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Dynamics of the seagrass *Zostera noltei* in a shallow Mediterranean lagoon exposed to chemical contamination and other stressors

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ABSTRACT

Seagrass decline due to a variety of stressors has been observed worldwide. In the shallow Vaccarès lagoon, Camargue, France, the dominant macrophyte species, *Zostera noltei*, has suffered two major declines since 1996. The first decline was well explained by salinity and turbidity variations, while the second one could not be explained by these parameters. Other stressors such as chemical contamination, eutrophication or temperature increase could be explanatory variables for this most recent decline. The aim of our study was to understand, *via* scientific monitoring from 2011 to 2015, the influence of chemical contamination and its possible interactions with other biological and environmental pressures, on seagrass physiology and population dynamics in the Vaccarès lagoon.

Multi-contamination by organic contaminants and trace metals was detected in the water and sediments, and their concentrations often exceeded environmental standards, particularly where seagrass regression was observed. Spatial variations in biological, environmental and chemical parameters in the lagoon were investigated by co-inertia analysis, which revealed significant relationships between environmental data, more particularly between contaminants, seagrass dynamics indices and biomarkers. Seagrass dynamics indices were negatively correlated with the concentrations of some herbicides in water (2,4-MCPA and bentazon) and with trace metals in sediments (arsenic). Rhizome starch contents in winter were negatively correlated with those herbicides and with several metals (arsenic, zinc, copper) in water and/or sediments. These results suggest that environmental contamination may play a role in seagrass decline. However, complementary investigations, such as monitoring over longer periods and additional toxicity tests, are required to address the causal link between contamination and seagrass decline.

1. Introduction

Seagrasses have a wide spatial distribution and are an important component of coastal ecosystems. They provide many essential ecosystem services such as sediment stabilization, nutrient cycling, a nursery for living organisms, high primary production (CO_2 fixation), support for commercial fisheries and a source of food (Costanza et al., 1997; Orth et al., 2006; Waycott et al., 2009). However, seagrass decline has been observed worldwide, with a 29% decrease in the known extent of meadows since 1879, and has been ongoing at a rate of 110 km^2 $year^{-1}$ since 1980 (Bernard et al., 2007; Waycott et al., 2009), potentially affecting the ecosystem services provided. Apart from the highly fluctuating natural dynamics of seagrass meadows, which can vary from enduring to transitory depending on the species' traits (Kilminster et al., 2015), a variety of stressors for seagrasses have been identified. These stressors can be divided in (1) natural processes such as storms, cold winters, sedimentation but also sediment resuspension caused by frequent wind, which reduces light availability, grazing by birds and urchins and diseases and (2) anthropogenic pressures including invasive species, eutrophication, dragging, aquaculture, dredging, brown tides, toxicants, global climate change and physical and hydrological ecosystem modifications (Charpentier et al., 2005; Orth et al., 2006; Waycott et al., 2009; Plus et al., 2010; Dos Santos et al., 2013; Diepens et al., 2017).

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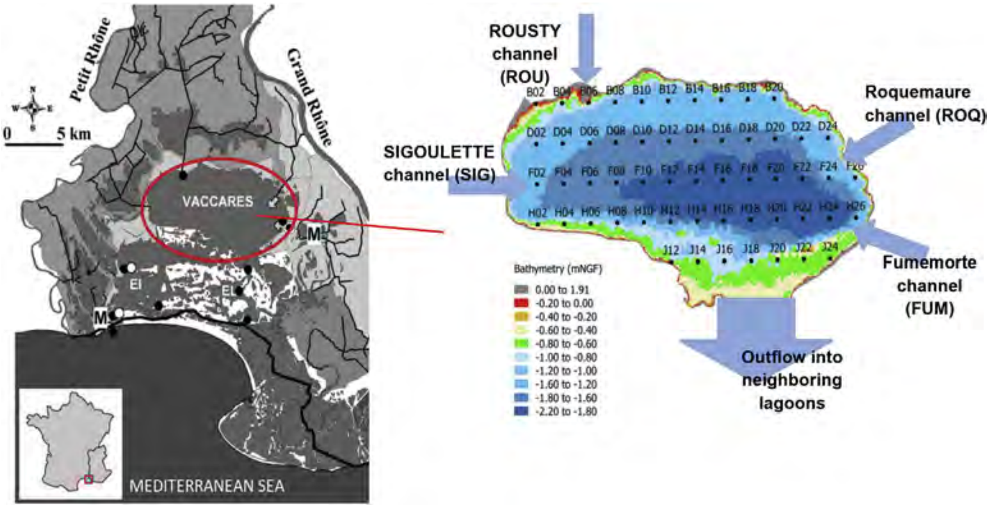

Fig. 1. Location of the Vaccarès lagoon and samples stations in the study area (Camargue, South of France). Figure has been modified from Boutron et al. (2015).

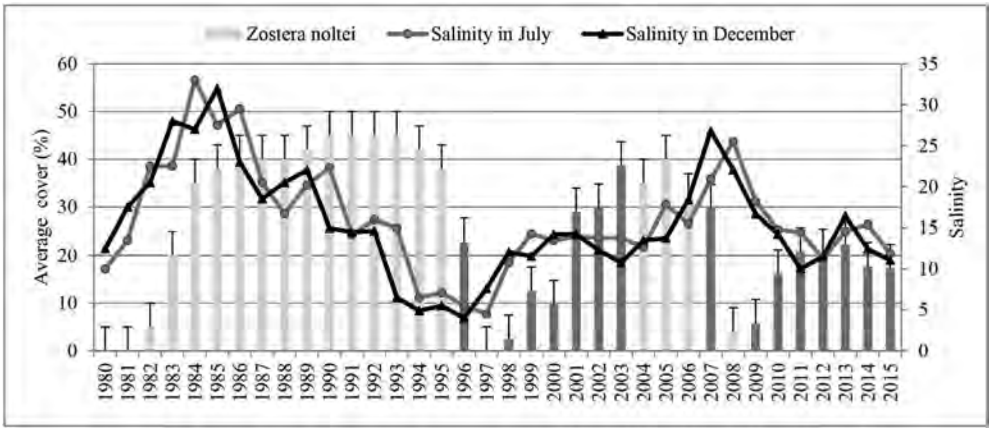

Fig. 2. Annual average cover (%) of *Zostera* meadow in the Vaccarès lagoon over all stations per year (grey bars), in comparison with salinity in July (circles) and December (triangles). Dark grey bars: average cover of 55 sampling stations, light grey bars: estimated cover using remote sensing.

In the south of France, seagrass decline has been reported in the river deltas along the Mediterranean coast, especially in the Vaccarès lagoon, Camargue (Fig. 1). This shallow lagoon is among the most disturbed coastal ecosystems because it is isolated by the Rhône River, dams and surrounding intensive agriculture (mainly rice). This is the main lagoon of the Ile de Camargue, internationally recognized as a biosphere reserve under the UNESCO's Man and the Biosphere Programme, and as a RAMSAR site. The Ile de Camargue is of global importance for nesting, staging, and wintering water birds (Scott and Rose, 1996). Since 1993, its dominant macrophyte species, *Zostera noltei* (a.k.a. *Z. noltei*, Hornemann, 1832), has shown extreme variations in its average cover with two major declines in 1997 and 2008 (Fig. 2). The first decline was explained by a decrease in salinity, probably due to significant rainfall and greater freshwater input, which caused an increase in water turbidity (Charpentier et al., 2005). The other main factors ruling the spatial distribution of *Z. noltei* are water depth and wave exposure. However, these abiotic factors could not explain the second decline (Charpentier et al., 2005).

It has been reported that poor water quality, and especially eutrophication, have a negative impact on *Z. noltei* at population and individual levels, and could lead to regime shifts towards an algae dominated state (Valiela et al., 1992; McGlathery, 2001; Brun et al., 2002, 2008; Cardoso et al., 2004; Cabaço et al., 2008; Plus et al., 2001, 2010). For example, there were fewer non-structural carbohydrates in rhizomes at stations with a poor ecological quality status along the Atlantic coast of Portugal and Spain (García-Marín et al., 2013).

Moreover, chemical mixtures, including trace metals and pesticides, do impact seagrasses at individual level (*i.e.* physiological and whole plant levels), and may be linked to population and community effects (Flores et al., 2013; Negri et al., 2015; Unsworth et al., 2015; Wilkinson et al., 2015; Diepens et al., 2017). Previous studies revealed the presence of 24 different pesticides in the surface water of the lagoon in 2004. Rice cultivation was the major contributor (90%) and the remaining 10% came from the Rhône River (Comoretto et al., 2007). Considering contaminant concentrations and their fate (*e.g.* adsorption to organic matter in sediment and bioaccumulation) and effects, poor water and sediment quality could be considered as a direct cause of *Zostera* meadow decrease in the Vaccarès lagoon. Understanding seagrass dynamics and the influence of multiple stressors on seagrasses at various levels of biological organisation is crucial for environmental risk assessment and for preserving these valuable ecosystems with integrated management strategies. The aim of the present study was to examine the role played by chemical contamination in the dynamics of *Zostera* meadows, taking into account possible interactions with other environmental stressors. It was hypothesised that some spatial patterns in the dynamics and physiology of *Zostera* could be identified across the Vaccarès lagoon and tied in with contamination gradients.

2. Material and methods

To study temporal variation in the parameters monitored, we present data from 1980 to 2015 (Table 1, for details see supporting

Table 1
Available data for the Fumemorte channel and the Vaccarès lagoon.

Available data	Vaccarès lagoon	Fumemorte channel	Inter-site comparison
Water data	2011–2013	2011–2015	2011–2013
Sediment data	2012–2015	2012–2014	2012–2014

information - Tables S1 and S2). The earliest years concern eelgrass cover (1980–2015). Subsequent attention was given to the potential effects of chemical contamination and environmental parameters focusing on the years 2011–2013 for inter-sites comparison.

2.1. Study site

The Vaccarès lagoon, part of the Rhône delta located in South-East France, has a surface area of 65 km^2 and a maximum depth of 2.2 m (Fig. 1). Hydrology, pesticide distribution and agriculture practices in this area have been described in detail before (Comoretto et al. 2007, 2008; Höhener et al., 2010; Boutron et al., 2015). Areas to the north and to the east of this lagoon consists of agricultural lands (420 km^2), mainly rice fields. Two main channels (Roquemaure and Fumemorte) drain runoff water from rice farming into the lagoon, which is indirectly connected to the Mediterranean through low-lying lagoons ("EI" and "EL" in Fig. 1). The hydrological contributions of these channels to the lagoon vary significantly according to different time scales: interannual and inter-seasonal depending on cropping cycles and evaporation. In case of heavy rainfall, two other channels (Rousty and Sigoulette) can flow into the lagoon. Comoretto et al. (2007) revealed that peaks in the amounts of several pesticides corresponded closely with the use in rice fields of pre-emergence herbicides in April and post-emergence herbicides in June.

Five main macrophytes are present in the lagoon: *Zostera noltei, Ruppia cirrhosa, Chara galioides, Stuckenia pectinata* and *Lamprothamnium papulosum. Zostera noltei*, the dominating species, is highly tolerant to variations in salinity, irradiance, nutrient concentrations and substrata (Vermaat et al., 2000; Charpentier et al., 2005; Fernández-Torquemada and Sánchez-Lizaso, 2011). The spatio-temporal dynamics of this seagrass community, and the factors influencing these dynamics, were described over the period 1993–2003 by Charpentier et al. (2005).

3. Seagrass parameters

3.1. Seagrass cover and dynamics

From 1980 to 2015, yearly macrophyte surveys were conducted across the lagoon based on a standardized sampling protocol. They were carried out by the SNPN (French National Society for Nature Protection, La Capelière), using a georeferenced sampling grid with 55 stations, that was defined in 1987 in collaboration with the Tour du Valat research institute (Fig. 1). At each station, the cover percentage of *Z. noltei* was evaluated in 10 quadrats (50 × 50 cm), randomly distributed along a transect. For the years 2004, 2005, 2006 and 2008 seagrass cover was not monitored but was estimated by means of remote sensing (unpublished results).

These data allowed us to compare local seagrass dynamics, especially at the eight sampling stations closest to the shore and to the mouth of the drainage channels (B06, B12, D24, F02, H08, J14, J18 and J22 in Fig. 3), included in the contamination survey. Three indices expressing the recent seagrass dynamics were calculated by averaging the seagrass cover on each of these stations for the two years of monitoring (2012 and 2013), corresponding to the period when seagrass traits and lagoon contamination were measured:

- Index 1 = (average cover in 2012–2013)/(average cover in the 2 years with the highest values since 1996)
- Index 2 = (average cover in 2012–2013)/(average of the last 10 years)
- Index 3 = (average cover in 2012–2013)/(average of the 8 years before 2012)

The first index gives an idea of the current development of seagrass compared with their full potential observed over the period (a value of 100% expressing optimal development). The other two represent the recent dynamics of the meadow (with values greater than 100% expressing an increase in the average cover; in contrast, values of less than 100% showing a regression).

3.2. Seagrass physiological data

Between 2011 and 2013, *Z. noltei* was collected by EcoLab at these eight stations in the Vaccarès lagoon in order to measure the photochemical efficiency of PSII, pigments, biomass and starch contents. in the Vaccarès lagoon.

3.2.1. Photochemical efficiency of PSII

The photochemical efficiency of PSII was determined *in situ*, based on the dark-adapted maximum quantum yield F_v:F_m measured monthly by fluorometry between July 2012 and July 2013 using a portable field device (Diving-PAM, Walz, Germany). This parameter is a generic stress marker (Maxwell and Johnson, 2000) and can notably be used as an indicator of sublethal toxicity of chemical contaminants on aquatic macrophytes (Scarlett et al., 1999).

At each sampling station, 15 distinct, visually-healthy individual ramets, were taken within a radius of 10 m in order to obtain a representative sample of the site and were left for 20 min in a plastic box filled with water from the site to allow the plants to acclimatise to the dark. Each second youngest leaf per individual was taken and fluorescence measurements were performed on the lower part of the leaf using metal clips to isolate the area.

3.2.2. Leaf pigment contents

Cut leaves, randomly taken from at least five healthy individual ramets devoid of necrosis and epiphytic coverage, were immediately frozen in liquid nitrogen in the field. In the laboratory, 20 mg leaf samples were stored at −80 °C to undergo subsequent pigment analyses. The pigment composition of *Z. noltei* leaves was determined on the plants collected in July 2012, in order to calculate the violaxanthin/zeaxanthin ratio, which can be used as a biomarker of herbicide contamination (Diepens et al., 2017). Pigments were extracted from the chloroplasts of the leaf cells and analysed by High Performance Liquid Chromatography (HPLC, Agilent Technologies 1200 series) as described by Diepens et al. (2017).

3.2.3. Rhizome carbohydrate reserves during the winter period

Rhizomes were collected to determine mass per unit length, soluble sugars and starch contents (carbohydrate storage) in December 2011 and 2012.

Soluble sugar and starch contents were extracted and measured using earlier published methods (Yemm and Willis, 1954; Zweens and Bouman, 1968). In short, soluble sugars were determined using anthrone reagent (2%) and measurements were done photospectrometrically at 585 nm. For starch contents, amyloglucosidase (3.4 mg/mL of citrate buffer) was used, and glucose was measured using a D-glucose kit (r-Biopham) according to manufacturer's protocol.

4. Environmental parameters

4.1. Physicochemical water parameters

Since 1996, water depth has been measured in the eastern part of

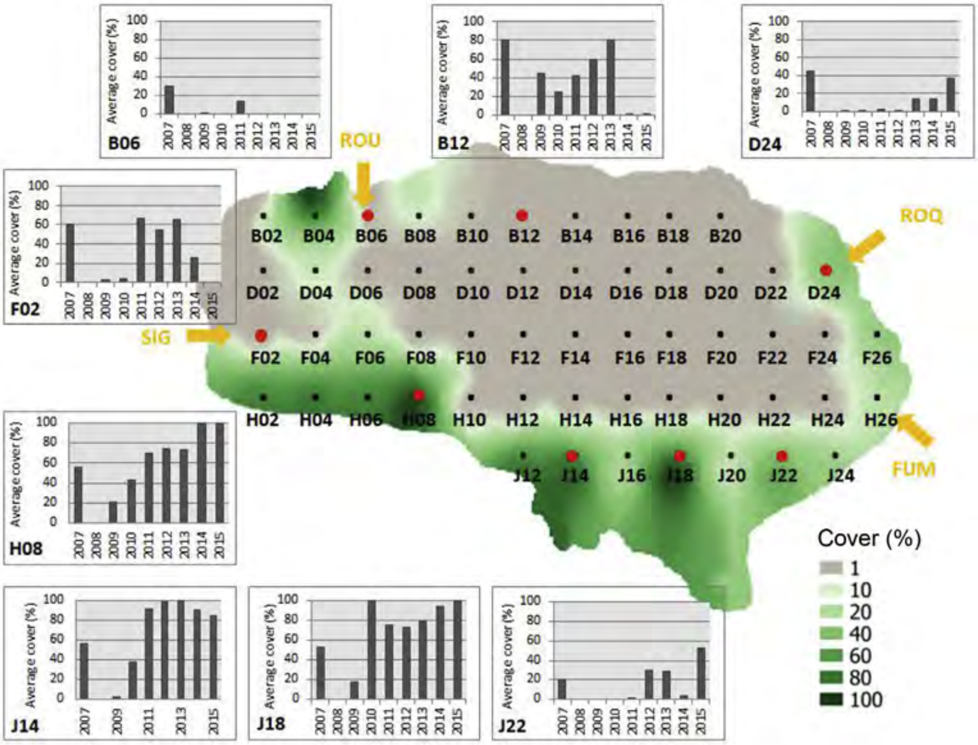

Fig. 3. *Zostera noltei* cover (%) interpolated over the whole lagoon in 2015. Data were interpolated using the Inverse distance method with a triangular mesh (elementary mesh size: 250 m). Histograms represent average seagrass cover at each station from 2007 to 2015. Year 2008 is missing. Yellow arrows symbolize the outlets of the drainage channels: Fumemorte (FUM), Roquemaure (ROQ), Sigoulette (SIG) and Rousty (ROU). Red points correspond to seagrass cover monitoring stations for which indices of dynamics have been calculated. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the Vaccarès lagoon close to the Fumemorte outflow (see Fig. 1:location 1 in Boutron et al., 2015) on a 15-min basis using a float-operated Thalimedes Shaft Encoder with integral data logger from OTT Hydrometry.

Since 1980, salinity has been evaluated monthly, using a conductivity meter (WTW LP 318) at the centre of the lagoon, based on the Practical Salinity Scale.

Over the period 2008–2015 water temperature, dissolved oxygen, and conductivity were measured with a multiparameter probe at the different sites monitored in this study.

All these parameters were measured by the SNPN.

Finally, using meteorological data from Météo France over the period 1996–2001, average wave exposure index (REI) was calculated for each station following the formula described by Charpentier et al. (2005).

4.2. Sediment characteristics

In July 2012 sediment samples were collected from 6 sites (close to F02, B06, B12, H06, J14, J24; Fig. 1) by EcoLab. For each site, 3 plots of 1 m^2 were delimited at 10–15 m from each other. Five regularly-spaced cores were taken from each plot using a Perspex corer of 5 cm in diameter (5 cm deep) and then pooled and homogenized for further analyses. These samples were divided into four subsamples. The first subsample was used to measure the nutrient contents of pore water (NH_4^+ and PO_4^{3-}) based on colorimetric methods. The second subsample was used to determine moisture and organic matter contents. For this purpose, this subsample was weighed, dried at 105 °C (48 h) and weighed again. It was then dried at 200 °C (2 h) followed by 500 °C (10 h) and weighed one last time to get the ash mass. The third subsample was used for grain size determination, which made it possible to determine the percentage of clay. The last subsample was used for subsequent trace metal analyses. For this purpose, it was quartered and sieved after drying at room temperature and 3 g of sediment (< 63 µm) were then collected.

4.3. Chemical contamination

Organic chemical (*i.e.* pesticides, PCBs and PAHs) and trace metal (TMs) concentrations in water and sediment were obtained from sampling campaigns carried out from 2011 to 2015 by the SNPN in the Vaccarès lagoon and the drain channels. Samples were analysed by the Health, Environment and Hygiene Laboratory (CARSO, Lyon) using widely accepted standard methods. The water samples used for trace metal analyses were pre-filtered (< 0.45 µm), whereas the organic chemical analyses were run on raw water (see Tables S1–S2 in SI for the list of parameters). A total of 74 organic chemicals and 19 TMs were investigated in water samples *vs.* 39 organic contaminants and 22 TMs in whole sediments. Organic contaminant concentrations were normalized to the total organic carbon fraction in sediments.

In parallel, the TM contents of the fine fraction of sediments (< 63 µm) were analysed by EcoLab in July 2012, after total digestion by the chemical mixture $HF/HNO_3/H_2O_2$ in a savillex (teflon bottle) following a well calibrated multi-step procedure (see N'Guessan et al., 2009). The exchangeable fraction of TMs (*i.e.* the potentially bioavailable fraction) was also analysed after extraction with 0.01 M $CaCl_2$ following the method of Menzies et al. (2007). After the dilution process, the TMs were analysed on an Inductively Coupled Plasma Mass Spectrometer (ICP-MS Perkin-Elmer ELAN 6000) at OMP (Observatory Midi-Pyrénées analytical platform Toulouse, France). The analytical data quality was checked by using the standard STSD 3 and SUD 1 simultaneously, to verify the accuracy and reproducibility of the mineralization procedure for TMs, and by calculating the average relative error, to check analytical precision. For most elements, the yielded recovery rates (QA/QC) ranged from 80% to 120%. The lower recovery rate obtained for Cr might be due to its refractory characteristics, which implied a strong adsorption onto siliceous materials (Tam and Yao, 1998; Liaghati et al., 2004). For each element, the Relative Standard Deviation (RSD) was lower than 15%.

5. Indicators for environmental risk assessment

5.1. Risk in the water column

5.1.1. Quality standards

To identify potential risks, concentrations of trace metals and organic contaminants were compared against environmental quality standards (EQS) for surface water, as defined by the Water Framework Directive (2000/60/EC) and implemented for each substance in the 2008/105/EC Directive (Table S3):

(1) AA-EQS, representing the annual average concentration not to be exceeded in surface water
(2) MAC-EQS, corresponding to the maximum permitted concentration in surface water

5.1.2. Risk quotients

Using Predicted No Effect Concentration (PNEC) values traken from a freshwater ecotoxicology database, four risk quotients (RQ) were calculated for the maximum and average concentrations of each substance detected in the water column from 2011 to 2014 and in 2015, using the following ratio:

$$RQ = MEC/\ PNEC$$

With the MEC (Measured Environmental Concentration) corresponding to:

(1) Maximum concentration of each substance in 2015
(2) Average concentration of each substance in 2015
(3) Maximum concentration of each substance from 2011 to 2014
(4) Average concentration of each substance from 2011 to 2014

If the ratio is greater than 1, there is a risk for aquatic ecosystems.

5.2. Risk in sediments

PAH, PCB, phthalates and TM concentrations in sediments were compared to several sediment quality guidelines defined by Macdonald et al. (1996), relative to dry weight of sediments, and by Swartz (1999) for Organic Carbon (OC)-normalized PAH concentrations.

5.3. Calculation of enrichment factors

One method used to evaluate sediment contamination by trace metals in the Vaccarès lagoon is to determine an enrichment factor (EF), defined by Ackerman (1980). It corresponds to the concentration ratio of a considered element to a reference element (naturally abundant on Earth) in a given sample. This was calculated for each trace metal from the Vaccarès sediments as follows:

$$EF = \frac{(X/Y)sample}{(X/Y)reference\ material}$$

X is the concentration of the considered element and Y is the concentration of the reference element, here aluminium. Local background noise data were not available. Thus, background data from the Upper Terrestrial Continental Crust (UCC) defined by Wedepohl in 1995 were used. It can be considered that trace elements with EF values noticeably higher than 2 do not originate from the local sediment background and can be attributed to chemical pollution.

6. Statistical analyses

To identify the factors involved in the regression of *Z. noltei*, environmental and seagrass data for eight study stations from 2011 to 2013 (see sections 2 and 3) were matched by co-inertia analysis, a multivariate method used for examining a possible co-structure between these two matrices, analysed beforehand by principal component analyses (PCA). A Monte-Carlo permutation test (RV test) was performed to measure the co-structure between the data tables, and thus identify possible statistical relationships between seagrass status and environmental parameters. Correlation tests were also carried out to study the relationships between environmental and biological variables taken two by two. To reduce the number of variables in the environmental matrix, only a subset of the chemical contaminants measured was considered a priori. This subset included bentazon and 2,4-MCPA, which were the herbicides with the highest concentrations in the Vacarrès lagoon during the study period, and the TM known for their potential toxicity to the aquatic biota (Al, As, Cu and Zn; both total and exchangeable fractions).

All statistical analyses were performed using Rstudio software version 3.1.2, with the ade4 package for multivariate analyses. The significance level was set to 0.05.

7. Results

7.1. Macrophyte dynamics

Seagrass cover varied temporally and spatially at and between stations from 1996 to 2015 (Figs. 2 and 3). Following the almost complete disappearance of the meadow in the Vaccarès from 1997 to 1999, *Z. noltei* recolonized, except at the centre of the lagoon. Since the second decline over the period 2008–2009, some spatial distribution patterns of seagrass have been identified in relation to their proximity to drainage channels (Fig. 3).

More precisely, seagrass was present only at the nearshore stations. From 2011 to 2013, a regression was observed at the mouths of the drainage channels (indices 2 and 3 $\ll$100%; Table S4), particularly at the outlets of the Rousty, Sigoulette, Roquemaure and Fumemorte channels, next to agricultural areas. At these stations, seagrass cover was either between 15 and 35% (H26 and D24), or had completely disappeared (B06, F02) (Table S4). In addition, the seagrass meadow was absent in the north of the lagoon (B14 to B20) and it appeared to be very poorly developed ($<$ 20%) along the east/south-east bank (Fig. 3; Table S4).

On the contrary, on the western shores of the lagoon seagrass cover reached up to 100% within some stations (*e.g.* B04, H08, J14 and J18) (Fig. 3) and presented elevated development indices (Table S4).

Finally, five stations (B12, F02, H08, J14 and J18) displayed very high development indices (index 1 $>$ 80%) which have increased over the last five years (indices 2 and 3 $>$ 100%) (Table S4).

8. Environmental parameters

8.1. Physical water parameters

Annual average water temperatures ranged from 16.3 °C to 19.3 °C over the period 2008–2015 showing seasonal variations with minimum and maximum temperatures of 2.2 °C in January and 32.2 °C in July.

Temporal variation in salinity is higher from year to year than from month to month within a year (Fig. 2). Over the period 1996–2015, average (SD) salinity in the lagoon was 13.7 (4.7). The lowest salinities were measured in 1996 and 1997 (3.7) and the highest values were detected in 2007 and 2008 (27.2).

The inter-annual variability of the wave exposure index (REI) for a given station was very low (all coefficients of variation below 3%). Consequently, six-year averaged REI values (1996–2001) for each station were used to characterize the stations in the present study in terms of relative exposure to hydrological disturbances.

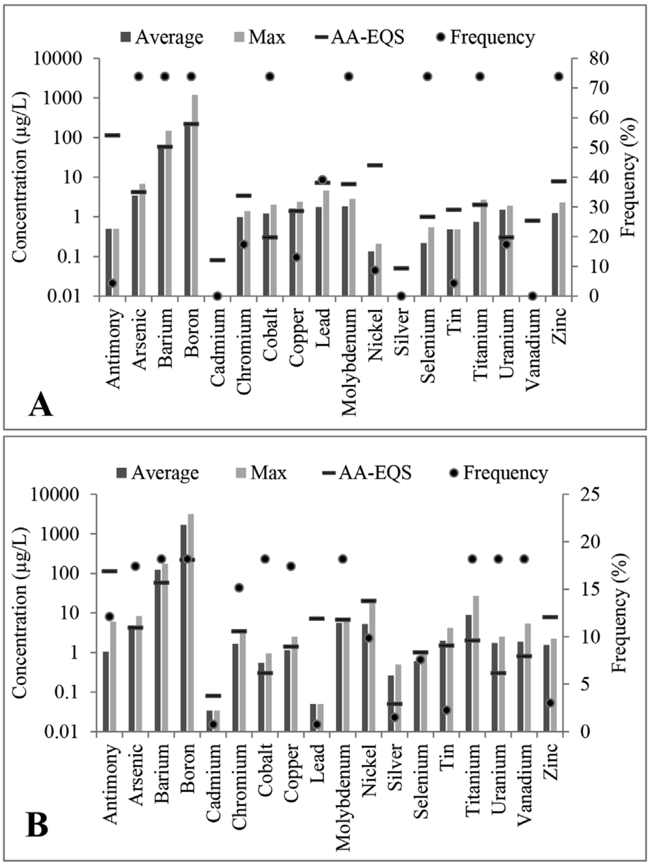

Fig. 4. Trace metals dissolved in waters of the Fumemorte channel (**A**) and in all stations in the Vaccarès lagoon (**B**) from 2011 to 2013. In 2014, 2015, no trace metals data is available concerning water of the Vaccarès lagoon. Average (dark grey bars) and maximal (light grey bars) correspond to trace metals concentrations ($\mu g.L^{-1}$) over the period 2011 to 2013. The Y-axis of concentrations is plotted on a logarithmic scale. Horizontal stripes indicate the annual average environmental quality standard limits (AA-EQS). Black circles represent the number of times a trace metal was detected during the period (frequency scale on the right).

8.2. *Chemical contamination*

8.2.1. *Trace metals*

In surface water from 2011 to 2013, 18 dissolved TMs were detected in filtered waters from the lagoon, and 16 in the Fumemorte channel (Fig. 4). Most of the metals were detected frequently and were around or above the AA-EQS values, except for antimony, cadmium, lead and zinc. Especially, barium and boron were detected in almost all samples and in concentrations above the AA-EQS values, with maximum concentrations in the lagoon of 174 $\mu g.L^{-1}$ (J22) for barium and 3143 $\mu g.L^{-1}$ (B06) for boron (Fig. 4).

In the Fumemorte channel, only arsenic, barium, boron, cobalt, titanium and uranium exceeded the AA-EQS (Fig. 4). Note that the average and maximum TM concentrations were higher in 2013 than in 2011, except for uranium.

In sediments, most trace metals were detected at both sites, except antimony, silver and cadmium (Fig. S1) from 2012 to 2014. The enrichment factors (EFs) of most TMs highlighted anthropogenic contributions (EFs > 20) except for barium, beryllium, tin and titanium (EFs < 2 see Fig. S2). In addition, strong elevated values of EFs (EFs > 40) for arsenic and thallium indicated extreme pollution. The TMs measured in 2015 showed similar patterns, except for tin which was detected in the Vaccarès lagoon and the EF of antimony which indicated extreme pollution. Moreover, thallium was no longer detected in 2015 (Fig. S2).

8.2.2. *Organic chemicals*

A total of 68 different organic chemicals were found in the surface water of Fumemorte and of the lagoon over the period 2011 to 2013, with 38 pesticides (28 herbicides, 4 insecticides, 4 fungicides and 2 multi-action pesticides), 13 PAHs, 4 PCBs, 4 solvents, 3 benzenes, 2 phenols and 4 others (Fig. 5). More chemicals were found in Fumemorte (47) than in the lagoon (29). At station J22, close to the Fumemorte inflow, 16 chemicals were found between 2011 and 2013, which was considerably less than in the channel.

Most pesticide concentrations were below the EQS values, with the exception of several PAHs (benzo (a) pyrene, dibenzo (a, h), anthracene, benz (a) anthracene, fluoranthene, naphthalene), a fungicide (azoxystrobin), a dichloroaniline derivative, and an insecticide (tebufenozide) (Fig. 5). Moreover, bentazon and 2,4-MCPA which were among the herbicides with the highest concentrations and the most frequently detected in the waters, were always below the AA-EQS limits. The maximum 2,4-MCPA concentration was an exception, but it still remained below the MAC-EQS value. Bentazon concentrations in the water were on average (SD) 1.7 (4.0) $\mu g.L^{-1}$ in Fumemorte and 0.3 (0.5) $\mu g.L^{-1}$ in the lagoon, with maximum values of 1.7 $\mu g.L^{-1}$ (*vs* AA-EQS: 7 $\mu g.L^{-1}$). From 2011 to 2015, these concentrations increased by a factor of 10. The 2,4-MCPA concentrations in the water were on average 0.2 (0.2) $\mu g.L^{-1}$ in Fumemorte and 0.2 (0.1) $\mu g.L^{\text{-}}1$ in the lagoon, with maximum values of 0.6 $\mu g.L^{-1}$ (*vs* AA-EQS: 0.5 $\mu g.L^{-1}$) (Fig. 5).

The spatial patterns of contamination were very clear, with a decreasing gradient of contamination from east to west for bentazon and 2,4-MCPA (Fig. 6). This suggested that these herbicides flowed in mainly through the Fumemorte channel to the east of the lagoon and perhaps also, particularly for 2,4-MCPA, through the Rousty channel (Fig. 6).

Finally, PCBs of the Fumemorte sediments were most numerous than in the Vaccarès lagoon. In contrast with the water phase, more pesticides were found in the sediments of the lagoon (10) than in those of the channel (4) (Fig. S3).

9. Relationships between the quality of the aquatic environment and seagrass dynamics

A co-inertia analysis (Fig. 7) revealed a significant relationship between environmental data and seagrass status matrices (RV = 0.72, *P-value* = 0.021), indicating that the spatial variability of the biological parameters in the lagoon is not independent of abiotic parameters.

Dynamics indices and starch contents in rhizomes were positively correlated with the second axis of the co-inertia analysis. The other biological variables appeared to be structured along the first axis with a positive correlation for the maximum quantum efficiency of PSII (F_v:F_m) in July and October and as well as for the violaxanthin/zeaxanthin ratio, and a negative correlation for F_v:F_m in May and April, for sugars and for rhizome mass per unit length. The ratio violaxanthin/zeaxanthin thus appeared to be independent of meadow dynamics and rhizome starch reserves (vector orthogonal to axis 2). There was also no clear link between the indices of seagrass dynamics and most F_v:F_m measurements either, which were highly variable from season to season (Fig. 7A).

- Spatial variation of seagrass characteristics

The co-inertia analysis discriminated the 8 study stations along axis 2 reasonably well, as a function of meadow dynamics (Fig. 7A and C). Among the stations with negative dynamics (B06, D24, J22), only B06, in a central position along this axis, remained close to some sites with positive dynamics (B12, F02, H08, J14 and J18). In addition to the high values of the three dynamics indices, it was noted that the stations located on the upper part of axis 2 were also characterized by high starch contents during the winter period (Fig. 7A and C). The study stations

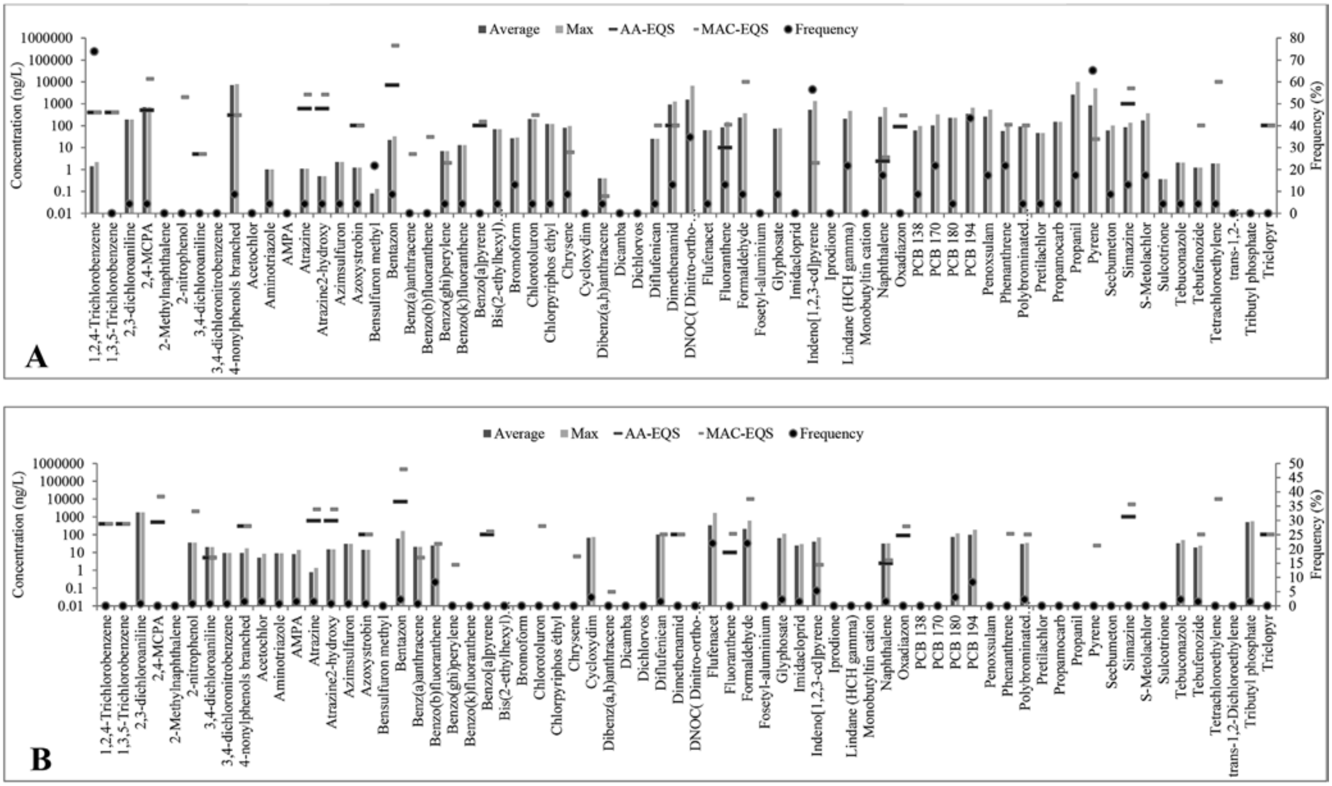

Fig. 5. Pesticides in raw waters of the Fumemorte channel (**A**) and in all stations in the Vaccarès lagoon (**B**) from 2011 to 2013. In 2014, 2015, no pesticides data is available concerning water of the Vaccarès lagoon. Average (dark grey bars) and maximal (light grey bars) correspond to pesticide concentrations (ng.L^{-1}) in surface water over the period 2011 to 2013. The Y-axis of concentrations is plotted on a logarithmic scale. Horizontal stripes indicate the annual average environmental quality standard limits (AA-EQS) for the dark one and the maximum acceptable concentration (MAC-EQS) for the light one. Black circles represent the number of times a pesticide was detected during the period (frequency scale on the right).

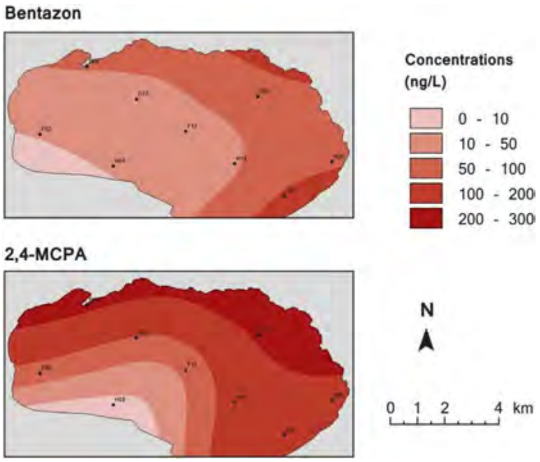

Fig. 6. Spatial variabilities in the contents of two herbicides (bentazon and 2,4-MCPA) in the Vaccarès lagoon. Spline spatial interpolation from average concentrations measured from early June to late July 2011 at 9 sampling stations (x 4 sampling dates).

also seemed to discriminate along axis 1 as a function of the violaxanthin/zeaxanthin ratio. Those located in the positive part of this axis would be characterized by seagrass with high concentrations of violaxanthin, whereas those in the negative part would present seagrass with high concentrations of zeaxanthin.

- Environment, seagrass dynamics and rhizome starch contents

By studying the link between the biological and environmental variables (Fig. 7A and B, Table S5), a negative correlation was noted between the dynamics indices and concentrations of 2,4-MCPA ($r = -0.69$ to - 0.73, $P = 0.040$ to 0.057) in water in 2011. To a lesser extent, dynamics indices were negatively correlated with the total arsenic content in sediments, with bentazon r concentrations in water in 2011 and 2012, with 2,4-MCPA concentrations in water in 2012, with exchangeable zinc and ammonium contents in sediments and with copper and arsenic concentrations in water (but none of these correlations were significant when tested individually). On the other hand, exchangeable arsenic and copper in sediments were positively correlated with seagrass dynamics indices ($r = 0.45$ to 0.56 and $r = 0.26$ to 0.38, respectively), but none of these correlations were significant at $P < 0.05$.

Similar results were observed concerning correlations with starch reserves (Fig. 7A and B), which were negatively correlated with herbicides and trace metals found in water and sediments, as well as with NH_4 contents in sediment pore water.

- Environment and PSII maximum quantum yields

The co-inertia analysis and correlation tests revealed significant negative correlations between (1) the photosynthetic potential in July 2012, October 2012 and July 2013 and the exchangeable aluminium in sediments, salinity, the wave exposure index, PO_4 contents in sediments and sedimentary organic matter; and between (2) the photosynthetic potential in April and May 2013 and the total aluminium, zinc and copper in sediments. Other negative correlations between the photosynthetic potential, water depth and clay percentage were observed but none of these correlations were significant at $P < 0.05$ (Fig. 7A and B, Table S5).

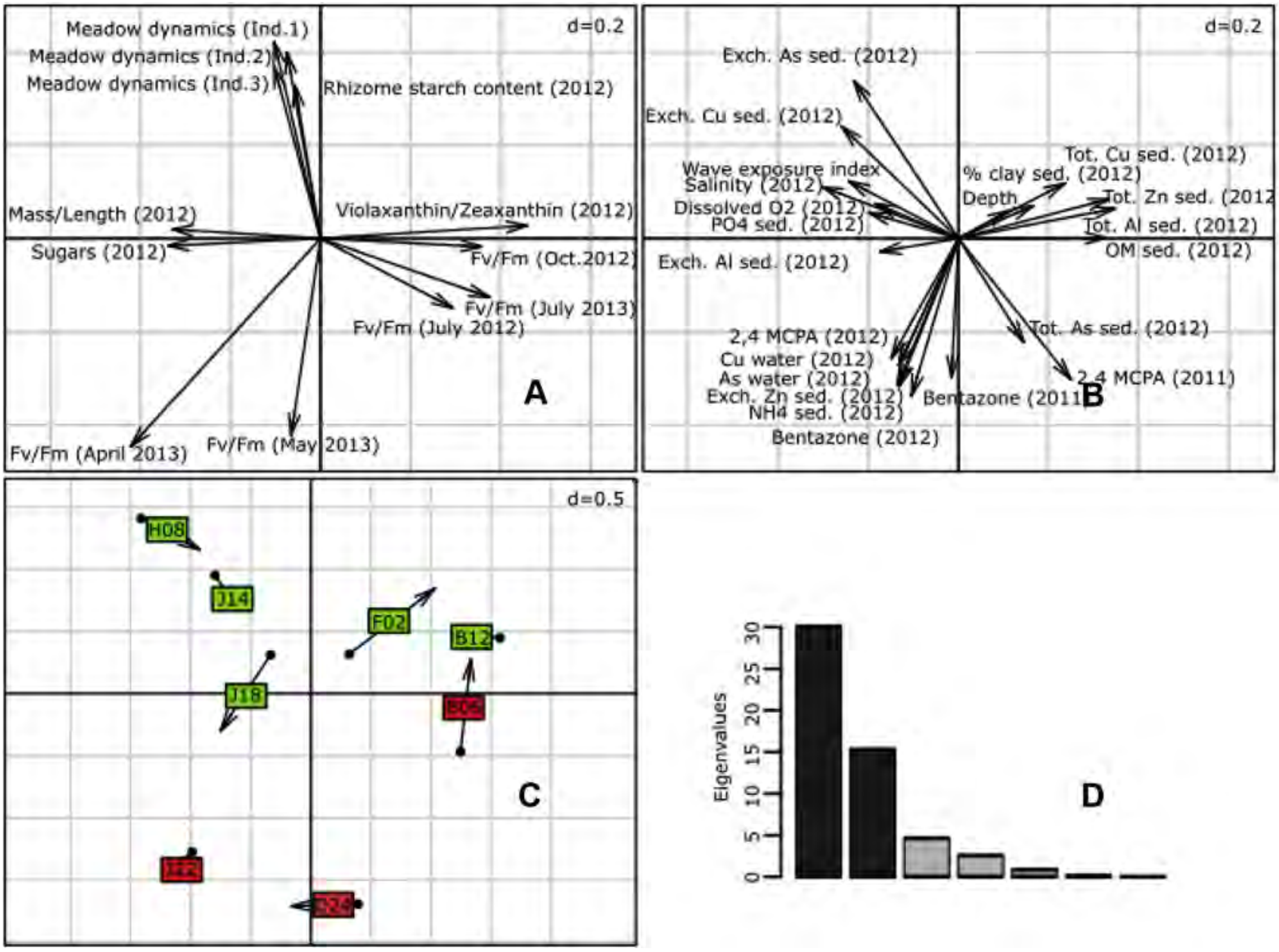

Fig. 7. Co-inertia analysis matching environmental and chemical data with biological variables expressing the state of seagrass. (**A**) Biological variables in the co-inertia plane. (**B**) Environmental variables in the co-inertia plane. (**C**) Study stations in the co-inertia plane, the basis of the vectors corresponding to the positioning from biological data and the arrow to the environmental data. Red boxes indicate stations with negative and green boxes positive seagrass dynamics. (**D**) Eigenvalue histogram of the co-inertia analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

- Environment and pigments

The violaxanthin/zeaxanthin pigment ratio appeared to be positively correlated with total aluminium in sediment ($r = 0.86$, $P = 0.007$), total zinc ($r = 0.83$, $P = 0.010$) and to a lesser extent, with total copper ($r = 0.63$, $P = 0.093$), and organic matter ($r = 0.62$, $P = 0.100$). In contrast, this ratio is negatively correlated with dissolved oxygen ($r = -0.75$, $P = 0.031$), salinity ($r = -0.90$, $P = 0.003$) and the wave exposure index ($r = -0.70$, $P = 0.05$), and with phosphates in sediment pore water ($r = -0.47$, $P = 0.24$) (Fig. 7A and B, Table S5).

10. Environmental risk associated to chemical contamination

10.1. In the water column

The four risk quotients (RQ) were used to analyse the evolution of risk over the period 2011–2015 (Table 2, Table S6).

When the 93 contaminants analysed were considered individually over the period 2011–2015, 40 of them (6 TMs and 34 organic molecules) did not present any risk for the aquatic environment (RQ $<$ 1) and 15 of them were no longer detected in 2015 in the Fumemorte channel (Table S6).

Since 2011, 15 chemical contaminants (8 TMs and 7 pesticides) in the water column have presented a real risk either due to their annual average concentrations or to their maximum concentrations over a year (Table 2). Although the maximum concentrations of copper, vanadium and 2,4-MCPA presented a risk for the environment, the average annual concentrations of these contaminants was actually relatively low. Two other contaminants (cobalt and chrysene) had shown high risk quotients since 2011 in the Vaccarès lagoon and Fumemorte channel, but these RQ appeared to decrease in 2015 (Table 2), suggesting this risk could possibly disappear in future years. However, since sampling in 2015 was carried out only in Fumemorte, it is likely that some of the contaminants had moved and accumulated in Vaccarès, especially in the sediment and biota in the case of the hydrophobic, less degradable chemicals.

In addition, 9 organic contaminants (*i.e.* pyrene, dichlorvos, acetochlor, propanil, diflufenican, azimsulfuron, PCB 138, PCB 180 and chlorotoluron) and one trace metal (tin) that presented a high risk in the Fumemorte channel from 2011 to 2014, were no longer detected in 2015 (Table 2).

Finally, chromium was the only contaminant that regularly exceeded the PNEC value, with risk quotients indicating potential toxic effects in the Fumemorte channel since 2015 (Table 2).

10.2. In sediments

The risk assessment methods developed by Macdonald et al. (1996) and Swartz (1999) gave a first overview of the potential chemical risks of TMs and the various organic contaminants detected in the Vaccarès sediments from 2012 to 2015.

Table 2
Trace metals and organic molecules in waters presenting a risk for the aquatic environment in the Vaccarès lagoon and the Fumemorte channel, according to calculated risk quotients.

Type of substance	Recent risk (2015)	Risk from 2011 to 2014	Risk from 2011 to 2014 but not detected in 2015	Risk 2011–2015	Not detected (2011–2015)
Trace metals	Chromium (Fum)	Cobalt (all) Silver (Vacc.)	Tin (Fum)	Arsenic (all) Barium (all) Boron (all) Copper (all) * Nickel (all) Titanium (all) Uranium (all) Vanadium (all)*	Tellurium (all) Toluene (all)
Organic contaminants		Benzo(a)anthracène (Vacc.) Benzo (ghi)perylene (Vacc.) Chrysene (Vacc.)* Dibenzo (a,h)anthracene (Vacc.) Indeno [1,2,3-cd]pyrene (all) Tebufenozide (Vacc.)	Acetochlor (Fum) Azimsulfuron (Fum) Chlorotoluron (Fum)* Dichlorvos (Fum) Diflufenican (Fum) PCB 138 (Fum) PCB 180 (Fum) Propanil (Fum) Pyrene (Fum)	2,4-MCPA (all)* 3,4 dichloroaniline (all) Bensulfuron methyl (all) Dimethenamid (all) Flufenacet (all) Oxadiazon (all) Tebuconazole (all)	Carbendazim (all) Chlorpyriphos ethyl (all) Dibromochloromethane (all) Dichlorobromomethane (all) Dichlorprop total (all) Fluorene (all) PCB 170 (all) PCB 194 (all) Penoxsulam (all) Secbumeton (all) 2,3-dichloroaniline (all) 1,2,4-trichlorobenzene (all) 1,3,5-trichlorobenzene (all) 3,4-dichloronitrobenzene (all) Polybrominated diphenyl ether (all) 2-nitrophenol (all) Monobutyltin cation (all)
Total of contaminants	1	8	10	15	19

* Probable risk (HQ close to 1)/risk for maximum concentrations.
Vacc.: Vaccarès only; Fum: Fumemorte only; All: Vaccarès + Fumemorte.

- For trace metals (TMs)

Since 2012, only arsenic and nickel concentrations exceeded the TEL sedimentary threshold values (below which toxicity very rarely occurs) (Fig. 8). In 2015 in particular, arsenic concentrations were above this threshold at most of the stations in the Vaccarès lagoon (*i.e.* B06, B12, B20, D24, H08, and H26) and nickel concentrations in the northern and south-eastern sectors (Fig. S4).

The same observations were made for arsenic and nickel in 2012, 2013 and 2014 (Fig. 8), particularly at the mouth of the Rousty channel (B06 and B06bis) and in the Fumemorte channel (Fig. S4).

- For organic contaminants

Since 2012, the concentrations of organic contaminants, either mixed (sum of PAHs) or considered separately, have been significantly lower than their respective sedimentary threshold values (TEL), below which toxicity very rarely occurs (Fig. 8).

As observed with the method of Macdonald et al. (1996), results indicate that contamination of these sediments by PAHs cannot have toxic effects on the benthos. Indeed, the concentrations of these mixed PAHs (normalized to total organic carbon in the sediment) are not even within the 95% confidence interval of the lowest sedimentary guide value - the TEC (Swartz, 1999) - and are even 5 to more than 200 times lower for the entire Vaccarès system (Fig. S5).

11. Discussion

The reasons for the decline of *Z. noltei,* in the Vaccarès lagoon, do not seem to be limited to salinity variations as previously stated, but could be due to multiple stressors including chemical contamination.

11.1. Seagrass dynamics and life history

The spatial distribution of submersed aquatic vegetation is governed by a wide variety of parameters. An important aspect is light availability, which depends on several factors such as depth, suspended solids, phytoplankton and epiphytes (Duarte, 1991; Koch, 2001). *Zostera* is absent from the centre of the Vaccarès lagoon due to greater water depths (> 1.60 m deep) and sediment resuspension caused by frequent wind (Charpentier et al., 2005). In contrast, *Z. noltei* rapidly recolonized the shallow borders between 1997 and 2008 due to increased water transparency and salinity levels higher than the optimum salinity for growth (Charpentier et al., 2005). However, the second decline of *Zostera* in 2008 led to its near extinction, especially in the eastern part of the lagoon, with only 4% of seagrass cover remaining. In 2015, seagrass regression still continued at the mouths of the two main agricultural drainage channels, Roquemaure and Fumemorte. Since 2009, however, some areas of recolonization/progression have been observed in the south-eastern and southern areas of the lagoon, especially in sites (*e.g.* on D14 and D18 since 2010) near the interconnections with other lagoons close to the coast: the average cover of the *Zostera* meadow seems now to be stabilized at 20% (*i.e.* $13\,\text{km}^2$ of the surface area of the lagoon). This positive dynamic could be due to the ability of *Z. noltei* to rapidly recover from disturbances owing to its opportunistic characteristics, such as high shoot turnover, short time to sexual reproduction, low physiological resistance and seed bank development (Kilminster et al., 2015).

A biological factor that can have an impact on seagrass development in shallow lakes is avian grazing (Jacobs et al., 1981; Thayer et al., 1984; Park, 1999; Auby et al., 2011; Wood et al., 2012; Dos Santos et al., 2013). Birds can be very selective in their consumption and have a targeted impact on seagrass (Dalloyau and Robin, 2013), as is the case with coots (*Fulica atra*) in the Vaccarès lagoon. Their occurrence is often seasonal and last for just a few months, particularly in the autumn-winter period following the peak of seagrass biomass, and they contribute to regulating the annual cycle of seagrass meadow (Jacobs et al., 1981). However, their population density in winter is not high enough to cause sufficient damage to explain the decline of the meadow. The biomass removed, amounting to 3–4% of *Z. noltei* production in the

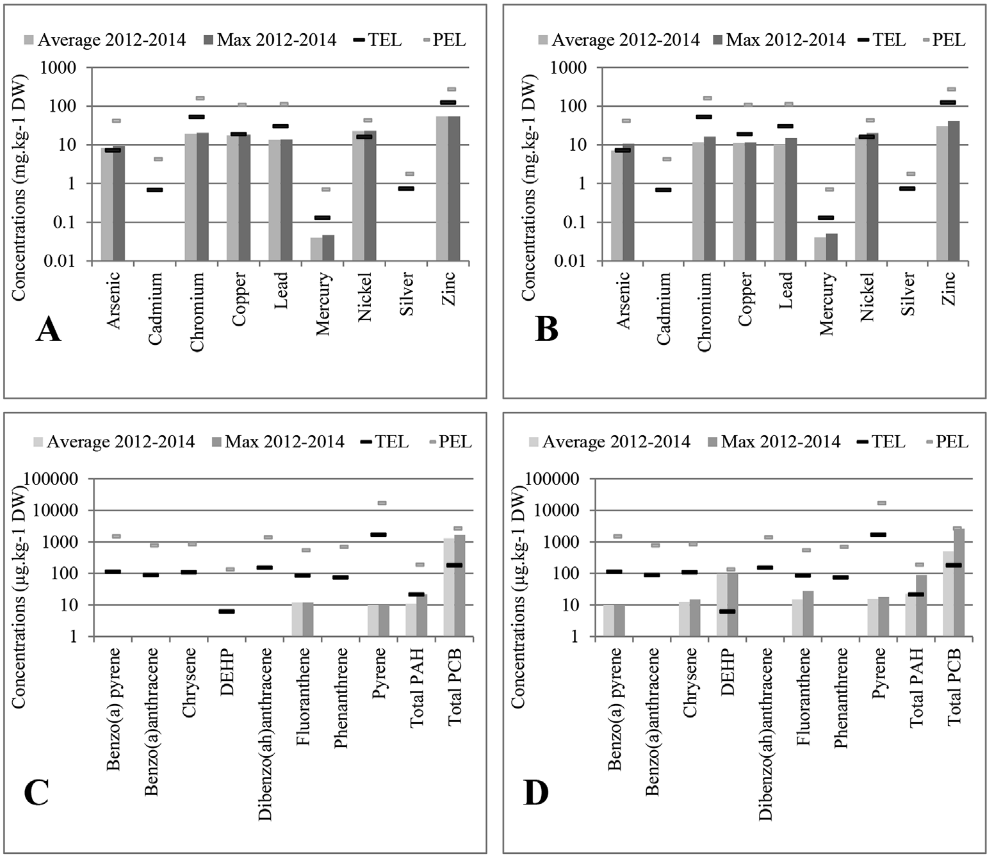

Fig. 8. Chemical risk assessment of the Fumemorte (A and C) and the Vaccarès (B and D) sediments for trace metals and organic contaminants using the method of Macdonald et al. (1996): comparison with the TEL (Threshold Effect Level) and PEL (Probable Effect Level), respectively, for the period 2012–2014. The Y-axis of concentrations is plotted on a logarithmic scale.

Vaccarès lagoon according to unpublished estimations, is probably compensated by macrophyte regrowth, dependent on the availability of resources (*e.g.* sufficient light and nutrients).

11.2. Seagrass and multiple abiotic stressors

The wide distribution range of *Z. noltei* demonstrates its ability to cope with wide variations in temperature. *Z. noltei* populations in Southern Europe were able to resist heat shocks of up to 35 °C for a few hours (Massa et al., 2009). Besides, the germination of *Z. noltei* seeds can occur between 10 and 30 °C, and is stimulated by higher values (Hoostmans et al., 1987). Similarly, optimal salinity ranges from 5 to 30 g.L^{-1} (Vermaat et al., 2000; Charpentier et al., 2005; Fernández-Torquemada and Sánchez-Lizaso, 2011). Thus, changes in salinity (3–30 g.L^{-1}) or temperature (2.2°C-32.2 °C) are not likely to be the most influential factors in population dynamics across the Vaccarès lagoon. However, our co-inertia analysis results showed a significant global link between numerous environmental parameters and seagrass variables, indicating that seagrass dynamics and physiology could be explained by the distribution and interaction of multiple abiotic factors.

11.2.1. Effect of physicochemical parameters

Among the physiological markers studied, the violaxanthin/zeaxanthin ratio in *Zostera* leaves varied significantly across the lagoon. Violaxanthin can be reversibly converted to zeaxanthin with energy being dissipated through heat. This conversion is a photoprotective mechanism, in case of high light intensity (Havaux and Niyogi, 1999; Tanaka et al., 2008) or exposure to photosynthesis inhibitors (Diepens et al., 2017). In contrast, violaxanthin has a light-harvesting role and its concentration increases in low light conditions. In the present case, spatial variations in the violaxanthin/zeaxanthin ratio are obviously related to the light intensity received by the macrophytes, depending on the depth and turbidity of the water column. This is supported by the positive correlations between this pigment ratio and water depth, and also by positive correlations with conditions likely to reduce water clarity *via* sediment resuspension (sediments with high clay and organic matter contents, low water salinity).

Water depth, percentage of clay and organic matter were also negatively correlated with soluble sugar in rhizomes and with their mass per unit length. This relationship, shown by the co-inertia analysis, may explain why the development of *Z. noltei* is limited beyond a certain depth, varying spatially and depending on turbidity (Charpentier et al., 2005). The ability of the plant to assimilate and store carbon is indeed a critical issue in its long-term persistence.

11.2.2. Effects of multi-contamination

Previous studies have shown that a decrease in water and sediment quality are the main causes of seagrass decline reported worldwide (Orth et al., 2006; Short et al., 2007, 2011; Waycott et al., 2009), as this affects seagrass meadow resilience (Unsworth et al., 2015). In the Vaccarès system, trace metals and organic concentrations have increased since 2011, particularly in channels draining into the lagoon. Very high total herbicide concentrations measured in the water of Fumemorte suggest preferential contamination from this channel. The regression of seagrass meadows at the mouth of the Roquemaure, Rousty and Sigoulette channels (Fig. 3) suggests that their infrequent outflow (Boutron et al., 2015) could also play a role in the multi-contamination evidenced in the lagoon, and in seagrass decline.

According to the co-inertia analysis, seagrass dynamics indices and rhizome starch contents were both negatively correlated with the concentrations of herbicides 2,4-MCPA and bentazon in water, with metals arsenic, zinc, copper in water and/or sediments, and with NH_4^+ in sediment pore water. While laboratory experiments had highlighted the potential or actual impact of these different chemical compounds (*e.g.* Nielsen and Dahllöf, 2007; Christianen et al., 2011; Diepens et al., 2017), the present results suggest a negative effect on *Z. noltei* dynamics *in situ.* Chemical contamination, especially by photosynthetic inhibitors such as bentazon, may reduce carbon storage at the end of the growing season, thus impeding post-winter restart, with effects at whole plant and population levels.

At physiology level, there are just a few studies that examine the combined effect of trace metal elements and pesticides on *Zostera* sp. and they are limited to the effects on photosynthesis (Macinnis-Ng and Ralph, 2004; Gamain et al., 2017). However, in the present study, 14 TMs and 22 pesticides were detected at least once in surface water and/or in sediments, at concentrations presenting a chemical risk for the aquatic environment during the summer peak (June–July) of seagrass meadow (Vermaat and Verhagen, 1996), which could result in a process of bioaccumulation (Auby et al., 2011). This is supported by Lyngby and Brix (1982) who demonstrated that the trace metal concentrations in *Zostera marina* reflected the trace metal concentrations in sediment or water of Limfjord, Denmark. More precisely, Lyngby and Brix (1984) found that metals could accumulate in the tissues of *Zostera marina* to attain concentrations of up to 1850 times higher than those found in water under controlled conditions. Similar results were recently obtained regarding a relationship between metal concentrations (*i.e.* cadmium and lead) in sediment and seagrass tissues (Lin et al., 2016). In addition, in our case study, cadmium, copper and zinc could have inhibited seagrass growth even though, Lyngby and Brix (1984) demonstrated that these metals have toxic effects on *Zostera marina* only at extremely high concentrations (*i.e.* at least 50 times higher than in the Vaccarès lagoon). However, this short-term experiment shows the toxicological potential of these elements, and raises the question of their long-term effects at low concentrations. Our results were sustained by strong negative correlations between exchangeable trace metals (*e.g.* copper) and the photosynthetic potential of seagrass. Concerning organic contaminants, the no-observed effect concentrations (NOEC) reported in the literature for vascular or non-vascular phototrophic organisms exposed to bentazon or 2,4-MCPA are much higher than the maximum values of these two contaminants found in the Vaccarès lagoon over the period 2011–2013 (*e.g.* maximum concentrations of 1.7 and 0.6 $\mu g.L^{-1}$ *vs.* NOEC: 5 and 470 $\mu g.L^{-1}$ for bentazon and 2,4-MCPA, respectively). Consequently, it is difficult to associate seagrass dynamics with the impact of these single molecules, particularly as no clear link was found between *in situ* contamination patterns and the photosynthetic potential of plants. In this regard, Gera et al. (2012) demonstrated that multiple environmental stressors could impact Fv:Fm values. The impact on seagrass dynamics is likely to be due to the sum of all herbicides. Indeed, a combined effect of these herbicides could be close to those causing a diminution of the photosynthetic potential of *Z. noltei* after 24 h of exposure (10 $\mu g.L^{-1}$) (Elger et al., 2012).

Many parameters can increase the bioavailability or toxicity of trace metals and organic contaminants, such as the decrease in pH at the entrance of the lagoon or the resuspension of sediment caused by the regression of *Zostera* and by the combined effects of the shallow depth and long fetch of the lagoon and strong waves; however, it is really difficult to predict the resulting effect of a mixture of contaminants in a given situation (*i.e.* an additive or even synergistic action) (Chesworth et al., 2004; Macinnis-Ng and Ralph, 2004; Nielsen and Dahllöf, 2007). Moreover, the degradation process of contaminants (*i.e.* photolytic activity) is rarely complete and most often leads to metabolites, which may be more toxic or persistent than the initial molecules (Srogi, 2007).

Finally, feedback loops from seagrass systems can also have an influence, such as less sediment trapped, higher turbidity, reduced light availability, negative impacts on seagrass growth. But also greater amounts of nutrients in the water and higher algae growth might outcompete the seagrass by absorbing most of the available light and even shading it (Van Der Heide et al., 2010). These loops can even lead to system collapse and regime shifts towards either a sand bar state or an algae dominated state. Though the latter was not observed in the field in 2015.

12. Conclusion

Years of surface water and sediment monitoring have demonstrated the presence and quantities of a mixture of contaminants comprising pesticides and trace metal elements in the Vaccarès lagoon. Some of them have concentrations well above environmental quality standards and present a risk for the biota. Spatial patterns of chemical contamination, particularly involving some herbicides used in rice cultures, coincide with the location of drainage channel outflows and with the areas of meadow decline. In these areas, seagrasses also had reduced winter rhizome reserves.

Nevertheless, natural ecosystems are complex and many inter-dependent parameters at different time scales may also influence the physiology and population dynamics of *Z. noltei.* This makes it difficult to pinpoint if a change in seagrass meadow is natural or due to anthropogenic stressors, or combination of the two, and, if so, which of these factors has the greatest impact. Complementary investigations, such as long term monitoring and additional toxicity tests, notably considering the impacts of mixture toxicity and chronic exposure, are required to address the causal link between contamination and seagrass decline. Measurement of the actual contaminants in *Z. noltei* tissues in the field (*e.g.* using liquid/gas chromatography for herbicides, or ICP Mass Spectrometry for TM) would also provide useful information. These different approaches will help to determine whether the system undergoes natural fluctuations and is resilient to natural and anthropogenic stressors or whether it is at risk.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2019.03.019.

References

Ackerman, F., 1980. A procedure for correcting grain size effect in heavy metal analysis of estuarine and coastal sediments. Environ. Technol. Lett. 1 518–257.

Auby, I., André Bost, C., Budzinski, H., 2011. Régression des herbiers de zostères dans le bassin d'Arcachon : état des lieux et recherche des causes. Rapport IFREMER ODE LER Arcachon, pp. 195.

Bernard, G., Boudouresque, C.F., Picon, P., 2007. Long term changes in Zostera meadows in the Berre lagoon (provence, Mediterranean Sea). Estuarine. Coastal and Shelf Science 73.

Boutron, O., Bertrand, O., Fiandrino, A., Höhener, P., Sandoz, A., Chérain, Y., Coulet, E., Chauvelon, P., 2015. An unstructured numerical model to study wind-driven circulation patterns in a managed coastal Mediterranean Wetland: the Vaccares lagoon system. Water 7, 5986.

Brun, F.G., Hernández, I., Vergara, J.J., Peralta, G., Pérez-Lloréns, J.L., 2002. Assessing the toxicity of ammonium pulses to the survival and growth of *Zostera noltei*. Mar. Ecol. Prog. Ser. 225, 177–187.

Brun, F.G., Olive, I., Malta, E.J., Vergara, J.J., Hernández, I., Pérez-Lloréns, J.L., 2008. Increased vulnerability of *Zostera noltei* to stress caused by low light and elevated ammonium levels under phosphate deficiency. Mar. Ecol. Prog. Ser. 365 67-55.

Cabaço, S., Macha's, R., Vieira, V., Santos, R., 2008. Impacts of urban wastewater discharge on seagrass meadows (Zostera noltei). Estuarine. Coastal and Shelf Science 78, 1–13.

Cardoso, P.G., Pardal, M.A., Lillebo, A.I., Ferreira, S.M., Raffaelli, D., Marques, J.C., 2004. Dynamic changes in seagrass assemblages under eutrophication and implications for recovery. J. Exp. Mar. Biol. Ecol. 302, 233–248.

Charpentier, A., Grillas, P., Lescuyer, F., Coulet, E., Auby, I., 2005. Spatio-temporal dynamics of a *Zostera noltei* dominated community over a period of fluctuating salinity in a shallow lagoon, Southern France. Estuarine. Coastal and Shelf Science 64, 307–315. https://doi.org/10.1016/j.ecss.2005.02.024.

Chesworth, J.C., Donkin, M.E., Brown, M.T., 2004. The interactive effects of the antifouling herbicides Irgarol 1051 and Diuron on the seagrass *Zostera marina* (L.). Aquat. Toxicol. 66, 293–305.

Christianen, M.J.A., Van der Heide, T., Bouma, T.J., Roelofs, J.G.M., Van Katwijk, M.M., Lamers, L.P.M., 2011. Limited toxicity of NHx pulses on an early and late successional tropical seagrass species: interactions with pH and light level. Aquat. Toxicol. 104, 73–79.

Comoretto, L., Arfib, B., Chiron, S., 2007. Pesticides in the Rhône river delta (France): Basic data for a field-based exposure assessment. Sci. Total Environ. 380, 124–132. https://doi.org/10.1016/j.scitotenv.2006.11.046.

Comoretto, L., Arfib, B., Talva, R., Chauvelon, P., Pichaud, M., Chiron, S., Höhener, P., 2008. Runoff of pesticides from rice fields in the Ile de Camargue (Rhône river delta, France): field study and modeling. Environ. Pollut. 151, 486–493. https://doi.org/10.1016/j.envpol.2007.04.021.

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neil, R.V., Paruelo, J., Raskin, R.G., Sutton, P., Van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. Nature 387, 253–260.

Dalloyau, S., Robin, F., 2013. Distribution des Bernaches cravants à ventre sombre (Branta bernicla bernicla) et disponibilité alimentaire des herbiers à Zostère naine (Zostera noltei) : vers une caractérisation de la qualité des habitats intertidaux des Pertuis Charentais. Rapport 2013, 79 SEP-LPO.

Diepens, N.J., Buffan-Dubau, E., Budzinski, H., Kallerhoff, J., Merlina, G., Silvestre, J., Auby, I., Tapie, N., Elger, A., 2017. Toxicity effects of an environmental realistic herbicide mixture on the seagrass *Zostera noltei*. Environ. Pollut. 222, 393–403.

Dos Santos, V.M., Matheson, F.E., Pilditch, C.A., Elger, A., 2013. Seagrass resilience to waterfowl grazing in a temperate estuary: a multi-site experimental study. J. Exp. Mar. Biol. Ecol. 446, 194–201.

Duarte, C.M., 1991. Seagrass depth limits. Aquat. Bot. 40, 363–377.

Elger, A., Auby, I., Belles, A., Budzinski, H., Buffan-Dubau, E., Dos Santos, V., Fidenci, M., Kallerhoff, J., Le Menach, K., Matheson, F., Merlina, G., Pardon, P., Plus, M., Silvestre, J., Tapie, N., Trut, G., 2012. Impact de CONtaminations diffuses par les herbicides sur les HErbiers de zostèeres (ICONHE): Rapport final.

Fernández-Torquemada, Y., Sánchez-Lizaso, J.L., 2011. Responses of two Mediterranean seagrasses to experimental changes in salinity. Hydrobiologia 669, 21–33. https://doi.org/10.1007/s10750-011-0644-1.

Flores, F., Collier, C.J., Mercurio, P., Negri, A.P., 2013. Phytotoxicity of four photosystem II herbicides to tropical seagrasses. PLoS One 8, e75798. https://doi.org/10.1371/journal.pone.0075798.

Gamain, P., Feurtet-Mazel, A., Maury-Brachet, R., Auby, I., Pierron, F., Belles, A., Budzinski, H., Daffe, G., Gonzalez, P., 2017. Can pesticides, copper and seasonal water temperature explain the seagrass *Zostera noltei* decline in the Arcachon bay? Mar. Pollut. Bull. 134.

García-Marín, P., Cabaço, S., Hernández, I., Vergara, J.J., Silva, J., Santos, R., 2013. Multi-metric index based on the seagrass *Zostera noltei* (ZoNI) for ecological quality assessment of coastal and estuarine systems in SW Iberian Peninsula. Mar. Pollut. Bull. 68, 46–54.

Gera, A., Alcoverro, T., Mascaró, O., Pérez, M., Romero, J., 2012. Exploring the utility of *Posidonia oceanica* chlorophyll fluorescence as an indicator of water quality within the European Water Framework Directive. Environ. Monit. Assess. 184 (6), 3675–3686.

Havaux, M., Niyogi, K.K., 1999. The violaxanthin cycle protects plants from photo-oxidative damage by more than one mechanism. Proc. Natl. Acad. Sci. Unit. States Am. 96, 8762–8767. https://doi.org/10.1073/pnas.96.15.8762.

Höhener, P., Comoretto, L., Housari, Fa, Chauvelon, P., Pichaud, M., Cherain, Y., Chiron, S., 2010. Modeling anthropogenic substances in coastal wetlands: application to herbicides in the Camargue (France). Environ. Model. Softw 25, 1837–1844. https://doi.org/10.1016/j.envsoft.2010.05.005.

Hoostmans, M.J.M., Vermaat, J.E., Van Vierseen, W., 1987. Seed-bank development, germination and early seedling survival of two seagrass species from The Netherlands: *Zostera marina* L., and *Zostera noltei* Hornem. Aquat. Bot. 28, 275–285.

Jacobs, R.P.W.M., Den Hartog, C., Braster, B.F., Carriere, F.C., 1981. Grazing of the seagrass *Zostera noltei* by birds at Terschelling (Dutch Wadden Sea). Aquat. Bot. 10, 241–529.

Kilminster, K., McMahon, K., Waycott, M., Kendrick, G., Scanes, P., McKenzie, L., O'Brien, K., Lyons, M., Ferguson, A., Maxwell, P., Glasby, T., Udy, J., 2015. Unravelling complexity in seagrass systems for management: Australia as a microcosm. Sci. Total Environ. 534, 97–109.

Koch, E.W., 2001. Beyond light: physical, biological, and geochemical parameters as possible submersed submersed aquatic vegetation habitat requirements. Estuaries 24, 1–17.

Liaghati, T., Preda, M., Cox, M., 2004. Heavy metal distribution and controlling factors within coastal plain sediments, Bells Creek catchment, southeast Queensland Australia. Environ. Int. 29, 935–948.

Lin, H., Sun, T., Xue, S., Jiangn, X., 2016. Heavy metal spatial variation, bioaccumulation, and risk assessment of *Zostera japonica* habitat in the Yellow River Estuary, China. Sci. Total Environ. 541, 435–443. https://doi.org/10.1016/j.scitotenv.2015.09.050.

Lyngby, J.E., Brix, H., 1982. Seasonal and environmental variation of Cd, Cu, Pb, and Zn concentrations in eelgrass (*Zostera marina* L.) in the Limfjord, Denmark. Aquat. Bot (in press).

Lyngby, J.E., Brix, H., 1984. The uptake of heavy metals in eelgrass *Zostera marina* and their effect on growth. Ecol. Bull. 36, 81–89.

Macdonald, D., Carr, R.S., Calder, F.D., Long, E.R., Ingersoll, C.G., 1996. Development and evaluation of sediment quality guidelines for Florida coastal waters. Ecotoxicology 5, 253–278.

Macinnis-Ng, C.M.O., Ralph, P.J., 2004. In situ impact of multiple pulses of metal and herbicide on the seagrass, *Zostera capricorni*. Aquat. Toxicol. 67 (3), 227–237.

Massa, S.I., Arnaud-Haond, S., Pearson, G.A., Serraõ, E.A., 2009. Temperature tolerance and survival of intertidal populations of the seagrass *Zostera noltei* (Hornemann) in southern Europe (Ria Formosa, Portugal). Hydrobiologia 619, 195–201. https://doi.org/10.1007/s10750-008-9609-4.

Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence - a practical guide. J. Exp. Bot. 51, 659–668.

McGlathery, K.J., 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. J. Phycol. 37 (4), 453–456.

Menzies, N.W., Donn, M.J., Kopittke, P.M., 2007. Evaluation of extractants for estimation of the phytoavailable trace metals in soils. Environ. Pollut. 145.

Negri, A.P., Flores, F., Mercurio, P., Mueller, J.F., Collier, C.J., 2015. Lethal and sublethal chronic effects of the herbicide diuron on seagrass. Aquat. Toxicol. 165, 73–83. https://doi.org/10.1016/j.aquatox.2015.05.007.

N'Guessan, M., Probst, J.L., Bur, T., Probst, A., 2009. Trace elements in stream bed sediments from agricultural catchments (Gascogne region, S-W France): where do they come from? Sci. Total Environ. 407, 2939–2952.

Nielsen, L.W., Dahllöf, I., 2007. Direct and indirect effects of the herbicides Glyphosate, Bentazon and MCPA on eelgrass (*Zostera marina*). Aquat. Toxicol. 82, 47–54.

Orth, R.J., Carruthers, T.J., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., 2006. A global crisis for seagrass ecosystems. Bioscience 56, 987–996 doi:10.1641/0006-3568(2006)56[987:agcfse]2.0.co;2.

Park, S.G., 1999. Changes in the abundance of seagrass (Zostera spp.) in Tauranga Harbour from 1956-96. In: Environmental Report 99/30. Environment Bay of Plenty, Tauranga, New Zealand.

Plus, M., Dalloau, S., Trut, G., Auby, I., de Montaudouin, X., Emery, E., 2010. Long-term evolution (1988–2008) of Zostera spp. meadows in arcachon bay (bay of Biscay). Estuarine. Coastal and Shelf Science 87, 357–366. https://doi.org/10.1016/j.ecss.2010.01.016.

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

Scarlett, A., Donkin, P., Fileman, T.W., Evans, S.V., Donkin, M.E., 1999. Risk posed by the antifouling agent Irgarol 1051 to the seagrass, *Zostera marina*. Aquat. Toxicol. 45, 159–170.

Scott, D., Rose, P.M., 1996. Atlas of Anatidae Populations in Africa and Western Eurasia, vol. 41 Wetlands International Publication. Wetlands International: Wageningen, The Netherlands.

Short, F., Carruthers, T.J.B., Dennison, W.C., Waycott, M., 2007. Global seagrass distribution and diversity: a bioregional model. J. Exp. Mar. Biol. Ecol. 350, 3–20.

Short, F., Polidoro, B., Livingstone, S.R., Carpenter, K.E., Bandeira, S., Bujang, J.S., Calumpong, H.P., Carruthers, T.J.B., Coles, R.G., Dennison, W.C., Erftmeijer, P.L.A., Fortes, M.D., Freeman, A.S., Jagtap, T.G., Kamal, A.H.M., Kendrick, G.A., Kenworthy, W.J., La Nafie, T.A., Nasution, I.M., Orth, R.J., Prathep, A., Sanciangco, J.C., van Tussenbroek, B., Vergara, S.G., Waycott, M., Zieman, J.C., 2011. Extinction risk assessment of the world's seagrass species. Biol. Conserv. 114, 1961–1971.

Srogi, K., 2007. Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. Environ. Chem. Lett. 5 (4), 169–195.

Swartz, R.C., 1999. Consensus sediment quality guidelines for polycyclic aromatic hydrocarbon mixture. Environ. Toxicol. Chem. 18, 780–787.

Tam, N.F.Y., Yao, M.W.Y., 1998. Normalization and heavy metal contamination in mangrove sediments. Sci. Total Environ. 216 33–29.

Tanaka, Y., Sasaki, N., Ohmiya, A., 2008. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J. 54, 733–749. https://doi.org/10.1111/j.1365-313X.2008.03447.x.

Thayer, G.W., Bjorndal, K.A., Ogden, J.C., Williams, S.I., Zieman, J.C., 1984. Role of large herbivores in seagrass communities. Estuaries 7, 351–376.

Unsworth, R.K.F., Collier, C.J., Waycott, M., Mckenzie, L.J., Cullen-Unsworth, L.C., 2015. A framework for the resilience of seagrass ecosystems. Mar. Pollut. Bull. 100, 34–46.

Valiela, I., Foreman, K., LaMontagne, M., Hersh, D., Costa, J., Peckol, P., Barbara DeMeo-Andreson, B., D'Avanzo, C., Babione, M., Sham, C.-H., Brawley, J., Lajtha, K., 1992. Couplings of watersheds and coastal waters: sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. Estuaries 15 (4), 443–457.

Van Der Heide, T., van Nes, E.H., Van Katwijk, M.M., Scheffer, M., Hendriks, A.J., Smolders, A.J.P., 2010. Al ternative stable states driven by density-dependent toxicity. Ecosystems 13, 841–850.

Vermaat, J.E., Verhagen, F.C.A., 1996. Seasonal variation in the intertidal seagrass *Zostera noltei* Hornem: coupling demographic and physiological patterns. Aquat. Bot. 52, 259–281.

Vermaat, J.E., Verhagen, F.C.A., Lindenburg, D., 2000. Contrasting responses in two populations of *Zostera noltei* Hornem. to experimental photoperiod manipulation at two salinities. Aquat. Bot. 67, 179–189.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes Jr., A.R., Kendrick, G.A., Judson Kenworthy, W.J., Frederick, T., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Natl. Acad. Sci. Unit. States Am. 106, 12377–12381. https://doi.org/10.1073/pnas.0905620106.

Wedepohl, K.H., 1995. The composition of the continental crust. Geochem. Cosmochim. Acta 59 (7), 1217–1232.

Wilkinson, A.D., Collier, C.J., Flores, F., Negri, A.P., 2015. Acute and additive toxicity of ten photosystem-II herbicides to seagrass. Sci. Rep. 5, 17443. https://doi.org/10.1038/srep17443.

Wood, K.A., Stillman, R.A., Clarke, R.T., Daunt, F., O'Hare, M.T., 2012. The impact of waterfowl herbivory on plant standing crop:a meta-analysis. Hydrobiologia 686, 157–167. https://doi.org/10.1007/s10750-012-1007-2.

Yemm, E.W., Willis, A.J., 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57, 508–514.

Zweens, J., Bouman, P.R., 1968. Simple and accurate determination of urinary glucose excretion with anthrone reagent. Diabetologia 4, 278–280. https://doi.org/10.1007/bf01309901.