Aquatic Botany
November 2013, Volume 111, Pages 16–25
http://dx.doi.org/10.1016/j.aquabot.2013.07.010

Algicidal effects of *Zostera marina* L. and *Zostera noltii* Hornem. extracts on the neuro-toxic bloom-forming dinoflagellate *Alexandrium catenella*

Mohamed Laabir[a, *], Micheline Grignon-Dubois[b], Estelle Masseret[a], Bernadette Rezzonico[b], Giselle Soteras[c], Manuel Rouquette[a], Fabien Rieuvilleneuve[a], Philippe Cecchi[d]

[a] Ecologie des Systèmes Marins côtiers, UMR5119, UM2, CNRS, IRD, Ifremer, UM1, Université Montpellier 2, CC 093, 34095 Montpellier, Cedex 5, France
[b] UMR 5805 EPOC, Université Bordeaux, 351, cours de la Libération, F-33405 Talence Cedex, France
[c] Conseil général du département de l'Hérault, 1000 rue d'Alco, 34087 Montpellier, France
[d] IRD UMR G-eau, 01 BP 182 Ouagadougou 01, Burkina Faso

*: Corresponding author : Mohamed Laabir, tel.: +33 4 67144766 ;
email address : mohamed.laabir@univ-montp2.fr

Abstract:

The inhibitory effects of crude extracts of *Zostera marina* L. and *Zostera noltii* Hornemann on the growth of the toxic red tide dinoflagellate *Alexandrium catenella* were investigated through bio-assays. Methanolic and aqueous extracts were prepared from fresh and detrital leaves of *Z. marina* and *Z. noltii* collected in the Thau lagoon and Arcachon bay (France). All the extracts exhibited significant inhibition of *A. catenella* growth, whatever the species and without the need of continuous addition of extracts. The effective concentrations (EC_{50}) varied in the range 0.036–0.199 g L^{-1} for *Z. noltii* and 0.036–0.239 g L^{-1} for *Z. marina*. Methanolic extracts prepared from fresh plant tissues were the most potent, with EC_{50} of 0.036–0.039 and 0.036–0.045 g L^{-1}, for *Z. noltii* and *Z. marina*, respectively. After exposure to the extracts, *Alexandrium* cells exhibited severe morphological anomalies. Hence many cells exhibited a lytic membrane and became necrotic. Cellular degradation appeared dose- and time-dependent. The observed algicidal activities demonstrated the existence of bioactive molecules in the tissues of *Zostera* leaves, which were stable in the culture medium. The chemical contents of the crude extracts were determined by NMR, LC/MS, and quantitative HPLC. Results showed the predominance of flavonoids and phenolic acids. The total phenolics concentrations correlated negatively with the EC_{50} values, suggesting that these secondary metabolites might be responsible for the observed algicidal effects.

Highlights

► We investigated the inhibitory effect of extracts from *Zostera marina* and *Zostera noltii* on the growth of *Alexandrium catenella*. ► Methanolic and aqueous extracts were prepared from fresh and detrital leaves of *Zostera* species. ► All the extracts exhibited dose-dependent significant inhibition of *Alexandrium* growth. ► Phenolic concentrations in extracts were found to correlate negatively with EC_{50} values. ► Phenolic compounds might be responsible of the observed algicidal effect.

Keywords: Growth inhibition ; Allelopathy ; *Alexandrium catenella* ; *Zostera marina* ; *Zostera noltii* ; Phenolics

1. Introduction

In recent years, the control of harmful algal blooms (HAB) has become an important issue for coastal ecosystem protection (Anderson et al., 2001; Kim, 2006). Aquatic macrophytes have long been suspected of limiting phytoplankton growth through the production and excretion of chemical substances (Gross et al., 2007). There is now strong evidence that chemical interferences occur in freshwater ecosystems with many organisms secreting inhibitory compounds (allelochemicals) against microalgae and cyanobacteria (Nakai et al., 2000; Thomas et al., 2000; Gross et al., 2007). Allelopathy refers to any direct or indirect, harmful or beneficial effect produced by plants, protists (e.g. microalgae, ciliates), bacteria, or viruses on another through the production of chemical compounds that leak into the environment (Rice, 1984). It has been suggested that growth inhibition of phytoplankton by submerged macrophytes may confer an advantage to the former in the competition for light, carbon, and nutrients (Gross, 2003; Gross et al., 2007). In the last decades, studies have shown that chemical interactions could play an important role in marine ecosystems in regulating the diversity, structure and seasonal variations of phytoplankton communities (Granéli and Hansen, 2006; Ianora et al., 2006; Granéli et al., 2008). The allelopathic effects of macroalgae against red tide microalgae have been reported (Jeong et al., 2000; Wang et al., 2007; Tang and Gobler, 2011), involving in particular the impacts of different species of *Ulva* on *A. tamarense* populations (Jin and Dong, 2003; Nan et al., 2008). In contrast, non-nutrient interactions between seagrass beds and HAB microalgae have never been reported.

Seagrass beds are thought to have the ability to improve water clarity in their local environment by promoting particle settlement, reducing resuspension and picking up nutrients (Kemp et al., 1984; Ward et al., 1984; Fonseca, 1996). It has also been suggested that the chemical content of *Zostera. marina* leaves might negatively affect the growth and photosynthetic carbon uptake of epiphytic diatoms (Harrison, 1982; Harrison and Durance, 1985). Water-soluble extracts of *Z. marina* from British Columbia have been shown to inhibit the growth of microalgae and marine bacteria and to control amphipod grazing (Harrison and Chan, 1980). Harrison (1982) suggested that water-soluble inhibitors may explain the low biomass of epiphytes on actively growing leaves, but other bioactive compounds may also play a role. Both European *Zostera* species contain bioactive substances, among which zosteric acid (Todd et al., 1993; Achamlale et al., 2009a, b), which may prevent the settlement of some marine bacteria, algae, barnacles and tube worms.

Across Europe, seagrass beds have declined, but at the same time the frequency of HAB events has increased in the last decades (Glibert et al., 2005). Many *Alexandrium* species produce potent neurotoxins that may potentially affect aquatic organisms and human consumers of shellfish which bioaccumulate these toxins (May et al., 2010; Anderson et al., 2012). In the Thau lagoon (French Mediterranean lagoon), the first major bloom of the toxic *A. catenella* (85 000 cells mL^{-1}) occurred in 1998 (Abadie et al., 1999; Lilly et al., 2002). Since then, recurrent episodes were reported during spring and fall reaching high cell concentrations (3-14 x 10^6 cells L^{-1}). Toxin contaminations in bivalves frequently exceeded the sanitary threshold and have induced frequent closing of the aquaculture zones with resulting economical damages. *Alexandrium* outbreaks initiated and developed exclusively in the Crique de l'Angle (Fig. 1), a small embayment, northeast of Thau lagoon, where the highest concentrations were usually registered (Genovesi et al., 2010; Chambouvet et al., 2011; Laabir et al., 2011). Depending on hydrological conditions, *Alexandrium* cells may further spread throughout the lagoon (Genovesi et al. 2013).

Interestingly, since 1990 the Crique de l'Angle has suffered an important regression of the *Zostera* bed which virtually disappeared from this area whereas it the periphery of the rest of the lagoon (Deslous-Paoli et al., 1998; Plus et al., 2001; 2003; 2005), where blooms did not develop. Coastal ecosystems are very complex, and many controlling factors are involved in

the occurrence of HABs (Anderson et al., 2012). Their initiation, duration and intensity may be related to a number of biological, chemical, and physical factors, although, many of these complex relationships have not yet been fully identified (Glibert et al., 2005; Collos et al., 2007; Genovesi et al., 2010; Chambouvet et al., 2011; Laabir et al., 2011; Anderson et al., 2012, Genovesi et al., 2013). In our case, among the possible causes of *Alexandrium* proliferations, the question arises if it is possible to draw conclusions from the chronology of events observed in the Thau lagoon, and to hypothesize that the disappearance of *Zostera* beds may have facilitated the settlement of *Alexandrium* blooms in the Crique de l'Angle. This hypothesis was supported by a cross-analysis of the French REPHY (phytoplankton and phycotoxins) and REBENT (benthic organisms) monitoring network databases that revealed low occurrences of *Alexandrium* blooms in the vicinity of extensive *Zostera* beds. In particular, in the Arcachon bay (French Atlantic Coast), a tidal ecosystem sheltering an important *Zostera* bed, low concentrations of *Alexandrium* spp. are observed and blooms never occurred (Auby et al., 2011).

In order to test this hypothesis, we have studied the effects of crude extracts of *Zostera* species on the growth of *A. catenella* to evaluate their potential negative impact. The main objectives of our study were (i) to assess algicidal activity of the metabolites produced by *Z. marina* and *Z. noltii*, (ii) compare the inhibitory effects of extracts from *Zostera* species collected in Mediterranean (Thau lagoon) and Atlantic marine waters (Arcachon bay), and (iii) to identify the causative bioactive substances.

2. Material and methods

2.1. Collection sites

The Thau lagoon is a brackish shallow lagoon located on the French Mediterranean coast (43°24'N-3°36'E) covering 75 km^2 with a mean-depth of 4.5 m (10 m maximum). Its catchment is drained by numerous little streams with intermittent and seasonal inflows. The lagoon is connected to the sea by two narrow openings. The two seagrass species, *Zostera marina* and *Z. noltii*, constitute about 22% of the total macrophytobenthos biomass that has been estimated at 8.74 ± 2.79 tons dry weight (DW) (Plus et al., 2005). The seagrass bed are located along the periphery of the lagoon between 0- and 5-m depth, except in the Crique de l'Angle, a shallow semi-eclosed area (2 km^2, men depth < 2.5 m) at the north of the lagoon, where *Zostera* has disappeared (Plus et al., 2001; 2003a; 2005).

The bay of Arcachon is a 155 km^2 mesotidal system located on the South-Western French Atlantic coast (44°40'N, 1°10'W). Tides are semidiurnal and enter the Bay through two channels. *Z. noltii* beds are extensive in Arcachon bay, and colonize approximately 40% of the exposed intertidal mudflats, between - 0.3 m and + 3.1 m above lowest tide. The period of meadow emersion during low tide is long (10-14 h). *Z. marina* is found in the subtidal area adjacent to the mudflats (Plus et al., 2010). The sampling stations were located in Andernos (inner part of the bay; 44°44'N, 01 05'W) for detrital leaves, and at Banc d'Arguin for fresh leaves.

Besides their ecological interest as breeding and transit zones for many marine species, both the Thau lagoon and the Arcachon bay hold important shellfish farming activities.

2.2. Zostera collection

Fresh leaves of *Z. noltii and Z. marina* were collected at two sites, Bouzigues (43°25'N, 03°35'E; Thau lagoon) at 0.40 and 1 meter deep respectively, and Arguin (44°35'N, 01°14'W; Arcachon bay) at low tide. Dead leaves of *Z. noltii and Z. marina* were collected from piles in

the intertidal zone at Andernos (44°43'N, 01°09'W, Arcachon bay). Only the green freshly-detached material was sampled. After collection, all the samples were quickly washed in freshwater for 1 to 2 min to remove sand and salt, air-dried at room temperature and then hand-picked to remove associated debris and separate *Z. noltii* from *Z. marina* when the two species were mixed. The collection data of the plant material are provided in Table 1.

2.3. Preparation of the extracts

Extracts were prepared from detrital or fresh leaves of *Z. noltii* and *Z. marina*. Dried leaves of plant material were cut into small pieces prior to extraction. Aqueous extracts were obtained by maceration of 10 g of plant material for 24 h in 200 mL of deionised water at room temperature. The extracts were filtered through a glass funnel then freeze-dried. Methanolic extracts were obtained by continuous extraction at reflux (100 mL for 10 g, 24 h). Extracts were evaporated to dryness under *vacuo.* The following abbreviations were adopted to distinguish the different extracts: (M) methanol, (W) water, (D) detrital, (F) fresh leaves, (Zn) *Z. noltii*, (Zm) *Z. marina*, (B) Bouzigues, (An) Andernos and (Ar) Arguin.

2.4. Structural analysis of the extract

The chemical content of the extracts was determined by nuclear magnetic resonance (NMR), ultraviolet (UV) and liquid chromatography–masss pectrometry (LC-MS) spectroscopies. Identification of the constituents was confirmed by comparison with authentic standards and LC/MS analyses. The solvents used were all HPLC-grade. Trifluoroactic acid (TFA) was purchased from Aldrich Chemical Company. NMR spectra were recorded on an AVANCE 300 MHz (Bruker) in DMSO d-6 (Euriso-Top, Gif-sur-Yvette). The purity of the standard used (P) was determined by quantitative NMR with sodium acetate as internal standard (Achamlale et al., 2009a, b). This led to the following P values: rosmarinic acid (RA) 99%, caffeic acid 99.2%, diosmetin 99.5%. The reference sample of rosmarinic acid was provided by Carbosynth Limited, Beedon - Newbury - RG20 8RY, UK. The reference of zosteric acid (ZA) was synthesized as previously described (Achamlale et al., 2009b). Flavonoid (F) standards were purchased from Extrasynthese (France).

Separation and quantification of the phenolics from*Zostera* dried crude extracts were performed using High-Performance Liquid Chromatography, consisting of a liquid chromatography system (Thermo electron) equipped with a SCM 1000 solvent degasser, a thermostatically controlled column apartment, an AS 3000 autosampler with a 100 µL loop, a PDA UV6000LP detector and a Chromquest Chromatography Workstation. Separations were carried out at 40 °C on a Hypersil GOLD C8 column (Thermo Finnigan), 175 A° pore size, 5 micron particle size, 250×4.6 mm i.d. column. The analytes were eluted at a flow rate of 1 mL min^{-1} using the binary gradient 0.1% (v/v) TFA in water (A) and methanol (B). The following linear gradient was used: 0 min, 1% B; 60 min, 99% B. Run time was 60 min; stop time: 60 min; post time: 10 min. UV spectra were collected over the range of 200-400 nm, and the quantification wavelengths of these chromatograms were set at 280, 328 nm and 350 nm. The injection volume was 20 µL. The data were integrated using Chromquest software. In addition, the data were processed to create a chromatogram, in which each peak represents the absorbance of the eluting substance at its λmax (max-plot chromatogram). Stock solutions of the dried methanolic extracts were prepared in dimethylsulfoxide (DMSO) at a concentration of 1 mg mL^{-1}. All solutions were filtered prior to analysis through a 0.20 µm syringe filter and injected three times into the HPLC.

Separation of the analytes was performed at 40°C. The method allowed a good separation of the compounds. Chromatographic peaks were checked for peak purity and identification was achieved by comparing retention times and UV spectra with those of the standards. Quantitative determinations of ZA were carried out by peak area measurements at 280 nm, using a calibration curve of coumaric acid which is the unsulphated analog of ZA, and has

the same response factor of the HPLC detector as ZA (Achamlale et al., 2009b).The square coefficient correlation (R^2) was 0.9998 (6 points). Quantitative determinations of RA were carried out by peak area measurements at 328 nm, using a calibration curve of the authentic sample at the same wavelength (R^2= 0.99, 6 points), and Flavonoids at 350 nm, using a calibration curve of diosmetin ($0R^2$= 0..99, 8 points).

2.5. Algae culture

The toxic *A. catenella* strain (ACT03) isolated in 2003 from the Thau lagoon was cultivated in Enriched Natural Sea Water (ENSW medium); (Harrison et al., 1980) at 38 psu, at a temperature of 20±1 °C and under a cool-white fluorescent illumination (100 µmol photons m^{-2} s^{-1}) on a 12 h:12 h light:dark cycle. Microalgae were cultured to exponential phase before subsequent inoculation.

2.6. Effect of Dimethyl Sulfoxide (DMSO) on the growth of A. catenella

The effect of increasing concentration of DMSO (0.05%, 0.1%, 0.25%, 0.5%, 1% and 5 % final concentration) on the growth of ACT03 strain was tested. There was no significant difference (t-test; N=20; t = 0.477; P = 0.636) between the growth of *A. catenella* cultivated in ENSW medium and those measured when DMSO was added with concentrations ranging between 0.05 and 1% of final volume of the cultures (Fig. 1), the growth rate values ranged from 0.53 to 0.59 day^{-1}. At 5%, *Alexandrium* cells did not divide and mortality occurred. Consequently, all the experiments were conducted with a maximum of 0.2% DMSO in final volume.

2.7. Effects of Zostera extracts on Alexandrium growth

The inhibitory effects of *Zostera* (detrital and fresh) leaves extracts were tested at different concentrations on the ACT03 strain in batch cultures (30 mL) through different experiments during a time-course experiment of 72 hours. The extracts were dissolved in DMSO *(Z. noltii)* or Ultrapure-water/DMSO (1:4 in v/v) *(Z. marina)*. The maximal concentration of DMSO which has no effect on the microalgae growth was previously determined (Fig . 1). Controls were performed by monitoring the growth of *A. catenella* in ENSW medium and in ENSW medium with DMSO or water/DMSO at the same concentrations as in the experiments with the dissolved extracts. Each flask was inoculated to obtain an initial concentration of about 800-1000 *A. catenella* cells mL^{-1}.All the experiments were conducted in triplicate. Algal growth was monitored daily by direct microscopic counts of cells. According to Guillard (1973), the maximum growth rate (µ; day^{-1}) was calculated from the slope of a linear regression over the entire exponential phase of growth by the least square method; $\mu = (LnN_t - LnN_o)/(t_1 - t_o)$ with No and Nt, the cell densities (cells mL^{-1}) at the beginning, (t_o) and the end (t_1) of the exponential phase, respectively.

The effective concentration inducing a 50 % reduction in *Alexandrium* growth (EC_{50}) was determined after 72 h of exposure using curves relating maximum growth rate to the experimental dosages. Aliquots were collected at 0; 24; 48 and 72 h during the experiment and fixed with formalin (2%) to further examine the effects of extracts on *A. catenella* cells. Description of cell morphology and structure was performed microscopically. At least twenty cells of each culture (controls and treatments) were photographed and analysed using a digital video camera connected to an optical microscope (AxioLab, Zeiss).*Alexandrium* cells were observed in fluorescent light using an Olympus AX70 microscope. The cells were dyed with DAPI (4',6-Diamidino-2-phenylindole dihydrochloride, specific for DNA, Sigma). The final concentration of the used fluorochrome in each examined sample was 2.5 µg mL-1. The main cellular characteristics used to identify anomalies were the shape of the cells and the dispersion of chromatin.

2.8. Statistical analyses

Comparative analyzes of the results were performed using the t-test or its non-parametric equivalent, the Mann-Whitney *U* test, when normality of data was not met. When the hypothesis of equality of variance was not answered, the dose-dependence of the different extracts has been approached in using the non-parametric Kruskal-Wallis one way analysis of variance on ranks (*H*-test). The differences in the median values among the treatment groups were compared at different times. When statistically significant, pairwise multiple comparison procedures (Tukey Test) were used to discriminate sub-groups ($P<0.05$). All the experiments were repeated three times for each independent assay.

3. Results

3.1. Phenolic content of the extract

All the extracts contained significant amounts of phenolics. The individual compounds identified were zosteric acid (ZA), rosmarinic acid (RA) and flavonoids (Table 1). Important amounts of flavonoids were found in all the crude extracts from the two species (52.2-82.9 mg g^{-1} DW in *Z. noltii* and 23.7-42.8 mg g^{-1} DW in *Z. marina)*, whereas ZA concentrations were substantially lower (0.35-0.83 mg g^{-1} DW in *Z. noltii* and 0.17-0.63 mg g^{-1} DW in *Z. marina*). In contrast, RA was abundant in the methanolic extracts (37.32 to 105.36 mg g^{-1} DW in *Z. noltii*, and 15.15 to 27.52 mg g^{-1} DW in *Z. marina*), but much less abundant (1.40±0.07 mg g^{-1} DW) or absent in the water extract of *Z. marina* and *Z. noltii*, respectively. Whatever the sampling site, the total amount of phenolics for a correspondingextract was higher in *Z. noltii* (62.51 to 188.59 mg g^{-1} DW) than in *Z. marina* (25.70 to 64.02 mg g^{-1} DW).

3.2. Effects of Zostera extracts on the growth of A. catenella

For *Z. noltii* extracts, a significant reduction of *A. catenella* densities occurred after 48h with heavy mortality when algal cells were exposed to the methanolic extracts prepared from fresh leaves (Zn-Ar-F-M, and Zn-B-F-M; H-tests: F = 15.79, P = 0.046; F = 16.35, P = 0.038, respectively), even at the lowest tested concentration (0.09 g L^{-1}). The cell yield measured at 72h decreased significantly for all *Z. noltii* extracts (H-tests: F = 23.15, P = 0.003 for Zn-An-D-W; F = 22.22, P = 0.005 for Zn-An-D-M; F = 16.72, P = 0.033 for Zn-Ar-F-M, and F = 16.17, P = 0.04 for Zn-B-F-M) when compared to the initial concentration of the inoculum, suggesting a high mortality rate (Fig. 2a) accompanied by cell damage (Fig. 4). For the methanolic and aqueous extracts prepared with *Z. noltii* detrital leaves and for most of the tested concentrations (0.065 to 3.30 g L^{-1}), the growth rates approached 0 indicating an important inhibition of cell division (Fig. 3). H-tests were systematically significant (H=18.717, P=0.009 and H=19.012, P=0.008 for Zn-An-D-W and Zn-An-D-M, respectively). However, the cells did reveal any lysis and the final cell density remained close to the initial one. The same inhibitory impact was observed after 48 h and was severe after 72 h exposure for extract concentrations of Zn-An-D-W higher than 0.32 g L-1. After 72 h, inhibition was established for cultures exposed to Zn-An-D-M extracts concentrations higher than 0.13 g L^{-1} and for concentrations higher than 0.09 g L^{-1} with water-soluble and methanolic extracts prepared from fresh leaves (Fig. 3).

Z. marina extracts led to similar results. With extracts from fresh leaves collected in Arguin (Zm-Ar-F-M), a significant decrease of the *A. catenella* cell concentration (H-test; F = 16.67, P = 0.038) was recorded after 24h of contact suggesting an important mortality accompanied with cell degradation for concentrations ≥0.09 g L^{-1} (Fig.2b). A significant decrease was observed after 48h of exposure to the extracts from detrital leaves (Zm-An-D-W and Zm-An-D-M; H-tests: F = 21.317, P = 0.006; F = 24.04, P = 0.002, respectively) at concentration ≥ 0.19 g L^{-1}. A significant decrease (H-test, H=21.293, P=0.003 and H=22.14,P=0.002 for Zm An-D-M and Zm-An-D-W respectively) in growth rate of *A. catenella* was systematically

observed after 72h with extracts prepared with detritus (Fig. 3). This generalized trend was observed with all the tested concentrations starting with the lowest concentration of 0.101 g L^{-1} for the extracts prepared from *Zostera* detritus from Andernos (Arcachon bay) including aqueous and methanolic extracts, and from 0.09 g L-1 for the methanolic extracts prepared from fresh leaves. For the extracts Zm-Ar-F-M and Zm-B-F-M, the algicidal activity was very strong and induced cell mortality even with the lowest concentrations tested (0.09 g L^{-1}).

3.3. Morphology of A. catenella cells exposed to Zostera extracts

A. catenella cells grown in culture medium prepared from natural seawater with and without DMSO, exhibited normal morphological characteristics with a symmetrical shape, normal and complete theca and membrane surrounding an intracellular organelles (Fig. 4A). Cell diameter ranged between 28 and 32 µm. Exposure to the crude methanolic/aqueous extracts of both detrital and fresh *Zostera* leavesinduced important morphological and structural cellular changes. After 24h, the cells became motionless while losing their theca and flagella; the intracellular content retracted and the space between the membrane and the wall widened (Fig. 4C, D). The number of cells stopping their division depended on the nature and the final concentration of each algicidal extract (Fig. 4B). *Alexandrium* cells showed an important degradation in their intracellular organelles when exposed to increasing concentrations of the tested extracts (Fig. 4E, F). These inhibited cells were observed under fluorescent light after staining the nucleus with DAPI and showed scattered and irregular DNA (Fig. 4H) in comparison to control cells which were characterized by regular U-shaped nucleus (Fig. 4G). The observed cell anomalies at lower *Zostera* extract concentrations (< 0.180 g L^{-1}) did not show up until 72 h.

3.4. Relationship between EC_{50} and the phenolic contents of Zostera species

All the tested extracts inhibited *A. catenella* growth, but the extracts prepared with fresh leaves were effective at lower concentrations than the extracts prepared from detritus (Fig. 3). A computational (SigmaPlot program) curve-fitting of the data using a sigmoidal function allowed calculation of the EC_{50} values. They correspond to the concentrations of *Zostera* extracts in g L^{-1} at which maximum algal growth rate determined on a 72 h growth period is reduced by 50% compared to the control. The EC50 values (Table 1) varied in the range 0.036-0.199 g L^{-1} for *Z. noltii* and 0.036-0.239 g L^{-1} with *Z. marina*. From these results, it appears that the two species led to very similar inhibition of *Alexandrium* growth, confirming the indications previously provided by growth rates comparisons. In both cases, all the extracts exhibited a strong inhibition effect. The highest EC50 values were found for the extracts prepared from detrital leaves, confirming also that extracts prepared from fresh leaves were more toxic. Methanolic extracts were about 2.5 times more active than aqueous extracts whatever the species: 0.079 g L^{-1} compared to 0.199 g L^{-1} for Zn-An-D-M and Zn-An-D-W, respectively and 0.092 g L^{-1} compared to 0.239 g L-1 for Zm-An-D-M and Zm-An-D-W, respectively. For the two *Zostera* species, the EC_{50} values of all of the extracts decreased when the total phenolic content (mg g^{-1} DW) increased (Figure 5; Table 1). The fitted regression appeared statistically significant for *Z. noltii* extracts ($P = 0.048$); the same tendency was observed for *Z. marina* extracts, but the fitted not significant ($P = 0.135$).

4. Discussion

We observed that *A. catenella,* was strongly inhibited by very low concentrations of crude extracts prepared from *Z. marina* or *Z. noltii* from subtidal (Thau lagoon) and intertidal beds (Arcachon Bay). Accross all the extracts studied, growth inhibition of *A. catenella* became apparent after only 24-48 h of exposure depending on the extract tested and its concentration. Inhibition persisted over three days without the need of continuous addition of

extract. The amplitude of the inhibition is time- and extract concentration-dependent. Our results show that the two eelgrass species led to very strong and similar inhibitions of *Alexandrium* growth. The EC_{50} varied from 0.04 to 0.2 g L^{-1} for *Z. noltii* and from 0.04 to 0.24 g L^{-1} for *Z. marina*. Extracts prepared from fresh leaves induced the strongest inhibitory effect and the highest EC_{50} values were found for the extracts prepared from detrital leaves. This was probably due to the loss of a part of the bioactive metabolites disloged leaves. Methanolic extracts from detritus were about 2.5 times more active than aqueous extracts. This is in agreement with previous studies (Gross et al., 2003; Erhard and Gross, 2006; Mulderij et al., 2007), which showed that solvents like methanol characterized by intermediate lipophilic properties do allow the extraction of watersoluble compounds from aquatic plants. In our study all of the *Zostera* extracts, including the aqueous ones, induced inhibition at low concentrations. Our results show clearly that high concentrations of phenolics correspond to low EC_{50} values (Figure 5), suggesting that these metabolites could be responsible for the observed algicidal activity.The correlation between EC_{50} values and phenolic contents of the extracts appears stronger for *Z. noltii* than for *Z. marina*. This might be linked to the difference in the flavonoid assemblage between the two species. The phenolics found in the *Zostera* extracts were zosteric acid (ZA), rosmarinic acid (RA) and flavonoids (F). RA is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid, which is mainly found in the plant species of *Boraginaceae* and *Lamiaceae*. A multitude of biological activities have been described for RA (Petersen and Simmonds, 2003). It is a potent phagodeterrent against the tobacco hornworm *Manduca sexta* (Petersen and Simmonds, 2003). The production of RA by *Z. noltii* and *Z. marina* is seasonally-dependent (Achamlale et al., 2009a; Ravn et al., 1994). ZA is a sulphated phenolic acid found in both *Z. noltii* and *Z. marina* (Todd et al., 1993; Achamlale et al., 2009b). ZA has been shown to have anti-fouling and anti-adhesion activities against algae, fungal spores and bacteria (Todd et al., 1993). *Z. noltii* and *Z. marina* differ in their individual flavonoid components. The 7-sulphates of luteolin, diosmetin and chrysoeriol and the 7,3"-disulphate of luteolin were identified for *Z. marina*, the last one being the major product, whereas only the 7-sulphates of luteolin and diosmetin are found in *Z. noltii* with this last one being dominant (Harborne and Williams, 1976; Grignon-Dubois and Rezzonico, 2012). These data joined to our experimental results suggest that the phenolic compounds present in the *Zostera* extracts are probably incriminated in the algicidal activity against *Alexandrium* cells.

The relatively lower EC_{50} values obtained with the crude extracts prepared from detrital leaves suggest that RA could play a significant role in the inhibition effect. Indeed, methanolic or aqueous extracts contained similar concentrations of F and ZA, but RA was found only in methanolic extracts which were highly active. This is true for both *Z. noltii* and *Z. marina*. However, flavonoids probably contribute to the inhibitory effects, as shown by the results observed with the methanolic extracts prepared from fresh leaves. Indeed, the lowest crude extract EC_{50} was not obtained for the highest concentration of RA (105.36 mg g^{-1}), which has been found with *Z. noltii* leaves collected in the Thau lagoon. The different constituents might differ in the amplitude of the functional response that they induce or they could act in synergy.

Toxicity mechanisms of action of toxic substances usually include inhibition of growth (Nakai et al. 2001), of PSII activity (Hagmann and Jüttner, 1996), and of cellular motility. Toxic compounds can also affect the membrane permeability and the protein and nucleic acids synthesis (Ianora et al. 2006). We observed severe structural anomalies of *Alexandrium* cells exposed to *Zostera* extracts and in acute cases membranes were lytic. Observations using photonic microscopy have revealed that after 24 h exposure to the crude extract, *A. catenella* cells underwent dramatic changes as they lost their flagella and settled. Our results indicated that extracts prepared from *Z. marina* and *Z. noltii* altered significantly the cellular structure of *Alexandrium* resulting in the observed high mortality rate (Fig. 3).

Recently, inhibitory effects of dry powder of *Ulva* spp. against *Alexandrium tamarense* have been reported. The biomass at which dried *Ulva* tissue inhibited the normal microalgal growth by 50% were 0.19 g DW L^{-1} for *U. lactuca* (Nan et al., 2008) and 0.6-0.8 g DW L^{-1} for *U. linza* (Wang et al., 2007) and *U. pertusa* (Jin and Dong, 2003). For comparison purpose, the dry weight of *Zostera* (DWP) equivalent to the EC50 has been calculated (Table 1). The obtained values were in the same order and varied in the range 0.12-0.77 g DW L^{-1} for *Z. noltii* and 0.09-0.83 g DW L^{-1} for *Z. marina*. They are lower than the plant biomass per volume found in moderately dense *Zostera* beds: 0.66 gDW L-1 for *Z. noltii* meadow and 0.97 gDW L-1 for *Z. marina* in Thau lagoon (Plus et al. 2003a,b). This is in favor of a possible natural negative effect of the allelochemicals extracted from *Zostera* on *Alexandrium*. However, to gain more insights into the potential effects of *Zostera* bio-active molecules *in situ*, further investigations have to determine if the allelopathic substances are effectively exudated in the water column (Harper, 1975; Planas et al., 1981, Hilt et al. 2012).

Acknowledgements

This work benefitted from financial supports from the European project ECOLAGUNESSOE1/P2/F153 (FEDER SUDOE-INTERREG VI B program) and from the French ASCOBAR program of the Region of Aquitaine.

References

Abadie, E., Amzil, Z., Belin, C., Comps, M.A., Alziere-Papayanni, P.,Lassus, P., Le Bec, C., Marcaillou-Le Baut, C., Nezan, E., Poggi, R., 1999.. Contamination de l'étang de Thau par *Alexandrium tamarense*. Episode de novembre à décembre 1998. Ifremer, 44 pp. Available online at http://www.ifremer.fr/docelec/doc/1999/rapport-884.pdf

Achamlale, S., Rezzonico, B., Grignon-Dubois, M., 2009a.Rosmarinic acid from beach waste: Isolation and HPLC quantification in *Zostera* detritus from Arcachon lagoon. Food. Chem. 113, 878-883.

Achamlale, S., Rezzonico, B., Grignon-Dubois, M., 2009b. Evaluation of *Zostera* detritus as a potential new source of Zosteric acid. J. Appl. Phycol. 21, 347-352.

Anderson, D.M., Andersen, P., Bricelj, V.M., Cullen, J.J., Rensel, J.E., 2001. Monitoring and management strategies for harmful algal blooms in coastal waters. APEC #201-MR-01.1, Asia Pacific Economic Program, Singapore, and IOC-UNESCO, Tech Series 59, Paris, pp 174-264.

Anderson, D.M., Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E., Montresor, M., 2012. The globally distributed genus *Alexandrium*: Multifaceted roles in marine ecosystems and impacts on human health. Harmful Algae. 14, 10-35.

Auby, I., D'Amico, F., Barbier, C., Duval, M., Neaud-Masson, N., Plus, M., Rumebe, M., Tournaire, M.P., Trut, G., Ryckaert, M., Derrien, A., Margat, S., Schmitt, A., Charpentier, G., Seugnet, J.L., Thomas, G., Guesdon, S., Llevot, G., Beaugrand, P., Carrere, C., Michel, V., Petrau, C., Bariou, J., Landier, P., Debinski, O., Le Mao, A., 2011. Suivi hydrologie et phytoplancton des masses d'eau du bassin hydrographique Adour-Garonne en 2005-2010. http://archimer.ifremer.fr/doc/00069/18021/15553.pdf.

Chambouvet, A., Laabir, M., Sengco, M., Vaquer, A., Guillou, L., 2011. Genetic diversity of Amoebophryidae (Syndiniales) during *Alexandrium catenella/tamarense* (Dinophyceae) blooms in Thau lagoon (Mediterranean Sea, France). Res. Microb. 162 (9), 959-968.

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, southern France. Harmful Algae. 6, 781-789.

Deslous-Paoli, J.M., Souchu, P., Mazouni, N., Juge, C., Dagault, F. 1998. Relations milieuressources : impact de la conchyliculture sur un environnement lagunaire méditerranéen (Thau). Oceanol. Acta. 21, 831-843.

Erhard, D., Gross, E.M., 2006. Allelopathic activity of *Elodea canadensis* and *E. nuttallii* against epiphytes and phytoplankton. Aquat. Bot. 85, 203-211.

Fonseca, M., 1996. The role of seagrasses in nearshore sedimentary processes: a review. In: Nordstrom, K.F., Roman, C.T., (Eds.) Estuarine Shores: Evolution, Environmental and Human Alterations. Wiley, New-York, pp. 261-286.

Genovesi, B., Laabir, M., Masseret, E., Collos, Y., Vaquer, A., Grzebyk, D., 2010. Dormancy and germination patterns in resting cysts of *Alexandrium tamarense* species complex (Dinophyceae) can facilitate bloom formation in a shallow lagoon (Thau, southern France). J. Plank. Res. 31, 1209-1224.

Genovesi, B., Mouillot, D., Laugier, T., Fiandrino, A., Laabir, M., Vaquer, A., Grzebyk, D., 2013. Influence of sedimentation and hydrodynamics on the spatial distribution of Alexandrium catenella/tamarense resting cysts in a shellfish farming lagoon hit by toxic blooms. Harmful Algae. 25, 15-25.

Glibert, P.M., Anderson, D.M., Gentien, P., Graneli, E., Sellner, K.G., 2005. The global complex phenomena of Harmful Algal Blooms. Oceanography 18, 136-147.

Granéli, E., Hansen, P.J., 2006. Allelopathy in harmful algae: a mechanism to compete for resources? In: Graneli, E., Turner, J.T., (Eds.), Ecology of Harmful Algae. Springer- Verlag, Berlin, pp. 189-201.

Granéli, E., Salomon, P.S., Fistarol, G.O., 2008. The Role of Allelopathy for Harmful Algae Bloom Formation. Algal Toxins: Nature, Occurrence, Effect and Detection. V. Evanglista et al. (Eds.), Springer science. NATO Science for Peace and Security Series A: Chem. Biol. 2008, 159-178.

Grignon-Dubois, M., Rezzonico, B., 2012. First phytochemical evidence of chemotypes for the seagrass *Zostera noltii*. Plants 1, 27-38.

Gross, E.M., 2003. Allelopathy of aquatic autotrophs. Crit. Rev. Plant. Sci. 22, 313–39.

Gross, E.M., Erhard, D., Ivanyi, E., 2003. Allelopathic activity of *Ceratophyllim demersum* L. and *Najas marina* ssp. *intermedia* (Wolfgang) Casper. Hydrobiologia. 506, 583-589.

Gross, E.M., Hilt, S., Lombardo, P., Mulderij, G., 2007. Searching for allelopathic effects of submerged macrophytes on phytoplankton - state of the art and open questions. Hydrobiologia. 584, 77-88.

Guillard, R.R.L., 1973. Division rates. In Stein, J. R. (ed.), Handbook of Phycological Methods. Culture Methods and Growth Measurements. Cambridge University Press, pp. 290-311.

Hagmann, L., Jüttner F., 1996. Fischerellin A, a novel photosystem-II-inhibiting allelochemical of the cyanobacterium *Fischerella muscicola* with antifungal and herbicidal activity. Tetrahedron Lett.36, 6539-6542.

Harborne, J.B., Williams, C., 1976. Occurrence of sulphated flavones and caffeic acid esters in members of the fluvials. Biochem. System. Ecol. 4, 37-41.

Harper, J.L., 1975. Review of 'Allelopathy. Physiological Ecology' by Rice, E.L. The Quarterly Review of Biology 50(4), 493-495.

Harrison, P.G., 1982. Control of microbial growth and of amphipod grazing by water-soluble compounds from leaves of *Zostera marina*. Mar. Biol.67,225-230.

Harrison P.G., Chan A.T., 1980. Inhibition of the growth of micro-algae and bacteria by extracts of eelgrass *(Zostera marina)* leaves. Mar. Biol. 61, 21-26.

Harrison, P.G., Durance, C.D. 1985. Reduction in photosynthetic carbon uptake in epiphytic diatoms by water-soluble extracts of leaves of *Zostera marina*. Mar. Biol. 90, 117-120.

Harrison, P.J., Waters, R.E. and Taylor, F.J.R., 1980. A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. J. Phycol. 16, 28-35.

Hilt, S., Butler, E., Bauer, N. 2012. Comparison of methods to detect allelopathic effects of submerged macrophytes on green algae. J. Phycol. 48, 40–44.

Ianora, A., Boersma, M., Casotti, R., Fontana, A., Harder, J., Hoffmann, F., Pavia, H., Potin, P., Poulet, S.A. and Toth, G. 2006. New trends in marine chemical ecology. The H.T. Odum synthesis essay. Estuar. Coasts. 29, 531-551.

Jeong, J.H., Jin, H.J., Sohn, C.H., Suh, K.H., Hong, Y.K., 2000. Algicidal activity of the seaweed *Corallinan pilulifera* against red tide microalgae. J. Appl. Phycol. 12, 37-43.

Jin, Q., Dong, S., 2003. Comparative studies on the allelopathic effects of two different strains of *Ulva pertusa* on *Heterosigma akashiwo* and *Alexandrium tamarense*. J. Exp. Mar. Biol. Ecol. 293, 41-55.

Kearns, K.D., Hunter, M.D., 2001. Toxin-producing *Anabaena flos-aquae* induces settling of *Chlamydomonas reinhardtii*, a competing motile alga. Microbial. Ecol. 42, 80-86.

Kemp, W.M., Boynton, W.R., Twilley, R.R., Stevenson, J.C., Ward, L.G., 1984. Influences of submersed vascular plants on ecological processes in Upper Chesapeake Bay. In: Kennedy, V.S., (Eds.) Estuaries as Filters. Academic Press, New York, pp. 367-394.

Kim, H.G., 2006. Mitigation and controls of HABs. In: Graneli, E., Turner, J.T., (Eds.), Ecology of Harmful Algae. Springer-Verlag, Berlin, pp. 327-338.

Laabir, M., Jauzein, C., Genovesi, B., Masseret, E., Grzebyk, D., Cecchi, P., Vaquer, A., Perrin, Y., Collos, Y., 2011. Influence of temperature, salinity and irradiance on the growth and cell yield of the harmful red tide dinoflagellate *Alexandrium catenella* colonising Mediterranean waters. J. Plank. Res. 33, 1550-1563.

Lilly, E.L., Kulis, D.M., Gentien, P., Anderson, D.M., 2002. Paralytic shellfish poisoning toxins in France linked to a human-introduced strain of *Alexandrium catenella* from the western Pacific: evidence from DNA and toxin analysis. J. Plank. Res. 24, 443-452.

May, S.P., Burkholder, J. M., Shumway, S.E., Hégaret, H., Wikfors, G.H., Frank, D., 2010. Effects of the toxic dinoflagellate *Alexandrium monilatum* on survival, grazing and behavioral response of three ecologically important bivalve molluscs. Harmful Algae. 9, 281-293.

Mulderij, G., Mau, B., Van Donk, E., Gross, E.M., 2007. Allelopathic activity of *Stratiotes aloides* on phytoplankton- towards identification of allelopathic substances. Hydrobiologia. 584, 89-100.

Nakai, S., Inoue, Y., Hosomi, M., Murakami, A., 2000. *Myriophyllum spicatum*-released allelopathic polyphenols inhibiting growth of blue-green algae *Microcystis aeruginosa*. Water. Res. 34, 3026-3032.

Nakai, S., Inoue, Y. Hosomi M., 2001. Algal growth inhibition effects and inducement modes by plant-producing phenols. Water. Res.35, 1855-1859.

Nan, C., Zhang, H., Lin, S., Zhao, G., Liu, X., 2008. Allelopathic effects of *Ulva lactuca* on selected species of harmful bloom-forming microalgae in laboratory cultures. Aquat. Bot. 89, 9-15.

Petersen, M., Simmonds, M.S.J., 2003. Rosmarinic acid. Phytochem.. 62, 121-125.

Planas, D., Sarhan, F., Dube, L., Godmaire, H., Cadieux, C., 1981. Ecological significance of phenolic compounds of *Myriopyllum spicatum*. Verh. Internat. Verein. Limnol. 21, 1492-1496.

Plus, M., Deslous-Paoli, J.M., Auby, I., Dagault, F., 2001. Factors influencing primary production of seagrass beds (*Zostera noltii* Hornem) in the Thau lagoon (French Mediterranean coast). J. Exp. Mar. Biol. Ecol. 259, 63-84.

Plus, M., Chapelle, A., Lazure, P., Auby, I., Levavasseur, G., Verlaque, M., Belsher, T., Deslous-Paoli, J.M., Zaldivar, J., Murray, C., 2003a. Modelling of oxygen and nitrogen cycling as a function of macrophyte community in the Thau lagoon. Cont. Shelf. Res. 23, 1877-1898.

Plus, M., Chapelle, A., Ménesguen, A., Deslous-Paoli, J.M., Auby, I. 2003b. Modelling seasonal dynamics of biomasses and nitrogen contents in a seagrass meadow (Zostera noltii Hornem.): application to the Thau lagoon (French Mediterranean coast). Ecological. Modelling. 161(3), 211-236

Plus, M., Auby, I., Verlaque, M., Levavasseur, G., 2005. Seasonal variations in photosynthetic irradiance response curves of macrophytes from a Mediterranean coastal lagoon. Aquat. Bot. 81, 157-173.

Plus, M., Dalloyau, S., Trut, G., Auby, I., De Montaudouin, X., Emery, E., Noel, C., Viala., C. 2010. Long-term evolution (1988-2008) of *Zostera* spp. meadows in Arcachon Bay (Bay of Biscay). Estuar. Coast. Shelf. Sci. 87, 357-366.

Ravn, H., Pedersen, M.F., Borum, J., Andary, C., Anthoni, U., Christophersen, C., Nielsen, P.H., 1994. Seasonal variation and distribution of two phenolic compounds, rosmarinic acid and caffeic acid, in leaves and roots-rhizomes of eelgrass (*Zostera marina* L.). Ophelia 40, 51-61.

Rice, E.L., 1984. Allelopathy. 2nd Ed. Academic Press, Orlando, Florida, USA pp 320-343.

Tang, Y.Z., Gobler, C.J., 2011. The green macroalga, *Ulva lactuca*, inhibits the growth of seven common harmful algal bloom species via allelopathy. Harmful Algae 10, 480-488.

Thomas, S., Cecchi, P., Corbin, D., Lemoalle, J., 2000. The different primary producers in a small African tropical reservoir during a drought: temporal changes and interactions. Freshwater Biol. 45, 43-56.

Todd, J., Zimmerman, R.C., Crews, P., Alberte, R.S., 1993. The antifouling activity of natural and synthetic phenolic acid sulfate esters. Phytochem.. 34, 401-404.

Wang, R., Xiao, H. Wang, Y. Zhou, W, Tang, X., 2007. Effects of three macroalgae, *Ulva linza* (Chlorophyta), *Corallina pilulifera* (Rhodophyta) and *Sargassum thunbergii* (Phaeophyta) on the growth of the red tide microalga *Prorocentrum donghaiense* under laboratory conditions. J. Sea. Res. 58, 189-197.

Ward, L.G., Kemp, W.M., Boynton, W.R., 1984. The influence of waves and seagrass communities on suspended particulates in an estuarine embayment.Mar. Geol. 59, 85-103.

Figures

Fig. 1. Time variation of *A. catenella* abundances (cells mL^{-1}) at different concentrations of DMSO. Data are the means ± SD of three independent experiments.

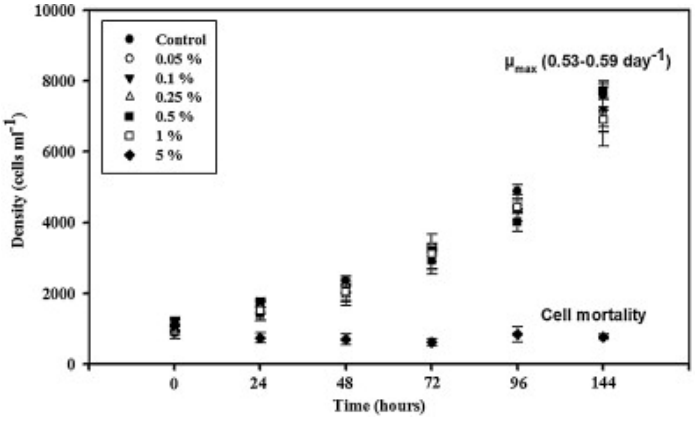

Fig. 2a Density (cells mL^{-1}) variation of *Alexandrium catenella* exposed to different concentrations (g L-1) of (M) methanolic or (W) water extracts of (F) fresh or (D) detrital leaves of (Zn) *Zostera noltii* from (B) Bouzigues, (An) Andernos and (Ar) Arguin. Controls were constituted of *A. catenella* grown with a determined volume of DMSO or Ultrapure-Water/DMSO (1:4 in v/v), DMSO corresponding to the highest DMSO concentration of the tested extract. Statistics of the non parametric Kruskal-Wallis one way analysis of variance on ranks (H), and associated probability (P) are provided. Values are the means of three replicates.

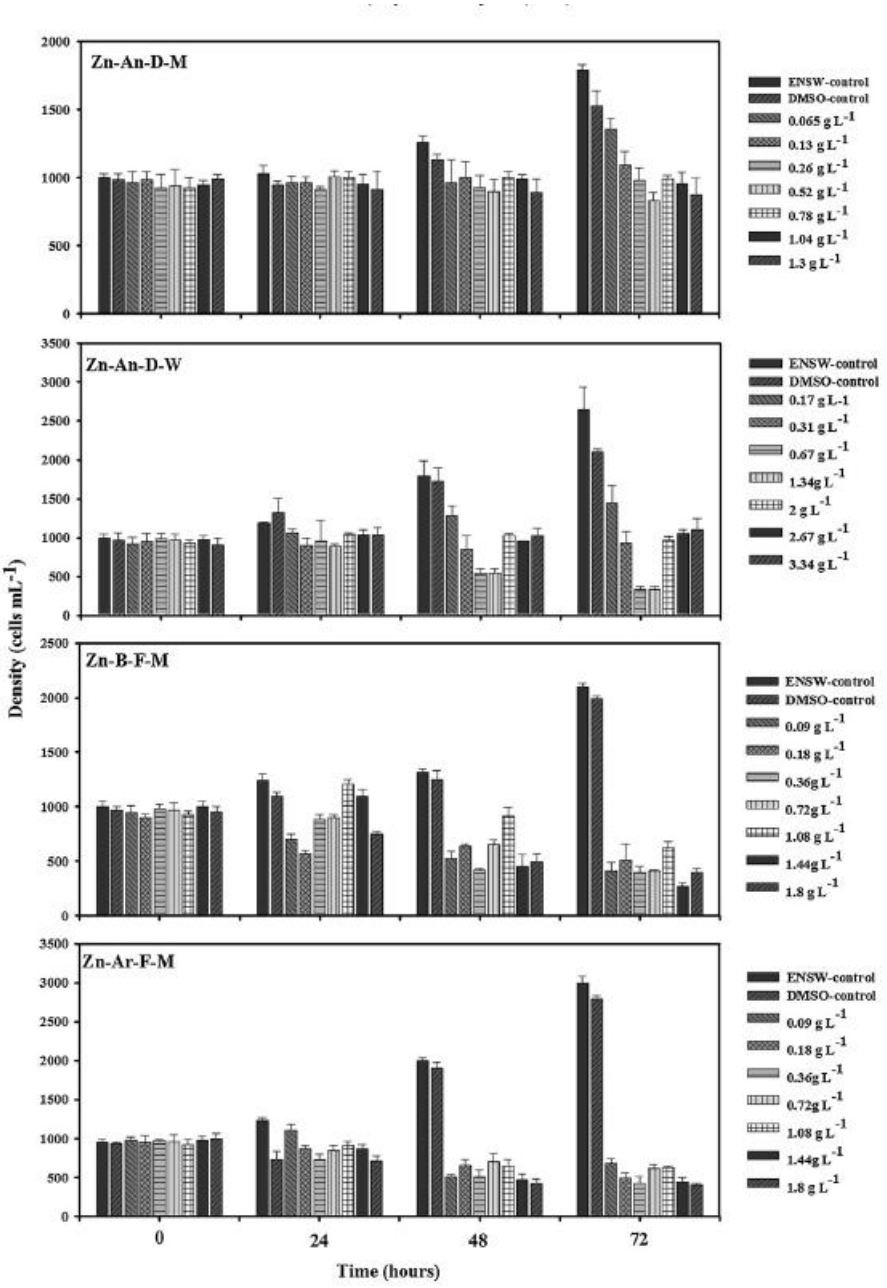

Fig. 2b Density (cells mL^{-1}) variation of *Alexandrium catenella* exposed to different concentrations (g L-1) of (M) methanolic or (W) water extracts of (F) fresh or (D) detrital leaves of (Zm) *Zostera marina* from (B) Bouzigues, (An) Andernos and (Ar) Arguin. Controls were constituted of *A. catenella* grown with a determined volume of DMSO or of Ultrapure-Water/DMSO (1:4 in v/v) corresponding to the highest Ultrapure-Water/DMSO concentration of the tested extract. Statistics of the non parametric Kruskal-Wallis one way analysis of variance on ranks (H), and associated probability (P) are provided. Values are the means of three replicates.

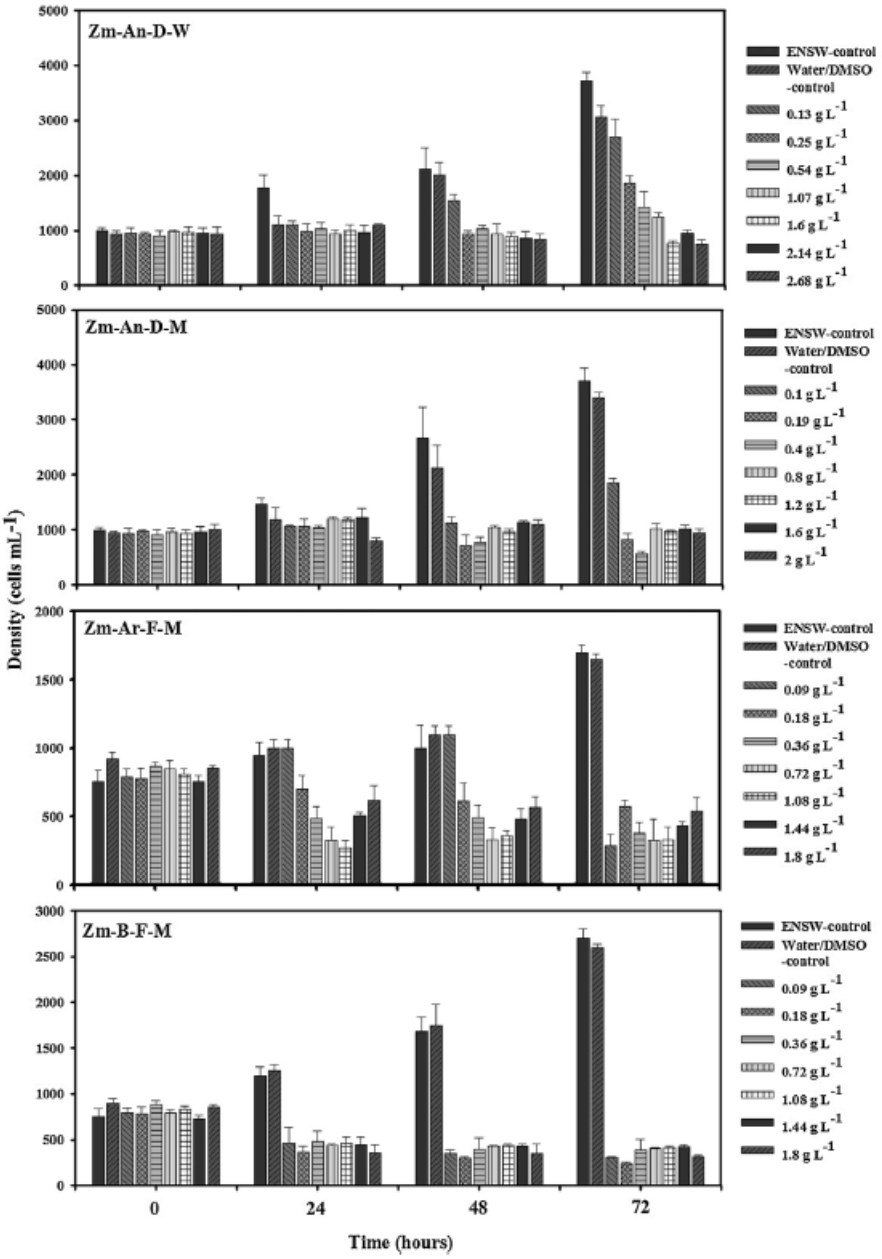

Fig. 3. Maximum growth rate (day^{-1}) of *Alexandrium catenella* exposed to (M) methanolic or (W) water extracts of (F) fresh or (D) detrital leaves of (Zn) *Z. noltii* or (Zm) *Z. marina* from (B) Bouzigues, (An) Andernos and (Ar) Arguin. Controls were constituted of *A. catenella* grown with determined volume of DMSO or Ultrapure-water/DMSO (1:4 in v/v). Growth values of 0 day^{-1} indicate an arrest of cell division and those below 0 day^{-1} indicate cell mortality. Statistics of the non parametric Kruskal-Wallis one way analysis of variance on ranks (H), and associated probability (P) are provided.

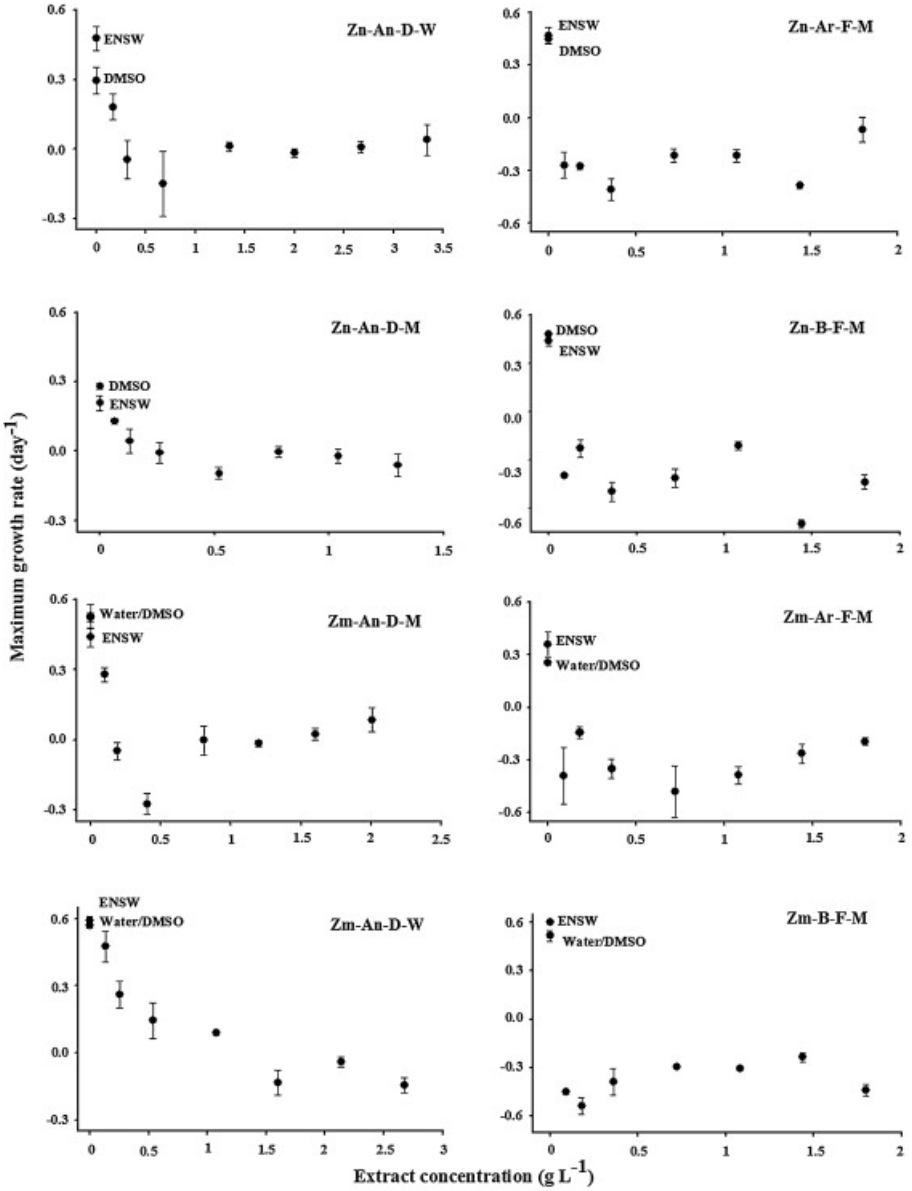

Fig. 4.*Alexandrium catenella*. Light microscope photographs of (A) control cells presenting normal morphology and (B-F) cells exposed to *Z. marina* or *Z. noltii* extracts. Affected microalgae failed to divide (B) or presented dense material and no flagella (C, D). When increasing extract concentration and exposure duration, *Alexandrium* cells (E, F) lose their intracellular organelles and their membranes were lytic. Epifluorescence microscope photographs of (G) control cells present a regular U-shaped nucleus while (H) inhibited cells show remaining irregular and scattered DNA stained with DAPI fluorochrome. The scale bar corresponds to 5µm.

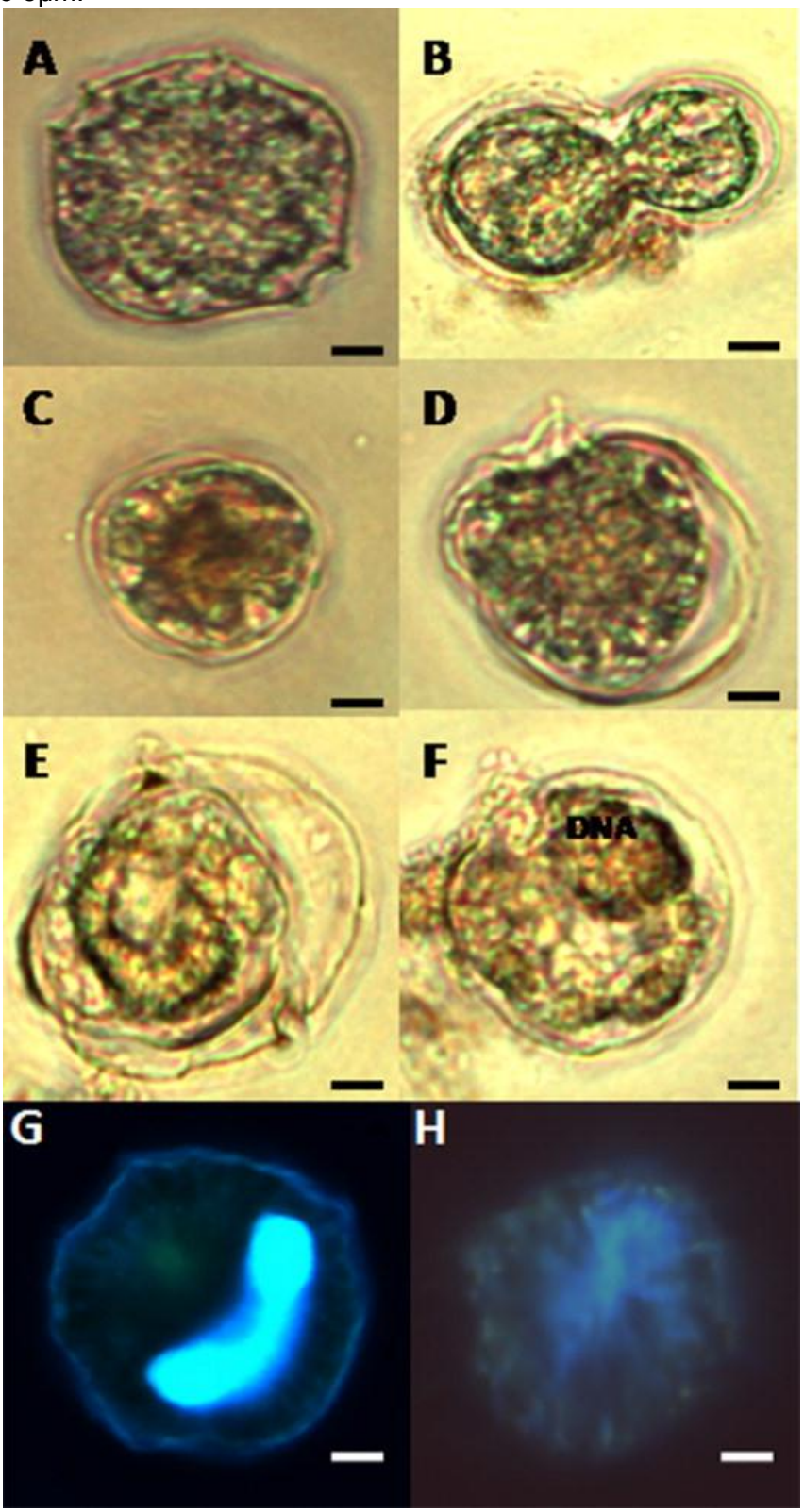

Fig. 5. Relationship between the phenolics contents of the different tested extracts (mg g^{-1} dry weight) and the associated EC50 (g L^{-1}) for the two eelgrass species considered separately. Correlation coefficients are indicated for both regressions. The equations are Y = 0,76 exp (-0,02 * X) and Y = 0,93 exp (-0,05 * X) for *Zostera noltii* and *Zostera marina*, respectively.

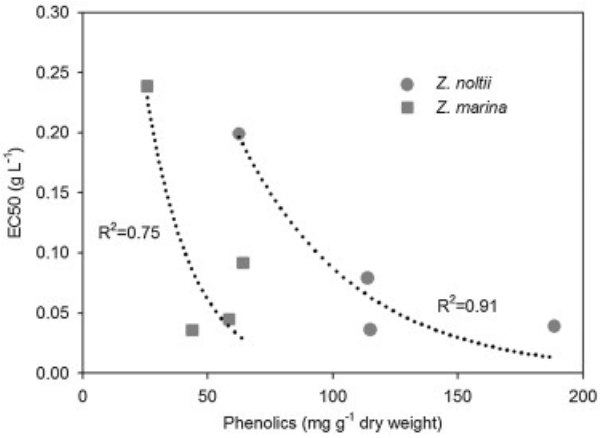

Table 1
Collected species, sampling sites, plant material, extraction method, EC_{50} ($g\,L^{-1}$) values of *Alexandrium catenella* growth inhibition, DWP ($g\,L^{-1}$) Dry Weight of *Zostera* spp. Plant equivalent to the EC_{50} and the amount of phenolics in the extracts ($mg\,g^{-1}$ dry weight).

Species	Site (date of collection)	Biomass material	Extraction method	Extract of *Zostera* code[a]	EC_{50} ($g\,L^{-1}$)	DWP ($g\,L^{-1}$)	Phenolic content $mg\,g^{-1}$ dry weight			
							ZA[b]	RA[b]	F[b]	Total
Zostera noltii	Andernos (23/01/05)	Detrital leaves	Water	Zn-An-D-W	0.2 ± 0.02	0.76 ± 0.09	0.62 ± 0.01	–	61.89 ± 0.59	62.51
			Methanol reflux	Zn-An-D-M	0.08 ± 0.1	0.42 ± 0.02	0.75 ± 0.01	60.93 ± 0.61	52.20 ± 0.61	113.88
	Arguin (20/11/09)	Fresh leaves	Methanol reflux	Zn-Ar-F-M	0.04 ± 0.01	0.12 ± 0.02	0.83 ± 0.02	37.32 ± 0.43	76.93 ± 0.77	115.08
	Bouzigues (17/12/09)	Fresh leaves	Methanol reflux	Zn-B-F-M	0.04 ± 0.01	0.12 ± 0.02	0.35 ± 0.015	105.36 ± 0.48	82.88 ± 0.83	188.59
Zostera marina	Andernos (02/08/05)	Detrital leaves	Water	Zm-An-D-W	0.24 ± 0.03	0.82 ± 0.11	0.60 ± 0.01	1.40 ± 0.07	23.70 ± 0.35	25.70
			Methanol reflux	Zm-An-D-M	0.09 ± 0.02	0.29 ± 0.06	0.63 ± 0.01	27.52 ± 0.30	35.87 ± 0.41	64.02
	Arguin (20/11/09)	Fresh leaves	Methanol reflux	Zm-Ar-F-M	0.05 ± 0.01	0.17 ± 0.02	0.61 ± 0.01	15.15 ± 0.39	42.75 ± 0.45	58.52
	Bouzigues (17/12/09)	Fresh leaves	Methanol reflux	Zm-B-F-M	0.04 ± 0.01	0.09 ± 0.02	0.17 ± 0.01	23.26 ± 0.41	20.33 ± 0.36	43.76

[a] Extract's characteristics: (M) methanolic extraction, (W) aqueous extraction, (D) detrital leaves, (F) fresh leaves, (An) Andernos and (Ar) Arguin in Arcachon bay, (B) Bouzigues in Thau lagoon.

[b] Phenolics: (ZA) zosteric acid, (RA) rosmarinic acid and (F) flavonoids. Values of EC50, DWP and phenolic contents are expressed in mean ± SD ($n = 3$).