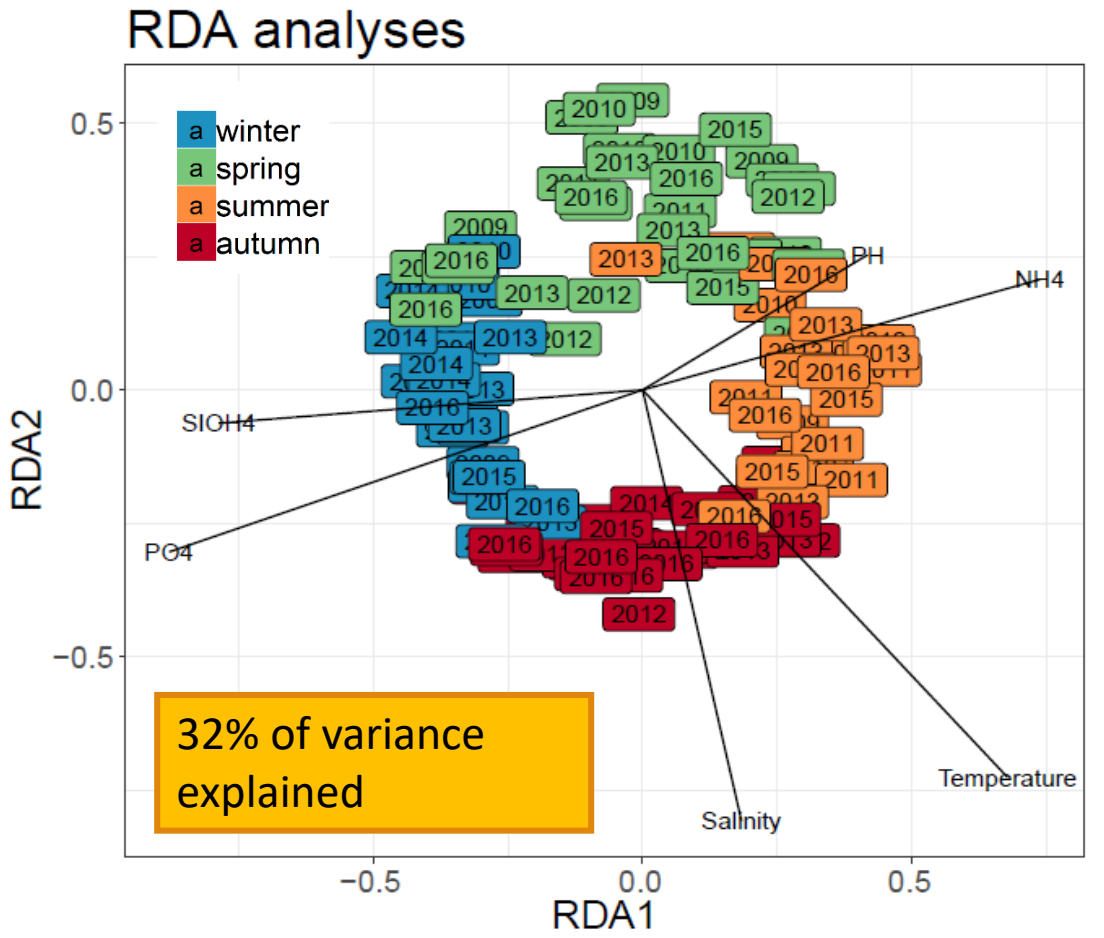
- Guinardia delicatula
- Minidiscus variabilisi
- Minidiscus comicus
- Thalassiosira concaviuscula
- Heterocapsa rotundata
- Gyrodinium cf gutrula/Gyrodinium dominans/Gyrodinium moestrupii
- Pentapharsodinium sp.
- Gyrodinium helveticum, Gyrodinium rubrum
- Warnowia sp.
- Dinophyceae
- Gyrodinium fusiforme
- Ostreococcus_lucimarinus
- Teleaulax amphioxeia
- Teleaulax gracilis
- Teleaulax acuta
- Cryothecomonas sp.
- Dino-Group-I-clade-1_X-sp.
- Parengyodontium/Engyodontium/Tritirachium/Beauveria

	Bacillariophyta
	Dinophyceae
	Chlorophyta
	Cryptophyta
	Cercozoa
	Dinoflagellata
	Fungi

RDA: Redundancy analyses

References:
- Numerical ecology - Legendre & Legendre
- Van den Wollenberg (1977)
- Legendre & Gallagher (2001)





- **<u>Multiple linear regression (MLR)</u>**
- Environment can predict 32% of the variance.
- Temperature is the parameter explaining the most. Macronutrients selected are instead SIOH4, PO4 and NH4.
- Repeating cycle indicative of the community's resilience.

Time-series….. Long term… Team work first of all !

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Eric Thiébaut Fabrice Not Manon Viprey Stéphanie Ristori
Mariarita Caracciolo Samuel Forsans
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Erwan Corre Léa Joret Olivier Quenez Christophe Caron
Maxime Manno Samuel Forsans Colomban de Vargas Sarah Romac
Dominique Marie Anne-Claire Baudoux Christian Jeanthon
Raffaele Siano Thierry Cariou Thierry Comtet Frédéric Mahé
Nicolas Henry Eric Macé Marc Vernet Marie Latimier Noël Guidal
Isabelle Gaillard Gilles Maron Jean-Michel Roallec François Le Ven…
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Merci!!

Station Biologique de Roscoff
Coastal Observatory

CNRS UPMC
Station Biologique
Roscoff

THANK YOU – QUESTIONS ?