Allelopathic effects of *Zostera spp* on the growth and photosynthetic activity of the toxic dinoflagellate *Alexandrium catenella*

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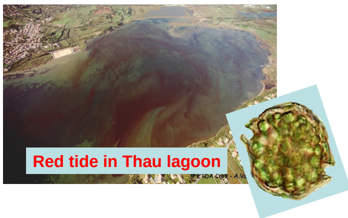
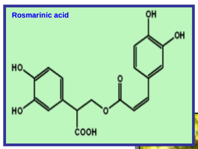
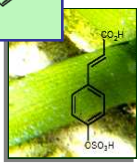
Allelopathy is a biological phenomenon by which an organism produces one or more biochemicals that influence the growth, survival, and reproduction of other organisms. These substances are known as allelochemicals and can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms. Aquatic macrophytes have long been suspected of suppressing phytoplankton growth through the production and excretion of chemical substances.

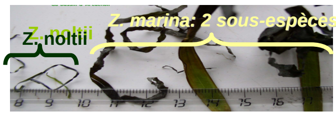
Alexandrium catenella **is a widespread PSP toxin-producing dinoflagellate species. Since 1998, recurrent *A. catenella* blooms have been observed in the Thau lagoon (French Mediterranean coast), leading to the closing of shellfish farms (Collos *et al.,* 2007), but never in Arcachon lagoon (French Atllantic coast). Analyses of REPHY and REBENT monitoring network database showed a low occurrence of *Alexandrium* blooms in the vicinity of extensive *Zostera* beds. This led us to investigate the non-nutrient relationship between *Zostera species* and *A. catenella*.**



- ***Zostera marina*** **(eelgrass) is found on both coasts of North America, as well as in Europe.**
- ***Zostera noltii*** **(dwarf eelgrass) occurs along the Atlantic European and northen African coasts and the Mediterranean sea. It s distinguished from *Z. marina* by its smaller size, and by the colour and shape of the leaves.**
- **The two species produce bioactive phenolics among which zosteric acid, rosmarinic acid and flavonoids (Achamlale et al., 2009)**







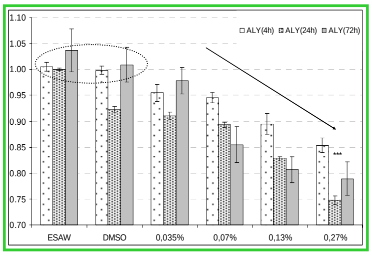
The ability of *Zostera* spp. to produce allelochemicals has been tested in laboratory using bioassays. Aqueous or methanolic extracts from leaves of *Zostera marina* and *Z. noltii* were assayed to determine their allelopathic effects on *A. catenella.*

Materials and Methods

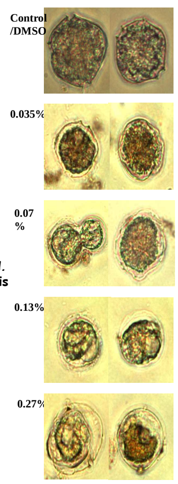
A. catenella **was grown in batch cultures using ESAW medium under appropriate light (µmol photons.m^{-2} s^{-1}) and temperature (20±1°C) conditions.**

Methanolic and aqueous extracts were prepared with *Zostera leaves* from the Bay of Arcachon and the Thau lagoon. They were analyzed for both the identity and quantity of phenolics present.

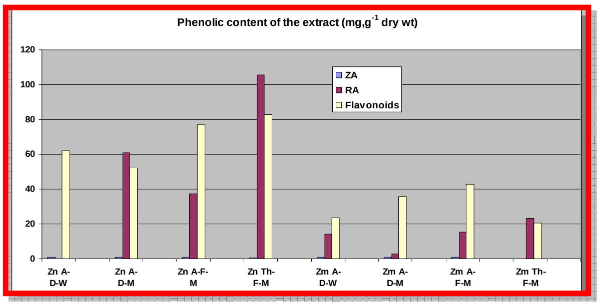
To test allelopathic affect of *Zostera species*, target species was cultivated in culture medium with different concentrations of the extracts. The inhibition tests were conducted in 6-well sterile plates (72 h). The nutritive medium was brought entirely at T0 time. *A. catenella* was added to each well (inoculation concentration of 800-1000 cells.ml^{-1}) and the growth was monitored by direct microscopic counts of cells. The effect of the crude extracts on the rate of photosynthesis II (PS II) in *A. catenella* was assessed by sensitive fluorimetric measurement of chlorophyll fluorescence of photosystem II (PS II) using Phyto-Pam.



Photosynthetic efficiency (reported to the control in Y axis) of *A. catenella* is negatively affected by *Zostera* extracts. This effect is cumulative, time and concentration dependent.



Photos showing heavy morphological anomalies affecting *A. catenella* cells exposed to increasing amounts (0.03-0.27%) of *Zostera* extracts.



Chemical analyses showed that Zostera leaves contain large amounts of phenolics. Rosmarinic acid (RA; 75-105.36 mg.g^{-1} dry weight) and flavonoids (Fl; 7.02-82.87 mg.g^{-1} dry weight) presented the higher concentrations, while Zosteric acid (ZA; 0.17-1.3 mg.g^{-1} dry weight) was always the minor phenolic acid. *Z. noltii* contains the higher amounts of phenolics (65.51-188.59 mg.g^{-1} dry wt). Z. marina was less rich in phenolic acids, with a maximum of 58.52 mg.g^{-1} dry weight for fresh leaves sampled in Arguin (Arcachon).

Extracts obtained from detrital and fresh leaves of *Z. marina* and *Z. noltii* strongly inhibited the growth of *A. catenella* at very low concentration whatever the extracts. *Alexandrium* cells showed severe morphological anomalies when exposed to the extracts. Significant reduction of their photosynthetic activity was systematically observed after 4h of exposure, which became more marked as time went on.

A long-term inhibition was observed without the need of continuous addition of extracts, suggesting that the allelochemicals produced by *Zostera* are stable in the medium. The significant amounts of phenolics contained in the extracts could be responsible of the inhibition effects observed.

This is the first report of the allelopathic potential of *Zostera* species against a dinoflagellate. Considering the deleterious impacts of HAB on public health and economic resources, the allelochemicals from *Zostera* detritus may be considered as potential candidates to mitigate the effects of *Alexandrium* blooms on aquaculture.

Species	Plant material	Extraction Method	Extract code*	IC50 (mg.L^{-1})
Z. noltii	detrital leaves	Water, rt	Zn A-D-W	199.7
		MeOH reflux	Zn A-D-M	79.8
	Fresh leaves	MeOH reflux	Zn A-F-M	36.1
	Fresh leaves	MeOH reflux	Zn Th-F-M	39.6
Z. marina	detrital leaves	Water, rt	Zm A-D-W	239.5
		MeOH reflux	Zm A-D-M	92.2
	Fresh leaves	MeOH reflux	Zm A-F-M	45
	Fresh leaves	MeOH reflux	Zm Th-F-M	36

ACHAMLALE S., REZZONICO B., GRIGNON-DUBOIS M. (2009a) - Rosmarinic acid from *Zostera* detritus from Arcachon lagoon. *Food Chem.,* 113: 878-883.
ACHAMLALE S., REZZONICO B., GRIGNON-DUBOIS M. (2009b) -Evaluation of detritus as a potential new source of Zosteric acid. *J. Appl. Phycol.,* 21: 347-352.
COLLOS Y., VAQUER A., LAABIR M., ABADIE E., LAUGIER T., PASTOUREAUD A. (2007). Contribution of several nitrogen sources to growth of *Alexandrium catenella* during blooms in Thau lagoon. *Harmful Algae,* 6, 781-789.
GROSS E.M., HILT S., LOMBARDO P., MULDERIJ G. (2007) - Searching for allelopathic effects of submerged macrophytes on phytoplankton - state of the art. *Hydrobiologia,* 584: 77-88.
HAGMANN L., JÜTTNER F. (1999) - Fischerellin A, a novel photosystem-II-inhibiting allelochemical of the cyanobacterium *Fischerella muscicola* with antifungal and herbicidal activity. *Tet. Lett.,* 36: 6539-6542.
KEARNS KD, HUNTER M.D. (2000) - Green algal extracellular products regulate antialgal toxin production in a *cyanobacterium*. *Environ. Microbiol.,* 2: 291–297.

Acknowledgements
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Les substances allélopathiques chez *Zostera marina* et *Zostera noltii*

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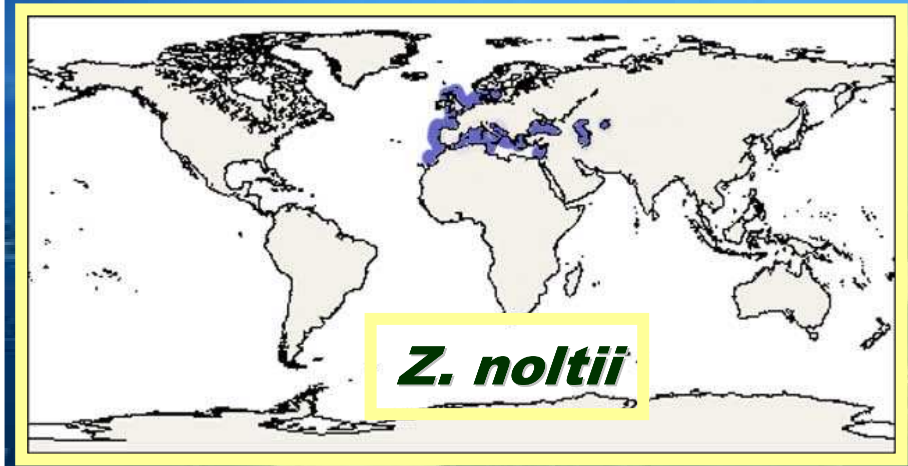
Zostera dans les mers du globe

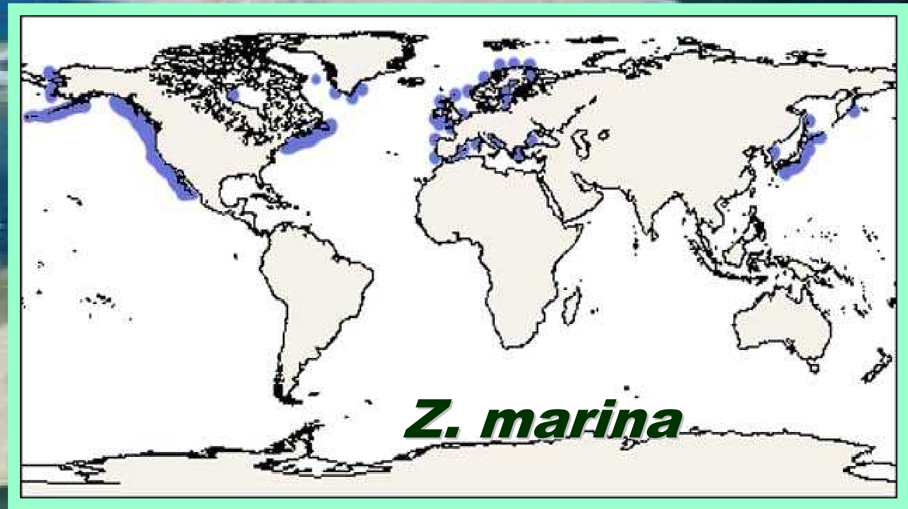


Z. noltii

espèce essentiellement Européenne : Atlantique, Iles britaniques, Mer Baltique, Méditerrannée, Mer Noire, Mer Caspienne, Mer d'Aral.
Petit peuplement au Maroc et en Mauritanie

Zostera marina

sur la plupart des rivages européens, la mer Noire et une partie des rivages asiatiques. Peuple également toutes les côtes d'Amérique du Nord.
2 sous-espèces: *Z. marina* Linnaeus et *Z. angustifolia* (Hornem.)



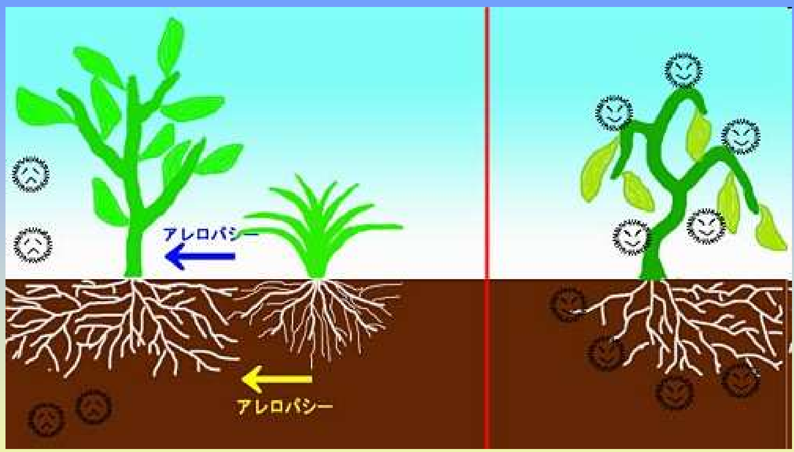
Les herbiers constituent des écosystèmes remarquables, qui présentent un fort intérêt écologique, patrimonial et économique.

Espèces ingenieurs peuplant les zones côtières :
contribuent à la structure physique des milieux littoraux (filtrent la colonne d'eau, stabilisent les sédiments, créent une zone tampon le long de la côte.
formations végétales clés: niveau de production primaire et biodiversité très élevé,es ce qui les classe parmi les écosystèmes les plus productifs de la planète.

Allélopathie *(allelon* du grec "mutuel" ou "réciproque" et *pathos* "souffrance "

Interaction entre organismes par l'intermédiaire de molécule chimiques, généralement des métabolites secondaires, capables d'affecter la croissance, la santé, la biologie ou le comportement d'une autre espèce. Ces signaux peuvent être inhibiteurs ou stimulants.

Les composés allélopathiques sont libérés dans l'air ou le sol (plante terrestre), l'eau ou le sédiment (plante aquatique). Allélopathie en milieu marin : peu étudiée



deux types de substances allélochimiques :

- **les allomones, qui procurent un avantage à l'organisme qui les émet (substances défensives)**
- **et les kairomones, qui procurent un avantage à l'organisme qui les perçoit.**

Potentiel allélopathique de *Zostera marina*

(données relatives à *Z. marina* d'Amérique du Nord)

<u>Extrait aqueux de feuilles :</u>

Inhibition de la croissance de micro-algues et de bactéries:

- **P. G. Harrison, A. T. Chan, Marine Biology 61, 21-26 (1980)**

Contrôle de la croissance microbienne et de la consommation par les amphipodes:

- **P.G. Harrison, Marine Biology 67, 225-230 (1982).**

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Rôle possible des phénoliques dans la résistance à *Labyrinthula zosterae,* agent pathogène primaire de la « wasting disease ».

- **R. N. Buchsbaum, F. T. Short, D. P. Cheney, Aquat. Bot. 37, 291 (1990).**
- **L. H. T. Vergeer, A. Develi, Aquat. Bot. 58, 65 (1997).**

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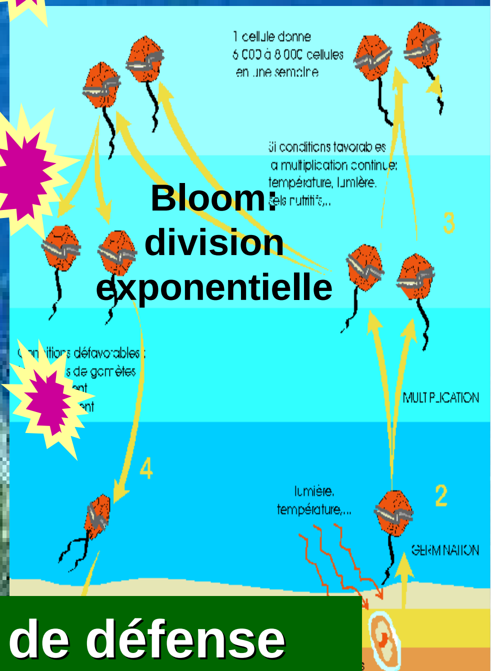
Caractérisation de l'acide Zostèrique (acide phénolique sulfaté) ayant des propriétés antifongiques et antibactériennes.

- **J.S. Todd, R.C. Zimmerman, P. Crews, R.S. Alberte, Phytochem. 34,401 (1993).**
- **Applications : agrochimie, antifouling**

Un constat **: pas de bloom d'Alexandrium dans les zones abritant des herbiers à Zostère denses : Bassin d'Arcachon**

Hypothèse de travail **: les Zostères sécrètent dans le milieu des substances allélopathique capables d'inhiber le développement *d'Alexandrium catenella***



Stratégie :

Extraire et identifier ces substances

Evaluer leur potentiel allélochimique

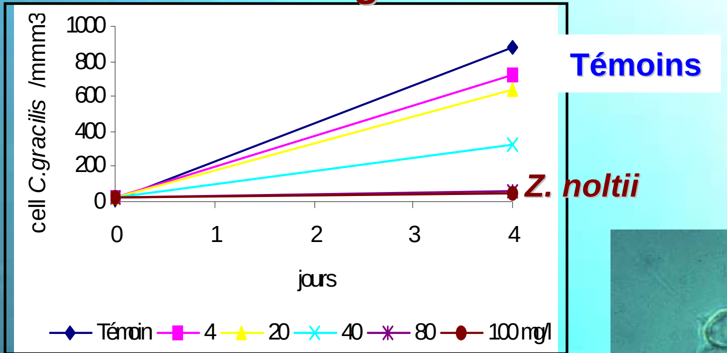
Communication chimique inter-espèce - stratégie de défense

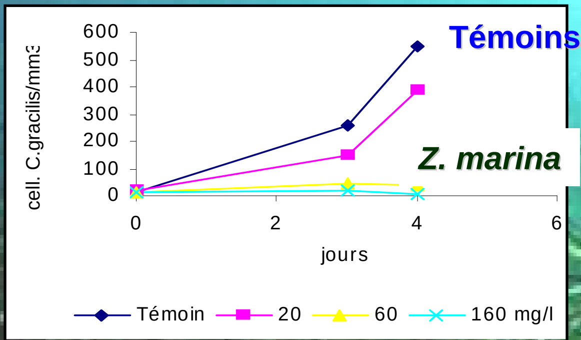
extraction / isolation processes

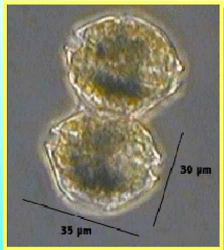
- *Seagrasses*
  - MeOH → Methanolic extract
  - $MeOH/H_2O$ → Aqueous methanolic extract
    - AcOEt → Phenolics
    - n-BuOH → Phenolics
    - sugars

Inhibition de la croissance d'espèces microphytoplanctoniques

Chaetoceros gracilis

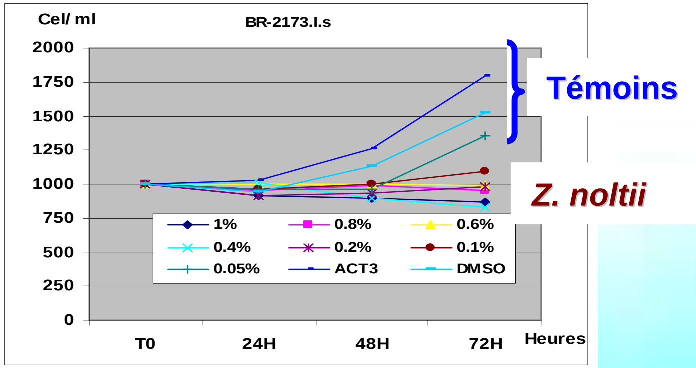


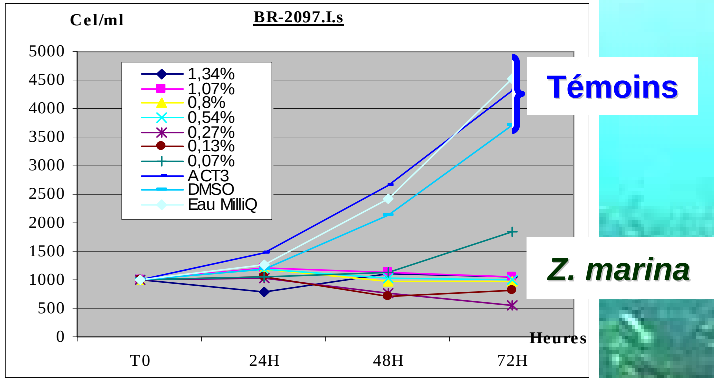




Coll. G. Arzul, Ifremer

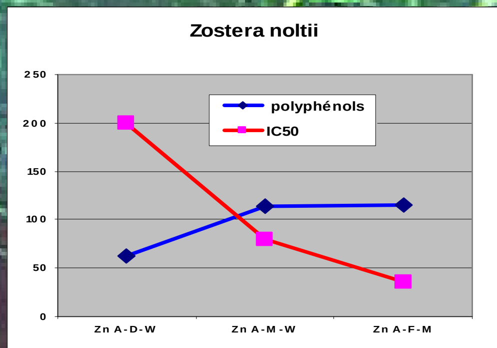
Alexandrium catenella

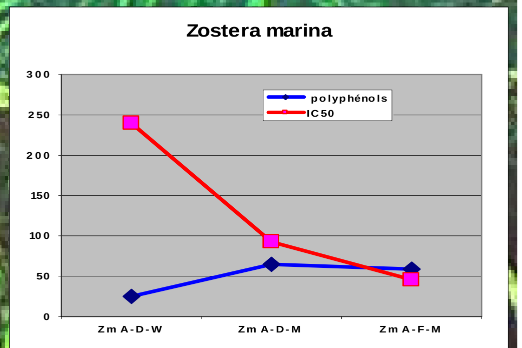




Coll. E. masseret & M. Laabir (UM2)

Tous les extraits montrent un fort pouvoir inhibiteur à faible concentration, y compris ceux préparés à partir des détritus.
Corrélation inverse significative entre concentration en polyphénols et IC 50, ce qui confirme leur rôle dans l'activité inhibitrice observée





Les substances phénoliques chez *Zostera*

Chez les plantes terrestres, les composés phénoliques jouent un rôle majeur dans l'interaction des plantes avec leur environnement. Ils peuvent être soumis à d'importantes fluctuations face aux agressions du milieu et ont de ce fait une grande importance en écophysiologie.

Objectifs : caractériser les polyphénols responsables de l'activité inhibitrice (*Z. noltii et Z. marina*)

Variation interspécifique

Variation saisonnière de leur production

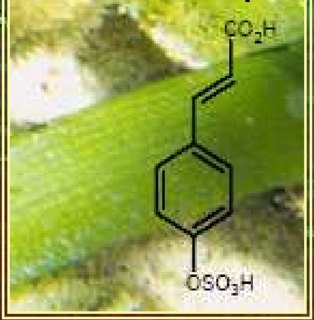
Sont-ils tous identiques quel que soit l'herbier : varaiabilité géographique?

Sont-ils exsudés dans le milieu marin? : Zostère vivante & échouages (laisses de mer)

Impact des facteurs anthropiques sur leur production

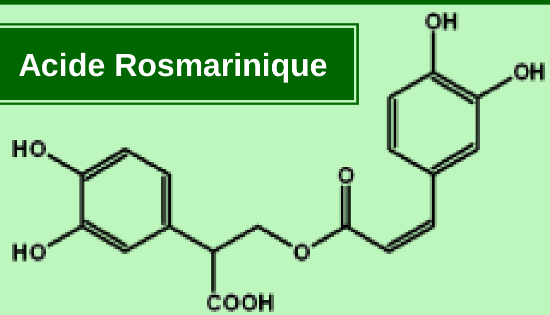
*c*aractérisation chimique : mise en évidence de substances bio-actives

Acide Zostérique



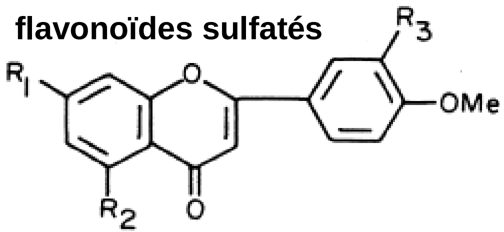
(antifongique, antibactérien, antisalissure)

Acide Rosmarinique



anti-oxydant puissant, antiviral
anti-asthénique, anti-inflammatoire,
antimutagène, antibactérien

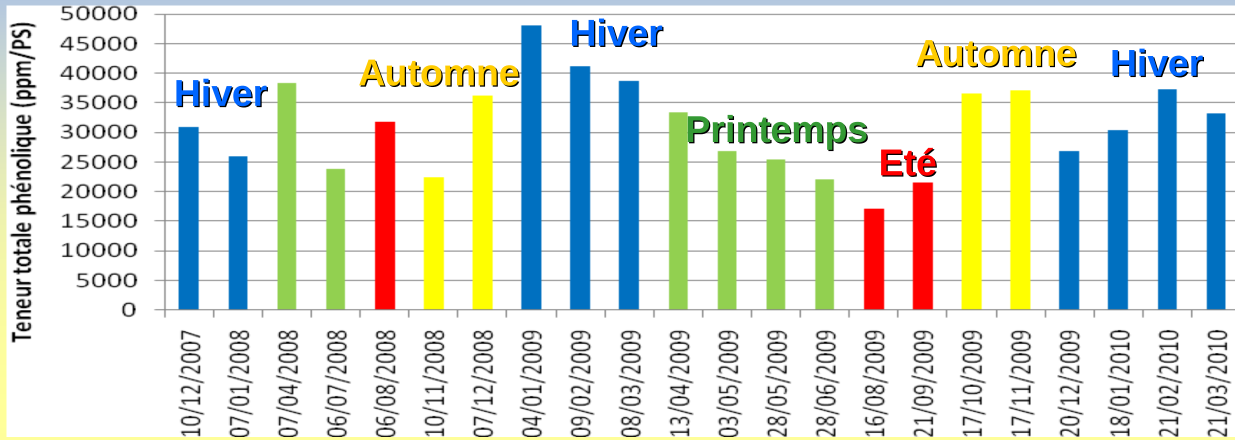
flavonoïdes sulfatés



Production des polyphénols: variation saisonnière chez *Z. noltii* (Bassin d'Arcachon)

-Suivi mensuel d'un herbier pendant 3 ans (2007-2010): 44 collectes, 76 extraits préparés et analysés (RMN, HPLC)

Teneurs importantes en polyphénols tout au long de l'année : 16000-46000 ppm/ps
Pics saisonniers : hiver et parfois automne

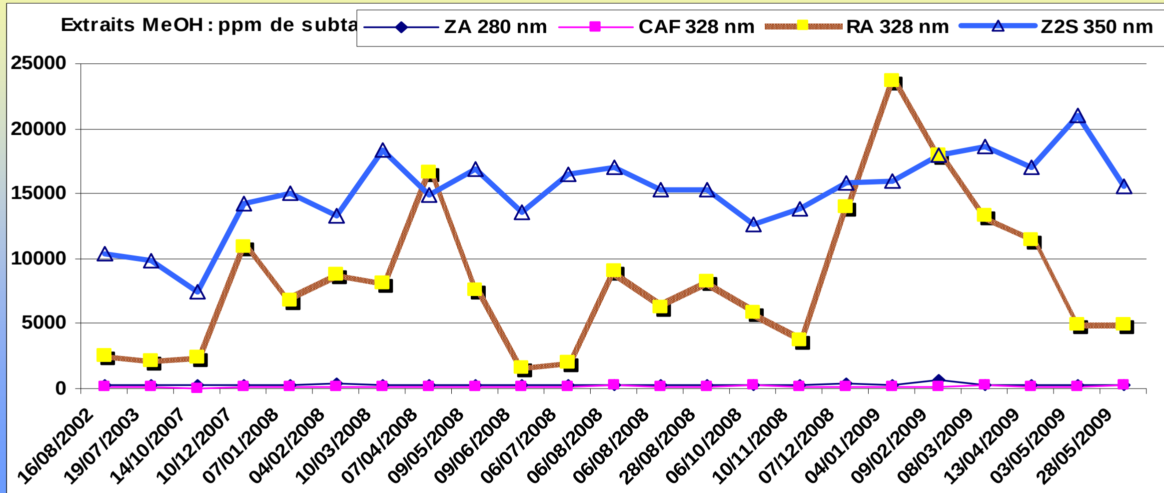


fortes teneurs de diosmétine sulfaté (Z2S) tout au long de l'année :
7500-21000 ppm

Ac. rosmarinique (RA) très variable selon saison: 2500-24000 ppm (pic hivernal)

Ac zostérique(ZA): faible teneur par rapport à RA et Z2S, mais relativement constante : 300-400 ppm

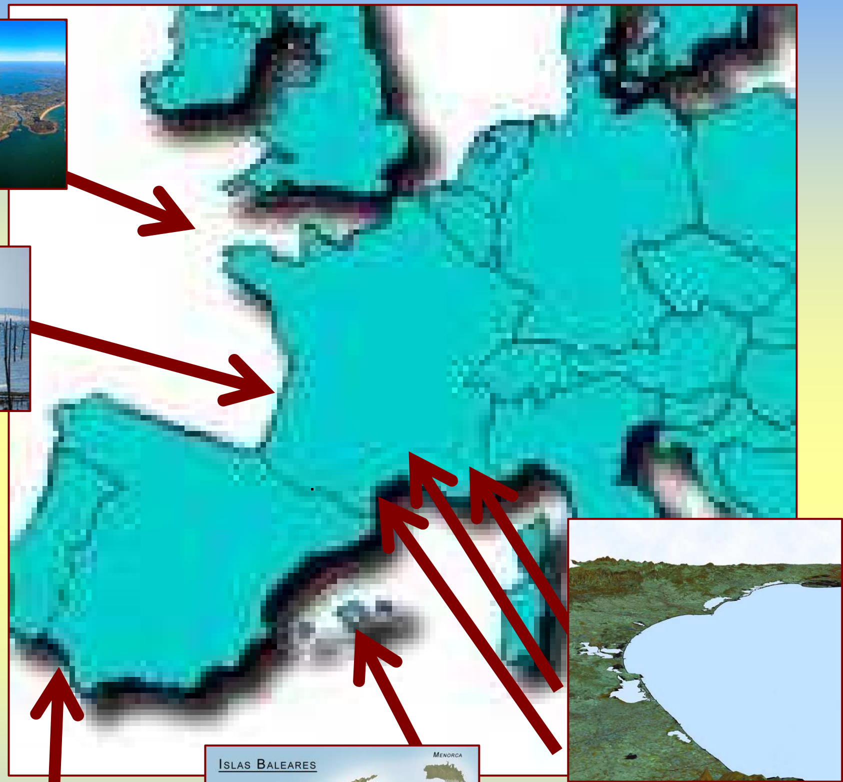
Production de biomasse : 30000 – 44000 T/an (ps)
Polyphenols : 10000-40000 ppm/ps soit 300 – 1700 T/an



Variabilité géographique chez *Z. noltii*

Golfe du Morbihan (56)

Bassin d'Arcachon (33)

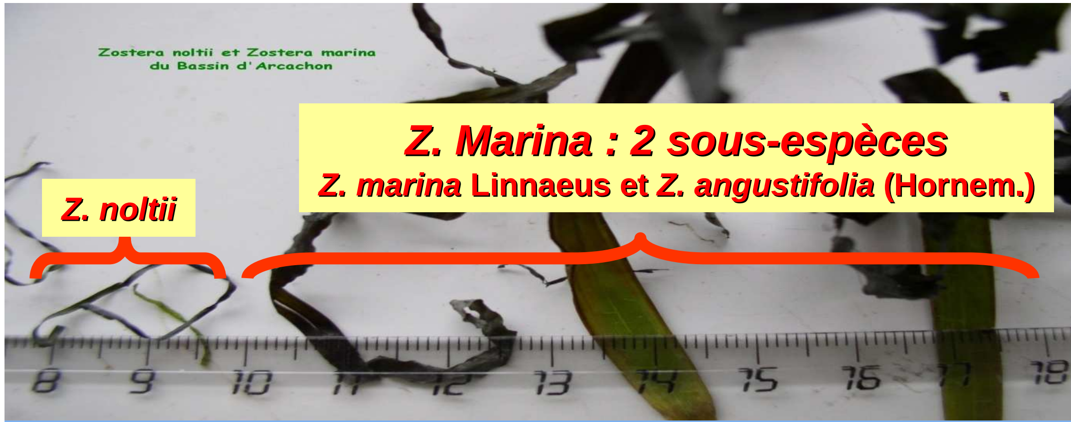


Ria Formosa (Pt)

Baie de Cadiz (Es)

Iles Baléares (Es)

Etangs Méditerranéens:
Salses Leucate (69)
Thau (34)
Berre (13)



Variabilité géographique chez *Z. marina?*

	Zone géographique	*Z. noltii*	*Z. marina*
France	Bassin d'Arcachon	x	x
	Golfe du Morbihan	x	x
	Etang de Thau	x	x
	Etang de Salses Leucate	x	x
	Etang de Berre	x	
Espagne	Delta de l'Ebre	x	
	Cadiz	x	
	Baléares	x	
Portugal	Ria Formosa	x	
Mer Baltique	Danemark (Baltique)		x
	Pologne (Baltique)		x
USA	Californie (côte ouest)		x
	Massachuset (côte est)		x

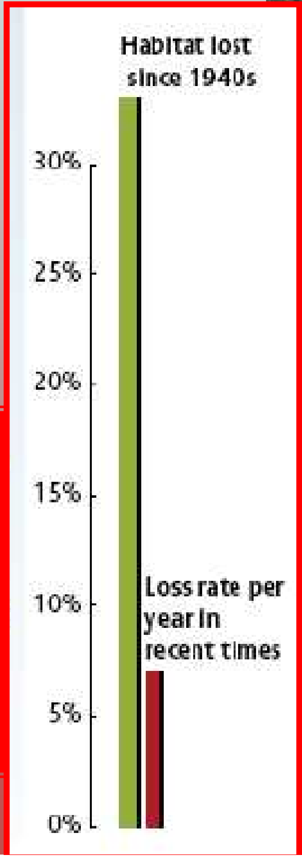
Z. angustifolia (Hornem.) domine en Mer Baltique, tandis que *Z. marina* Linnaeus domine sur la façade Atlantique

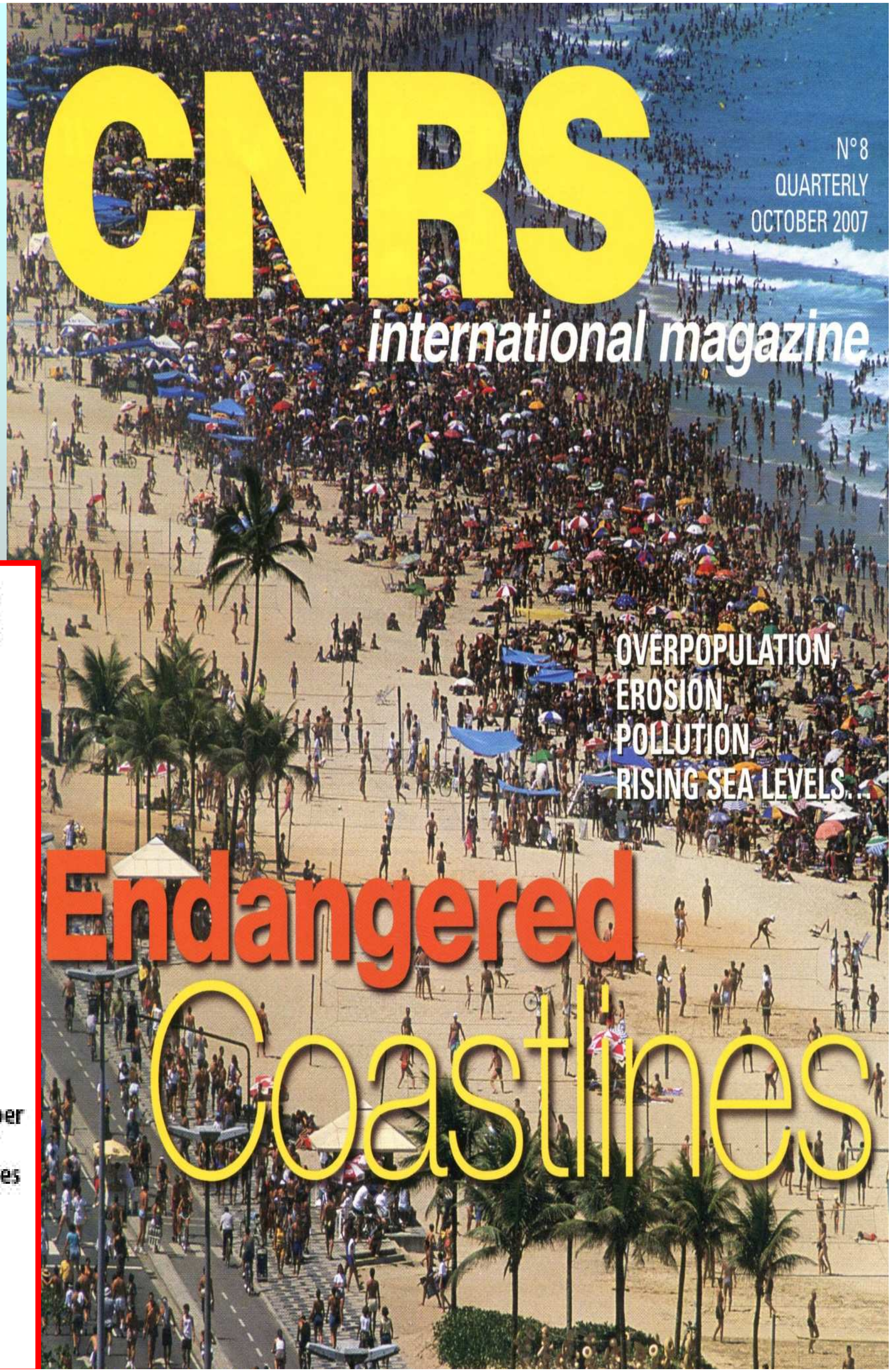
➢ Herbiers marins: particulièrement fragiles et sensibles à la détérioration de la qualité des eaux et à la concentration d'activités humaines

➢ 60 % de la population mondiale vit sur le littoral. Si rien ne change : 80% attendu en 2030

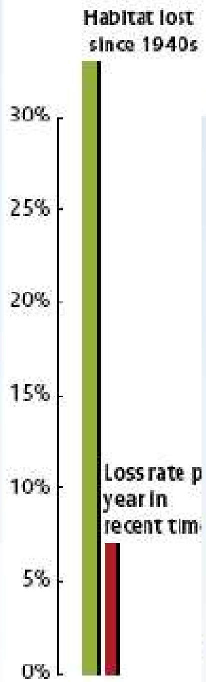
➢ Le littoral est le réceptacle de toutes les pollutions d'origine continentale.

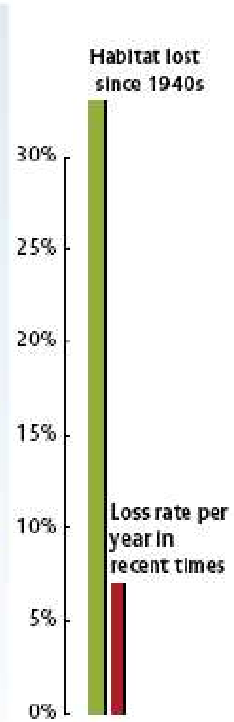
Régression des herbiers marins ⟶ perturbation de l'ensemble de l'écosystème côtier





Régression des herbiers





Régression des herbiers marins ⟶ perte de biodiversité

Herbier : environ 150 espèces   Sédiment nu ~ 20

La biodiversité augmente la capacité des écosystèmes à réaliser différentes fonctions écologiques, qui sont à l'origine des services écosystémiques (bénéfices directs ou indirects l'homme).

La Directive Cadre sur la Stratégie pour le Milieu Marin (DCSMM ; 2008/56/CE) établit une politique communautaire visant à atteindre ou maintenir un « bon état écologique » du milieu marin au plus tard en 2020.

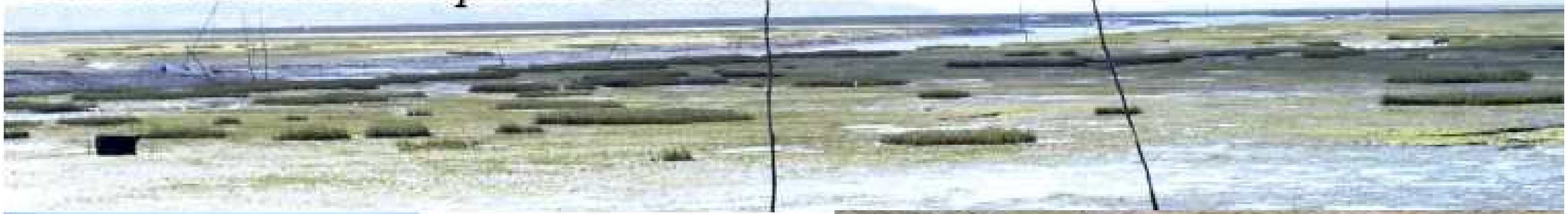
Deux types de descripteurs sont utilisés pour qualifier les herbiers marins: leur superficie et les caractéristiques démographiques et biométriques des populations. Ils permettent de mettre en évidence l'évolution des milieux, mais pas d'anticiper les destructions d'habitats, alors qu'il serait primordial d'agir à un stade précoce.

En matière de dysfonctionnement, le lien causes-effets est difficilement identifiable à l'échelle macroscopique.

Parallèlement à ces descripteurs, nous proposons d'utiliser un outil « bio-marqueur capable de décrire l'état de la plante à l'échelle moléculaire.

L'objectif est de pouvoir détecter au niveau du métabolisme, les signes avant-coureurs de la régression pour permettre une gestion préventive de ses effets dévastateurs.

Bassin d'Arcachon : partie orientale



Quelques exemples de stress subi par les herbiers

Spartine anglaise, dépôts algues vertes sur les herbiers

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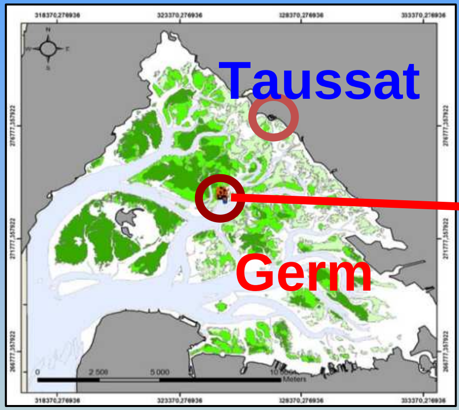
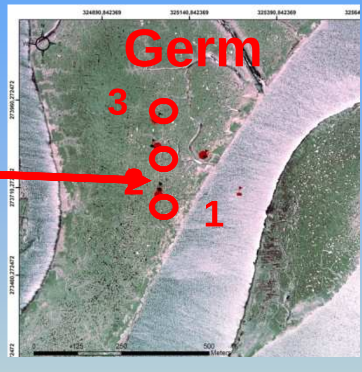
Dépérissement, disparition

Impact des facteurs anthropiques sur la production des polyphénols (*Zostera noltii, Bassin d'Arcachon*)

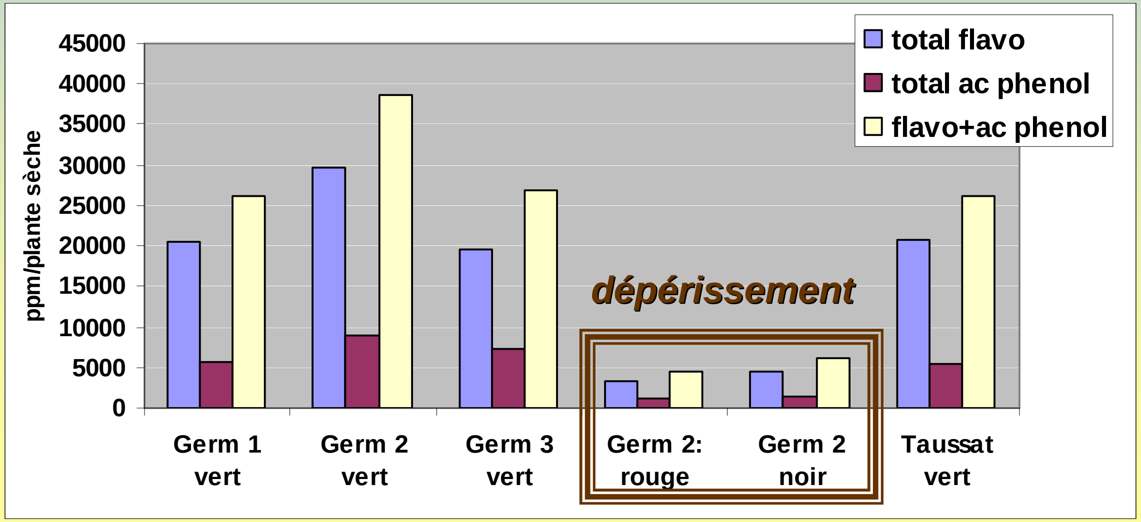


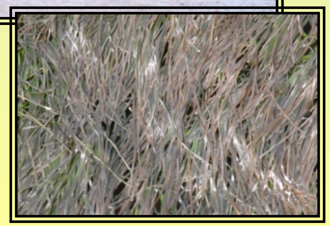


Apparition de zones de
« dépérissement » :
Vert, devient noirâtre, puis rougeâtre

Comparaison **Germanan/Taussat**
- échantillons du 28/05/09

Dépérissement : perte des polyphénols



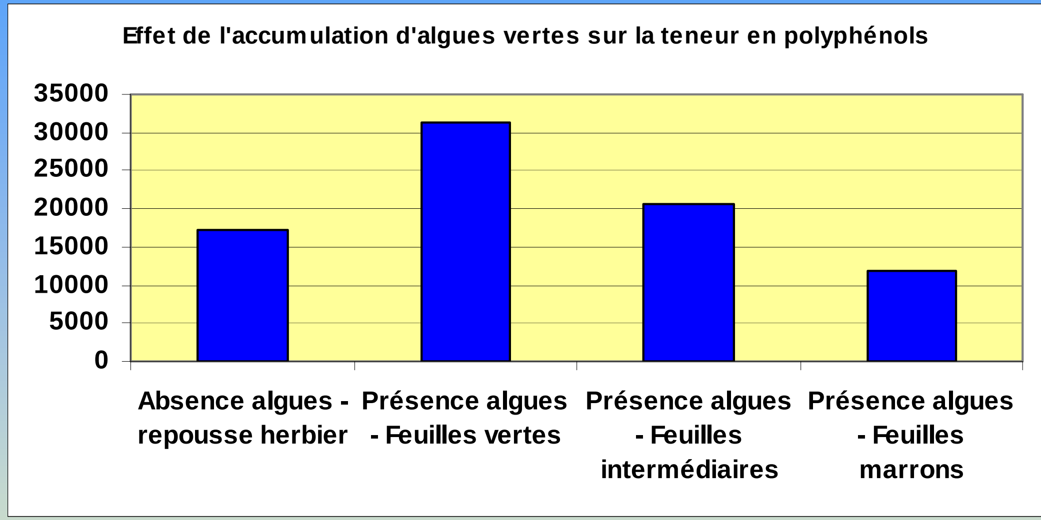


Dépérissement : perte des défenses chimiques

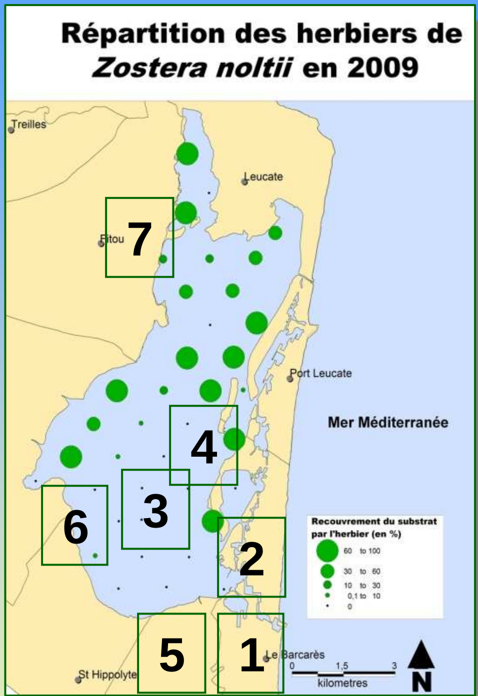
Impact des facteurs anthropiques sur la production des polyphénols (*Zostera noltii, Arcachon*)

Impact d'algues nitrophiles (*Cladophora agaegropila* et *Enteromorpha intestinalis*) sur les herbiers à Z. noltii (site d'Arcachon):

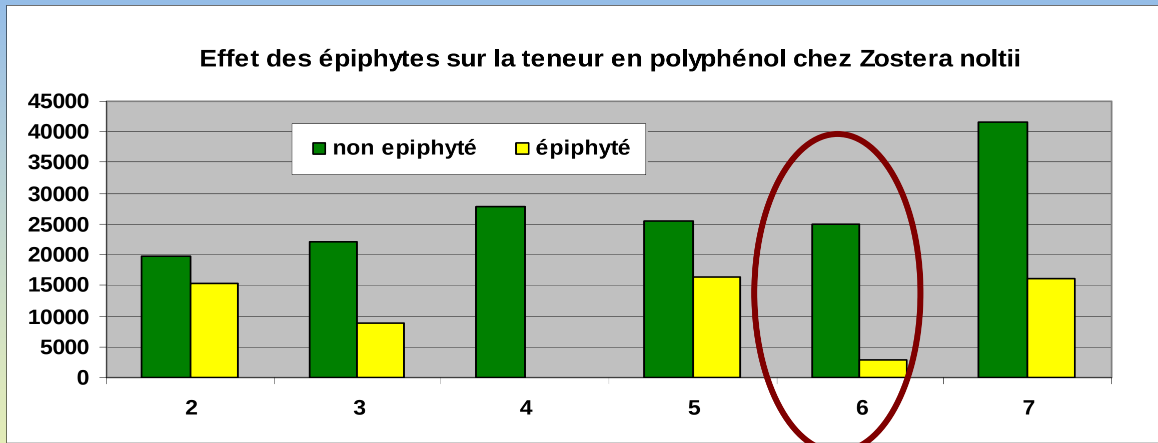
L'accumulation d'algues vertes sur l'herbier conduit à la diminution du contenu phénolique des feuilles de Zostère.



Impact des facteurs anthropiques sur la production des polyphénols (*Zostera noltii,* étang de Salses-Leucate)

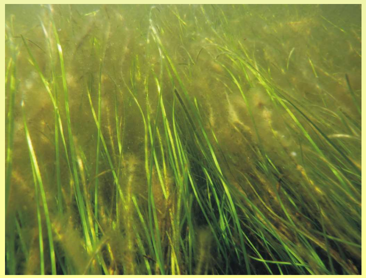


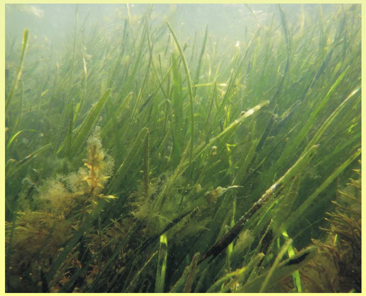
L'apport excessif de nutriments (azote, phosphore) est directement toxique pour les herbiers, mais plus grave, il stimule la croissance des *épiphytes* et des algues flottantes macroscopiques et microscopiques. Cela se traduit par une diminution de la quantité de lumière solaire pouvant atteindre les feuilles des plantes, ce qui réduit leur photosynthèse et donc leur *production primaire*.



Quantité d'épiphytes sur les feuilles: 6>>3>2=5>>7

Les épiphytes (du grec ἐπί «sur», φυτόν «végétal»; littéralement «à la surface d'un végétal») sont des plantes qui poussent en se servant d'autres plantes comme support.



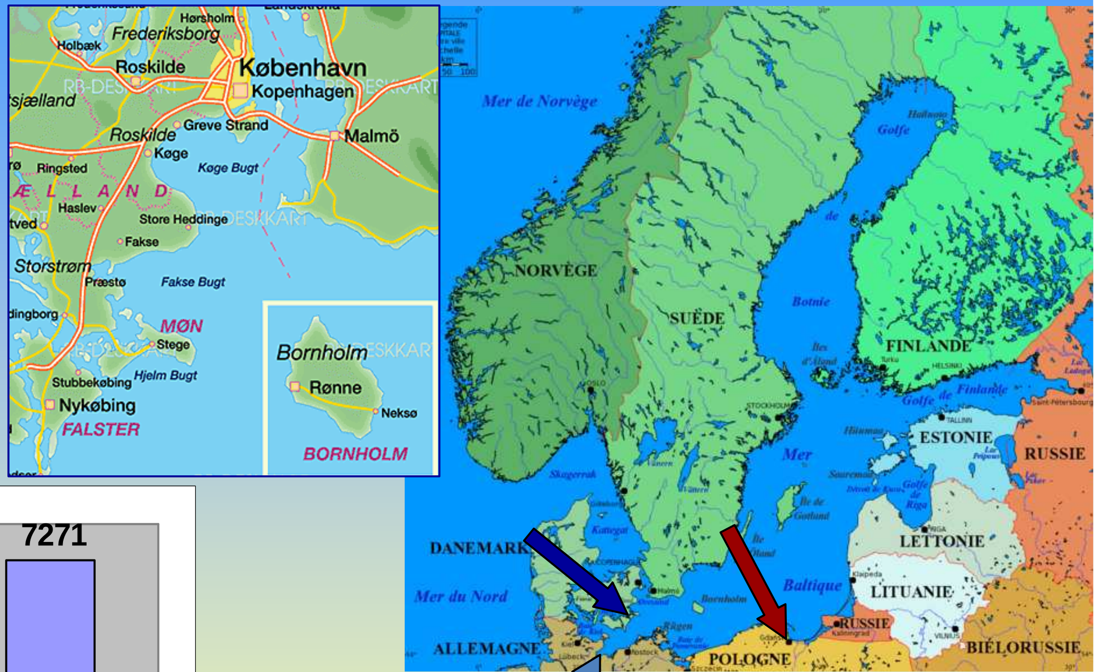


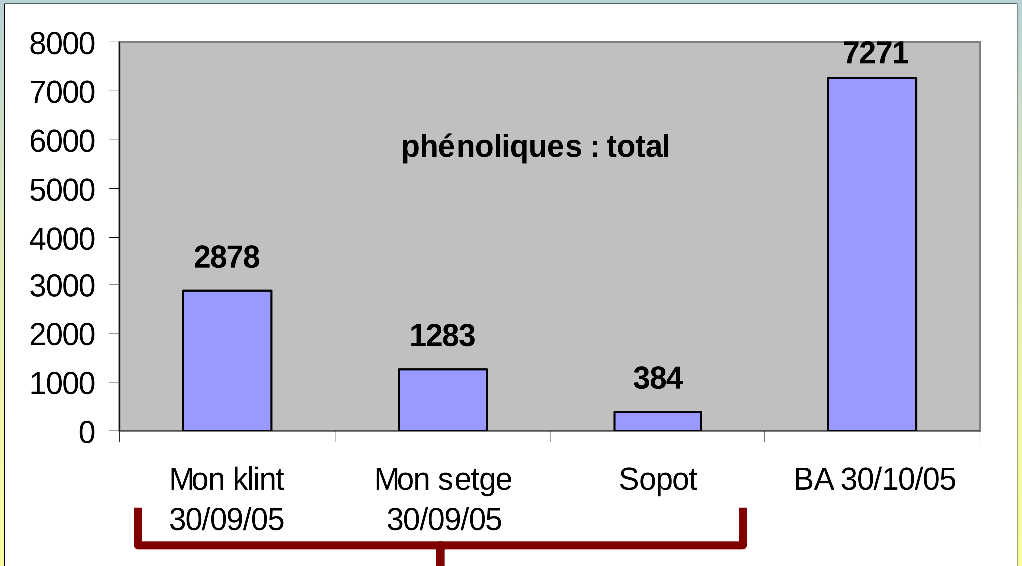
Impact des facteurs anthropiques sur la production des polyphénols

(Z. marina, Mer Baltique)

Baltique

- **Klutz (De)**
- **Sopot (Pl)**
- **Mϕn (Dk)**





Baltique **Arcachon**

Effets

conditions climatiques

Pollution

Mon klint : peu de rejets
Mon Setge : rejets industriels à proximité de l'herbier
Sopot : pollution importante

Les herbiers marins au cœur d’un projet européen

Gestion environnementale des zones lagunaires à vocation aquacole- Eco-lagunes

2009-2011 :
France – Espagne – Portugal
7 partenaires

Les objectifs:

Démontrer qu’une bonne gestion environnementale des milieux aquatiques lagunaires, en préservant la biodiversité, peut garantir le développement d’une activité économique durable

Harmoniser les outils de suivi des herbiers
Favoriser le développement des herbiers par l’enlèvement des espèces envahissantes

Montrer que le développement des herbiers permet la restauration de la biodiversité

Démontrer que la restauration de la biodiversité favorise le maintien de l’équilibre écologique et permet de limiter le développement du micro-phytoplancton toxique

Un herbier en déclin est le signe d’une lagune malade


AVANT DE COMMETTRE L'IRRÉPARABLE, JE VOUS DEMANDE DE CONSIDÉRER LE PROBLÈME DE LA BIODIVERSITÉ
Merci pour votre attention

Phenolic fingerprints of *Zostera noltii* from Atlantic and Mediterranean coasts

Micheline Grignon-Dubois and Bernadette Rezzonico

UMR 5805, EPOC, Université Bordeaux 1, 351, cours de la Libération, 33405 Talence cedex, France

One of the most interesting and fast developing fields in phytochemistry is chemical taxonomy, also called biochemical systematic. Some of the most useful chemical markers are the secondary metabolites. They have been demonstrated to play an ecological role, being involved in (pathogenic) interactions between plants and microorganisms or in communication with a symbiont. In the interaction of a plant with its abiotic and biotic environment, secondary metabolites provide protection against abiotic stress (such as ozone, UV light, cold, drought, heavy metals, or nutrient deficiency) or selective defense against herbivory and pathogen infection.

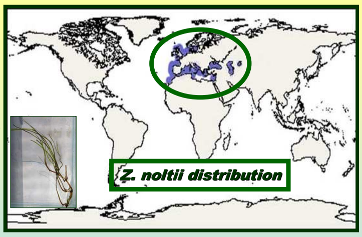
Phenolics are of a great importance in seagrass-environment relationships due to their involvement in the response of the plant to stress. Only a few studies have investigated the concentration of phenolics in *Zostera noltii*, aside from our papers related to rosmarinic and zosteric acid. Our ongoing studies have shown that the phenolics contained in *Z. noltii* from the bay of Arcachon and Thau lagoon (France) significantly inhibited the growth of microphytoplancton spp. like *Chaetoceros gracilis* and *Alexandrium catenella (1,2)*.

Documenting the presence of phenolics in living tissues of *Z. noltii*, and how these compounds vary in abundance between seagrass meadows across large geographical scales and growing in different habitats conditions is crucial to understanding the landscape-level adaptability of the plant to environmental factors..

***Zostera noltii* Hornem.** (Zosteraceae, common name dwarf eelgrass, synonym *Zostera nana* (Roth) is an important species of eelgrass occurring along European and North African coasts.

Classification:

Empire	Eukaryota
Kingdom	Plantae
Subkingdom	Viridaeplantae
Phylum	Magnoliophyta
Class	Monocots
Order	Potamogetonales
Family	Zosteraceae
Genus	Zostera



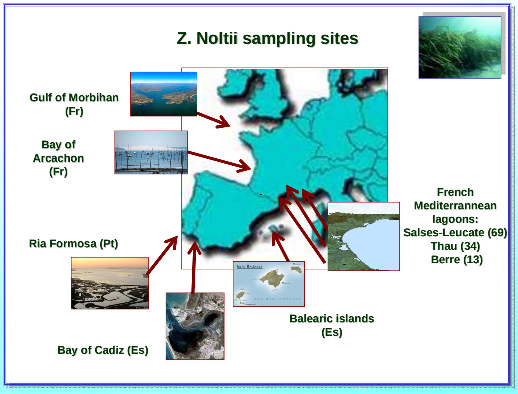
Identifying features

- Leaves grass green in colour.
- Leaf tips blunt and emarginate (notched), becoming indented in older leaves.
- Leaves 6 -30 cm long, 0.5-1.8 cm wide with 3 irregularly spaced veins.
- Leaf sheath short, 0.54 cm, and open with two membranous flaps.
- Reproductive shoots lateral. Seeds, 1.5-2 mm long (excluding style), white, and smooth.
- Rhizome 0.5-2 mm thick with 1-4 roots per node; fibre bundles in the innermost layers of the outer cortex

Like most of *Zostera* sp. this species may exhibit morphological variation depending on location tidal zone and age of plant (Phillips & Menez, 1988).

	Z. noltii bed	*seasons*	*collections*
France	Bay of Arcachon	W, Sp, Su, Au	24
	Gulf of Morbihan	Su, Au	2
	Thau lagoon	W, Sp, Su, Au	4
	Salses Leucate	Su	2
	Berre	Su	1
Spain	Ebro	Su	1
	Cadiz	Sp, Su	2
	Balearic island	Su	1
Portugal	Ria Formosa	Su	1

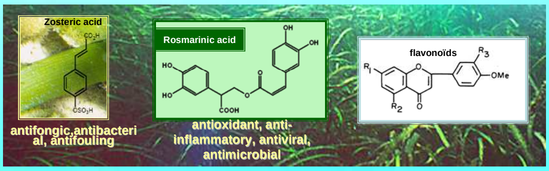
W = winter, Sp = spring, Su = summer, Au = autumn



Z. noltii leaves were collected from eight seagrass beds representative of three types of coastal ecosystems:

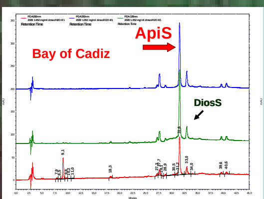
- **four mesotidal bays on the Atlantic coast (France, Portugal, Spain),**
- **three Mediterranean coastal lagoons (France),**
- **an open-sea Mediterranean Zostera bed (Spain).**

Methanolic and aqueous extracts were prepared from the plant material, and analyzed for both the identity and quantity of phenolics present using NMR and HPLC. These techniques has been proven to be method of choice for secondary metabolites profiling of plant material. They are non-destructive and can be performed at micro- scale on samples of 10-50 µg.

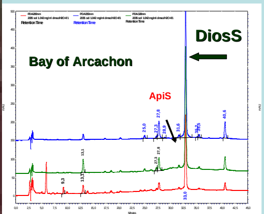


Chemical analyses showed the sampled seagrasses leaves contain flavonoids and phenolic acids including rosmarinic acid (RA), and zosteric acid (ZA) (3-5). RA and ZA were found at all the locations. The concentration of RA is seasonally dependent (2.75-105.36 $mg.g^{-1}$ dry weight), while the variations in the ZA remains low (300-400 µg.g-1 dry weight).

The three types of phenolics are present in methanolic extracts, while water allows selective extraction of flavonoids and ZA.

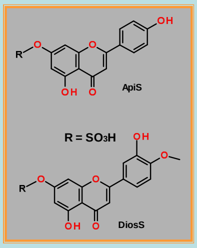


***Zostera noltii* : 2 chemotypes**



Chemotype = a chemically distinct entity in a plant or microorganism with differences in the composition of the secondary metabolites.

7-sulfated-apigénin chemotype - (ApiS)
Cadiz, Ria Formosa



7-sulfated-diosmétin chemotype - (DiosS)
Arcachon, Morbihan, Balearic islands, French Mediterranean lagoons

High performance liquid chromatography (HPLC) combined with diode array detection (DAD) was used for both qualitative and quantitative analyses of the extract composition. As expected on the basis of NMR data, the HPLC flavonoid profiles were largely dominated by a single product, which was respectively eluted at 31.6 min (λmax, 336 nm; Cadiz, Faro) and 33.0 min (λmax, 347 nm; all the other sites). Minor flavonoids were also found and identified to luteolin-7-glucose (λmax, 349 nm), and luteoline-7 sulphate (λmax, 349 nm). In addition, small amounts of apigenin-7-glucose (λmax, 335 nm) were detected in the samples from Cadiz. All the UV absorptions are in agreement with the literature. These assignments were supported by HPLC comparison with standards and LC-ESI-MS analysis in positive mode. In particular, the mass spectra clearly show for all the flavonoids detected, the [M+1] molecular peak and the characteristic ion peak at [M+1-80] for sulphated flavonoids or [M+1-162] for gluco-flavonoids.

Our results were confirmed by acid hydrolysis of the crude extracts, which led to apigenin (Cadiz, Ria Formosa) or diosmetin (all the other sites). In addition, authentic samples of the 7-sulphated-flavonoids were synthesized by sulfation of luteolin, apigenin and diosmetin with TBAHS. Comparison of the NMR, MS and UV spectra and HPLC retention time allows unambiguous identification of the sulphated flavonoid content of *Z. noltii* from the different seagrass beds.

Flavonoids content of Z, noltii (mg.g-1 dry wt, calculated as diosmetin equivalent): comparison Cadiz/Arcachon

	7-Gluco		7-sulfated			unsubstituted		
total	Lu-	Api-	Lu-	ApiS	DiosS	Lu	Api	Dios
8.41	0.17±0.01	0.56±0.01	1.15±0.07	5.08±0.	0.64±0.01	0.63±0.01	0.18±0.01	-
9.26	0.15±0.01	0.89±0.01	0.44±0.01	6.85±0.	0.64±0.01	0.48±0.01	0.21±0.01	-
11.32	0.47±0.02	tr	1.63±0.09	-	8.43±0.	0.51±0.01	-	0.28±0.01
16.78	0.61±0.01	tr	1.57±0.08	-	13.41±0	0.30±0.01	-	0.89±0.02

Apigenin sulphate had never been reported for *Z. noltii* before. Based on these data, our results showed that *Z. noltii* grown in the Bay of Cadiz and in Ria Formosa are chemically distinct from specimen grown in the other sites. This is the first report of a possible chemotype for a marine magnophytes. Understanding the geographic variation of *Z. noltii* sulphated flavonoid content and its possible link with ecological factors is now under progress.

1. M. Grignon-Dubois, G. Arzul, Results to be published.
2. M. Laabir, M. Grignon-Dubois, P. Cecchi, B. Rezzonico, F. Rieuvilleneuve, E. Masseret, in : *Proceeding of the 4th mediterranean symposium on marine vegetation.* El Asmi, S., Langar, H., Belgacem, Tunis, 251 pp. 2010, 187-188.
3. S. Achamlale, B. Rezzonico, M. Grignon-Dubois, *Food Chem.* 113, 878-883 (2009).
4. S. Achamlale, B. Rezzonico, M. Grignon-Dubois, *J. Applied Phyc.*, 21, 347-352 (2009)..
5. M. Grignon-Dubois, B. Rezzonico, T. Alcoverro, Estuarine, Coastal and Shelf Science, under press.

Acknowledgments

This work was supported by the SUDOE program (ECO-LAGUNES project) and the Region of Aquitaine.

Effect of environmental stress on the phenolic content of *Zostera noltii*

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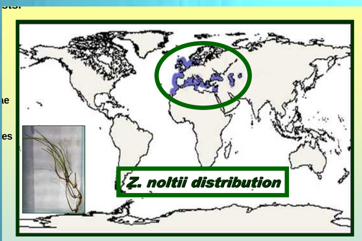
Seagrass meadows are susceptible to coastal environmental impacts and can serve as early warning systems of ecosystem degradation. *Zostera noltii* Hornemann is an important species of eelgrass occurring along European, North Africa, Mediterranean, Black Sea and Azov Sea coasts. The coastal habitats favoured by *Z. noltii* are under increasing threat from coastal development, pollution and other forms of human disturbance. The role of excess epiphyte, macroalgae or phytoplankton growth in shading of seagrass leaves and negatively affecting seagrass health is generally agreed to be a prevalent mechanism in seagrass decline worldwide.

Plants are provided with a large arsenal of chemicals that are utilised as defensive mechanisms. It is of interest to develop and validate biomarkers based on these chemical defences for monitoring conservation status and ecotoxicological impact in seagrass meadows. In this context, phenolic compounds constitute good candidates for providing insights into water quality. Our previous work have decrypted the phenolic-fingerprints of *Zostera* spp. and highlighted the potential of polyphenols as indicators of the coastal lagoon environmental quality. Documenting the presence of those compounds in living tissues, and how they vary in abundance in presence of environmental stress would be helpful to understand how human activities influence marine communities

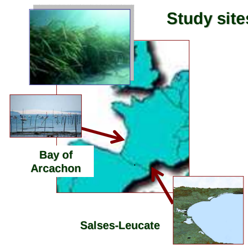


Little attention has been directed towards understanding the effects of environmental variables on the phenolic content of *Z. noltii*.

In this study, we have compared the phenolic content of healthy green leaf tissue of *Z. noltii* to specimens under the pressure of environmental stress:

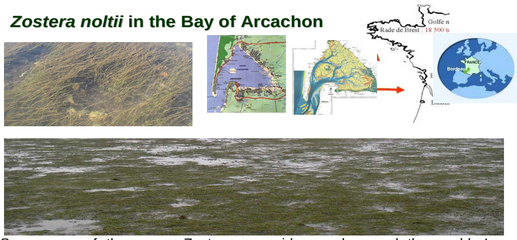
- **the accumulation of opportunistic macro-algae,**
- **the excessive development of epiphytes,**
- **the recurrent apparition of withered patches in *Z. noltii* beds.**

Study sites



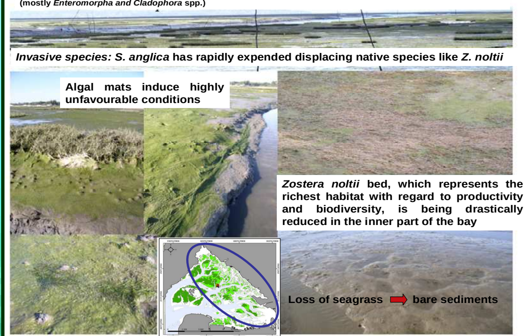
The study was conducted in the Bay of Arcachon (French Atlantic coast) and at Salses-Leucate (French Mediterranean coastal lagoon).
Leaves of Z. noltii were collected in the seagrass beds. Methanolic and aqueous extracts were prepared from the plant material, and analyzed for both the identity and quantity of phenolics present using NMR and HPLC.

Zostera noltii in the Bay of Arcachon

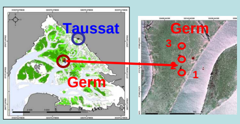


Seagrasses of the genus Zostera are widespread around the world. In Europe, there are two representatives, *Zostera noltii* Hornem and *Z. marina*. Both are present in Arcachon Lagoon, France, especially *Z. noltii* which constitutes 95% of the largest Zostera bed in Europe.
Extensive meadows of *Z. noltii* are found in the intertidal zone, covering about 40% of the lagoon

Bay of Arcachon : effects of invasive species and blooms of opportunistic macroalgae (mostly *Enteromorpha and Cladophora* spp.)



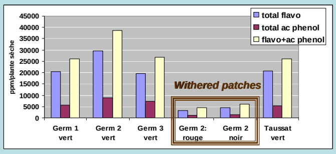
Effects of stressors on the production of polyphenols
(*Zostera noltii, Bay of Arcachon*)



Recurrent apparitions of withered patches in *Z. noltii* beds

Comparison Germanan/Taussat 28/05/09

Withering : decrease of the phenolic content



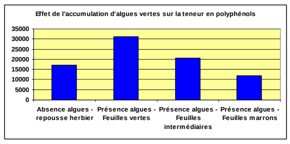
Withering : loss of the chemical defences

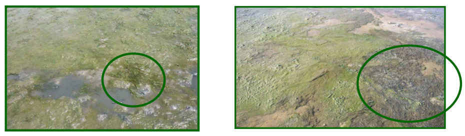
The effects of macroalgae accumulations and withering were investigated in Arcachon bay (French Atlantic coast).

Suffocation by accumulations of opportunistic macro-algae

Blooms of Enteromorpha and Cladophora spp. occur all along the year leading to algal mats, which induce highly unfavourable conditions for Zostera noltii

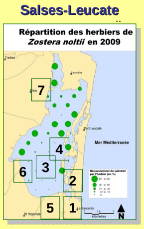
detrimental effects of macroalgal mats on the phenolic content



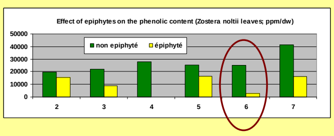


Nutrient enrichment encourages rapid growth of epiphytic algae which may cause severe shading of *Zostera noltii*

Salses-Leucate

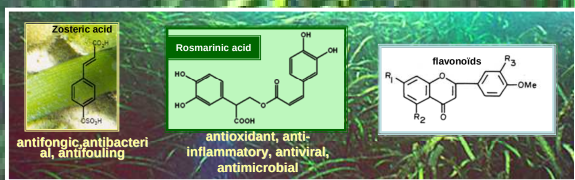


Samples were collected on August 29th 2009 at 7 stations (S1-S7) providing a large gradient of physico-chemical and environmental parameters. After collection, the seagrass material was sorted and divided into all green leaves (green bars) without visible epiphytes and leaves still loaded with epiphytes and macroalgae (yellow bars).



Phenolic content in "green" specimen is always higher than in epiphyted (yellow), showing the negative effect of epiphytes. The highest values were found at S7 (northern part of the lagoon), which is less eutrophicated than S3 and -6 (Ifremer, 2010).

Amount of epiphytes on the leaves: 6>3>2=5>>7



In all cases studied, the phenolic content of impacted specimens was found significantly lower than for the healthy ones.

These results show that these environmental stresses negatively affect the metabolism of *Zostera noltii*, and that polyphenols are good indicators of *Zostera* health conditions.

Acknowledgments
This work was supported by the SUDOE program (ECO-LAGUNES project) and the Region of Aquitaine.

Spatial variability in the phenolic content of *Zostera* spp from Salses-Leucate lagoon

M. Grignon-Dubois[a], B. Rezzonico[a] and L. Fonbonne[b]

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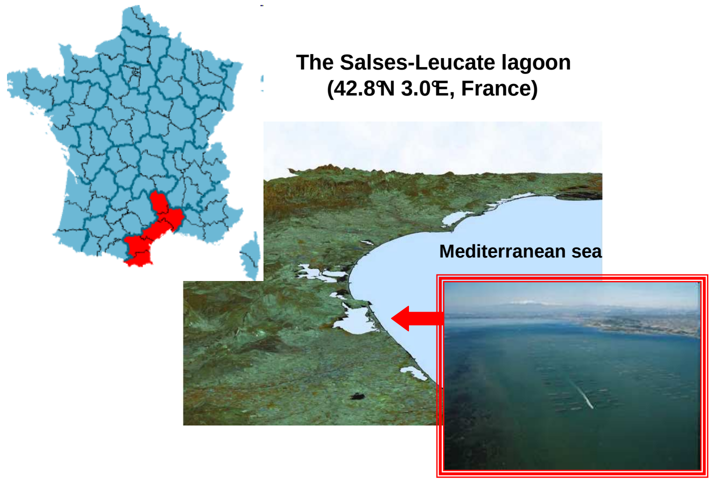
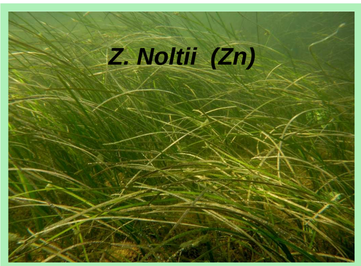
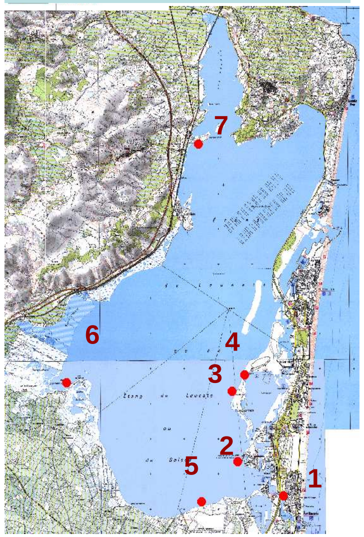
b. RIVAGE Salses-Leucate, Mairie de Leucate, rue du Dr Sidras, F 11370 Leucate

Mediterranean lagoons are highly productive areas representing more than 50% of the coastline in Languedoc-Roussillon (South of France). Many of them suffer from eutrophication and concomitant deterioration of water quality. Seagrass meadows are susceptible to coastal environmental impacts and can serve as early indicators of system-wide degradation. It is of interest to develop and validate chemical biomarkers for monitoring conservation status and ecotoxicological impact in *Zostera* meadows. In this context, phenolic compounds constitute good candidates, which may provide insights into water quality. They play several important functions in plants, and they are common in marine ecosystems. Documenting the presence of those compounds in alive tissues, and how they vary in abundance becomes crucial to understand how human activities influence marine communities. To do this we analyzed the abundance of specific phenolic compounds (rosmarinic (RA), caffeic (CAF), zosteric acids (ZA) and flavonoids (F)) of *Zostera spp.* from different sites located across the Salses Leucate lagoon (Achamlale *et al.,* 2009a, b).





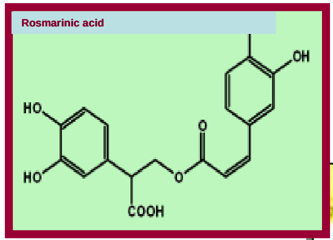
Sampling sites

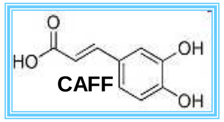


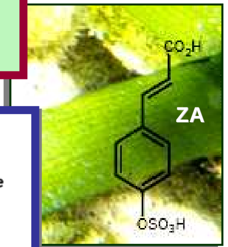
Batch & sites	S1	S2	S3	S4	S5	S6	S7
Zn (A)	-	SL2A-Zn	-	SL4A-Zn	SL5A-Zn	SL6A-Zn	SL7A-Zn
Zn (B)	-	SL2B-Zn	SL3B-Zn	-	SL5B-Zn	SL6B-Zn	SL7B-Zn
Za (A)	SL1A-Za	-	SL3A-Za	-	SL5A-Za	-	-
Za (B)	SL1B-Za	-	SL3B-Za	-		-	-

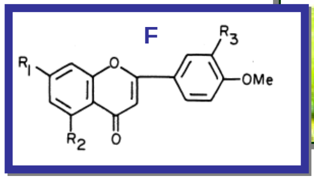
Materials and Methods

Samples were collected at Salses-Leucate lagoon (42.8°N 3.0°E, France) on August 29th 2009 a t 7 stations (S1-S7) providing a large gradient of physico-chemical and environmental parameters. After collection, the seagrass material was sorted and divided into all green leaves (A) without visible epiphytes and leaves still loaded with epiphytes and macroalgae (B). This led to 11 samples of *Z. noltii* and 5 samples of *Z. marina var. angustifolia* (Table 1). Methanolic extracts were prepared from the plant material, and analyzed for both the identity and quantity of phenolics present using NMR and HPLC (Table 1, data are expressed in mg (gdw-1) of dry matter of *Zostera*).







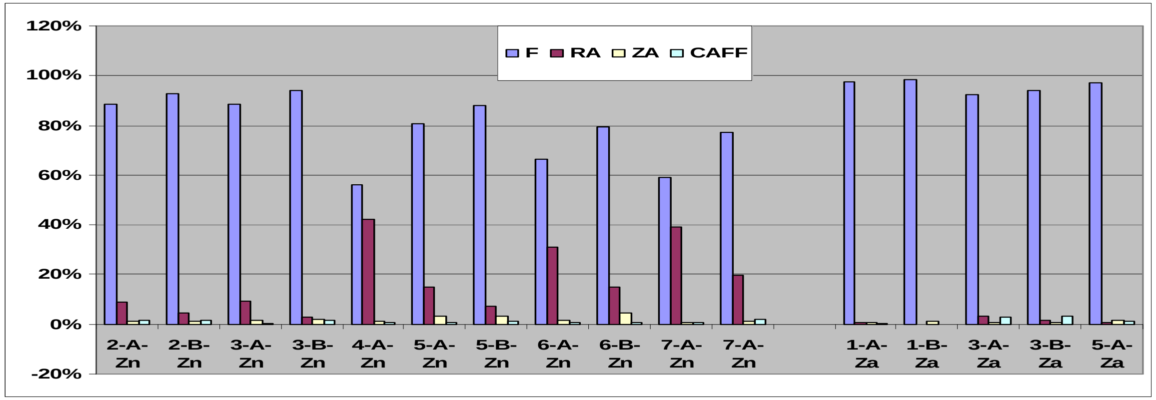


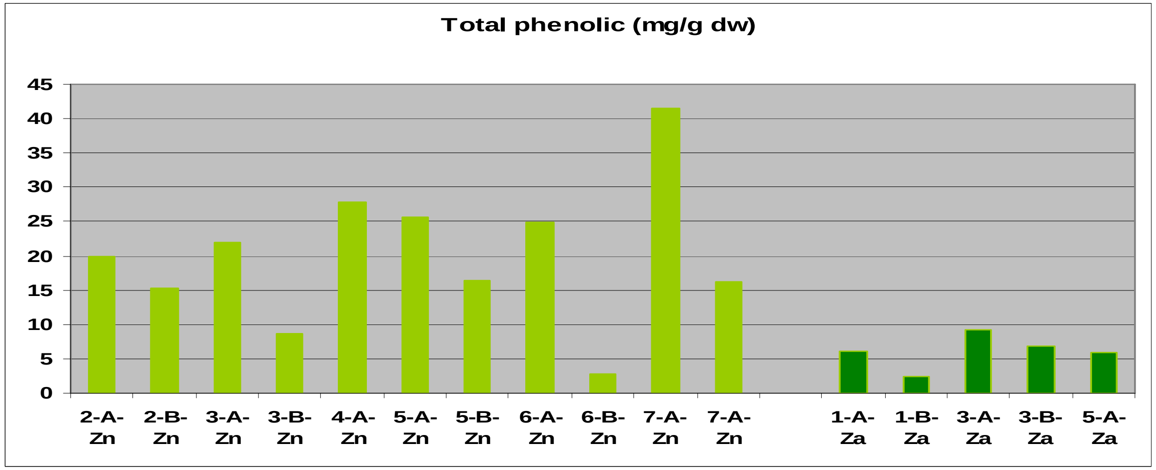
The phenolic profile was largely dominated by flavonoids whatever the species, but the concentration mean values are greater in *Z. noltii* (20 mg.g-1) than in *Z. marina var. angustifolia* (6 mg.g-1 dw). The proportions remain constant with *Z. marina var. angustifolia,* for which only traces of RA, ZA and CAFF were found. In contrast, important variations of the amount of RA were observed with specimen (A) of *Z. noltii :* 42, 31 and 39% respectively at S4, -6 and -7, but only 9% at S2 and -3.

Concentrations in specimen A are always higher than in B, showing the negative effect of macroalgae and epiphytes, which were particularly abundant at S3 and S6. Interestingly, the lowest values were found for the specimen B from these two stations. The highest values were found at S7 (northern part of the lagoon), which is less eutrophicated than S5 and -6 (Ifremer, 2010). The lowest content with *Z. marina var. angustifolia* was found at S1, an artificial channel affected by pollution and physical disturbance due to the passage of boats. Understanding the specific functional role of each of these compounds is now in progress.

This preliminary study highlights the potential of polyphenols as indicators of the coastal lagoon environmental quality

Species	Sites	Extract codes	Phenolic proportions (% of the total)				Total mg.g-1 dw
			F	RA	ZA	CAFF	
Z. noltii	S2	2-A-Zn	88	9	1	1	19.84
		2-B-Zn	93	4	1	2	15.35
	S3	3-A-Zn	89	9	1	1	21.98
		3-B-Zn	94	3	2	1	8.742
	S4	4-A- Zn	56	42	1	1	27.83
	S5	5-A-Zn	81	15	3	1	25.54
		5-B- Zn	88	7	3	1	16.45
	S6	6-A- Zn	66	31	2	1	24.86
		6-B- Zn	79	15	5	1	2.76
	S7	7-A- Zn	59	39	1	1	41.57
		7-A- Zn	77	20	1	2	16.20
Z. marina var angustifolia	S1	1-A- Za	98	1	1	0	6.02
		1-B-Za	99	0	1	0	2.42
	S3	3-A-Za	92	3	1	3	9.17
		3-B-Za	94	2	1	4	6.80
	S5	5-A-Za	97	1	1	1	5.92





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