



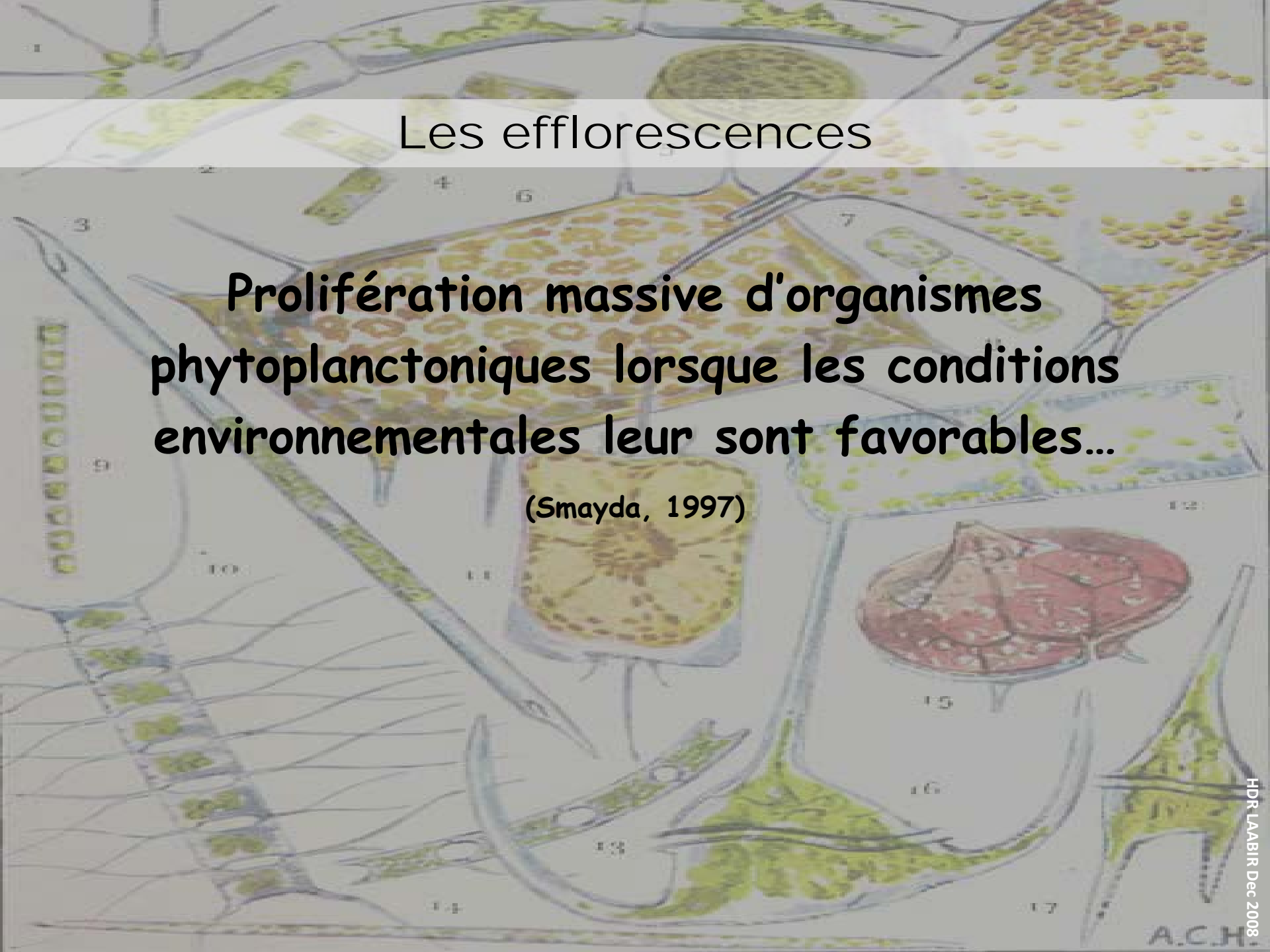
Equipe ETDA (Efflorescences Toxiques et Diversité Algale)
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- 3 Chercheurs CNRS
- 1 Chercheur IRD
- 3 Techniciens
- Etudiants en Master et Doctorat

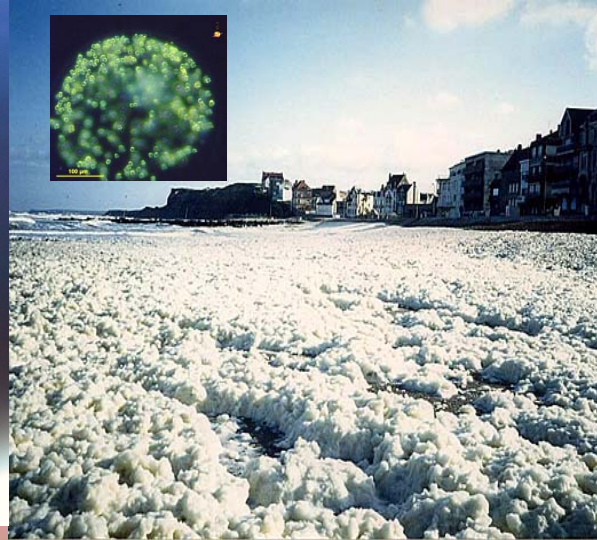
Les efflorescences

**Prolifération massive d'organismes
phytoplanctoniques lorsque les conditions
environnementales leur sont favorables...**

(Smayda, 1997)



Cette prolifération peut aboutir à des phénomènes d'eaux rouges



Les efflorescences de microalgues nuisibles (Harmful Algal Blooms)

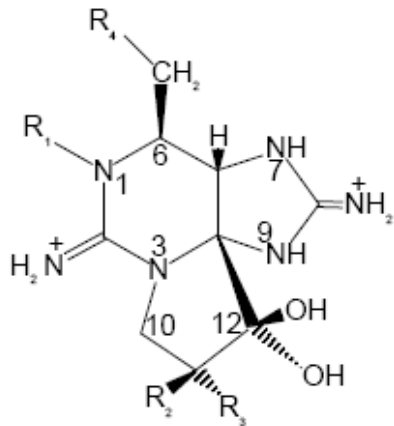
1- anoxies, NH_3 , H_2S

2- Intoxications (PSP, ASP, DSP... nécrose, mortalité)





Les toxines paralysantes

Alexandrium spp.



Saxitoxine

R ₁	R ₂	R ₃	R ₄	Toxines Carbamate		Toxines N-Sulfocarbamoyle		Toxines Décarbamoyle (dc)	
					Poids moléculaire		Poids moléculaire	—OH	Poids moléculaire
H	H	H		STX	301,31	B1	380,36	dcSTX	258,28
H	H	OSO ₃		GTX2	396,36	C1	475,41	dcGTX2	353,33
H	OSO ₃	H		GTX3	396,36	C2	475,41	dcGTX3	353,33
OH	H	H		NEO	317,31	B2(GTX6)	396,36	dcNEO	274,28
OH	H	OSO ₃		GTX1	412,36	C3	491,41	dcGTX1	369,33
OH	OSO ₃	H		GTX4	412,36	C4	491,41	dcGTX4	369,33

- Ingestion de coquillages contaminés (seuil à 80µEq STX/100g chair)

- Symptômes : 5-20 min après ingestion, peut entraîner la mort par paralysie des muscles respiratoires

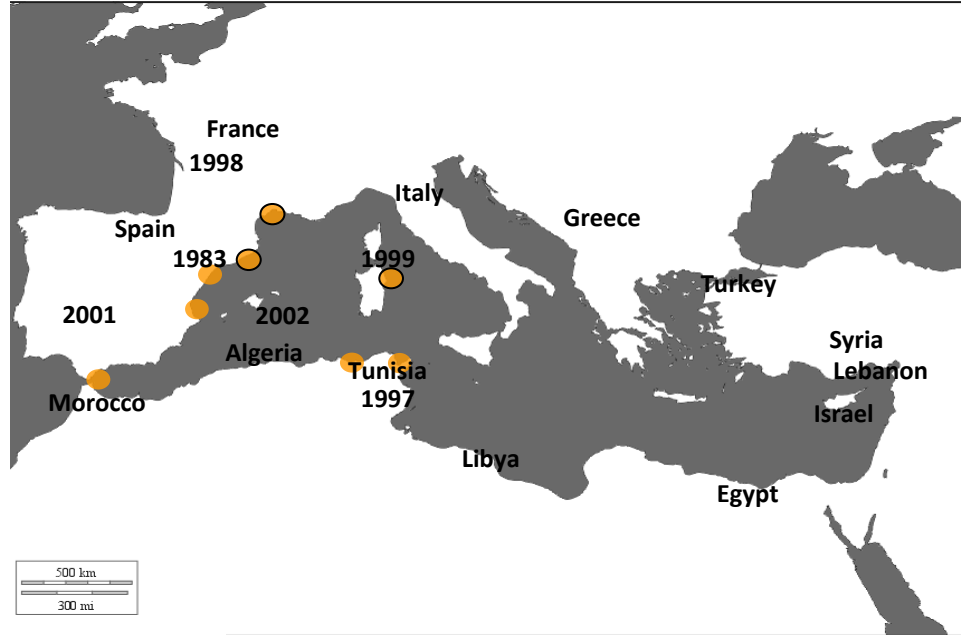
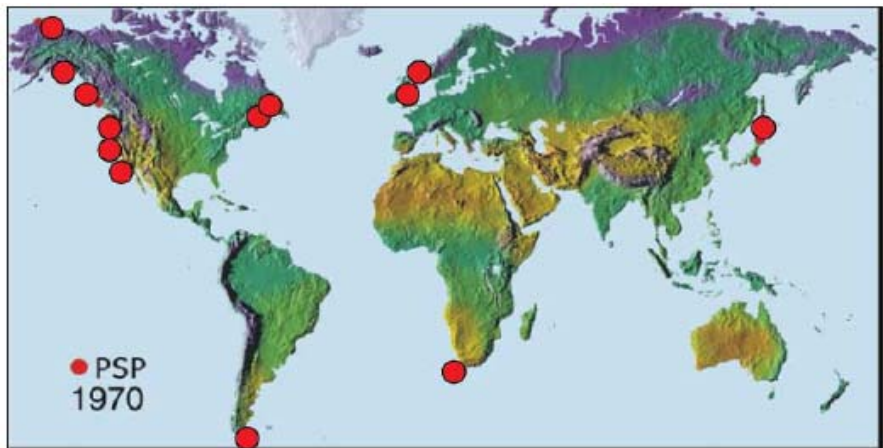
Action directe sur la transmission des influx nerveux (blocage des canaux à Na⁺)



Expansion géographique des événements PSP

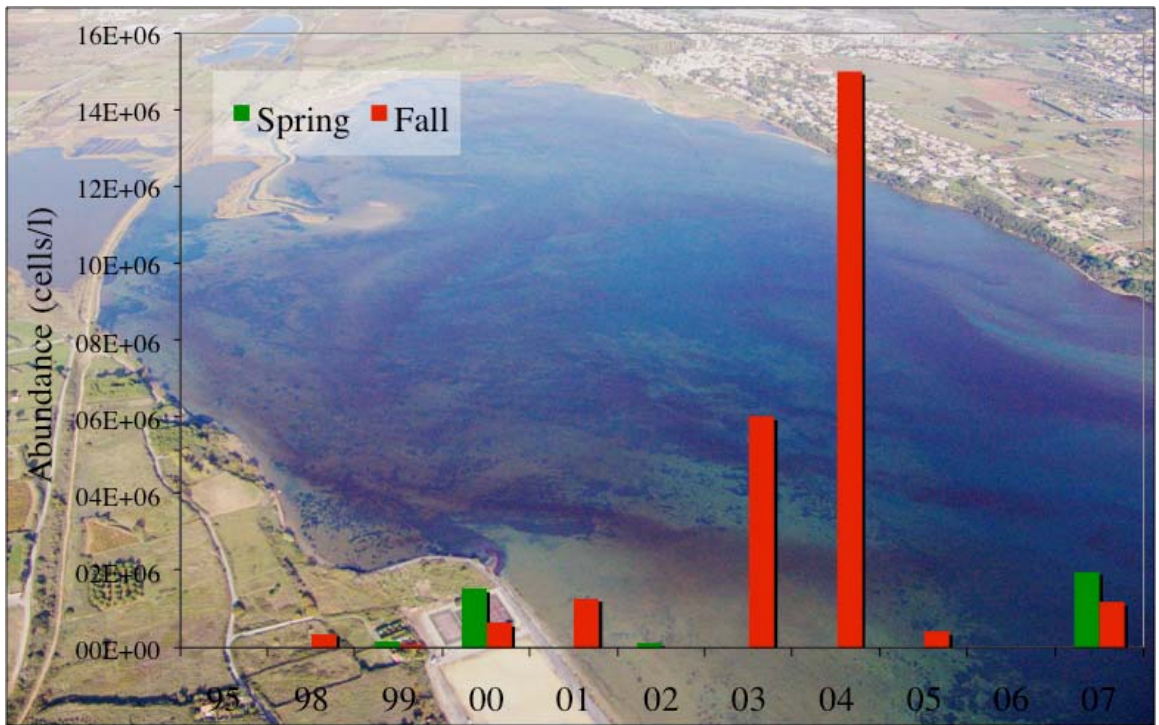


Etude biogéographique: *Alexandrium catenella/tamarense*



Penna et al 2008, modified; Frehi et al 2007



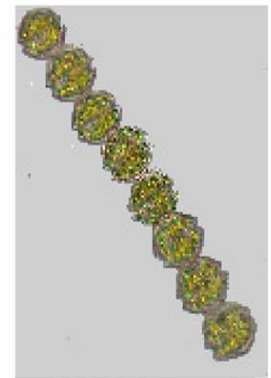


**Phénomène récurrent
Bloom printanier et
automnal**

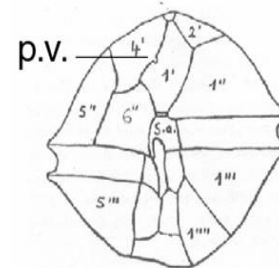
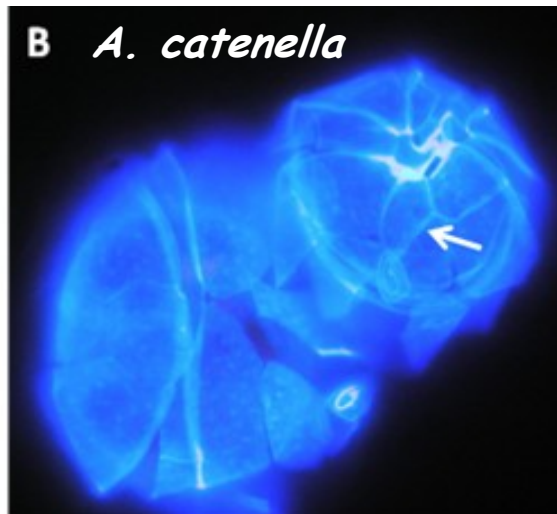
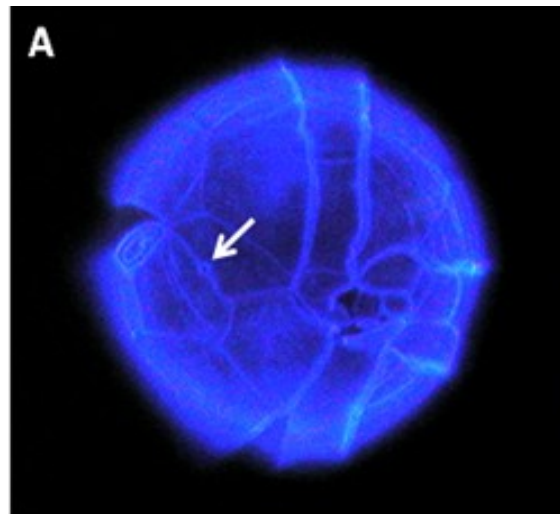
Intoxication PSP



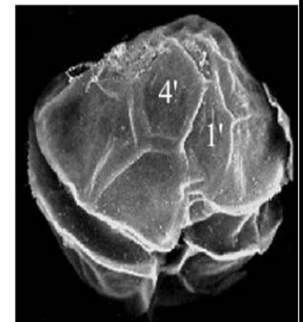
Cellule végétative
d'*Alexandrium catenella*



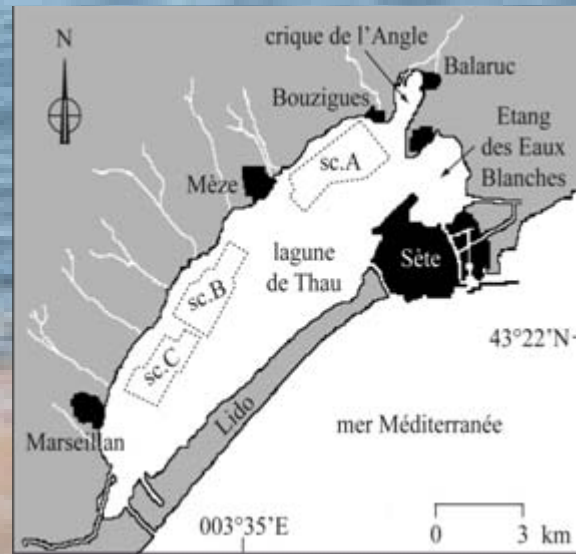
Chaîne de 8 cellules



Position du pore ventral
(p.v.) d'*A. tamarense*

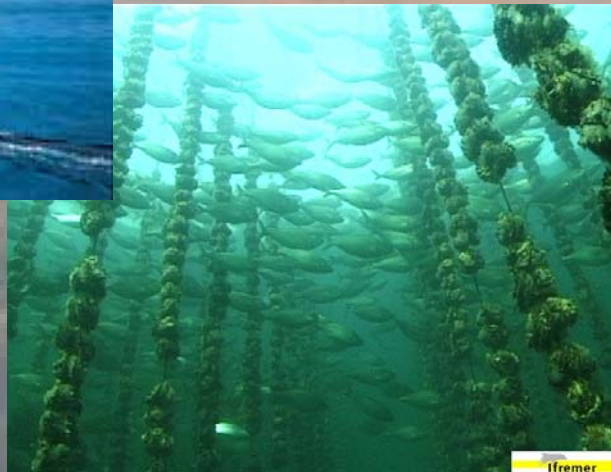


Absence de pore ventral
sur la thèque *A. catenella*



Lagune de Thau 10 % de la production nationale d'huîtres

Demande économique
et sociétale



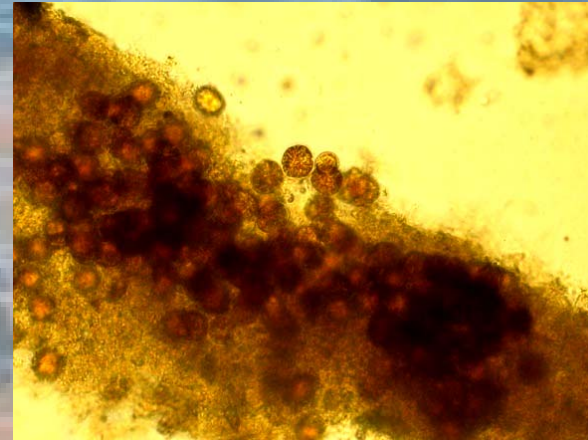


Quelques résultats marquants

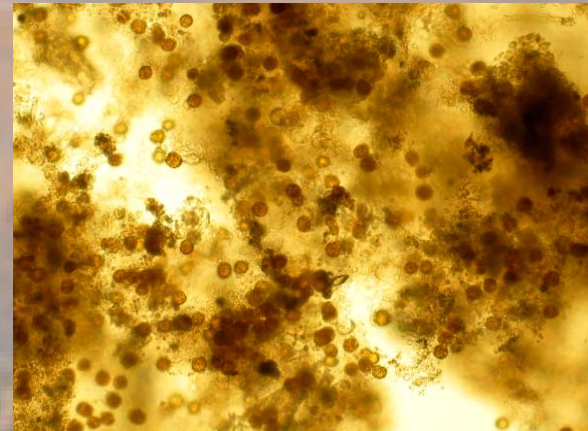
Survie des algues toxiques au transit stomacal chez *Crassostrea gigas*



Fèces

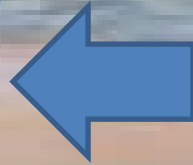
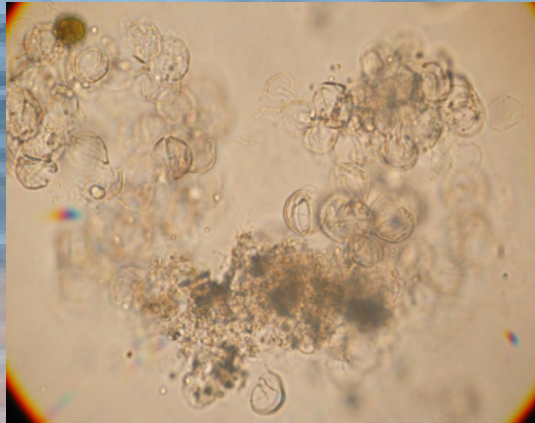


Pseudofèces



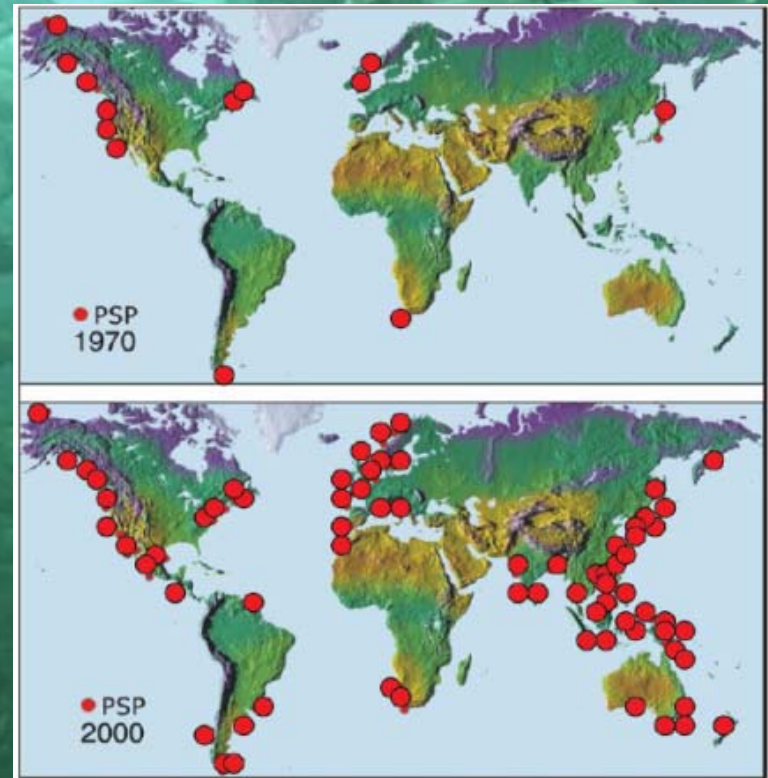
Alexandrium catenella

Production de kystes pelliculaires
(temporaires) intacts et immobiles



les kystes pelliculaires
se libèrent de leurs thèques
et donnent des cellules
végétatives viables qui se
divisent

Risque d'émergence dans Thau de nouvelles espèces
(*A. tamarense*, *Dinophysis sp.*, *Pseudo-nitzschia sp....*)
Intoxications ASP, DSP.....



Effet des facteurs environnementaux sur la croissance d'*A. catenella*



Cette espèce est:

Euryhaline

Eurytherme

Lumière saturante à partir de $90 \mu\text{mol.m}^{-2}.\text{s}^{-1}$

Température facteur important

Optimum de croissance se situe autour de 20°C

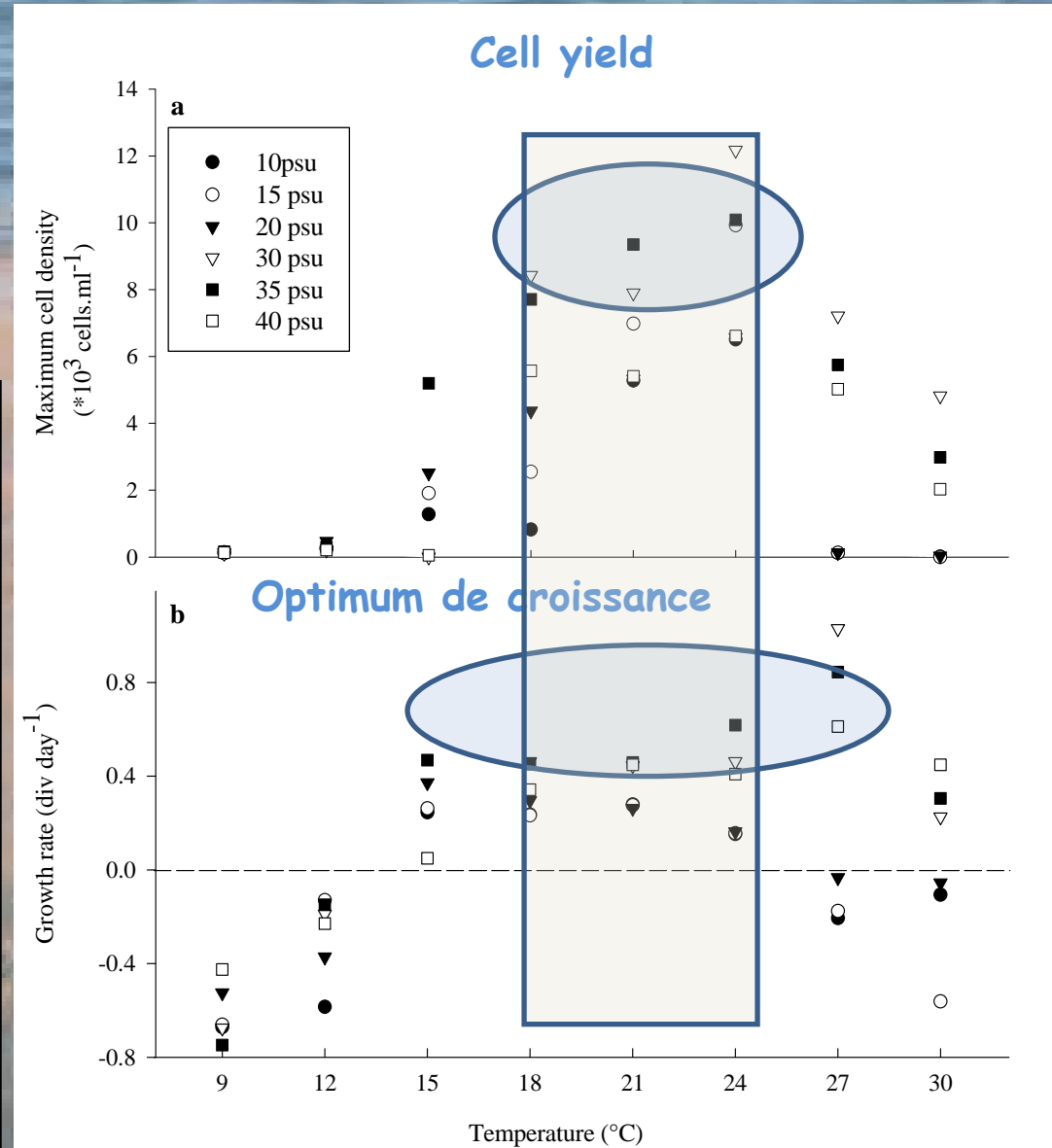


Fig. 4. Influence of salinity and temperature on (a) the final concentration and (b) the growth rate of *A. catenella*, in the 48 combined of salinity and temperature.

Contribution of known and unknown nitrogen sources to PN-based growth of *A. catenella* during blooms

N source	% total H growth	% total T growth
Nitrate	0.1–3	5–14
Nitrite	0.1–1	1–5
Ammonium	30–68	32–100
Urea	2–36	48–59
X	0–67	0–13

Principales sources d'azote

N organique ?
Mixotrophie ?

Range of values from six series of measurements over 3 years. In the H series with full enrichment, the contribution of NH_4 corresponds to $20 \mu\text{mol N l}^{-1}$ and other N sources at *in situ* concentrations. In the T series without any enrichment, the contributions of all known N sources correspond to *in situ* concentrations.

Différences entre les souches pour l'utilisation de l'azote

Rôle du kyste dormant dans l'initiation, le maintien et la récurrence des efflorescences d'*A. catenella*

Au cours de la phase de croissance exponentielle ; jusqu'à 8 cellules en chaîne

Chaîne cellulaire

Fusion des gamètes

Planozygote

Cellule végétative
Thèque bien visible ; plus large que long

Division ?

?

Cellule mobile, légèrement plus grosse et foncée (chargée de réserve)

Perte de mobilité et sédimentation

Expulsion de la thèque

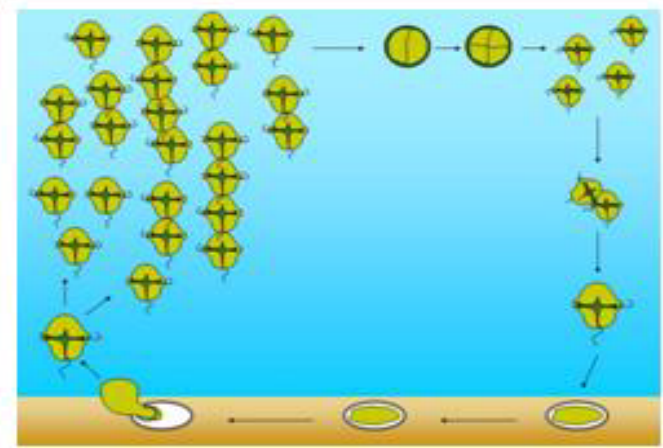
Le kyste de résistance s'extrait à travers un pore en moins de 60 secondes. Il est toujours flagellé

Thèque vide

Division ?

Planoméiocyte

Absence de thèque ; plus long que large ; sa nage atypique



Germination

La cellule s'extrait du kyste à travers un opercule

Domance & maturation

Pré-germination

La forme de la future cellule se dessine

Kyste de résistance mature

Eclaircissement du cytoplasme: des vacuoles se creusent du fait de la consommation des grains de réserves au cours de la période de dominance

Kyste de résistance immature

Double paroi visible, cytoplasme foncée et remplis de grains de réserve



Kyste vide avec opercule visible

Distribution & abundance ?

Temps de survie ?

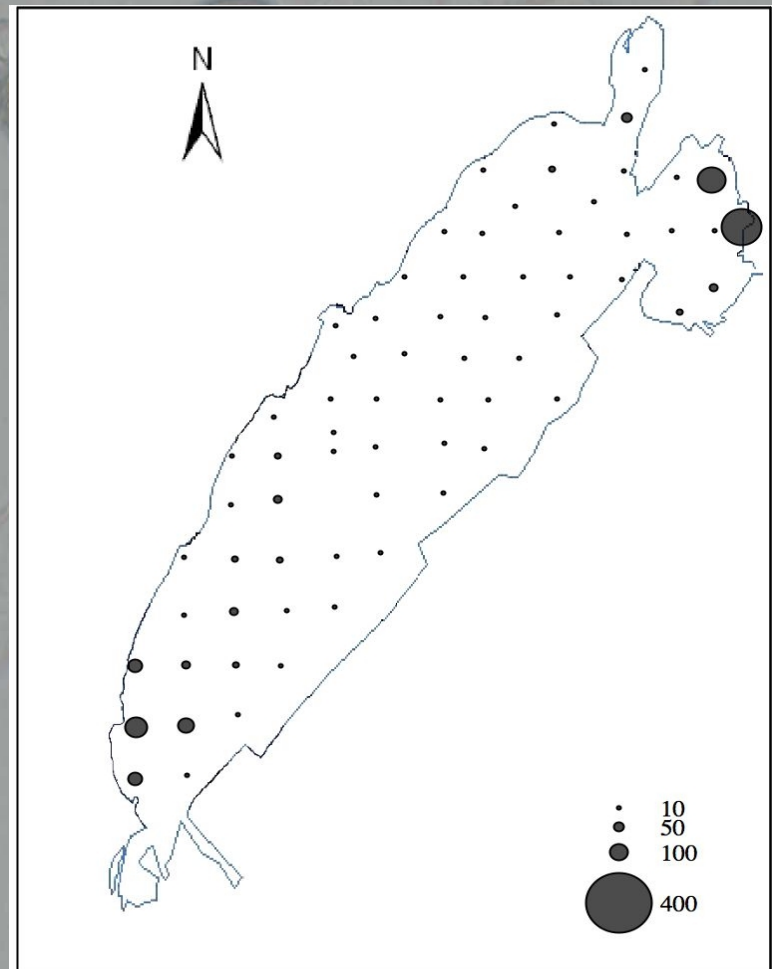
Germination & ensemencement ?

Distribution d'*A. catenella*



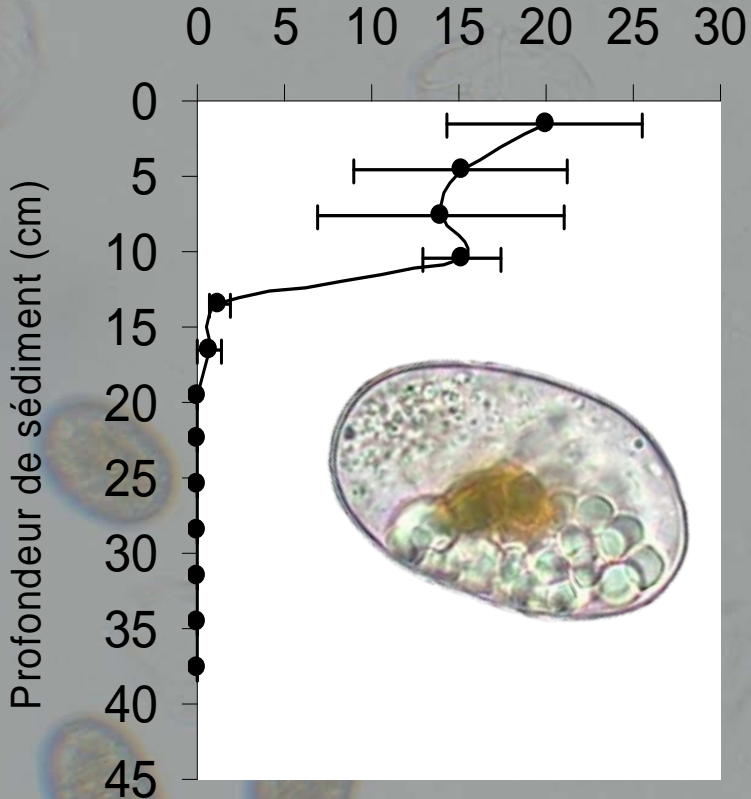
Mise en évidence de foyers
de kystes de résistance

Corrélation avec les
caractéristiques
du sédiment

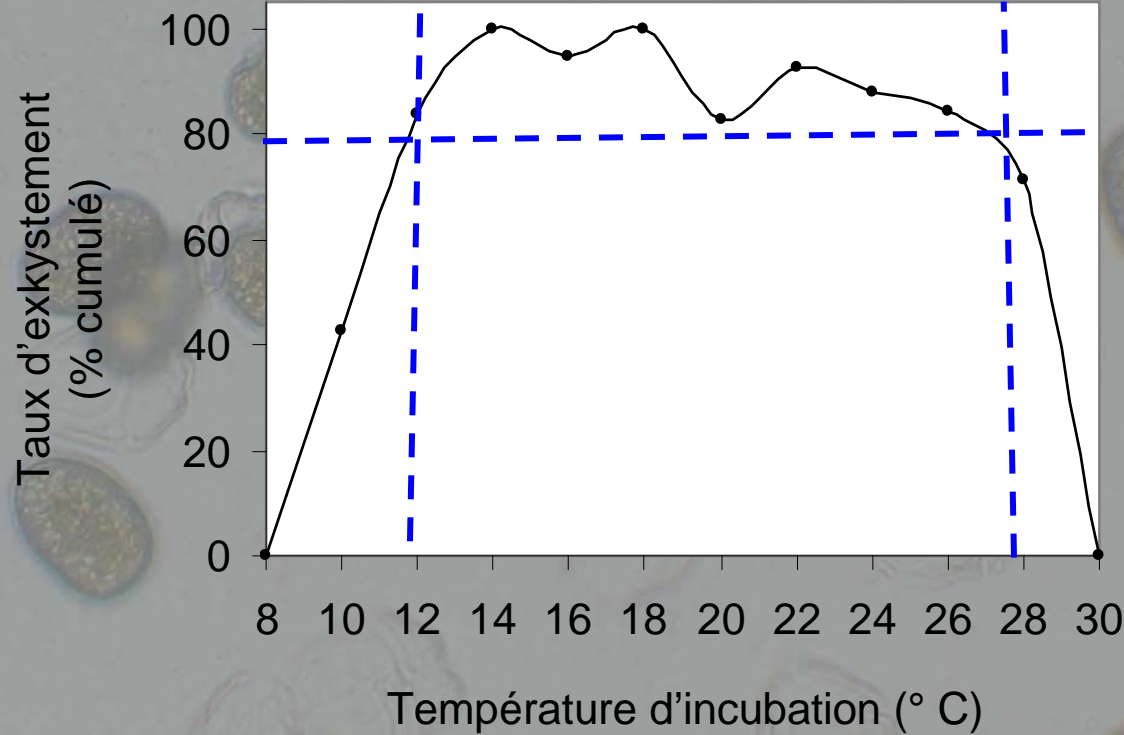


Distribution et Biologie du kyste dormant

Kystes g⁻¹ sédiment sec



Répartition verticale



Biologie du kyste
(fenêtre de germination)

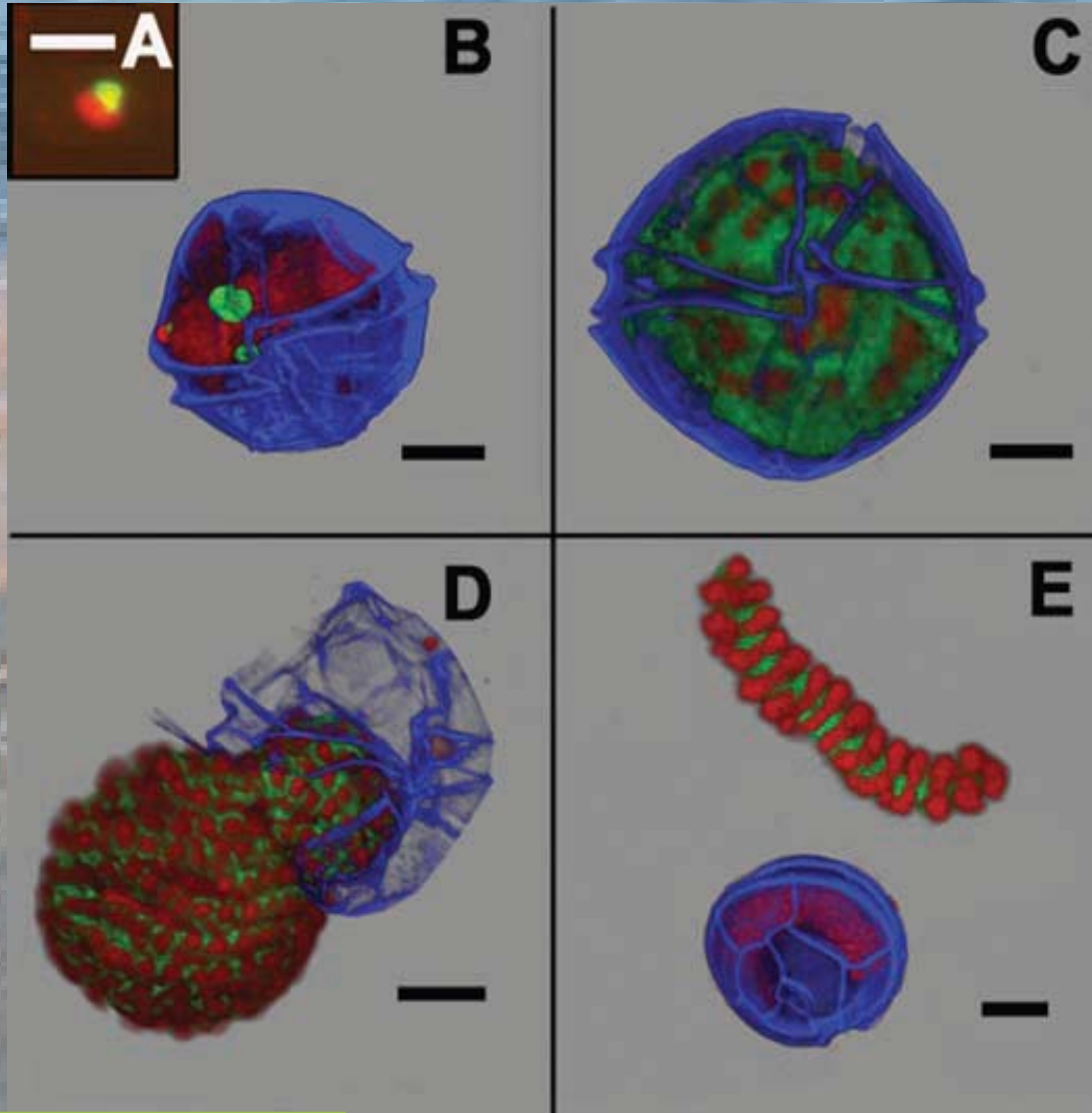
A. minutum (Estuaire de Penzé) avec son parasite *Amoebophrya* spp. (Syndiniales)

Spécificité marquée

Dinospore libre

1^{er} stade d'infection

Rupture de la thèque



Parasite mature
« trophont »

Stade
vermiforme



60-400
dinospores

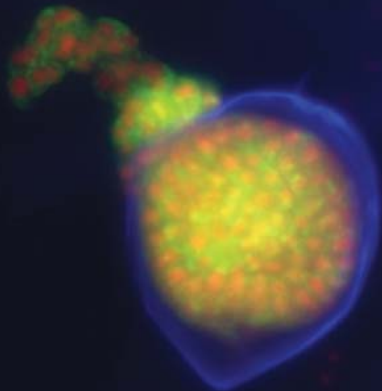
Sonde oligonucléotidique ALVO1
Dirigée contre SSU rRNA
des parasitoides

Fluorescence rouge
(ADN-propidium iodure)

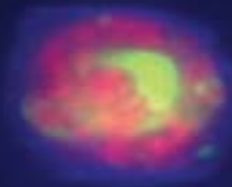
Thèque colorée
par le calcofluore

A. catenella non infecté jusqu'à présent...

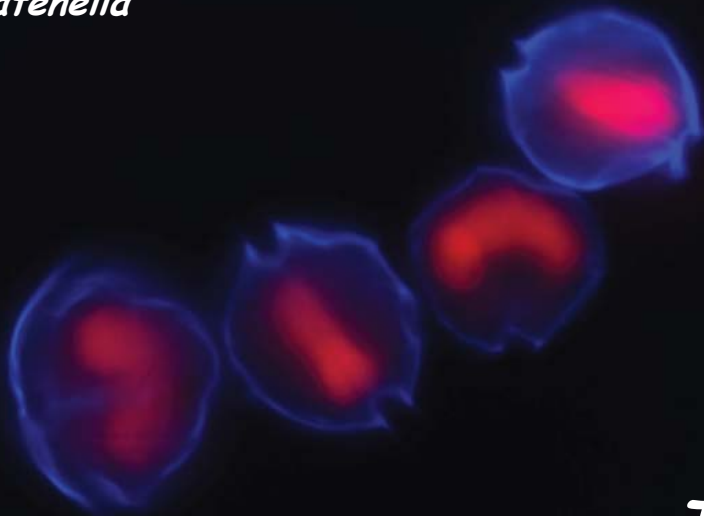
Prorocentrum sp.



Scrippsiella sp.



A. catenella



Thau

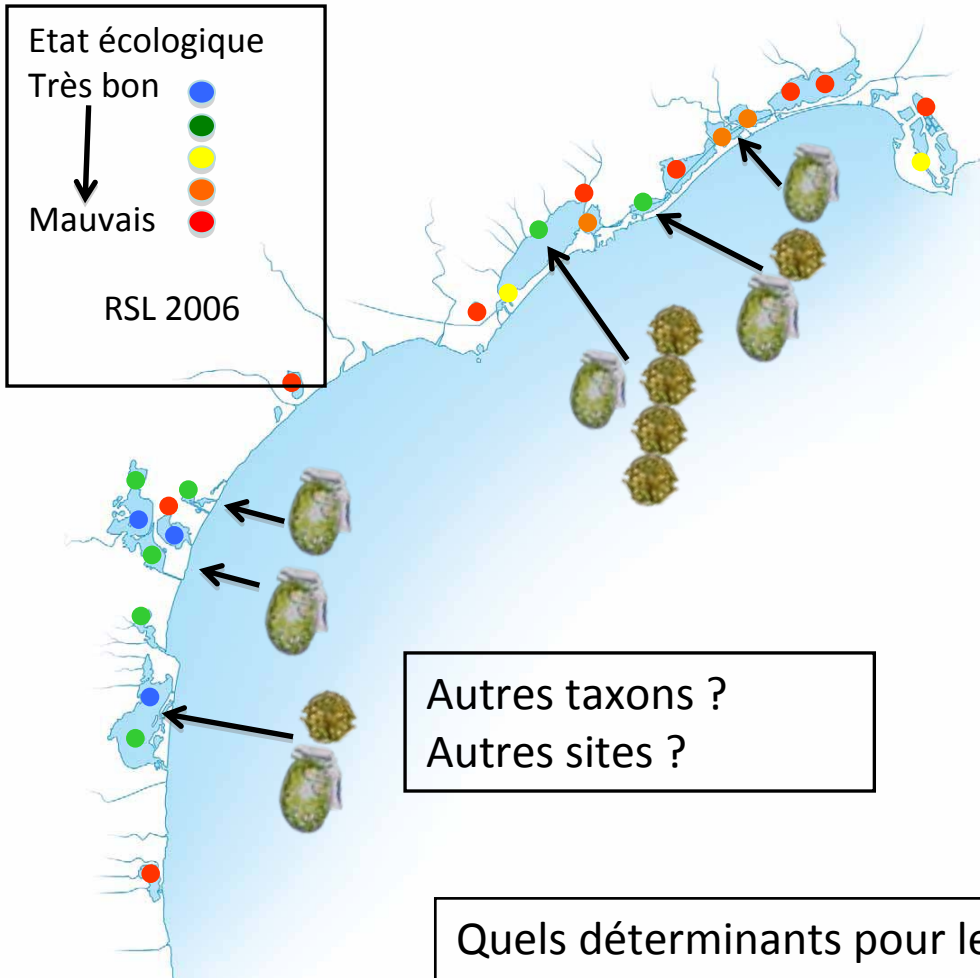
Ce qui peut expliquer...

- La persistance d'*A. catenella* *in situ* pendant plusieurs semaines
- La récurrence des blooms toxiques depuis 1998

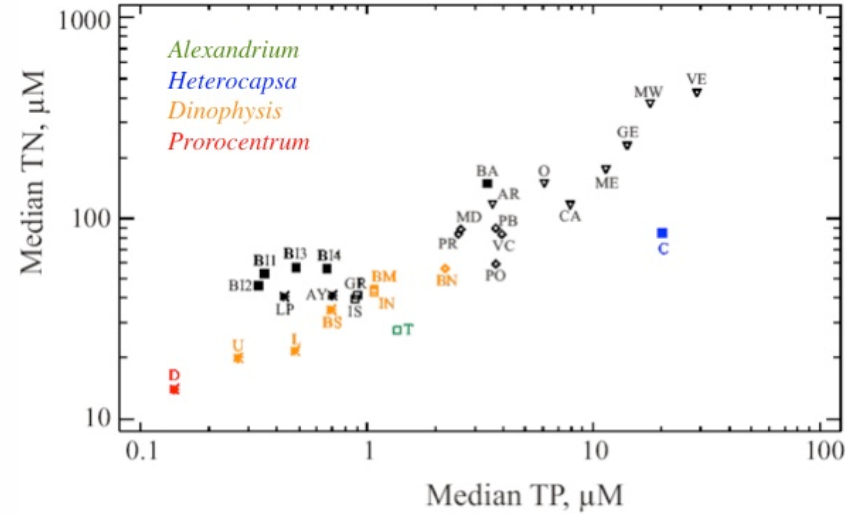
Possible adaptation des parasites à *A. catenella* (espèce invasive) 10^{aine} d'années....

Continuer le suivi *in situ*

Efflorescences toxiques et Diversité algale



Dinoflagellates and eutrophication

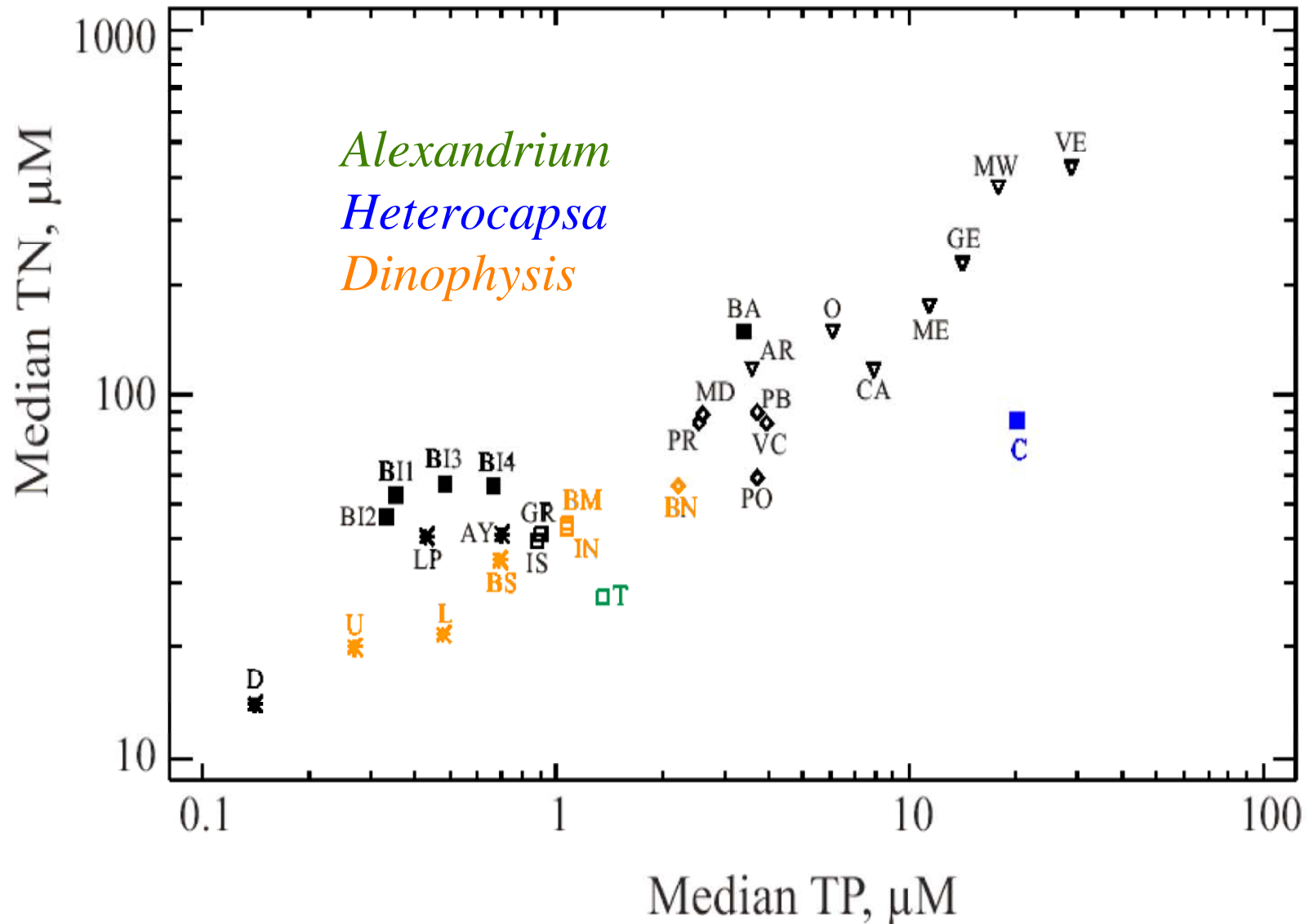


Phytoplancton lagunaire :
diversité vs biomasse ?

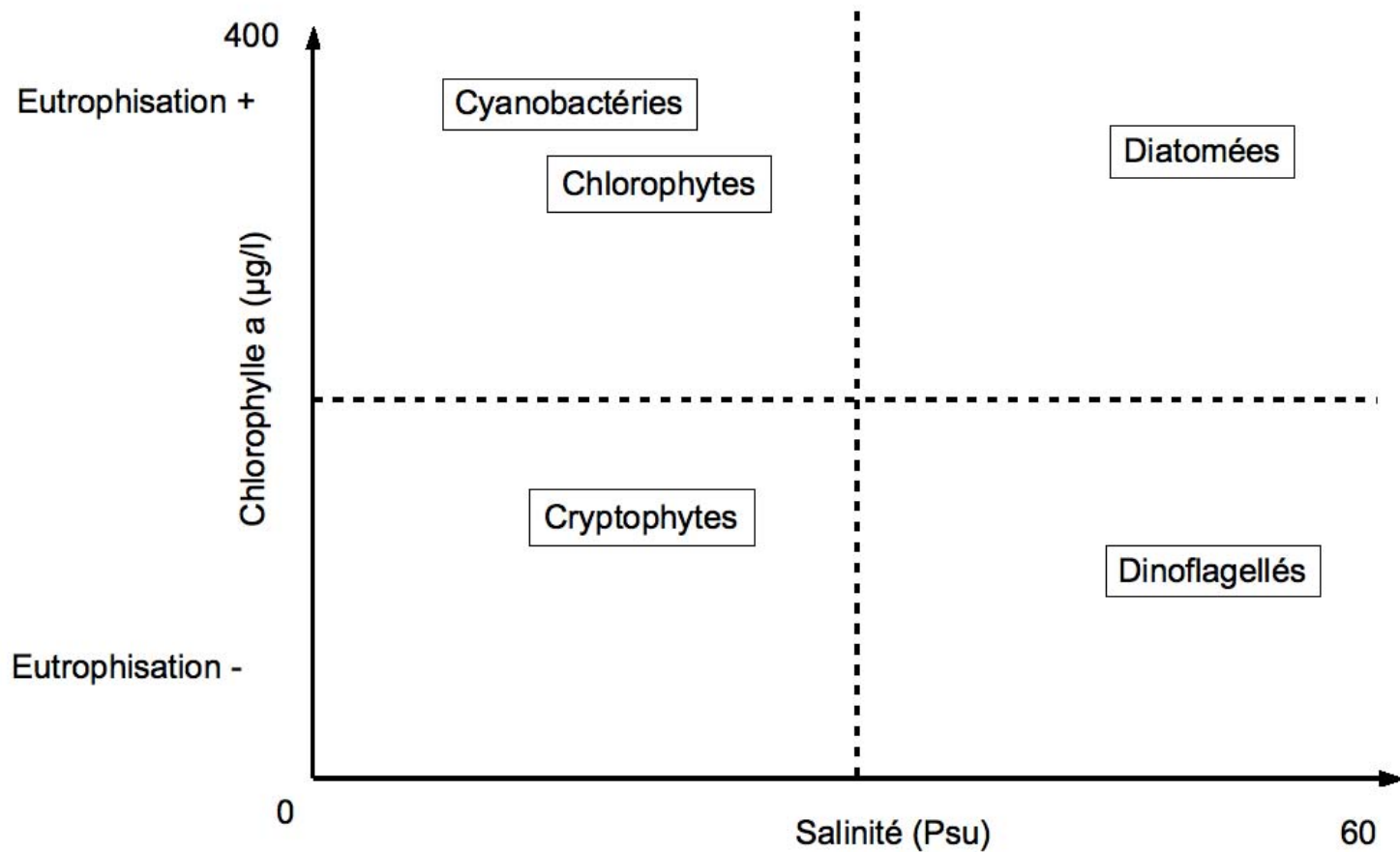
Quels déterminants pour les HABs ?

- Diversité génétique inter et intraspécifique ?
- Contrôles environnementaux ?
- Contrôles biologiques ?

Dinoflagellés et eutrophisation

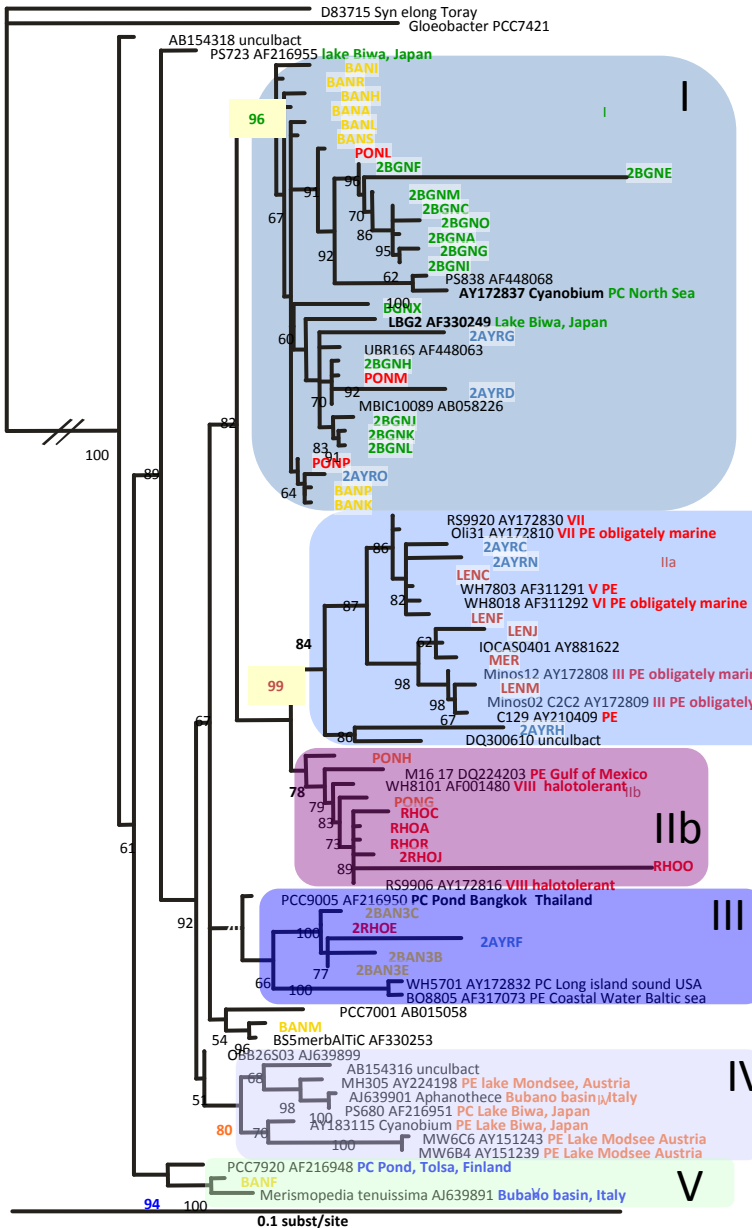


Relations entre groupes phytoplanctoniques, salinité et eutrophisation





Diversité génétique de *Synechococcus* et diversité lagunaire



Halotolerant
Synechococcus-PC

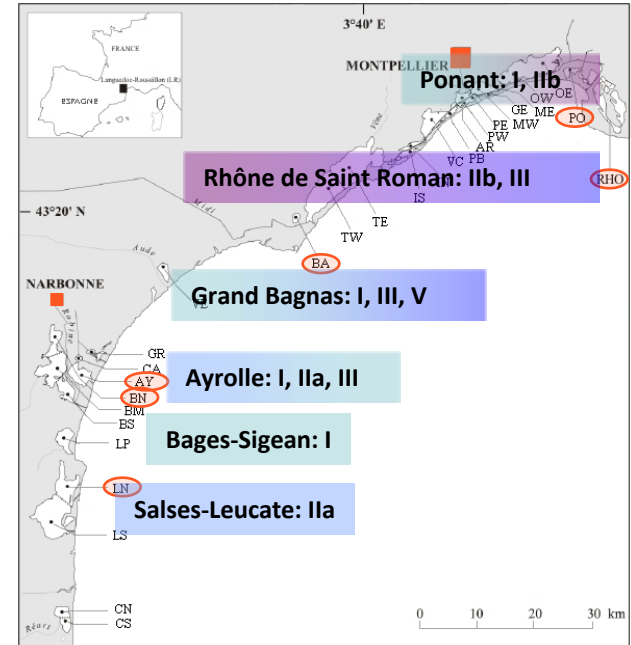
Marine
Synechococcus-PE
(sub-cluster 5.1)

Halotolerant
Synechococcus PE

Halotolerant
Synechococcus-PC
(sub-cluster 5.2)
+ PE

Synechococcus from Freshwater
PC-PE

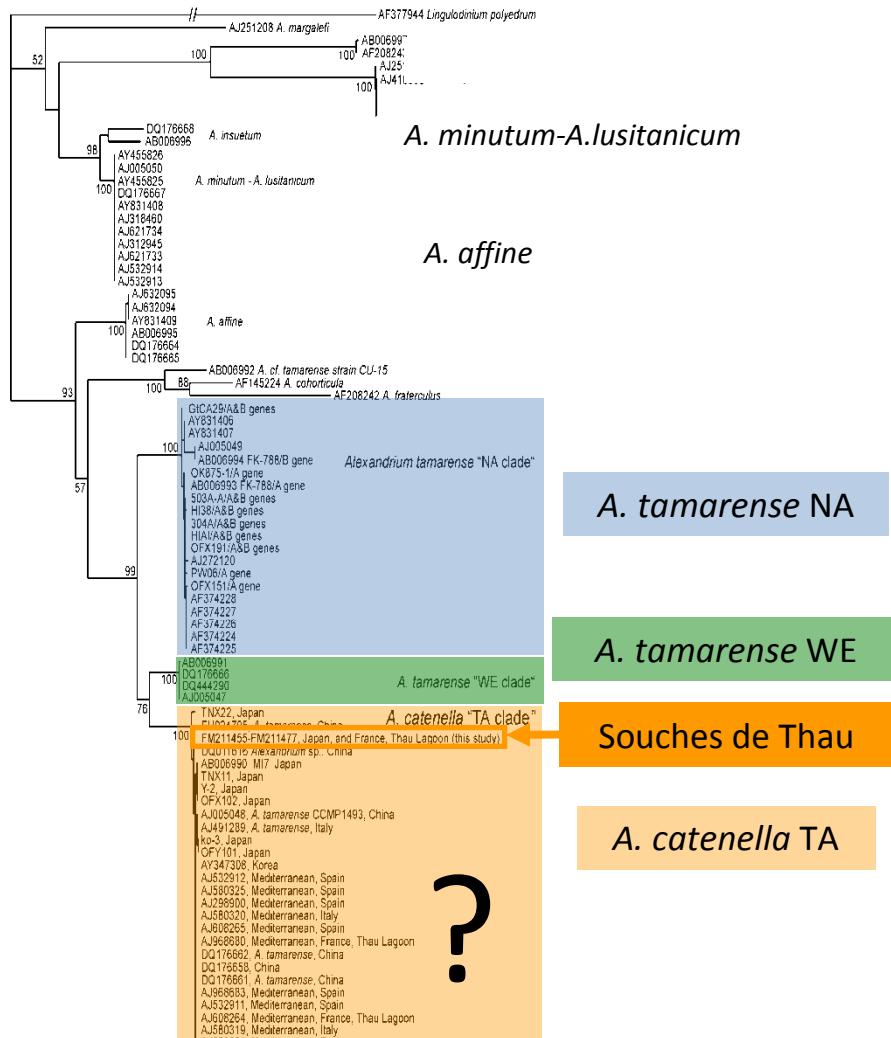
Synechococcus from Freshwater-PC



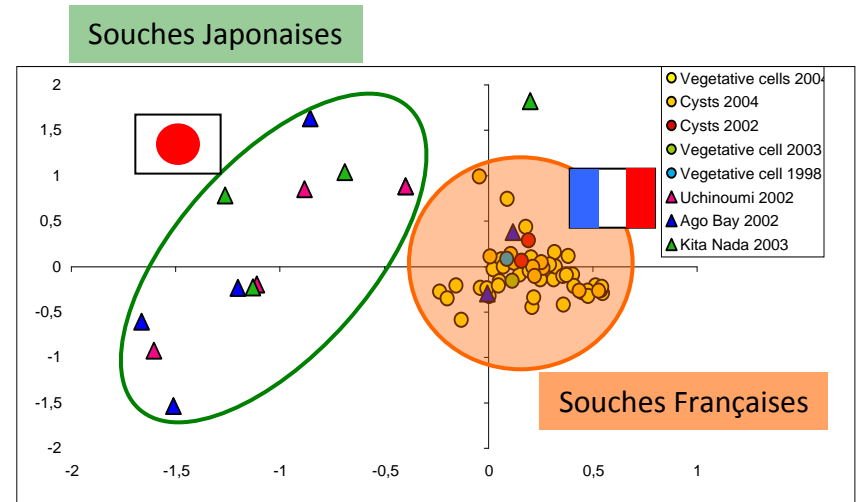
- Très large diversité d'écotypes:
mer -> eau douce
- Forte structuration des populations
- Importance : communications avec la mer: salinité, canaux, bassin versant, statut trophique

Diversité et structure des populations d'*Alexandrium catenella*

Diversité phylogénétique du genre *Alexandrium*



Structure génétique des populations française et japonaise



Mise en évidence de trois lignées distinctes au sein de l'espèce *A. catenella* grâce aux marqueurs microsatellites



L'opéron ribosomal ne peut pas élucider la variabilité intraspécifique parmi les populations d'*A. catenella* du clade géographique Asie Tempérée



Mise en place de la souchothèque au sein d'ETDA-ECOLAG

- Isolement de plusieurs centaines de souches monoclonales
- Culture de cellules issues de kystes de résistance
- A terme constituer une souchothèque de référence des microalgues toxiques apparaissant en Méditerranée



Les souches seront utilisées
dans les études génétiques,
toxiques et écophysiologiques

Allelopathic effects of *Zostera marina* and *Zostera noltii* on the growth of the toxic dinoflagellate *Alexandrium catenella*



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²Phytochimie et Valorisation de la Biomasse (PhyValBio), Université Bordeaux I, 351, cours de la Libération, 33405 Talence cedex, France.

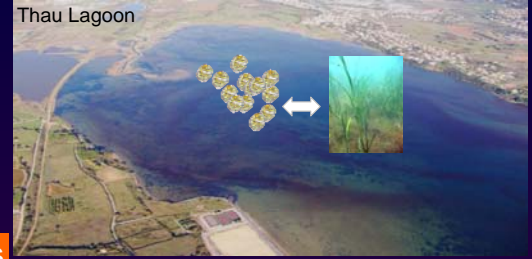
*Mohamed.laabir@univ-montp2.fr

ECOLAGUNES Project

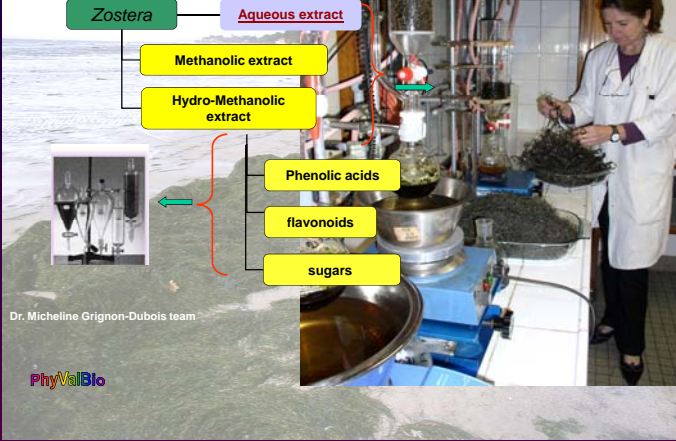
Introduction

SUDOE

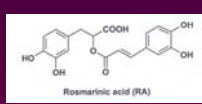
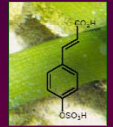
In the Thau lagoon (a French Mediterranean coastal lagoon), a bloom (85000 cellules.l⁻¹) of *Alexandrium catenella* occurred for the first time in November 1998. Later similar episodes appeared regularly in spring and autumn reaching high cell concentrations (5*10⁶ cells.l⁻¹) that induced the closing of shellfish farms. Allelopathy is a process implying secondary metabolites excreted by plants and micro-organisms which affects the competitive efficiency of the coexistent organisms. *Zostera marina* and *Zostera noltii* produce bioactive molecules as zosteric acid, a sulphated natural product able to prevent settlement of some marine bacteria, algae, barnacles and tube worms at non-toxic concentrations. These seagrass species contain also rosmarinic acid which have been reported to have a number of biological activities *in vitro*, such as antiviral, including human immunodeficiency virus 1, antibacterial, antioxydant, anti-inflammatory and anticarcinogenic. Water-soluble extract of *Z. marina* leaves inhibited the growth of micro-algae and many marine bacteria.



Preparation of the tested extracts



Dr. Micheline Grignon-Dubois team



Sampling of *Zostera* sp. leaves

Identification

Extraction

Isolation and purification

Isolation and purification

Biological test

Detritus of the sea grasses *Z. noltii* and *Z. marina* collected on the beaches of Arcachon Lagoon over a 3-year period contain zosteric acid, a natural sulphated phenolic acid and rosmarinic acid, a phenolic acid an economically important metabolite. (Achamlale et al. 2009a, b)

Objectives

The present study investigates non-nutrient relationship between two dominant seagrass species and *A. catenella* a toxic dinoflagellate blooming worldwide which is often responsible of paralytic shellfish poisoning events. Aqueous and methanolic crude extracts were prepared from two seagrasses, *Z. marina* and *Z. noltii* which were collected on the beaches of Arcachon lagoon. The potential allelopathic effects of different concentrations of these extracts have been tested on the growth of *A. catenella* (strain collected in Thau in 2003) by using bio-essays.

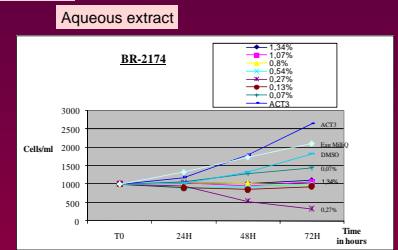
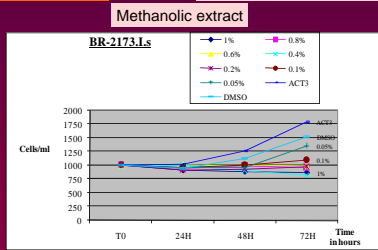
Methods

A. catenella was grown in batch cultures using ESAW medium (Harrison et al. 1980) under appropriate light (100 μmoles.m⁻².s⁻¹) and temperature (20 ± 1 C) conditions. To test allelopathic effect of *Zostera* species, target species was cultivated in culture medium with different concentrations of the extracts. Nutrients were added at (t0) in order to control the nutrient concentrations in the different tested treatments, including controls.

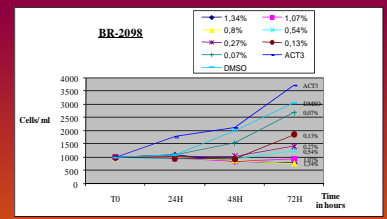
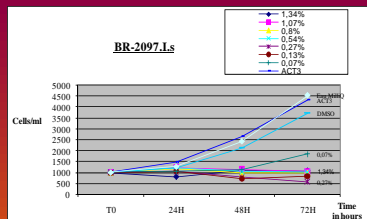
Results

Extracts from *Zostera noltii*

Inhibition of *A. catenella* growth



Extracts from *Zostera marina*



Our observations using photonic microscope showed that cells which stopped to divide in the presence of very low concentrations of the tested extracts presented morphological anomalies and lysis.

Inhibition of photosynthetic activity

Recent results (no shown here) clearly indicate that methanolic extract (BR.2173.I.S) of *Z. Noltii* have also a significant negative effect on the photosynthetic activity of *A. Catenella* (Phytopam fluorescence measurements during microcosm's experiments).

These results highlighted the strong inhibition effect exerted by natural extracts of *Zostera* sp. on the growth of *A. catenella*, even at very low concentrations. Extracts from leaves of *Zostera* species originated from Thau lagoon will be tested. Additional experiments will be dedicated to test the effect of continuous release of allelochemicals from the fresh seagrass tissues on the growth and the physiological activity of *A. catenella*. Because of the severe economic and public health caused by harmful microalgae, the allelopathic substances from *Zostera* detritus may be considered as potential candidates to mitigate the effects of harmful algal blooms on shellfish aquaculture in lagoons.

Achamlale S., Rezzonico B., Grignon-Dubois M. 2009a. Evaluation of *Zostera* detritus as a potential new source of zosteric acid. *J. App. Phy.* 21:347-352.
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 Buchsbaum R.N., Short F.T., Cheney D.P. 1990. Phenolic-nitrogen interactions in eelgrass *Zostera marina* L.: possible implications for disease resistance. *Aquat. Bot.* 37: 291-297.
 Collos Y., Gagne C., Laabir M., Vaquer A., Cecchi P., Souchu P., 2004. Nitrogenous nutrition of *Alexandrium catenella* (Dinophyceae) in cultures and in Thau lagoon, southern France. *J. Phycology.*, 40 : 96-103.
 Harrison P.G., Chan A.T. 1980. Inhibition of the Growth of Micro-Algae and Bacteria by Extracts of Eelgrass (*Zostera marina*) Leaves. *Marine Biology* 61: 21-26.
 Harrison P.G. 1982. Control of Microbial Growth and of Amphipod Grazing by Water-Soluble Compounds from Leaves of *Zostera marina*. *Marine Biology* 67: 225-230.
 Todd J.S., Zimmerman R.C., Crews P., Alberte R.S. 1993. The antifouling activity of natural and synthetic phenol acid sulphate esters. *Phytochem.* 34: 401-404.

Discussion

Bibliography