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WISER

Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery

DELIVERABLE

Deliverable D4.2-2: Responses of macroalgae and seagrass indicators to drivers of deterioration

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Content

Non-technical summary

The increasing human pressure on the coastal zone is rapidly deteriorating coastal environmental quality, particularly since year 1950. Policies aiming at improving coastal water and ecosystem quality are a priority in European countries (Water Framework Directive, Marine Strategy Framework Directive, Habitats Directive) as well as in other countries and regions in the Globe (e.g., USA, Clean Water Act). Well-developed seagrass and macroalgal beds provide many important services to coastal ecosystems, such as increased biodiversity and coastal protection, which disappear when seagrass and macroalgal distribution and abundance decline in response to human pressure. This study provides examples of how seagrass and macroalgal indicators respond to anthropogenic pressure in the European coastal zone.

Introduction

Human activities exert large pressures on the coastal zone, resulting in deterioration of coastal marine ecosystems (Millenium Ecosystem Assessment 2005, Halpern et al 2008, Lotze et al. 2008). The global human population has doubled during the last four decades of the 20th Century and increasingly concentrated into cities. At present, 23% of the global human population inhabits areas located within the closest 100 km to shore, and the highest population density occurs within the closest 10 km (Nicholls and Small 2002). The concentration of the global human population in the coastal zone is transforming coastal areas, encompassing both land and marine environments. Natural ecosystems are replaced by urban areas, artificial structures (e.g., harbours, dikes, etc) and installations to produce resources (e.g aquaculture farms, desalination plants). In addition, inputs of nutrients, organic matter and contaminants to the coastal zone have increased worldwide and are a major problem for coastal marine ecosystems (Nixon 2009, Howarth et al. 2011). The same is true for intense fishery (Jackson et al. 2001).

The intense pressure on the coastal zone has resulted in a widespread deterioration of coastal water quality, evidenced by e.g. eutrophication effects such as declines in water transparency and increased frequency of anoxic events causing declines of coastal key ecosystems, such as seagrass meadows and shifted the balance from benthic to pelagic dominance of primary production (Cloern et al. 2001, Duarte 1995, Duarte et al. 2008, Waycott et al. 2009, Howarth et al. 2011, Krause-Jensen et al. 2011a). Assessments of potential cumulated impact of multiple pressures on coastal ecosystems are scarce, but information on the spatial distribution and magnitude of combined pressures are now emerging and improve the basis for conducting such studies (Halpern et al., 2008, Foden et al., 2010, Helcom 2010).

Policies aiming at improving coastal water and ecosystem quality are a priority in European countries as well as in other countries and regions in the Globe (e.g. USA: Clean Water Act (CWA, 2002/P.L. 107–303/USA), National Estuary Program (www.epa.gov/nep)). In Europe, the Habitats Directive (HD, 92/43/EEC) sets standards to guarantee biodiversity by preserving the natural habitats of flora and fauna in the territory of European member states, and implementation of the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC) set a mutual platform and obligations to ensure “good ecological status” of coastal and marine waters (Borja *et al.,* 2010). In the WFD and MSFD the assessment of ecological status of European water bodies must be conducted using indicators of biological elements that are sensitive to water quality. Seagrasses and macroalgae compose the biological quality element “benthic vegetation" used by both the WFD and the MSFD, and meadows of the Seagrass species (*Posidonia oceanica*) are one of the habitats included in the HD.

Seagrass meadows are the dominant marine ecosystem of shallow sandy coastal areas, extending from the tropics to the poles except in Antarctica, while macroalgae colonize rocky bottoms and other hard, stable substratum in the intertidal as well as the subtidal zone. Seagrass meadows and kelp forests are extremely valuable ecosystem because they provide many ecological services to the coastal zone. They are highly productive, influence the structural complexity of habitats, enhance biodiversity, play important roles in global carbon and nutrient cycling, stabilize water flow and promote sedimentation, thereby reducing particle loads in the water as well as coastal erosion (Jones et al., 1994; Hemminga and Duarte, 2000; Steneck et al. 2002, Orth et al., 2006). Seagrass meadows have, in fact, been estimated to deliver the highest value, in terms of ecosystem services, of all natural ecosystems (Costanza et al., 1997). However, seagrass ecosystems rank amongst the most vulnerable ones on the biosphere and experience marked global decline to a large extent due the strong human pressure to the coastal zone (Short and Wyllie-Echeverria, 1996; Green and Short, 2003; Orth et al., 2006; Duarte, 2009; Waycott et al., 2009). Macroalgal ecosystems are also threatened by e.g. eutrophication which can cause reduction in depth extension and shift the composition from dominance late-successional species towards opportunistic species (Duarte 1995, Nielsen et al. 2002, Cloern 2001, Kemp et al. 2005, Schramm and Nienhuis, 1996; Walker and Kendrick, 1998). The decline of benthic marine plants is a nonlinear process that accelerates, through cascading effects, after reaching a certain level of disturbance (e.g., Duarte, 1995).

The requirements for the benthic marine vegetation to fulfill "Good ecological status" according to the WFD are that most disturbance sensitive macroalgal and angiosperm taxa associated with undisturbed conditions are present and that the level of macroalgal cover and angiosperm abundance shows only slight signs of disturbance. In order for an indicator to be accepted for use in the Water Framework Directive it must show a documented response to human pressure.

Objectives

The objective of this study is to explore relationships between seagrass and macroalgal indicators and anthropogenic pressures in the coastal zone through case studies.

Case study 1: Posidonia multimetric index (POMI) along gradients in anthropogenic pressure in the Mediterranean Sea

By Rosa Garcia Novoa, Núria Marbà (CSIC-IMEDEA), Teresa Alcoverro (CSIC-CEAB)

Background

POMI is a *Posidonia oceanica* multivariate index based on the combination, through principal component analysis (PCA), of physiological, morphological, structural and community level seagrass metrics, and that is used to reflect ecological status of coastal waters as required by the WFD (Romero et al. 2007). The scores obtained for the first PCA axis are then normalised to a 0-1 EQR scale using reference and worse sites. The WFD indicates the EQRs should show significant relationship with anthropogenic pressures. Therefore in this case study we explore the relationships of the metrics used to obtain POMI, and the EQRs obtained from the POMI index itself, with the anthropogenic pressures available for both the Catalonian and Balearic coastal zones, in order to explore the relationships over this broad coastal Mediterranean region.

Methods

Antropogenic pressures for the Catalonian coastal zone were obtained from an official WFD report of the Agència Catalana de l'Aigua (2005), and pressures for the Balearic Islands coastal zone were also obtained from an official WFD report on environmental impact assessment of the hydrological strategy of the Balearic Islands Government (Barón Périz et al 2008). From both reports ten common pressures for Catalonian and Balearic Islands coastal zones were identified, and from these only the units of five pressures (Pressure of coastal rigidification, Pressure of beach regeneration, Pressure of marinas, Pressure of shipping traffic, Chemical oxygen demand) could be unified for both regions under the same unit system (Table 1). Nutrients loads were measured differently in both regions. For Catalonia only nitrate (NO_3^-) data was available, while for the Balearic coastal zones total nitrogen (TN) and phosphorus (TP) were measured. Hence, the analysis of nutrients pressure was done separately for each region. The pressure of urban soil use, agricultural soil use and tourism could not be transformed under the same unit system. Extra data is needed and is currently being searched.

Table 1: Anthropogenic pressures used to assess relationships with *Posidonia oceanica* metrics and EQRs obtained from POMI, the formula to obtain the pressures and the units. * Pressures that could be unified for both regions under the same unit system.

Pressure	**Formula**	**Units**
*Pressure Coastal Rigidification	$\left(\mathit{Artificial\ coast\ length\ in\ water\ body} \big/ \mathit{Length\ of\ coast\ in\ water\ body}\right)\times 100$	%
*Pressure Beach Regeneration	$\left(\mathit{Volume\ of\ sand\ deposited} \big/ \mathit{Length\ of\ coast\ in\ water\ body}\right)$	m^3 of sand km^{-1} of the WB coastal line
*Pressure Marinas	$\left(\mathit{Number\ of\ moorings} \big/ \mathit{Length\ of\ coast\ in\ water\ body}\right)$	moorings $km^{-1}$of the WB coastal line
*Pressure Shipping Traffic	$\left(\mathit{Number\ of\ ship\ trips} \big/ \mathit{Year\ and\ Length\ of\ coast\ in\ water\ body}\right)$	ships km^{-1} of the WB coastal line $year^{-1}$
*Chemical Oxygen Demand	$\left(\sum \mathit{Outflow\ daily\ volume\ flow} \times \mathit{Chemical\ Oxygen\ Demand} \big/ \mathit{Length\ of\ coast\ in\ water\ body}\right)$	kg km^{-1} of the WB coastal line day^{-1}
Nitrate	$\left(\sum \mathit{River's\ daily\ volume\ flow} \times \mathit{Nitrate\ concentration} \big/ \mathit{Length\ of\ coast\ in\ water\ body}\right)$	kg km^{-1} of the WB coastal line day^{-1}
Total Nitrogen	$\left(\sum \mathit{River's\ daily\ volume\ flow} \times \mathit{Total\ nitrogen\ concentration} \big/ \mathit{Length\ of\ coast\ in\ water\ body}\right)$	kg km^{-1} of the WB coastal line day^{-1}
Total Phosphorus	$\left(\sum \mathit{River's\ daily\ volume\ flow} \times \mathit{Total\ phosphorus\ concentration} \big/ \mathit{Length\ of\ coast\ in\ water\ body}\right)$	kg km^{-1} of the WB coastal line day^{-1}
Pressure of Urban Soil Use	$\left(\mathit{Urban\ area} \times \mathit{Average\ annual\ precipitation} \big/ \mathit{Length\ of\ coast\ in\ water\ body}\right)$	ha km^{-1} of the WB coastal line
Pressure of Agricultural Soil Use	$\left(\mathit{Agricultural\ area} \big/ \mathit{Length\ of\ coast\ in\ water\ body}\right)$	ha km^{-1} of the WB coastal line
Pressure of Tourism	$\left(\sum \mathit{Number\ of\ tourism\ vacants} \big/ \mathit{Length\ of\ coast\ in\ water\ body}\right)$	vacants km^{-1} of the WB coastal line

Type II regressions were applied to pressures and *Posidonia oceanica* metrics from a total of 58 stations distributed among 30 water bodies for the Balearic Islands coastal zone, and from a total of 29 stations distributed among 16 water bodies for Catalonian coastal zone. The metrics assed were the metrics commonly used to obtain POMI (Table 2). The EQRs obtained from the POMI indexes for each coastal zone were also related to the anthropogenic pressures. Stepwise regression analysis was applied to the data to explore the contribution of each pressure to the metrics and POMI?s EQRs variability.

Table 2. *Posidonia oceanica* metrics used in type II regressions to assess the relationships with the anthropogenic pressures, and the metrics included in the calculation of the official POMI for each region.

***Posidonia oceanica* metrics**	**Pomi 5 Balearic I.**	**Pomi 9 Catalonia**
N epiphytes (% DW)	-	✓
Pb rhizome (µg/g DW)	-	✓
δ^{15}N rhizome (‰)	✓	✓
Leaves with Nechrosis (%)	-	✓
Shoot Leaf Area (cm^2 shoot^{-1})	-	-
Sacarose (% DW)	-	-
δ^{34}S rhizome (‰)	✓	✓
Density (shoot m^{-2})	-	✓
Cover (%)	✓	✓
Cu rhizome (µg/g DW)	-	-
Zn rhizome (µg/g DW)	-	-
N rhizome (% DW)	✓	✓
P rhizome (% DW)	✓	✓

Results

Relationships between Posidonia oceanica metrics and environmental common pressures

When considering Catalonia and Balearic Islands coastal zones globally three *P. oceanica* metrics were significantly related to the pressure of coastal rigidification (Fig. 1a,c,e), but with an $R^2 < 0.2$ (Table 3). The nitrogen content of epiphytes and the δ^{15}N signature in rhizome tissue increased with increasing coastal rigidification, while shoot leaf area decreased. There was a significant and positive relationship between coastal rigidification pressure and the lead content of rhizome tissue at Catalonia coastal zone (Fig. 1b, Table 3), and a negative significant relationship with *P. oceanica* density at Balearic Islands coastal zone (Fig. 1h). The R^2 were also low (Table 3). The POMI's EQRs from Catalonia coastal zone also showed a significant and negative relationship with increasing coastal rigidification (Fig. 2f, Table 3).

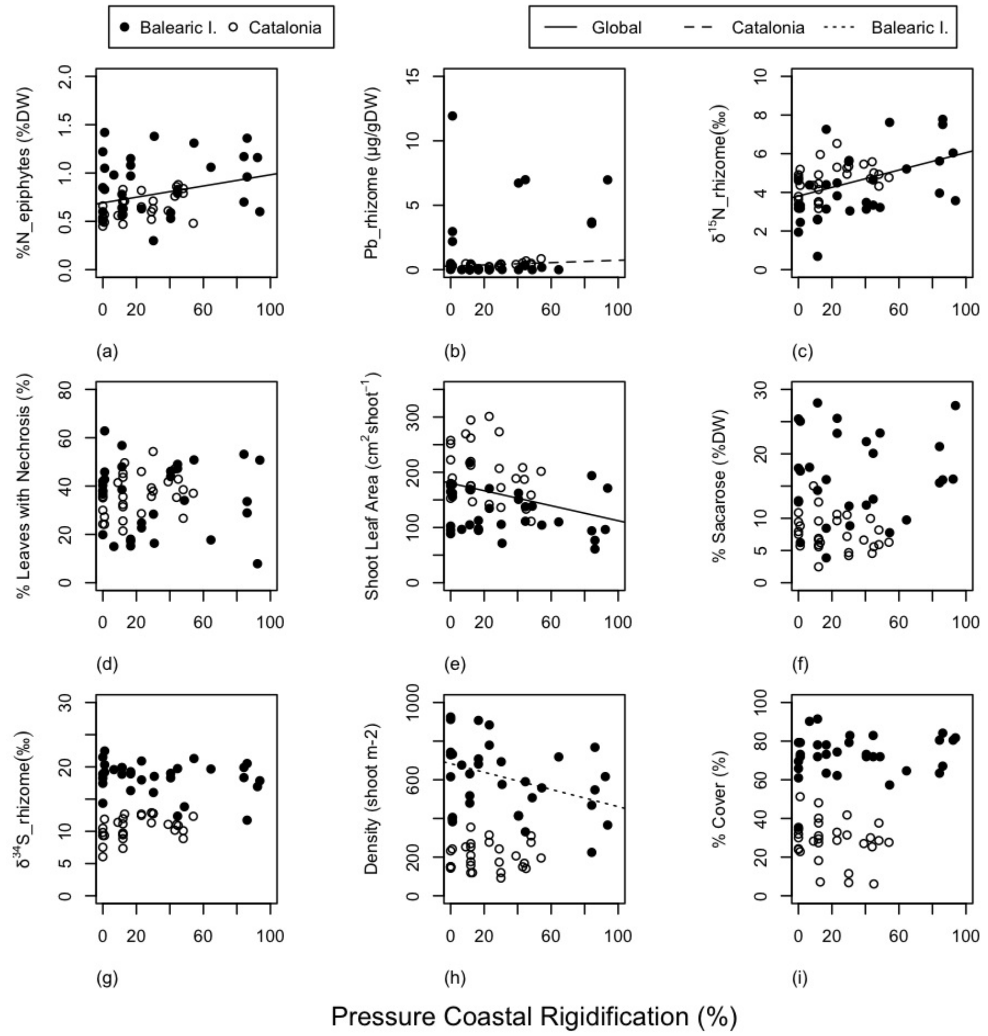


Figure 1. Relationship of pressure of coastal rigidification (%) *vs. Posidonia oceanica* metrics a) nitrogen content in epiphytes (%DW), b) lead content in rhizomes (ug/g DW), c) $\delta^{15}N$ signature in rhizomes (‰), d) percentage of leaves with nechrosis (%), e) shoot leaf area (cm^{2} shoot^{-1}), f) sacarose content (%DW), g) $\delta^{34}S$ signature in rhizomes (‰), h) density (shoot m^{-2}), i) percentage cover (%).

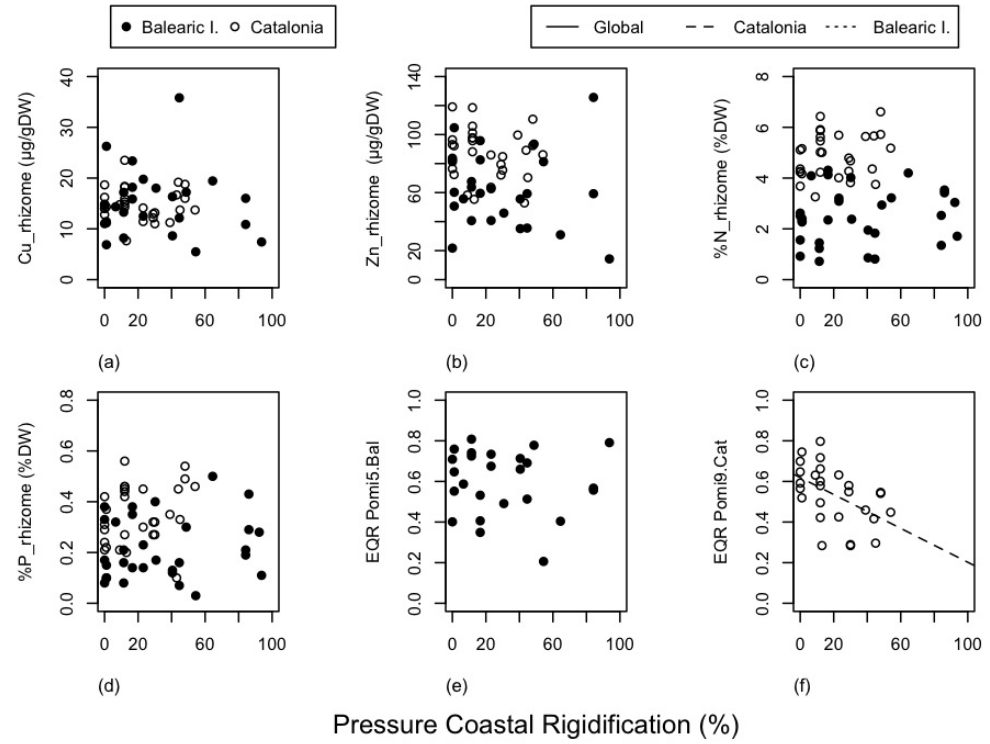


Figure 2. Relationship of pressure of coastal rigidification (%) *vs. Posidonia oceanica* metrics a) copper content in rhizomes (ug/g DW), b) zinc content in rhizomes (ug/g DW), c) nitrogen content in rhizomes (%DW), d) phosphorus content in rhizomes (%DW), e) EQR using for Balearic Island using index Pomi5, f) EQR for Catalonia using index Pomi9.

The pressure of beach regeneration of Catalonia and Balearic Islands coastal zones combined showed significant relationships with the nitrogen content of epiphytes, the $\delta^{15}N$ signature in rhizome tissue, shoot leaf area, the $\delta^{34}S$ signature in rhizome tissue and percentage cover (Fig. 3a,c,e,g,i). The R^2 were low, $R^2 < 0.15$ and the slope close to zero (Table 3), indicating slight changes in the metrics with increasing pressure with an small percentage (<15%) of the variance being explained by the regression model. The POMI's EQRs from Catalonia coastal zone showed a negative and significant relationship with increasing beach regeneration pressure, with an $R^2 = 0.16$ (Fig. 2f, Table 3).

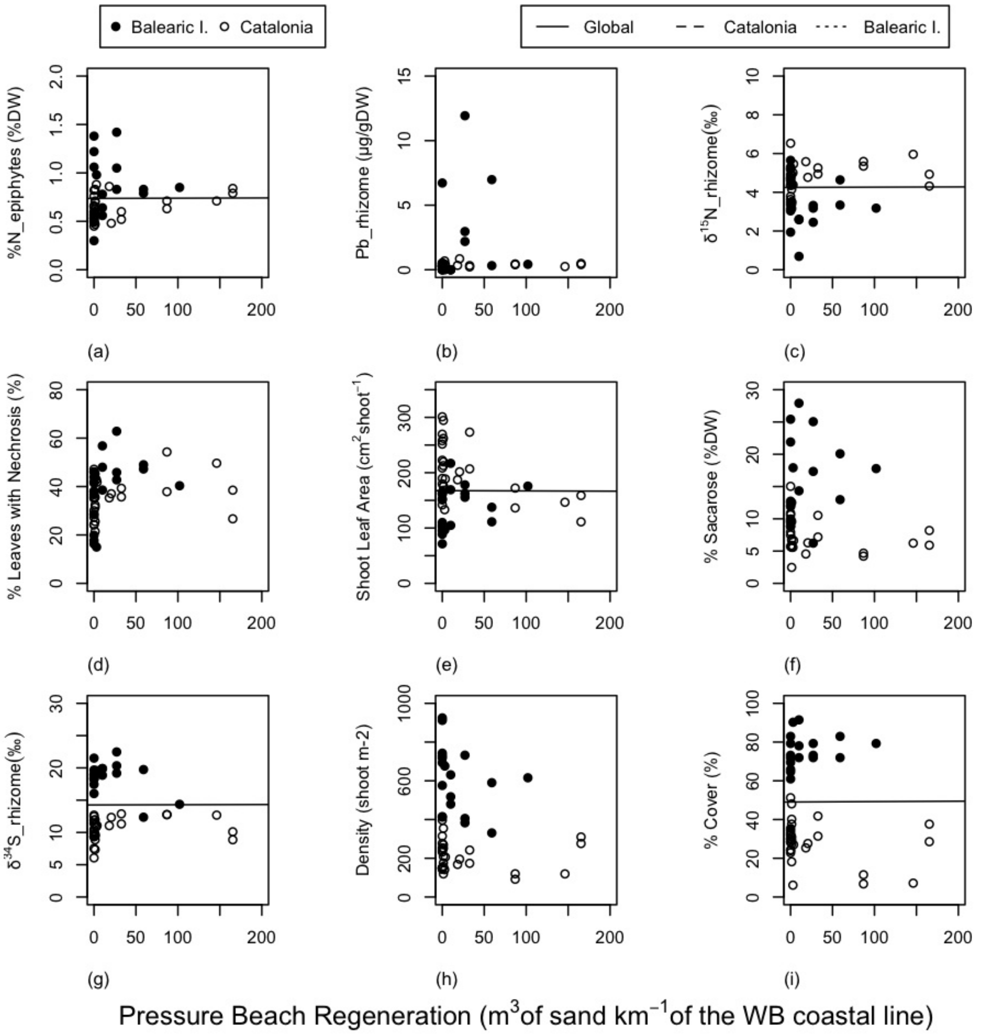


Figure 3. Relationship of pressure of beach regeneration (m^3 of sand km^{-1} of the water body coastal line) *vs. Posidonia oceanica* metrics a) nitrogen content in epiphytes (%DW), b) lead content in rhizomes (ug/g DW), c) δ^{15}N signature in rhizomes (‰), d) percentage of leaves with nechrosis (%), e) shoot leaf area (cm^2 shoot^{-1}), f) sacarose content (%DW), g) δ^{34}S signature in rhizomes (‰), h) density (shoot m^{-2}), i) percentage cover (%).

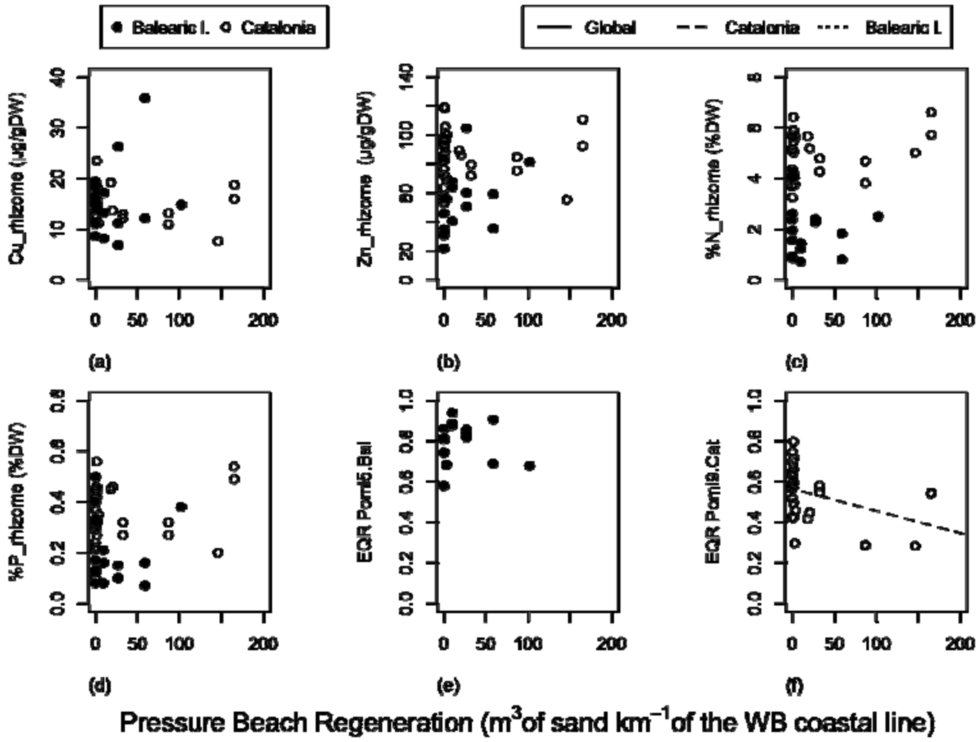


Figure 4. Relationship of pressure of beach regeneration (m^3 of sand km^{-1} of the water body coastal line) *vs. Posidonia oceanica* metrics a) copper content in rhizomes (ug/g DW), b) zinc content in rhizomes (ug/g DW), c) nitrogen content in rhizomes (%DW), d) phosphorus content in rhizomes (%DW), e) EQR using for Balearic Island using index Pomi5, f) EQR for Catalonia using index Pomi9.

P. oceanica density and percentage cover showed a significant and negative relationship with the pressure of marinas (Fig 5h,i) when considering Catalonian and Balearic Islands coastal zones globally. The R^2 however was < 0.1 (Table 3) indicating a weak relationship in both cases. There was a significant and positive relationship between the pressure of marinas and the nitrogen content of epiphytes (Fig. 5a) for the Catalonian coastal zone. For the Balearic coastal zone there also was a significant positive relationship of the $\delta^{15}N$ signature in *P. oceanica* rhizome tissue with the pressure of marinas (Fig. 5c). Both cases showed stronger R^2 (Table 3). None of the POMI's EQRs from Catalonia or Balearic Island showed a relationship with increasing pressure of marinas (Fig. 6e,f).

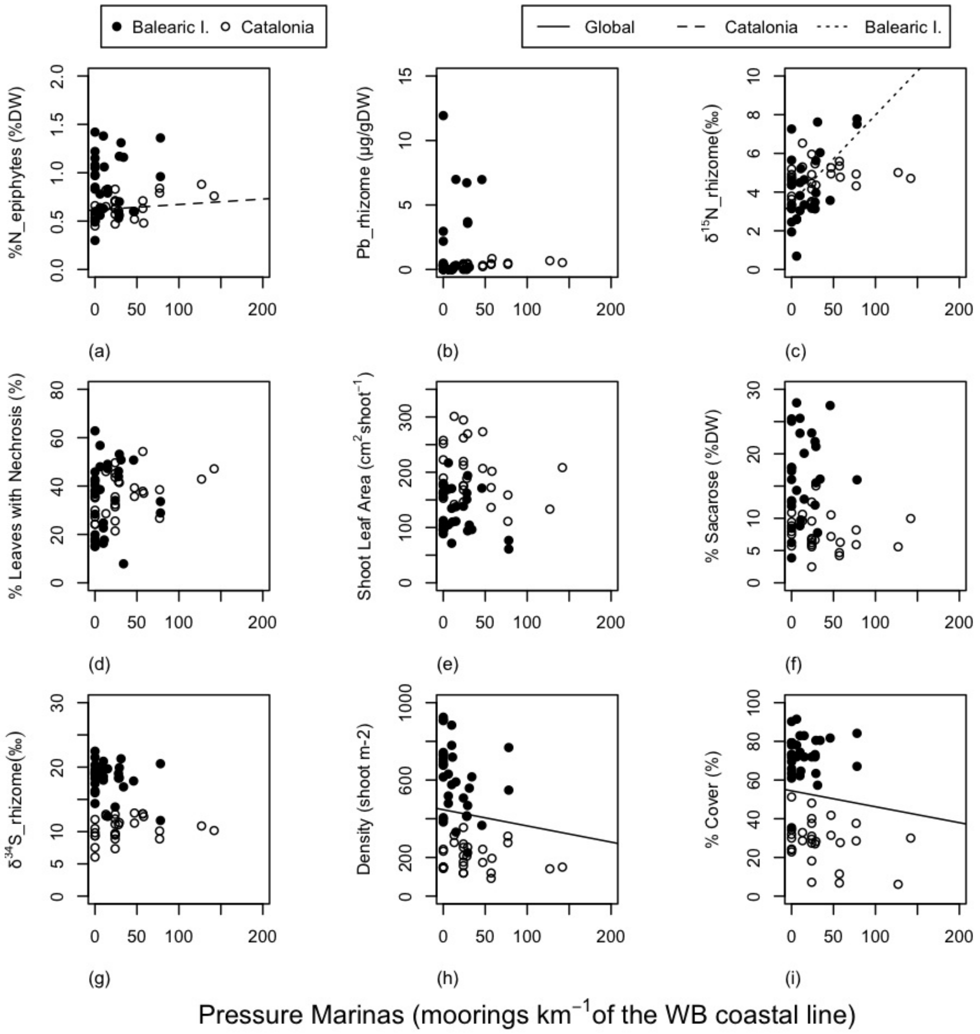


Figure 5. Relationship of pressure of marinas (moorings km^{-1} of the water body coastal line) *vs. Posidonia oceanica* metrics a) nitrogen content in epiphytes (%DW), b) lead content in rhizomes (ug/g DW), c) δ^{15}N signature in rhizomes (‰), d) percentage of leaves with nechrosis (%), e) shoot leaf area (cm^2 shoot^{-1}), f) sacarose content (%DW), g) δ^{34}S signature in rhizomes (‰), h) density (shoot m^{-2}), i) percentage cover (%).

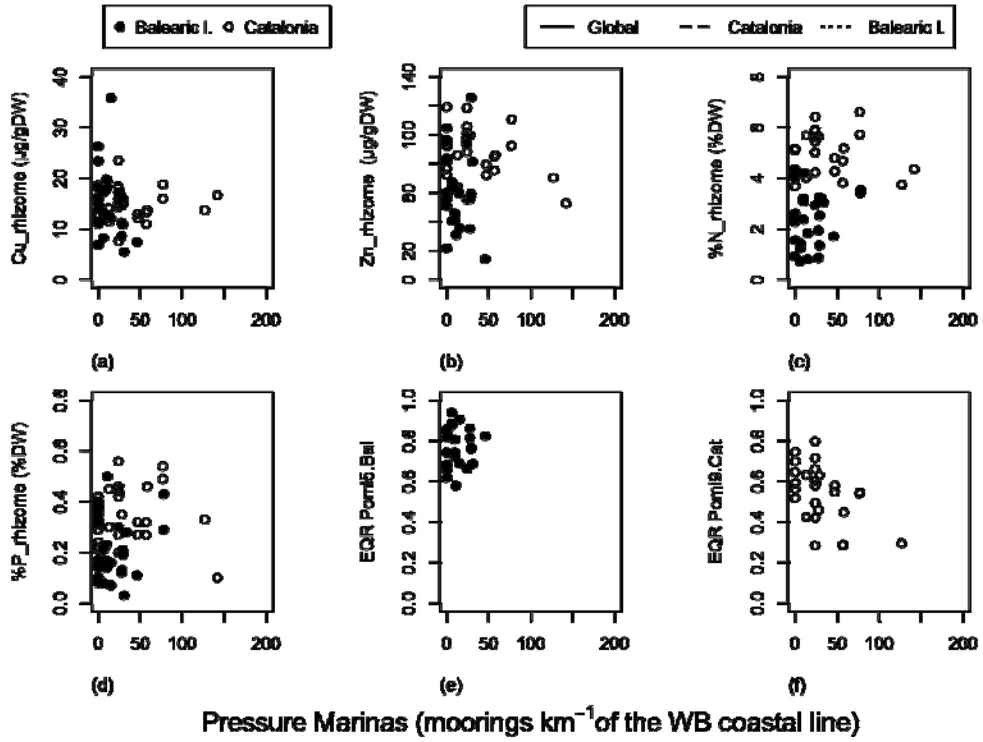


Figure 6. Relationship of pressure of marinas (moorings km^{-1} of the water body coastal line) *vs. Posidonia oceanica* metrics a) copper content in rhizomes (ug/g DW), b) zinc content in rhizomes (ug/g DW), c) nitrogen content in rhizomes (%DW), d) phosphorus content in rhizomes (%DW), e) EQR using for Balearic Island using index Pomi5, f) EQR for Catalonia using index Pomi9.

When analysing Catalonian and Balearic Islands coastal zones globally the pressure due to increasing shipping traffic showed significant and positive relationships with lead concentration in *P. oceanica* rhizome tissue, with percentage of sacarose in the plant and with percentage cover (Fig. 7b,f,i), and a negative relationship with the concentration of zinc (Fig. 8b). All these relationships were weak with $R^2 < 0.16$ (Table 3). The POMI's EQRs from Catalonia or Balearic Island did not relate to increasing shipping traffic (Fig. 8e,f).

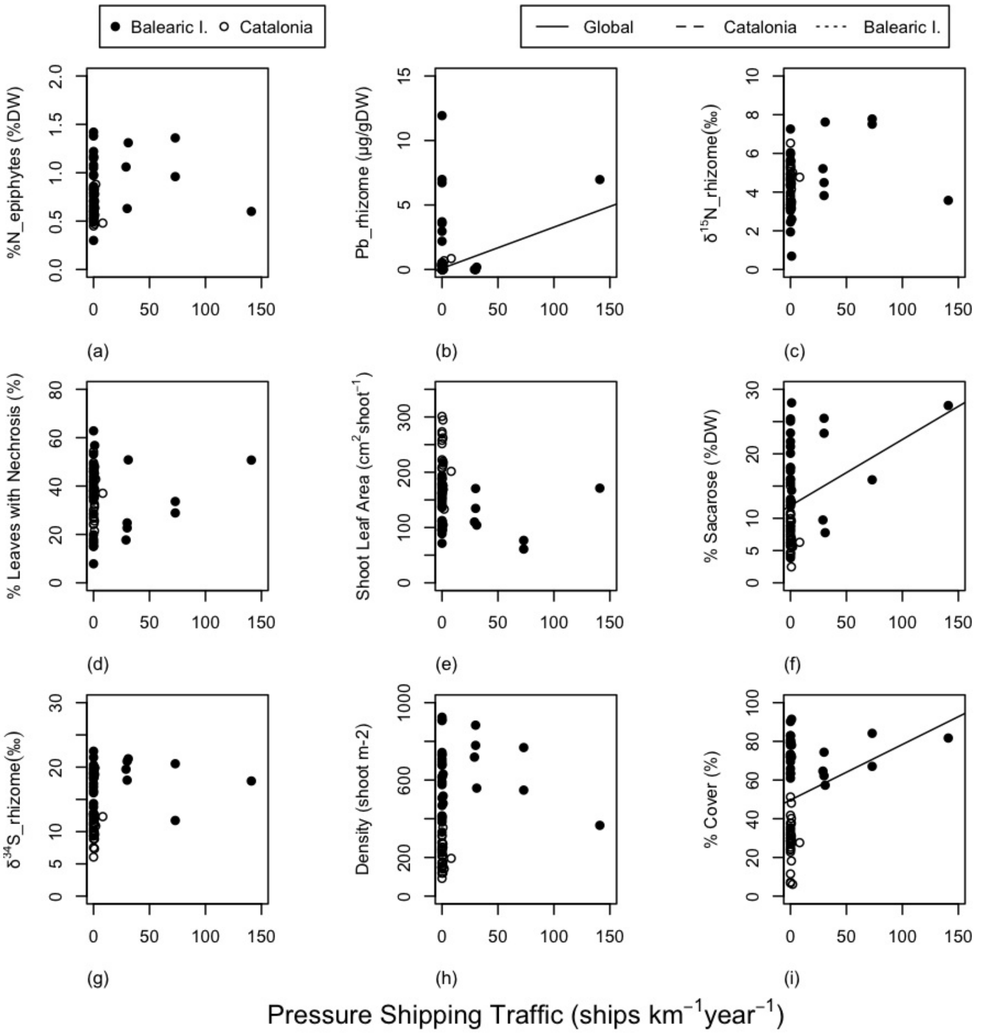


Figure 7. Relationship of pressure of shipping traffic (ships km^{-1} of the water body coastal line year^{-1}) *vs. Posidonia oceanica* metrics a) nitrogen content in epiphytes (%DW), b) lead content in rhizomes (ug/g DW), c) δ^{15}N signature in rhizomes (‰), d) percentage of leaves with nechrosis (%), e) shoot leaf area (cm^{2} shoot^{-1}), f) sacarose content (%DW), g) δ^{34}S signature in rhizomes (‰), h) density (shoot m^{-2}), i) percentage cover (%).

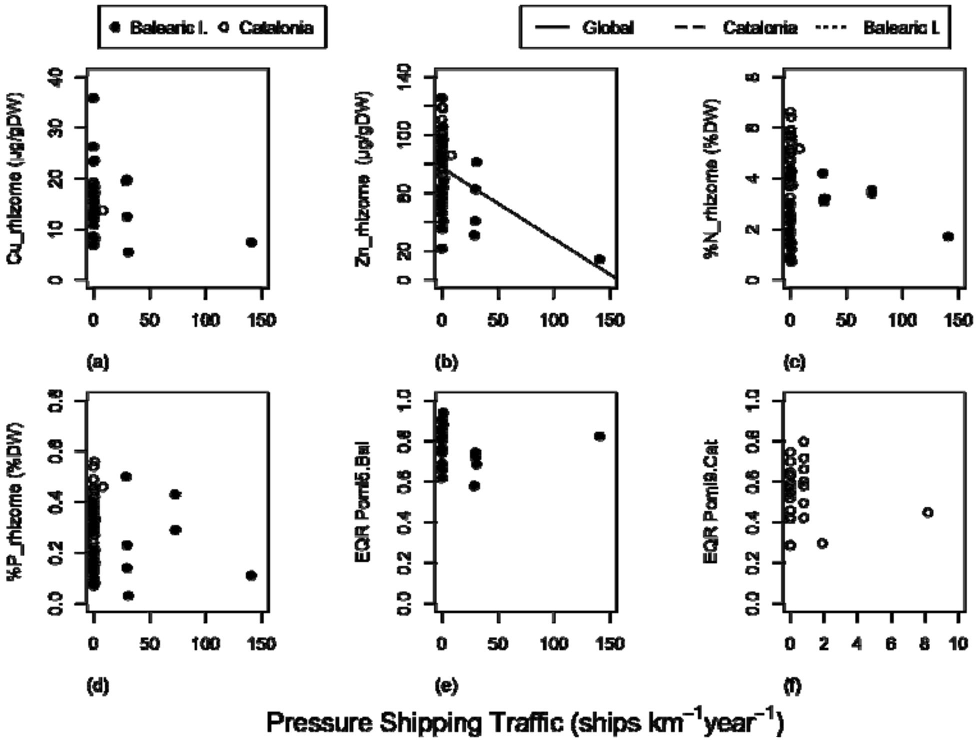


Figure 8. Relationship of pressure of shipping traffic (ships km^{-1} of the water body coastal line year^{-1}) *vs. Posidonia oceanica* metrics a) copper content in rhizomes (ug/g DW), b) zinc content in rhizomes (ug/g DW), c) nitrogen content in rhizomes (%DW), d) phosphorus content in rhizomes (%DW), e) EQR using for Balearic Island using index Pomi5, f) EQR for Catalonia using index Pomi9.

The $\delta^{15}N$ signature in rhizome tissue significantly increased with increasing chemical oxygen demand when analysing Catalonian and Balearic Islands coastal zones globally (Fig 9c). Shoot leaf area in the Balearic coastal zone had a negative and significant relationship with increasing chemical oxygen demand (Fig 9e). In the Catalonian coastal zone there was a significant and positive relationship between $\delta^{34}S$ signature in rhizomes and increasing chemical oxygen demand (Fig. 9g). The R^2 ranged between 0.14 and 0.27 (Table 3). The POMI's EQRs did not show relationships with the chemical oxygen demand pressure (Fig. 10).

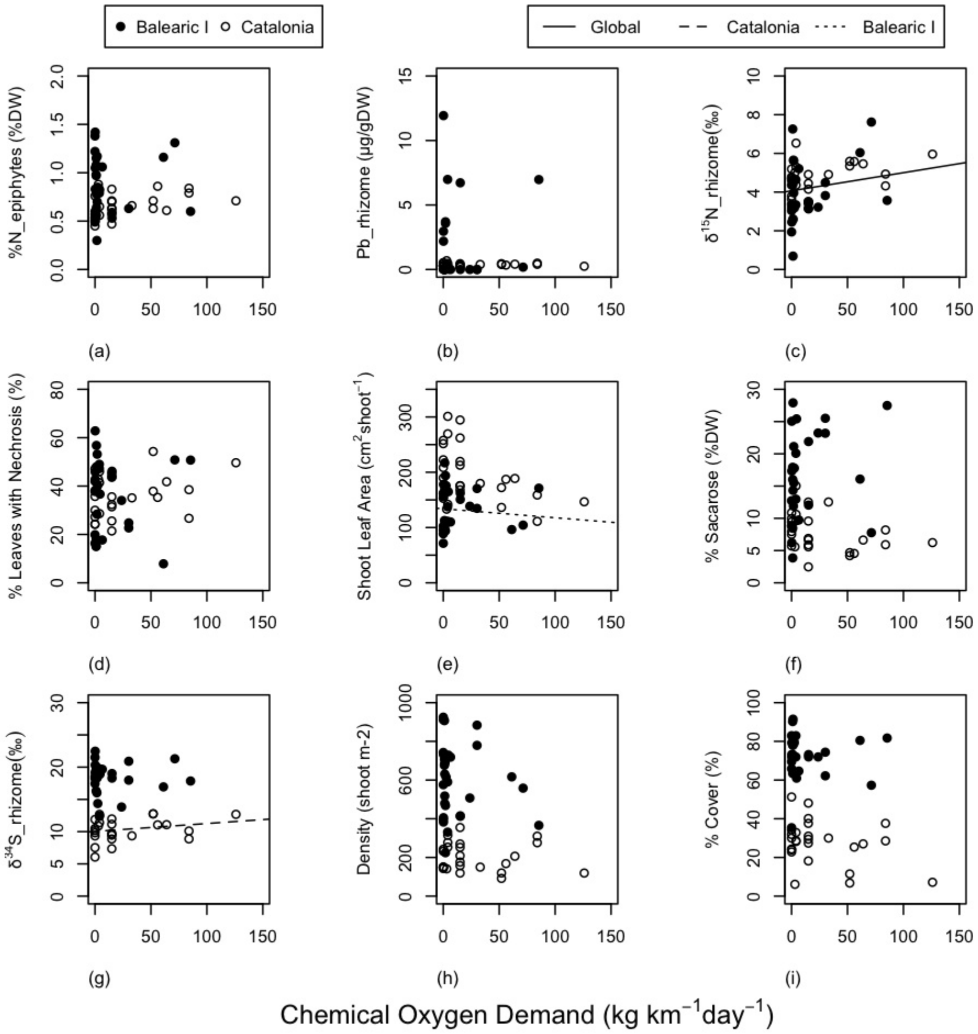


Figure 9. Relationship of the chemical oxygen demand pressure (kg km^{-1} of the water body coastal line day^{-1}) *vs. Posidonia oceanica* metrics a) nitrogen content in epiphytes (%DW), b) lead content in rhizomes (ug/g DW), c) δ^{15}N signature in rhizomes (‰), d) percentage of leaves with nechrosis (%), e) shoot leaf area (cm^{2} shoot^{-1}), f) sacarose content (%DW), g) δ^{34}S signature in rhizomes (‰), h) density (shoot m^{-2}), i) percentage cover (%).

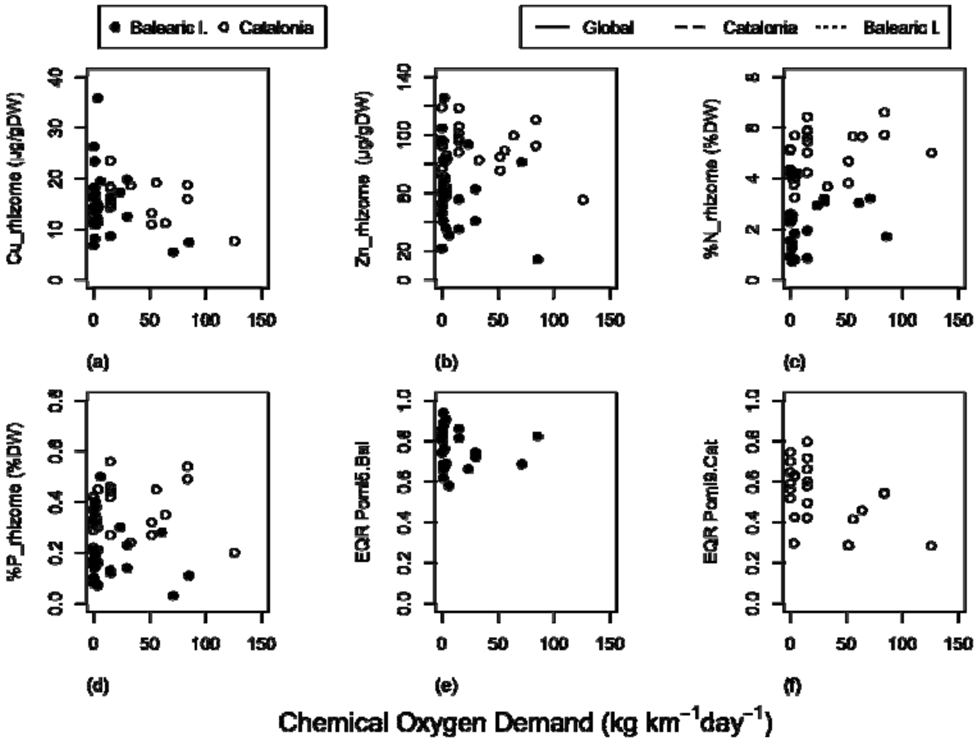


Figure 10. Relationship of the chemical oxygen demand pressure (kg km^{-1} of the water body coastal line day^{-1}) *vs. Posidonia oceanica* metrics a) copper content in rhizomes (ug/g DW), b) zinc content in rhizomes (ug/g DW), c) nitrogen content in rhizomes (%DW), d) phosphorus content in rhizomes (%DW), e) EQR using for Balearic Island using index Pomi5, f) EQR for Catalonia using index Pomi9.

Table 3. Type II significant regression equations fitted between environmental common pressures and *Posidonia oceanica* metrics and POMI's EQRs.

Region	Pressure	Metric	n	r^2	p	Intercept	Slope	Intercept Sterr	Slope Sterr
GLOBAL	Pressure Coastal Rigidification	N_epiphytes	58	0.09	≤0.05	0.69	0.003	0.09	0.002
CATALONIA	Pressure Coastal Rigidification	Pb_rhizome	29	0.29	≤0.01	0.28	0.005	0.07	0.003
GLOBAL	Pressure Coastal Rigidification	d15N_rhizome	58	0.19	≤0.001	3.81	0.02	0.47	0.01
GLOBAL	Pressure Coastal Rigidification	Shoot Leaf Area	61	0.10	≤0.05	180.22	-0.68	20.08	0.53
BALEARIC I.	Pressure Coastal Rigidification	Density	32	0.15	≤0.05	683.36	-2.22	88.72	1.94
CATALONIA	Pressure Coastal Rigidification	Pomi9.Cat	27	0.25	≤0.01	0.62	-0.004	0.08	0.003
GLOBAL	Pressure Beach Regeneration	N_epiphytes	58	0.14	≤0.01	0.74	0.00003	0.07	0.00002
GLOBAL	Pressure Beach Regeneration	d15N_rhizome	60	0.14	≤0.01	4.25	0.0001	0.35	0.0001
GLOBAL	Pressure Beach Regeneration	Shoot Leaf Area	61	0.09	≤0.05	167.62	-0.005	14.97	0.004
GLOBAL	Pressure Beach Regeneration	d34S_rhizome	61	0.06	≤0.05	14.26	0.0003	1.19	0.0003
GLOBAL	Pressure Beach Regeneration	Cover	61	0.09	≤0.05	49.04	0.002	6.49	0.002
CATALONIA	Pressure Beach Regeneration	Pomi9.Cat	27	0.16	≤0.05	0.57	-0.001	0.06	0.001
CATALONIA	Pressure Marinas	N_epiphytes	29	0.23	≤0.01	0.62	0.001	0.05	0.0004
BALEARIC I.	Pressure Marinas	d15N_rhizome	31	0.31	≤0.001	3.45	0.05	0.68	0.03
GLOBAL	Pressure Marinas	Density	61	0.07	≤0.05	446.67	-0.84	66.82	0.80
GLOBAL	Pressure Marinas	Cover	61	0.07	≤0.05	54.44	-0.08	6.78	0.08
GLOBAL	Pressure Shipping Traffic	Pb_rhizome	55	0.08	≤0.05	0.87	0.03	0.60	0.03
GLOBAL	Pressure Shipping Traffic	Sacarose	58	0.09	≤0.05	11.95	0.10	1.93	0.09
GLOBAL	Pressure Shipping Traffic	Cover	61	0.07	≤0.05	49.68	0.29	6.49	0.27
GLOBAL	Pressure Shipping Traffic	Zn_rhizome	55	0.16	≤0.01	77.41	-0.49	6.51	0.31
GLOBAL	Chemical Oxygen Demand	d15N_rhizome	60	0.27	≤0.001	4.07	0.01	0.34	0.004
BALEARIC I.	Chemical Oxygen Demand	Shoot Leaf Area	32	0.14	≤0.05	133.92	-0.16	14.51	0.15
CATALONIA	Chemical Oxygen Demand	d34S_rhizome	29	0.16	≤0.05	10.04	0.01	0.79	0.01

Relationships between Posidonia oceanica metrics and nutrients environmental pressures

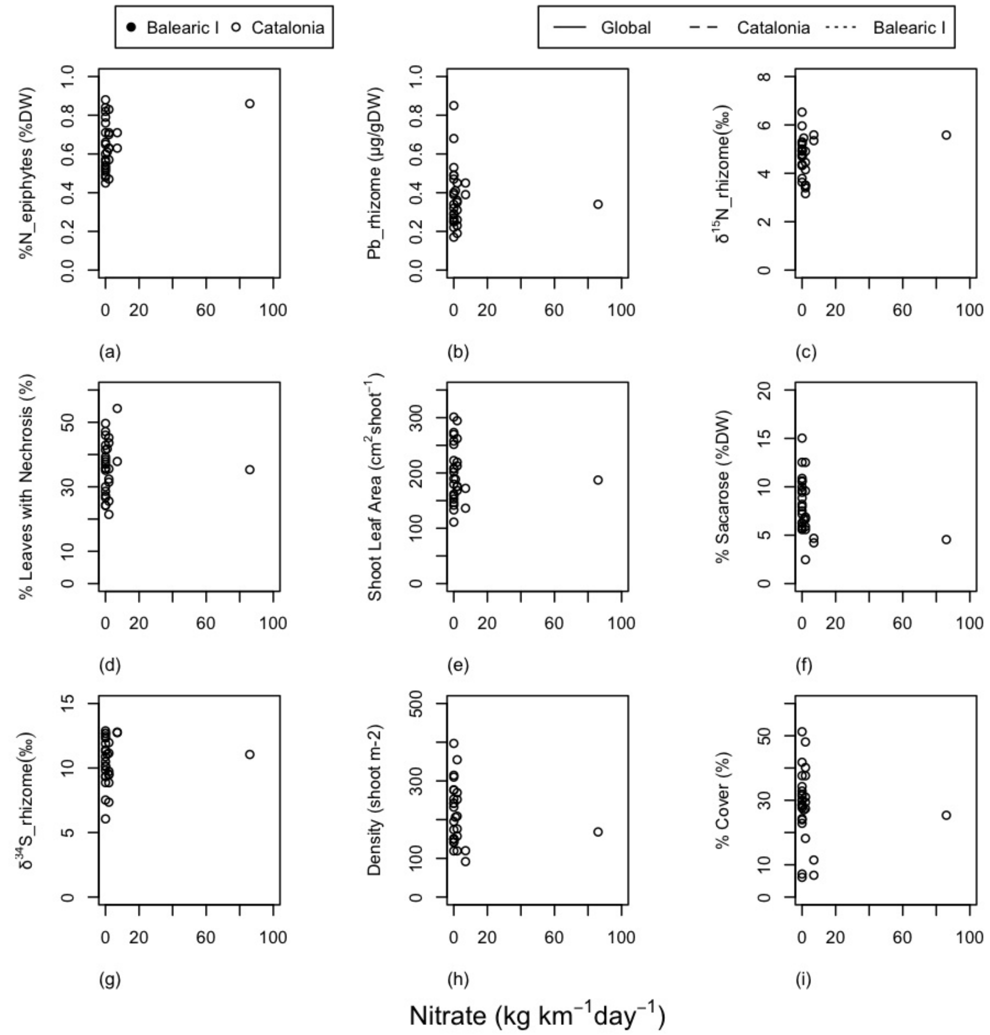


Figure 11. Relationship of pressure of nitrate concentration (kg km^{-1} of the water body coastal line day^{-1}) in Catalonian coastal zone *vs. Posidonia oceanica* metrics a) nitrogen content in epiphytes (%DW), b) lead content in rhizomes (ug/g DW), c) δ^{15}N signature in rhizomes (‰), d) percentage of leaves with nechrosis (%), e) shoot leaf area (cm^2 shoot^{-1}), f) sacarose content (%DW), g) δ^{34}S signature in rhizomes (‰), h) density (shoot m^{-2}), i) percentage cover (%).

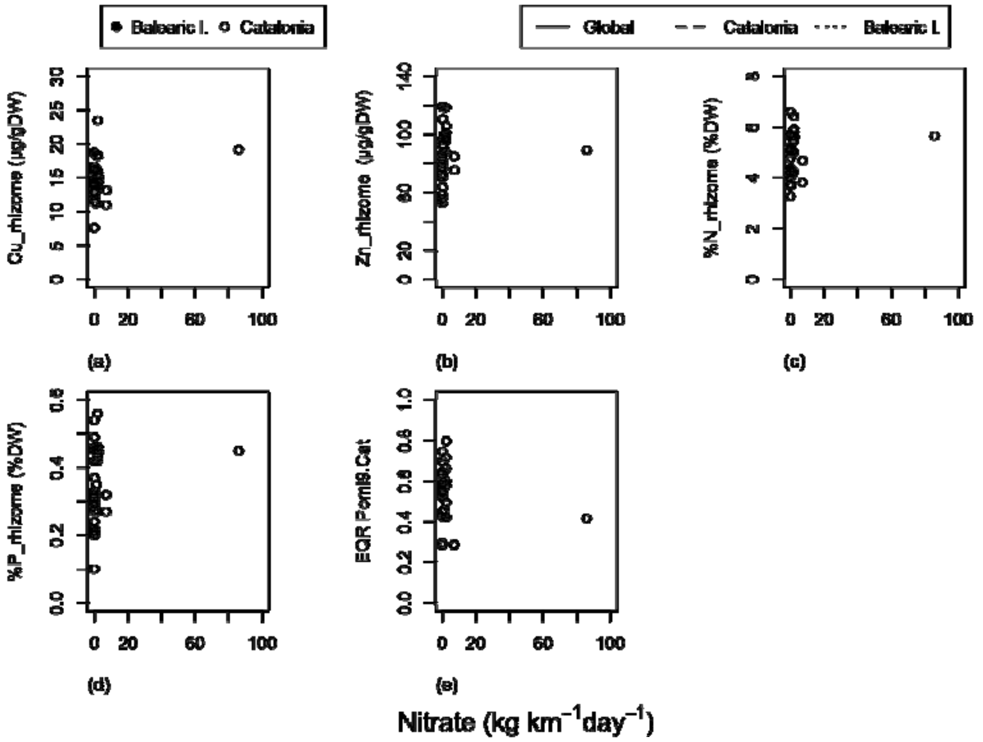


Figure 12. Relationship of pressure of nitrate concentration (kg km^{-1} of the water body coastal line day^{-1}) in Catalonian coastal zone *vs. Posidonia oceanica* metrics a) copper content in rhizomes (ug/g DW), b) zinc content in rhizomes (ug/g DW), c) nitrogen content in rhizomes (%DW), d) phosphorus content in rhizomes (%DW), e) EQR for Catalonia using index Pomi9.

In the Catalonian coastal zones the nutrient pressure was measured as concentration of nitrate. The *P. oceanica* metrics and the POMI's ERQs did not show significant relationships with increasing levels of this pressure (Fig. 11 and 12).

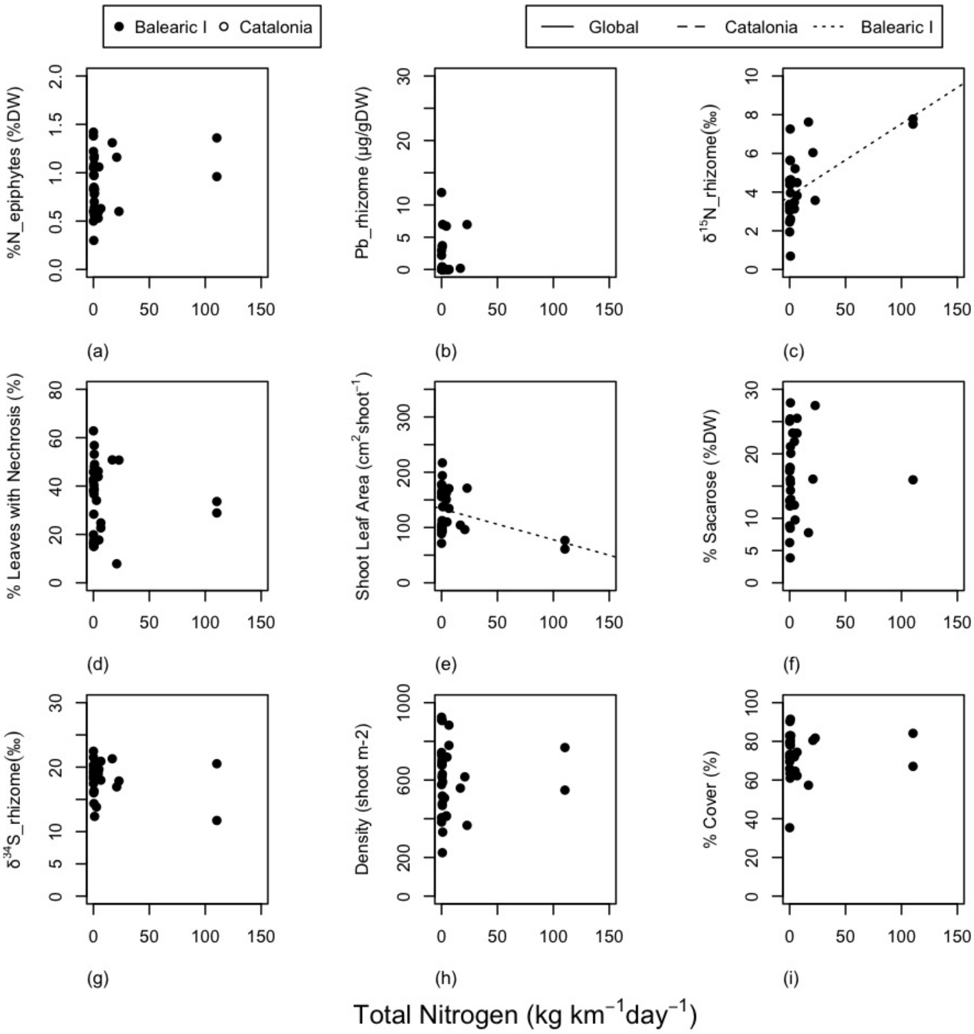


Figure 13. Relationship of pressure of total nitrogen concentration (kg km^{-1} of the water body coastal line day^{-1}) in Balearic Islands coastal zone *vs. Posidonia oceanica* metrics a) nitrogen content in epiphytes (%DW), b) lead content in rhizomes (ug/g DW), c) δ^{15}N signature in rhizomes (‰), d) percentage of leaves with nechrosis (%), e) shoot leaf area (cm^2 shoot^{-1}), f) sacarose content (%DW), g) δ^{34}S signature in rhizomes (‰), h) density (shoot m^{-2}), i) percentage cover (%).

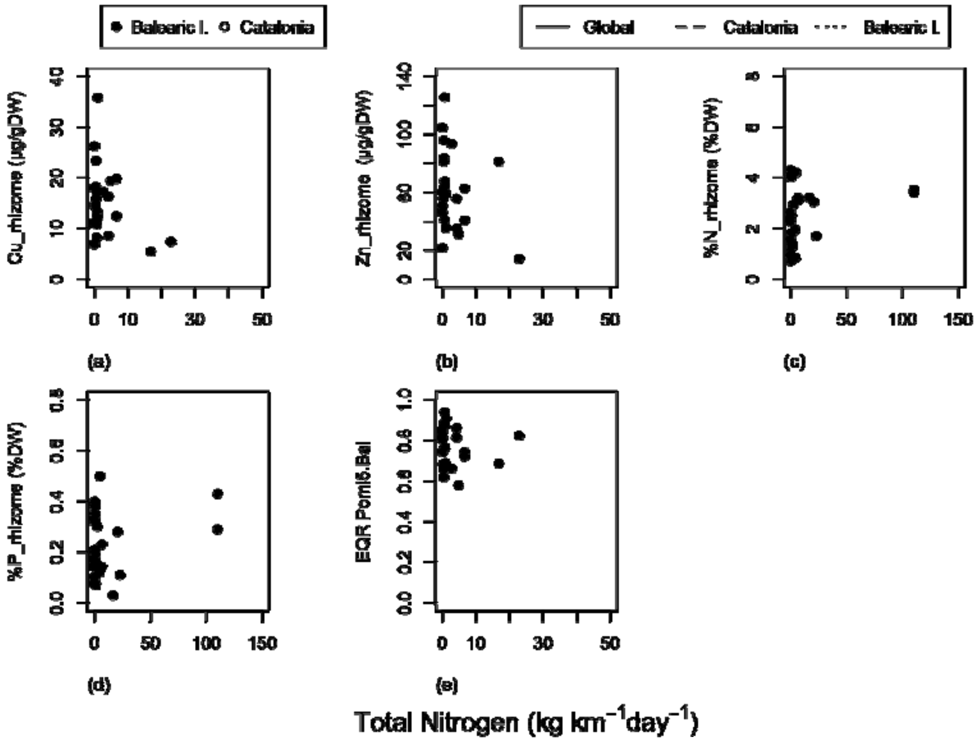


Figure 14. Relationship of pressure of total nitrogen concentration (kg km^{-1} of the water body coastal line day^{-1}) in Balearic Islands coastal zone *vs. Posidonia oceanica* metrics a) copper content in rhizomes (ug/g DW), b) zinc content in rhizomes (ug/g DW), c) nitrogen content in rhizomes (%DW), d) phosphorus content in rhizomes (%DW), e) EQR for Balearic Island using index Pomi5.

In the Balearic Islands coastal zone nutrient pressures were measured as concentrations of total nitrogen and phosphorus. The $\delta^{15}N$ signature in *P. oceanica* rhizomes showed a significant and positive relationship with increasing nitrogen pressure (Figure 13c), and the R^2 was > 0.3 (Table 4). Shoot leaf area showed a significant and negative relationship with a lower R^2 (Fig. 13e, Table 4). The POMI's EQR for the Balearic Islands coastal zone did not show a relationship with the nitrogen pressure (Fig. 14e).

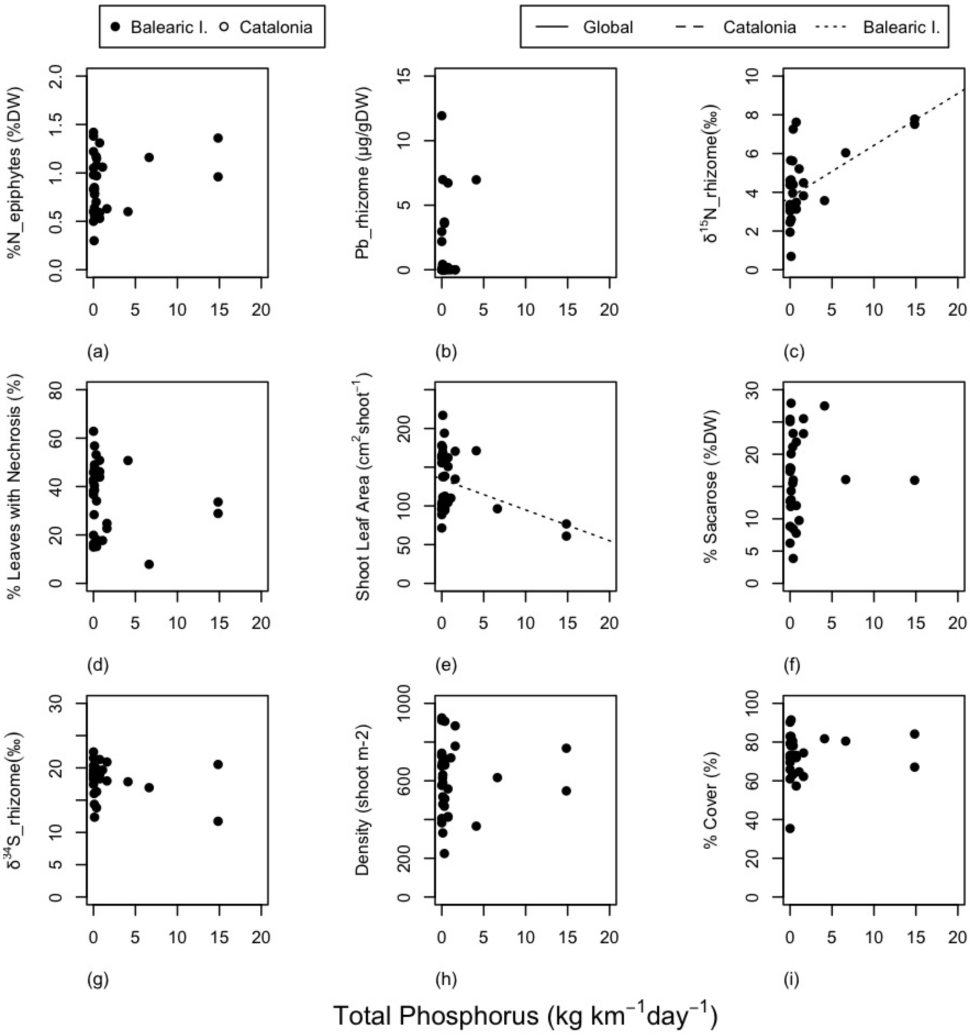


Figure 15. Relationship of pressure of total phosphorus concentration (kg km^{-1} of the water body coastal line day^{-1}) in Balearic Islands coastal zone *vs. Posidonia oceanica* metrics a) nitrogen content in epiphytes (%DW), b) lead content in rhizomes (ug/g DW), c) δ^{15}N signature in rhizomes (‰), d) percentage of leaves with nechrosis (%), e) shoot leaf area (cm^2 shoot^{-1}), f) sacarose content (%DW), g) δ^{34}S signature in rhizomes (‰), h) density (shoot m^{-2}), i) percentage cover (%).

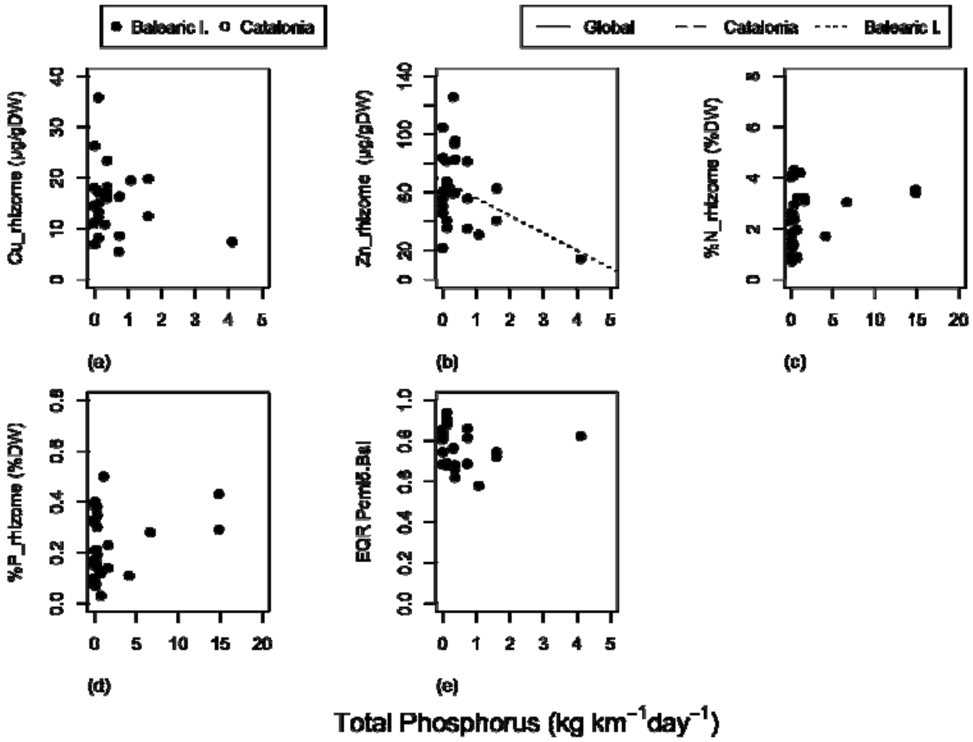
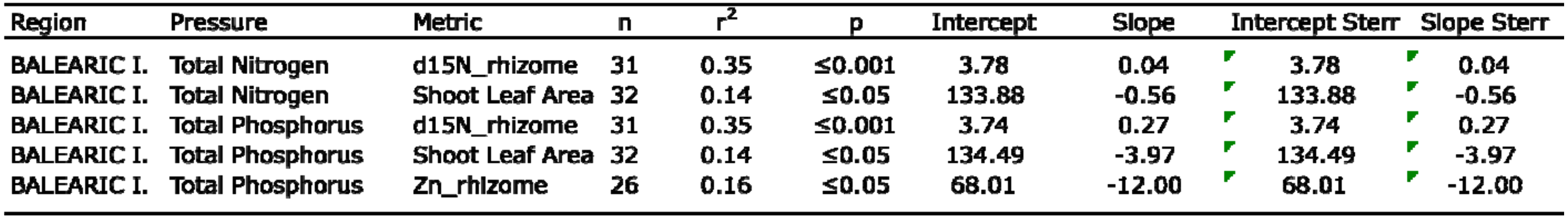


Figure 16. Relationship of pressure of total phosphorus concentration (kg km^{-1} of the water body coastal line day^{-1}) in Balearic Islands coastal zone *vs. Posidonia oceanica* metrics a) copper content in rhizomes (ug/g DW), b) zinc content in rhizomes (ug/g DW), c) nitrogen content in rhizomes (%DW), d) phosphorus content in rhizomes (%DW), e) EQR for Balearic Island using index Pomi5.

The δ^{15}N signature in *P. oceanica* rhizomes also showed a significant and positive relationship with increasing phosphorus pressure (Figure 15c), and the R^2 was also > 0.3 (Table 4). Shoot leaf area showed a significant and negative relationship with phosphorus and a lower R^2 (Fig. 15e, Table 4). The concentration of zinc in rhizome tissue significantly and negatively related with total phosphorus pressure (Fig. 16b), being the R^2 = 0.16. The POMI's EQR also did not show a relationship with phosphorus pressure (Fig. 16e).

Table 4. Type II significant regression equations fitted between nutrient pressures and *Posidonia oceanica* metrics



Region	Pressure	Metric	n	r^2	p	Intercept	Slope	Intercept Sterr	Slope Sterr
BALEARIC I.	Total Nitrogen	d15N_rhizome	31	0.35	≤0.001	3.78	0.04	3.78	0.04
BALEARIC I.	Total Nitrogen	Shoot Leaf Area	32	0.14	≤0.05	133.88	-0.56	133.88	-0.56
BALEARIC I.	Total Phosphorus	d15N_rhizome	31	0.35	≤0.001	3.74	0.27	3.74	0.27
BALEARIC I.	Total Phosphorus	Shoot Leaf Area	32	0.14	≤0.05	134.49	-3.97	134.49	-3.97
BALEARIC I.	Total Phosphorus	Zn_rhlzome	26	0.16	≤0.05	68.01	-12.00	68.01	-12.00

Environmental pressures contribution to Posidonia oceanica metrics variability

The stepwise regression analysis showed that when considering Balearic Islands and Catalonia coastal zones together, the pressures contributing the most to explain the variance within the metrics data were the pressure of coastal rigidification (PCR), the pressure of marinas (PM), the pressure of beach regeneration (PBR) and the pressure of chemical oxygen demand (COB). The $r^2 < 0.1$ (Table 5) indicated that less than 10% of the metrics variance was explained by the regression models between metrics and pressures. In the regression model between the metric $\delta^{15}N$ signature in rhizomes and the pressures of coastal rigidification and chemical oxygen demand the $r^2 = 0.29$, thus indicating a stronger relationship.

When applying the stepwise regression analysis to the Balearic Islands and Catalonia coastal zones separately, the pressures that contributed the most to explain the variance in the metrics in the Balearic Islands coastal zone were the pressure of coastal rigidification (PCR), the pressure of marinas (PM), the pressure of shipping traffic (PST), the pressure chemical oxygen demand (COB), and the nutrients pressures: total nitrogen (TN) and total phosphorus (TP). The highest r^2 was for the regression model between the metric $\delta^{15}N$ signature in rhizomes and the environmental pressures ($r^2 = 0.42$). The other regression models had $0.06 < r^2 < 0.14$ (Table 5), thus explaining little percentage of the metrics' variance. For Catalonia coastal zone, the pressures contributed to explain the variability in the metrics were the pressure of coastal rigidification (PCR), the pressure of shipping traffic (PST) and the pressure chemical oxygen demand (COB). The regression models between metrics and pressures had higher r^2 values, ranging between $0.21 < r^2 < 0.49$ (Table 5).

Table 5. Stepwise regression analysis of metrics as dependent variables and pressures as independent variables in Catalonia and Balearic Islands together, and separately. The excluded variables are shown in the order of exclusion. PCR = pressure of coastal rigidification, PM= the pressure of marinas, PBR= the pressure of beach regeneration, PST =the pressure of shipping traffic, COB= the pressure chemical oxygen demand, NO3= nitrate pressure, TN= total nitrogen pressure, and TP= total phosphorus pressure.

Region	Metrics	Ajusted r^2	F	p	Predictor variable	Beta	p	Excluded variables	Lack relationship
Global	N_epiphytes	0.04	4.77	<0.05	PM	0.24	<0.05	PCR, PBR, PST, COB	necrosis, Zn_rhizome, Cu_rhizome
	Pb_rhizome	0.07	6.82	<0.05	PCR	0.28	<0.06	PST, PBR, PM,COB	
	d15N_rhizome	0.29	8.11	<0.01	COB	0.33	<0.01	PM, PBR, PST	
					PCR	0.31	<0.01		
	Shoot Leaf Area	0.04	4.74	<0.05	PCR	-0.23	<0.05	PST, COB, PBR, PM	
	Sacarose	0.06	5.81	<0.05	PM	-0.26	<0.05	PCR, COB, PBR, PST	
	d34S_rhizome	0.05	5.02	<0.05	PM	-0.26	<0.05	PCR, PST, COB	
					PBR	0.23	<0.05		
	Density	0.07	7.67	<0.01	PM	-0.29	<0.01	PCR, COB, PBR, PST	
	Cover	0.07	7.85	<0.01	PM	-0.29	<0.02	COB, PST, PBR, PCR	
	N_rhizome	0.06	6.78	<0.05	PM	0.28	<0.05	PCR, PST, COB, PBR	
	P_rhizome	0.04	4.77	<0.05	PM	0.24	<0.05	PCR, PBR, PST, COB	
Balearic I.	Pb_rhizome	0.06	4.12	<0.05	PCR	0.28	<0.05	PM, PST, PBR, TN, COB, TP	N_epiphytes, necrosis, sacarose, d34S_rhizome, Cover, Zn_riz, Cu_rhizome, N_riz, Pomi5
	d15N_rhizome	0.42	11.35	<0.001	COB	2.11	<0.001	TN, PBR	
					PCR	1.30	<0.001		
					PM	-1.20	<0.01		
					TP	-1.14	<0.01		
					PST	-0.32	<0.05		
	Shoot Leaf Area	0.08	4.56	<0.05	TN	-0.27	<0.05	COB, TP, PST, PM, PBR, PCR	
	Density	0.09	6.80	<0.05	PCR	-0.33	<0.05	TP, TN, COB, PBR, PST, PM	
	P_rhizome	0.14	5.29	<0.01	TP	0.75	<0.01	PCR, COB, TN, PBR, PST	
					PM	-0.54	<0.05		
Catalonia	N_epiphytes	0.38	9.53	<0.001	PCR	0.77	<0.001	PBR, PST, PM, NO3	d15N_rhizome necrosis, sacarose Density, Cover Zn_rhizome, Cu_rhizome, N_rhizome, P_rhizome
					COB	-0.48	<0.05		
	Pb_rhizome	0.50	14.97	<0.001	PST	0.53	<0.001	COB, NO3, PM, PBR	
					PCR	0.34	<0.05		
	Shoot Leaf Area	0.22	7.45	<0.05	PBR	-0.47	<0.05	COB, PCR, PM, NO3, PST	
	d34S_rhizome	0.16	5.21	<0.05	COB	0.40	<0.05	PCR, PM, NO3, PST, PBR	
	Pomi9	0.22	8.30	<0.01	PCR	-0.50	<0.01	PBR, PST, NO3, PM, COB	

Summary

As a general pattern the relationships between the *P. oceanica* metrics and environmental pressures were weak, the regression models explaining in the best of the cases less than 50% of the variance within the metrics. In most of the regressions typical values for r^2 ranged between $0.05 < r^2 < 0.2$ (Table 3,4 and 5). The r^2 in the Catalonian coastal zone however were higher ($0.14 < r^2 < 0.35$) when compared to the Balearic Island coastal zone, or to both regions together. Furthermore, only in Catalonia the EQR obtained from the POMI index showed relationships with the pressures (Fig. 4, Table 3 and 5). Hence, in general the lack of strong relationships would indicate that the variance of *P. oceanica* metrics might be more strongly dependent on other non-tested environmental parameters, and/or that the intricate interactions of ecological processes and anthropogenic pressures affecting the metrics in the environment are too complex to show clear relationships.

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Case study 2: Ecological evaluation index (EEI) along gradients of anthropogenic pressures in Lesina lagoon, Italy and in Varna bay and lake, Bulgaria

By Sotiris Orfanidis (USalento), Kristina Dencheva (IO-BAS), Valentina Doncheva (IO-BAS), Galina Stereva (IO-BAS), Kremena Stefanova (IO-BAS)

2 Materials and methods

2.1. Sites description and anthropogenic pressures

In Italy (Puglia region, southeastern Italy, Adriatic Sea), the Lesina lagoon is a shallow (maximum water depth 2 m), large (surface area 51 km^2), non-tidal and mesohaline transitional water body (Figure 1a). It is connected with the sea through two narrow and relatively long canals (Acquarotta on the west side and Schiapparo on the east one), and geomorphologically divided into three water basins: a western, a central and an eastern basin. Potentially the lagoon has a low vulnerability to human activities, since its watershed is only 8 times larger than the lagoon surface. However, urban and agricultural wastewater discharges enter the lagoon particularly in the western basin (Table 1), leading to pulse eutrophication events (Vignes *et al.,* 2009). During summer 2008 a strong dystrophic crisis occurred in the western basin, determining hypoxic conditions for a few weeks over an area up to 2.0 km^2, significantly affecting all ecosystem compartments (Specchiulli *et al*., 2009; Vadrucci *et al*., 2009). Nutrient load from wastewaters, reduced hydrodynamism and extreme climate events were advocated as major causes of the dystrophic event (Vignes *et al.,* 2009).

The Bulgarian site is located in the western part of the Black Sea coast (microtidal, mesohaline water system) and includes the Varna lake, the south navigation canal between the lake and Varna bay (Figure 1b). The canal is 6 km long with a maximum depth of 12.5 m. Varna bay is the second largest bay in Bulgaria with a surface area of 20 km^2 and a maximum depth of 18.5 m. It is 4.5 km long and 8 km wide. Varna is the largest Bulgarian city on the Black Sea; it is a regional centre, accommodating more than 300,000 inhabitants, and an important holiday destination, attracting large numbers of tourists, particularly during the summer season. The spatial distribution of urban and industrial infrastructures determines a well defined gradient of decreasing pressures from the inner part of the lake towards the Bay (Table 1). Varna Lake is one of the important Black Sea coastal lakes affected by human activities. It is situated in NE part of Bulgaria and it is one of the biggest coastal lakes with average depth 9.5 m. It covers about 17 km^2 area. Hydrochemical peculiarities of Varna Lake are determined by its connections with Beloslav Lake and Varna Bay. The first one is very important for the recent state of the lake water quality because Beloslav Lake receives contaminated industrial and domestic waste waters. The other main factors for water deterioration are agriculture and port activities, maritime transport shipping, nutrients and organic matter enrichment. The lake eutrophication which is a significant and increasing problem has been well documented (Rozhdestvensky, 1986; Stoyanov, 1991; Shtereva et al. 2004 , Dencheva,2010). The events of hypoxia/anoxia and fish mortality as consequences of water contamination and eutrophication have been reported (Rozhdestvensky, 1992).

Figure 1. Map of study sites in two locations: (a) Lesina lagoon, (b) Bulgaria-Varna bay and lake.

a)

b)

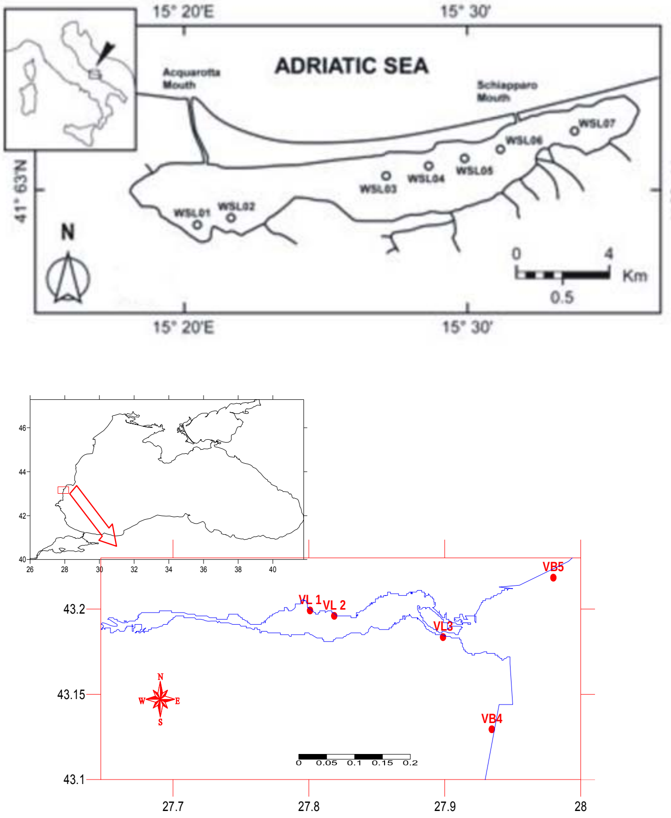


Table 1. Pressures determined at each location and sampling site (see Figure 1), showing the pressure gradient in the total value. Values: 1- low pressure; 2- moderate pressure; 3- high pressure.

Type of pressures	**Non-point sources**		**Pollution**			**Habitat loss**	**Industry**			**Ports**			**Fisheries**		**Physico-chemical**					
Pressures	**Agricultural diffuse inputs**	**Freshwater input**	**Domestic discharges**	**Domestic/industrial discharges**	**Industrial discharges**	**Land-claim**	**Industrial area**	**Water abstraction**	**Power generation**	**Port activity**	**Navigation**	**Dredging**	**Fin-Fisheries**	**Shell-fisheries**	**Chlorophyll**	**Nutrients DIN**	**Nutrients P**	**Oxygen**	**Turbidity**	**TOTAL**
Lesina lagoon																				
WSL01	2		2		3								3							10
WSL02	2		2		2								3							9
WSL06	2		1		1								3							7
WSL07	1		1		1								3							6
WSL03	1												3							4
WSL04	1												3							4
WSL05	1												3							4
Varna bay and lake																				
VL1	1		3		3		3		3							3	3	3	3	25
VL2	1		3		1											3	3	3	3	18
VL3			2		2				1	1						3	3	3	3	17
VB4	2		1													2	2	2	2	11
VB5			2													1	1	1	1	6

2.2. Definition of anthropogenic pressure indices

Pressures were quantified (low, medium and high) for each location and sampling site, as partial pressure and total pressure. The total pressure is the sum of partial pressures.

When available, quantitative data used for defining pressures were obtained from the systems' time-series. Physico-chemical parameters correspond to averaged monthly measurements (surface and/or bottom). Other types of pressures were defined based on expert judgment.

2.3. Sampling sites and analyses

Seven (7) sampling sites were selected in Lesina lagoon (Figure 1a). The sites were selected following the experience achieved with the monitoring programme and the analysis of a dystrophic crisis event in 2008, by setting the sites along a gradient from the western to the eastern basin of the lagoon. Sampling survey of the 7 stations was undertaken between 21st and 23rd September 2009. A 0.0225 m^2 Ekman grab was used to collect twelve replicates samples in each site.

In the laboratory, the formalin preserved samples were first washed in tap water for a few seconds, passed through a sieve of 500 µm and then transferred to sea water. Benthic macrophytes were then very carefully sorted out and species were identified to functional group level and as much as possible to species level by using a stereoscope and a binocular microscope. Taxonomically difficult taxa were consistently summarized to genus level as spp. No detailed taxonomic analysis of Cyanobacteria colonies was undertaken.

In order to estimate % coverage, a transparent double bottom square PVC container, filled with sea water and having at its bottom a square 15x15 cm matrix divided in 100 squares was used. The surface covered by each sorted taxon in vertical projection floating in sea water was quantified as % of coverage (2.25 cm^2 = 1% sampling surface). The % coverage of epiphytes on seagrass leaves was roughly assessed without removal of the epiphytes from the host plants. The total coverage often exceeded 100% due to the presence of different layers in the vegetation i.e. mainly canopy and understorey layers. For species present with insignificant abundance a coverage value of 0.01 % was allocated. From each sample, voucher specimens of taxonomically difficult taxa were fixed in 3-5% formalin sea water, which were deposited in the Fisheries Research Institute for future study.

Varna bay and lake were sampled on 8th -10th September 2009. Five sampling sites were selected (Figure1 b), specifically taking into account the environmental gradient of nutrients, whose concentrations decrease from the lake to the bay. The main current from lake to the south part of the bay is one of the main reasons for worse environmental conditions in this part in comparison with the north part. At each transect (sampling site) different number (from 7 to 42) square frame (0.01 m^2) samples and an additional sample with corer, for the sediment analysis were collected. Samples were taken from 0-2 m depth with the help of diving technique. Totally, 122 samples were processed. Visual assessment of total percent cover of the communities of every depth layer was carried out.

Macrophyte samples were frozen for preservation to -20°C till the processing for biomass estimation, without damage of cellular structure. In laboratory conditions all benthic macrophyte samples were washed and sieved to remove sediments. Macrophytes were sorted and identified to the lowest possible taxonomic level under microscope when needed. Species were dried for a while on a filter paper and weighted (fresh weight). Taxonomy was standardized using Algae base: http: //www.algabase.org

Sediment samples were processed at the Institute of Oceanology-Varna. Particle size distribution was determined for fractions less than 2 mm and dry sieving through a nest of sieves for coarser particles. To determine the organic content, the samples are dried at room temperature. Chemical oxidation of organic Carbon (C org) is carried out by “wet ashing” of the dried sediments using sulphuric acid mixture of dichromate at high temperature followed by photometric measurement. Organic content was expressed as TOC and all data were incorporated into a database.

2.4. Metrics and methods calculation

Eight metrics related to community structure [species number, Shannon-Weaver index (H’, $\log_2$), Pielou’s evenness index (J’), % of total coverage, and dry biomass (g/m^{-2})] and function (ESG I % coverage, ESG II % coverage, and EEI) were estimated. Fresh weight biomass data from Bulgarian coasts were multiplied by factor 0.09 to be transformed to dry biomass. The calculation of diversity indices was based on coverage measurements of vegetated samples and was performed using the PRIMER software. The abundance of the two Ecological State groups (ESG I, ESG II) and the Ecological Evaluation Index (EEI-c) for each site were calculated according to Orfanidis *et al*. (2001, 2003, 2011). The calculation of EEI-c in site 7 was modified by introducing a new group (ESG IIC) that included species of fresh water affinity such as *Potamogeton* sp. This species is valued similarly to opportunistic species (ESG IIB) since its existence in the site 7 is explained by low salinity (close to 10 PSU) that prohibits their growth. A modification of EEI index (Orfanidis et al., 2001, 2011) in conformity with Black Sea peculiarities was also developed. Index values are represented as biomass percent ratio of late-successional (sensitive species) divided by biomass of sensitive and opportunistic-tolerant species. Total cover value of macrophyte communities from every site was multiplied by percent biomass values to obtain final EI, presented as continuous numerical values from 0 to 10. For example: for 80% sensitive species biomass, EI is equal to “8”; 65% biomass is “6.5” EI value. EQR value has been calculated as current obtained EI value divided by referent value (10). EQR values for different ecological state classes are following: (0-0.2- “bad”; 0.2-0.4- “poor”; 0.4-0.6- “moderate”, 0.6-0.8 - “ good” and 0.8-1- “high status”.

2.5. Statistical treatment

Only samples with a % coverage >10% were analyzed. The response of metrics and assessment method to the pressure gradient was evaluated using Pearson correlation (r) and Spearman rank correlation coefficients (ρ) at p=0.01 after a log (x+1) transformation of the data. All metrics variation across the sampling sites were analysed using one-way ANOVA. Since not any transformation of the data undertaken the significance level boundary was set at 0.01. All statistical analyses were undertaken using “Statistica v. 7 and 7.1” software package. PCA analyses were performed on log (x+1) transformed data using the PRIMER software.

3. Results

The total pressures and the main environmental characteristics of each sampled site can be seen in Tables 1 and 2, respectively. From the PCA, components 1 and 2, which explains 86.2 % of the total variability (component 1=65.7%, component 2=20.5%) is inferred that the sampled sites belong in a gradient rather than in distinct environments and water types, in terms of depth, salinity, grain size etc (Figure 2).

Key biotic metrics of the benthic marophyte communities in the sampled sites are presented in Table 3. When studying the metrics correlation with the total pressures and the abiotic factors quite similar results as regards the two different statistical approaches were found (Tables 4 and 5). The strongest correlations were found between the total pressures and the functional indices EEI and EEI-c (-0.88 to -0.92 for Pearson correlation, -0.9 to -0.95 for Spearman rank correlation). In Figure 3 the non-linear relationships between the total pressures and the EEI and EEI-c are presented. These indices were also correlated with % organic content (0.77 to 0.71 for Pearson correlation, 0.76 to 0.73 for Spearman rank correlation). Strong correlations were also found between the total pressures and the functional index ESG I (-0.88 for Pearson correlation, -0.83 for Spearman rank correlation). The ESG I index was also correlated with distance to the pressures (0.72 for Pearson correlation, 0.62 for Spearman rank correlation). The structural indices J’ and H’ were significantly correlated with the organic content (-0.77 to -0.83 for Pearson correlation, -0.65 to -0.71 for Spearman rank correlation) and with the percent of mud (-0.78 to -0.81 for Pearson correlation, -0.72 to -0.70 for Spearman rank correlation). While the index H’ was also correlated with the distance to pressures (0.61 for Spearman rank correlation) the J’ index was correlated with salinity (-0.61 for Spearman rank correlation). The structural index Species No was correlated with depth (0.82 for Pearson correlation, 0.63 for Spearman rank correlation), with turbidity (0.77 for Pearson correlation, 0.63 for Spearman rank correlation) and with % of organic content (-0.75 for Pearson and for Spearman rank correlation). The total dry biomass was correlated with % oxygen saturation (0.67 for Pearson correlation) and with distance to the pressures (0.67 for Spearman rank correlation). The variation of selected biotic indices across the sampled sites in Lesina and Bulgarian coasts are presented in Figure 4.

Using the EEI methodology as modified for Lesina lagoon and Varna lake and bay (see above) the studied sites were classified as: VB4 - "bad" ESC; VL1, VL2, VL3, WSL01, WSL02 - “low” ESC; VB5 - “moderate” ESC; WSL03, WSL04, WSL07 - “good” ESC; and WSL5 -“high” ESC. While the mean value of EEI-c index (0.56) classifies the Lesina lagoon in “good” ESC, the mean value of EI (0.13) classifies the Varna bay and lake coasts in “bad” ESC.

Table 2. Key environmental characteristics of the sampling sites.

Country and water type (name of the site)	**Site**	**Depth (m)**	**Distance to the pressure (km)**	**Temperature (ºC)**	**Salinity PSU**	**Oxygen Saturation (%)**	**Turbidity (Secchi disk) (cm)**	**Redox potential (mV)**	**Gravel (%)**	**Sand (%)**	**Mud (%)**	**Organic Content (%)**
Italian lagoon	WSL01	-1	0.60	24	18,06	89.3	70	-429	6.1	55.4	38.5	4.7
(Lesina)	WSL02	-1	0.98	25	18.46	92	100	-384	4.3	46.1	49.6	5.6
	WSL03	-1.1	7.76	25.8	17.36	84.7	110	-382	1.9	58.4	39.8	10.4
	WSL04	-1.1	9.30	24.6	17.02	117.7	110	-360	4.9	66.7	28.4	9.4
	WSL05	-1.2	11.27	24.4	17.28	73.5	120	-393	3.6	63.2	33.2	14.0
	WSL06	-1.05	12.79	24.4	16.55	114.1	105	-384	4.4	70.0	25.5	8.7
	WSL07	-0.6	15.88	24.4	13.06	150	60	-333	0.3	63.5	36.3	9.8
Bulgarian bay and lake	VL1	-0.5	1.31	24.9	14.4	76	50	na	57.6	41.7	0.6	0.5
(Varna)	VL2	-1	2.09	24.5	15.2	83	100	na	55.9	43.2	0.9	0.43
	VL3	-1.5	9.68	24.3	15.1	89	150	na	0	55	45	0.4
	VB4	-2	17.4	24.8	16.9	98	180	na	26.4	72.7	0.9	0.1
	VB5	-2	19.18	24.1	16.1	118	250	na	4.6	94.4	1	0.05

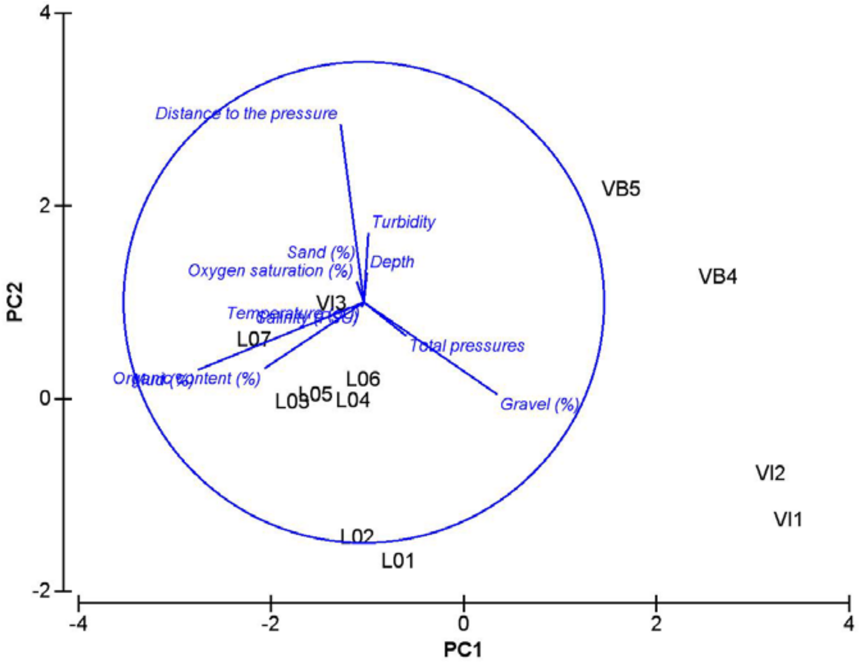


Figure 2. Principal Component Analysis using the total pressure (Table 1) and the abiotic data (Table 2) together with the sampling sites of Lesina lagoon and Bulgarian coasts.

Table 3. Key biotic metrics of the benthic marophyte communities in the sampling sites. NV=no vegetation.

Country and water type (name of the site)	**Site**	**Total Coverage (%)**	**Total dry Biomass (g/m^2)**	**Species No**	**J'**	**H'**	**ESG I (%)**	**ESG II (%)**	**EQR EEI (EEI-c, EI)**	**Dominant species**
Italian lagoon	WSL01	27.21	45.60	4.60	0.18	0.43	0.00	27.21	0.25	*Gracilaria* sp.
(Lesina)	WSL02	73.97	49.33	4.40	0.25	0.59	0.00	73.97	0.08	*Cladophora* spp.
	WSL03	42.06	51.26	5.50	0.27	0.63	35.00	7.06	0.76	*Nanozostera noltii*
	WSL04	232.46	347.74	4.92	0.42	0.94	156.67	75.79	0.69	*Ruppia cirrhosa*
	WSL05	94.20	107.68	3.36	0.51	0.83	71.33	22.87	0.87	*Nanonzostera noltii*
	WSL06	NV	NV	NV	NV	NV	NV	NV	NV	
	WSL07	79.31	199.48	3.00	0.39	0.64	56.88	22.43	0.73	*R.cirrhosa/ Potamogeton*
Bulgarian bay and lake	VL1	70.00	75.75	5.00	0.74	1.75	0.00	70.00	0.06	*Cladophora vagabunda*
(Varna)	VL2	67.50	93.16	5.00	0.63	1.12	0.00	67.50	0.07	*Cladophora vagabunda*
	VL3	65.00	64.97	7.00	0.66	1.39	0.00	65.00	0.07	*Cladophora vagabunda*
	VB4	61.67	79.90	13.00	0.89	2.39	4.00	57.00	0.01	*Ulva rigida*
	VB5	68.00	171.49	12.00	0.73	2.19	44.00	31.00	0.44	*Cystoseira barbata*

Table 4. Pearson correlations between key abiotic (Tables 1 and 2) and biotic (Table 3) metrics in Lesina lagoon (a) and Bulgarian coasts (b). Values in red show significant correlation for $p<0.01$.

	Total coverage (%)	*Total dry biomass (g/m^2)*	*Species No*	*J'*	*H'*	*ESG I%*	*ESG II%*	*EI*	*EEI-c*
Depth (m)	-0.04	0.02	0.82	0.41	0.54	0.14	0.02	-0.12	-0.01
Temperature (°C)	-0.03	-0.31	-0.04	-0.17	-0.12	0.06	-0.30	0.07	0.04
Salinity (PSU)	-0.14	-0.37	0.15	-0.42	-0.26	-0.02	-0.10	0.03	0.08
Oxygen saturation (%)	0.32	0.67	0.07	-0.06	0.00	0.50	-0.04	0.32	0.38
Turbidity	0.11	0.14	0.77	0.42	0.54	0.25	0.01	-0.02	0.09
Organic content (%)	0.20	0.14	-0.75	-0.77	-0.83	0.53	-0.50	0.77	0.71
Gravel (%)	-0.03	-0.11	0.22	0.43	0.42	-0.39	0.43	-0.52	-0.51
Sand (%)	0.16	0.51	0.53	0.22	0.35	0.69	-0.31	0.45	0.57
Mud (%)	0.02	-0.12	-0.58	-0.78	-0.81	0.20	-0.34	0.47	0.41
Total pressures	-0.30	-0.42	0.17	0.44	0.38	-0.88	0.60	-0.88	-0.92
Distance to the pressures (km)	0.36	0.57	0.39	0.43	0.42	0.72	-0.28	0.47	0.52

Table 5. Spearman rank correlation coefficient between key abiotic (Tables 1 and 2) and biotic (Table 3) metrics in Lesina lagoon (a) and Bulgarian coasts (b). For more information see Table 4.

	Total coverage (%)	*Total dry biomass (g/m^2)*	*Species No*	*J'*	*H'*	*ESG I%*	*ESG II%*	*EEI*	*EEI-c*
Depth (m)	-0.18	0.16	0.63	0.41	0.48	0.31	-0.13	0.05	0.13
Temperature (°C)	0.10	-0.17	0.06	0.00	-0.04	-0.05	0.24	-0.13	-0.02
Salinity (PSU)	-0.18	-0.50	-0.15	-0.61	-0.59	-0.01	-0.02	0.28	0.36
Oxygen saturation (%)	0.14	0.38	0.04	-0.09	0.05	0.35	0.01	0.07	0.17
Turbidity	-0.12	0.21	0.63	0.42	0.50	0.32	-0.09	0.05	0.12
Organic content (%)	0.40	0.05	-0.75	-0.65	-0.71	0.46	-0.35	0.76	0.73
Gravel (%)	-0.12	-0.03	0.22	0.35	0.34	-0.31	0.47	-0.62	-0.49
Sand (%)	0.14	0.56	0.21	0.15	0.26	0.76	-0.33	0.37	0.56
Mud (%)	-0.12	-0.48	-0.32	-0.72	-0.70	-0.10	-0.22	0.42	0.29
Total pressures	-0.37	-0.35	0.32	0.46	0.40	-0.83	0.40	-0.90	-0.95
Distance to the pressures (km)	0.21	0.67	0.34	0.55	0.61	0.62	-0.33	0.18	0.29

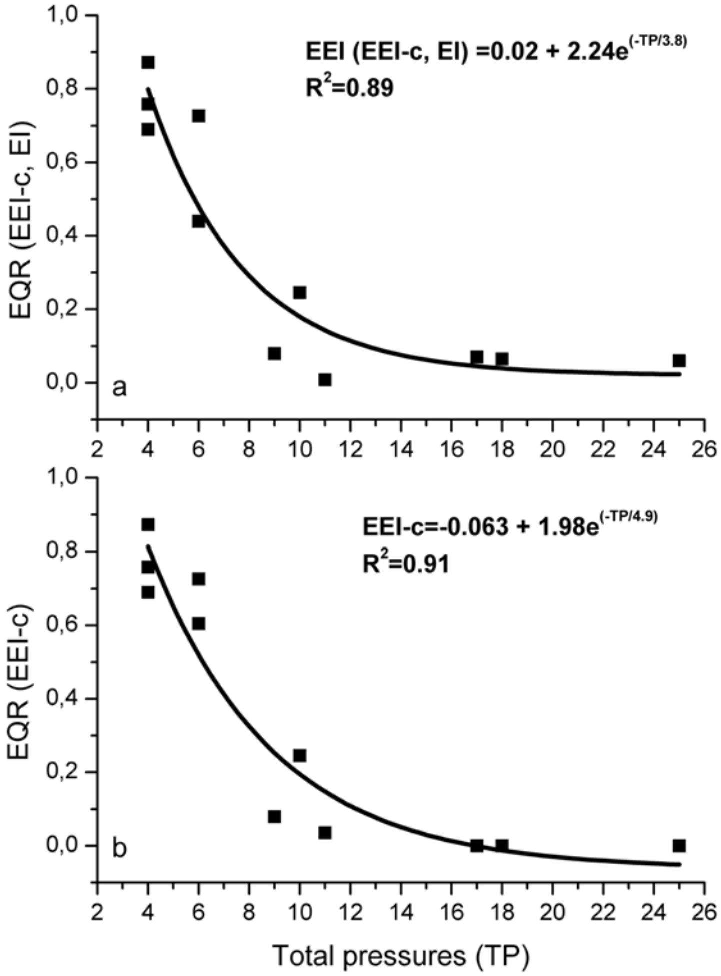
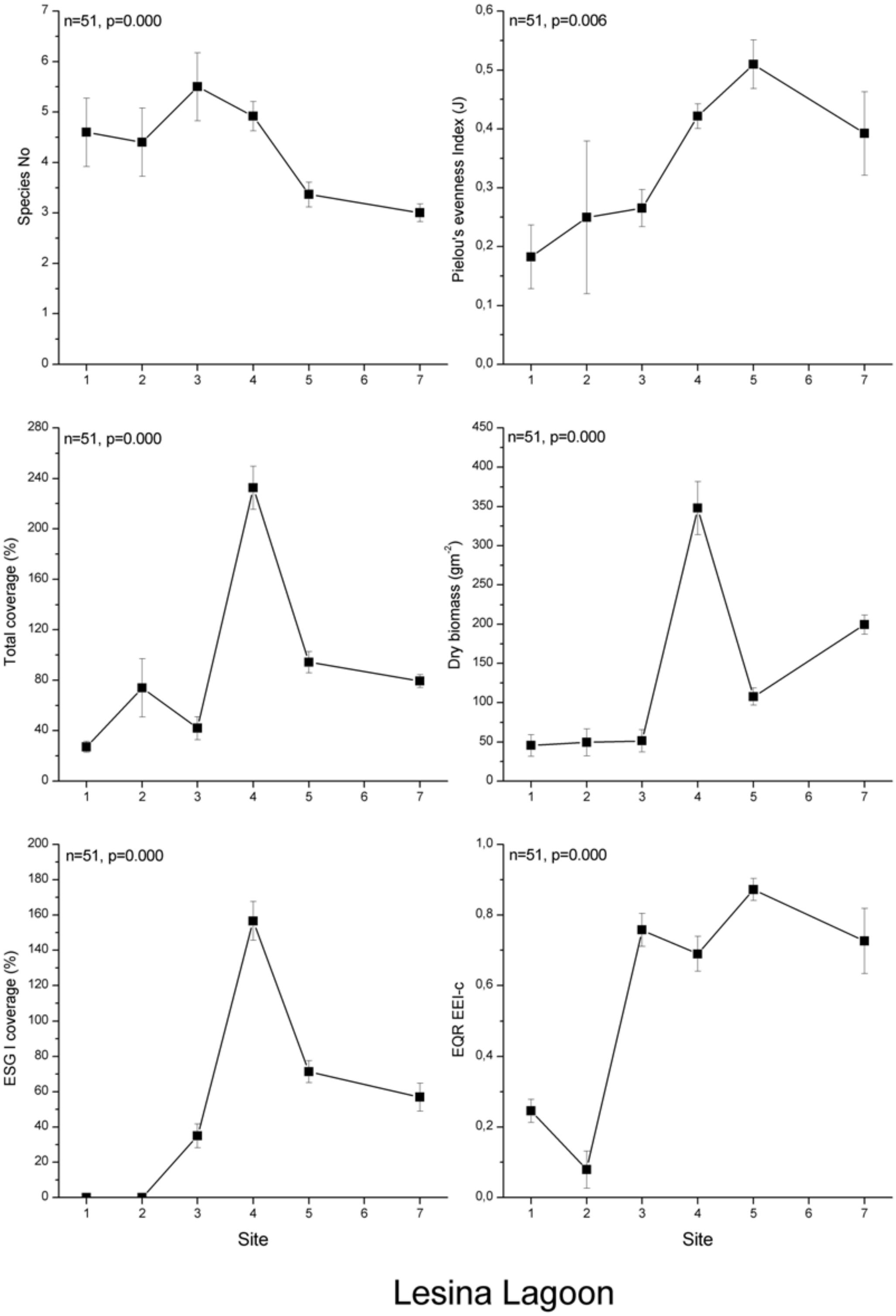


Figure 3. The relationship between a) EEI (EEI-c, EI), and b) EEI-c EQR's with total pressures across the sampling sites of two locations (Lesina lagoon, Varna lake and bay).

a) Lesina lagoon



a) Varna bay and lake

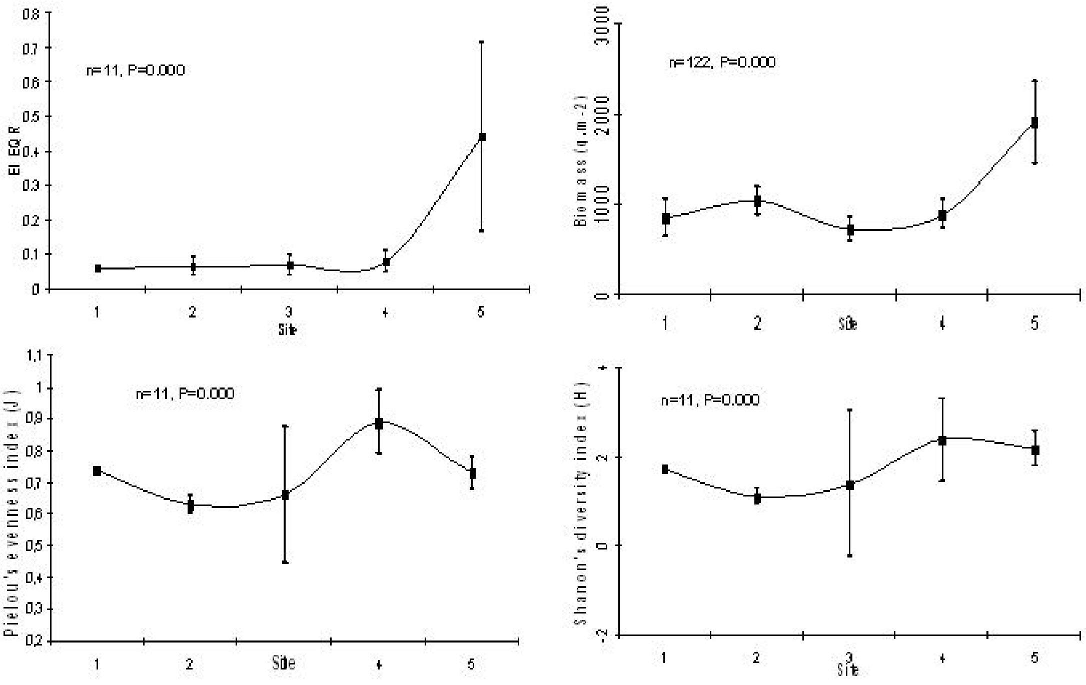


Figure 4. Mean values (±SE) of selected metrics in Lesina lagoon (a) and Bulgarian coasts (b). For Bulgarian coasts 1-VL=1 ;2-VL=2 ;3-VL=3 ;4-VB=4; 5-VB=5.

4 Discussion

The sampled sites of this study belong in a gradient enabling correlation of key structural and functional biotic indices of benthic macrophyte community with pressures and abiotic metrics at an international scale. Evidently, there is a considerable difference between the behaviour of structural and the functional indices in the transitional waters as has been also indicated by others (Orfanidis et al. 2008). The functional indices are better indicating the pressures of the water systems and therefore the ecological status, while the structural indices are better indicating the confinement of the lagoon.

From all functional metrics tested the biotic index EEI classified the sampling sites into five different ESC from “bad” to “high” ESC. The Lesina lagoon was classified in a higher ESC than the Varna lake and bay. Indeed the Lagoon of Lesina in general experience a low vulnerability to human activities, especially the central and eastern bases (Vignes *et al.,* 2009). On the opposite it is well documented (Rozhdestvensky, 1986; Stoyanov, 1991; Shtereva et al. 2004 Dencheva, 2010) that the Varna lake is very eutrophicated and polluted ecosystem and both biotic and abiotic parameters advocate for worse conditions. These contaminated waters enter the bay and the main current in south direction contributes to the deterioration in this part of the bay too.

Beside EEI the growth and dominance of late successional species (*Nanozostera noltii*, *Ruppia cirrhosa* and *Cystoseira barbata*) belonging in ESG I group has been restricted to less impacted areas. Input of nutrients and changes in light transparency are considered among the processes affecting the growth of sensitive members of ESG I in coastal and transitional waters (De Jonge *et al.*, 2002). Under nutrient excess and turbid conditions, species composition shift from angiosperms to dominance of opportunistic and often bloom forming macroalgae (Viaroli *et al*., 2008). This may be due to the efficient nutrient assimilation of opportunistic macroalgae and their non-linear and self-accelerating response after crossing certain nutrient boundaries (Duarte, 1995). Furthermore, opportunistic macroalgae demand lower light quantities for growth than rooted angiosperms (Lüning, 1990; Hemminga and Duarte, 2000). Under oligotrophic and highly transparent conditions angiosperms take advantage over seaweeds by using nutrients from the sediment (Hemminga and Duarte, 2000). Other factors that can trigger this switch e.g. hydrographic change, grazing etc. cannot be excluded, especially when interactions with other stressors are considered (Cloern, 2001).

The structural diversity indices, as in other lagoons (Middelboe *et al*., 1998; Kunii and Minamoto, 2000; Curiel *et al*., 2004; Mannino and Sarà, 2006; Orfanidis et al. 2008), were in general low. A decrease in macrophytes diversity of from the entrance to the inner parts of estuaries (Kautsky, 1995) and coastal lagoons (Coutino and Seeliger, 1984; Orfanidis et al. 2008) suggests either the existence of physiological stress due to strong salinity gradients (Coutino and Seeliger, 1984) or spore, fragment or propagule dispersal restriction (confinement) or interactions between them. An extensive study of macroalgal species diversity in the Danish estuaries indicated a rather complex pattern where species number of macroalgae increased with salinity and declined with nutrient concentrations (Middelboe *et* *al*., 1998). Surfaces provided by hard substrata and by seagrass leaves increases the colonization ability of macroalgae thereby increasing their diversity (Coutino and Seeliger, 1984; Middelboe *et al*., 1998; Mannino and Sarà, 2006) and affecting community composition (Nedwell *et al*., 2002). These results are in agreement with Thiebaut *et al*. (2002) who indicated that species diversity indices are inadequate to evaluate water quality in fresh water ecosystems.

5 Conclusions

From the analyses undertaken it was demonstrated the different responses of structural and functional indices to the abiotic metrics and to the pressures at an international scale. While structural indices are relevant to indicate the water system confinement, the functional indices, especially EEI (including EEI-c and EI modifications), are relevant to indicate the anthropogenic impact and the ecological status.

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Case study 3: Macroalgal cover along physicochemical gradients in in North European Seas

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Abstract

Macroalgae have large potentials as indicators of ecological quality as they are sensitive to anthropogenic pressures, ecologically important and have a long life span and an attached growth form over a potentially wide depth range. Large-scale studies of pressure-response relationships are, however, limited, probably because the diversity of macroalgal indicators is so large that only few indicators are common across regions. These have only to a limited extent been compiled and associated with information on physico-chemical variables. This study compiles data on the indicator 'macroalgal cover' and associated information on physico-chemistry across North European seas from Skagerrak to the Gulf of Finland, and explores whether macroalgal cover responds to eutrophication on this large geographic scale. Our database represents 7 countries: Norway, Sweden, Finland, Estonia, Lithuania, Germany and Denmark, with a total of 163 stations covering the depth range from shallow waters and down to 7-30 m and time series of 2-21 years. Our analyses identified significant negative effects from eutrophication on the total macroalgal cover index across the entire region from

the open Skagerrak coast to the inner Baltic Sea. Differences in salinity also affected macroalgal cover, though the effects and the interaction with nutrients differed between areas. The identified relationships were associated with considerable variations which could probably be reduced through inclusion of additional explanatory variables such as temperature in future analyses. Adjustments of national monitoring programs to apply more uniform sampling methods would also improve the explanatory power of future analyses. This would also increase the potential for mapping and quantifying this important habitat and its potential cover and establish protective measures.

Introduction

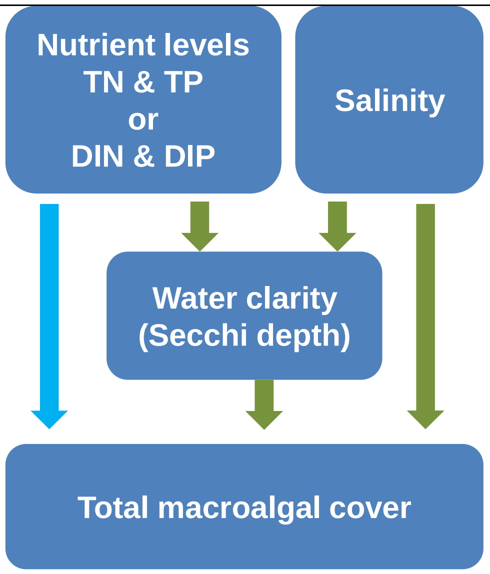
Macroalgae form ecologically important habitats on hard sea bottoms in the intertidal and subtidal coastal zone as well as on reefs in open waters (McRoy and Lloyd 1981, Steneck et al 2002). Such macroalgal habitats can be highly productive (e.g. Mann 1972) and their complex 3-dimensional structure with large canopy-forming kelps and an understory of smaller species create a diversity of niches and a resulting large biodiversity (Christie et al. 2003, 2009). The distribution and abundance of macroalgae depend on an array of environmental factors affecting recruitment and growth as well as mortality of the algae. Light is a major growth regulating factor, and light attenuation in the water column is therefore an important predictor of the range of depth distribution of macroalgae (Nielsen et al. 2002). Light attenuation in the water column is caused by phytoplankton and other particles as well as dissolved organic matter and the water itself (Kirk 1994, Aksnes et al. 2009). Drifting algae and particles sedimenting on the surface of the algae may also contribute to shading the macroalgal community (Moy et al. 2008). Eutrophication increases the level of these light attenuating components and also enhances the risk of anoxia, thereby constituting a major threat for macroalgal communities (Duarte 1995, Schramm and Nienhuis 1996, Moy et al. 2008, Cloern 2001, Howarth et al. 2011). Other anthropogenic pressures such as direct degradation or destruction of habitat (Mangialajo et al. 2008) can also cause physical damage to seaweed forests, and over-fishing may further affect macrophyte communities indirectly by producing cascading perturbations down the food web which interact with effects of eutrophication (Moksnes et al. 2008, Baden et al. 2010). Over-fishing is probably the major cause of incidences of mass occurrence of the northern sea urchin (*Strongylocentrotus droebachiensis*), which may graze kelp forests down to “barren grounds” (Jackson et al. 2001, Steneck et al. 2002). On the local scale, additional anthropogenic pressures such as exploitation of stone and gravel as well as sediment spill from dredging operations and chemical pollution e.g. from antifouling paint on ships may also affect the quality of the macroalgal habitat (Halpern et al. 2008, HELCOM 2010).

Macroalgae have large potentials as indicators of ecological quality because of their sensitivity to anthropogenic pressures in combination with their ecological importance, their long life span and attached growth form across potentially wide depth ranges. The list of macroalgal indicators used to assess ecological quality is long, but apparently only few common indicators of macroalgal distribution and abundance are measured across large European scales (e.g. http://www.wiser.eu /program-and-results/data-and-guidelines/method-database). This is likely the main reason why most pressure-response relationships for macroalgae have been conducted at local scales. In such local analyses, nutrient enrichment has been found to change the composition and structure of rocky shore macroalgal communities in the Mediterranean (Arévalo et al. 2007), and reduce water clarity and macroalgal cover in Danish coastal waters (Krause-Jensen et al. 2007, 2008, Carstensen et al. 2008) and stone reefs (Dahl and Carstensen 2008), and urbanization has also been found to cause a loss of fucoid species (Bokn et al. 1992; Mangialajo et al. 2008). A local analysis in the Baltic Proper has also documented significant relationships between macroalgal abundance and environmental variables such as exposure and water clarity (Eriksson and Bergström 2005). By contrast, there are only few large scale analyses of how a given macroalgal indicator responds to environmental factors. Another reason for this is that existing common macroalgal data sets have only to a limited extent been compiled and associated with information on physico-chemical variables. A recent study makes the first attempt to classify coastal North-East Atlantic waters based on macroalgae in combination with physical attributes (Ramos et al. 2010), and analyses of macroalgal cover initiated in Danish coastal waters have also been tested in a Danish-Finnish comparison (Krause-Jensen et al. 2009).

In order to identify and distinguish possible effects of anthropogenic pressures on macroalgae through analyses of large data sets, it is important to include not only anthropogenic pressures, such as increased nutrient levels, as independent variable in the analyses but also other environmental factors affecting macroalgae, such as salinity, substratum type and presence of grazers. The natural decline in salinity from Skagerrak to the Bothnian Bay reduces the biodiversity of seaweed forests as many red- and brown algal species cannot cope with the low salinity (Nielsen et al. 1995). This pattern of declining diversity towards the inner Baltic Sea is further reinforced by the narrow Danish straits and long spreading distances acting as a dispersal barrier for colonisation of macroalgal species from the source area in Kattegat to the inner Baltic Sea (Middelboe et al. 1997). In addition to the large-scale changes in salinity across the Skagerrak-Baltic Sea region, there are also estuarine salinity gradients at more local scales. Other important gradients in the region of relevance to macroalgae are water temperature, affecting the performance of the algae as well as determining their biogeographic distribution (Lüning 1990), wave exposure (Hurd 2000), affecting the transport of nutrients and gasses between the algae and the surroundings as well as ice scour that may physically destroy the canopies in shallow waters (Kiirikki & Ruuskanen1996) and sea-ice which may shade the algal community (Krause-Jensen et al. 2007).

The aim of our study was to 1) explore how eutrophication pressures, expressed in terms of nutrient concentrations and water clarity, affect the total cover of the macroalgal communities in North European seas from the Skagerrak region to the inner Baltic Sea and 2) identify how the drastically changing salinity across the region affects the relationships between eutrophication and algal cover. More specifically, we hypothesize that 1) high nutrient concentrations and low water clarity result in reduced macroalgal cover, and 2) that the large scale salinity gradients (across the Baltic Sea as well as estuarine salinity gradients) affect these relationships through positive effects of high salinity on water clarity, macroalgal diversity and eventually macroalgal cover (Fig. 1). We expect that by including eutrophication pressures and salinity, central factors affecting the macroalgal community, as explanatory variables in the analyses, we will be able to distinguish their possible effect on algal cover even though we are aware that several other variables of natural and anthropogenic origin contribute to regulating algal cover.

Figure 1. Conceptual model for effects of physico-chemistry on total macroalgal cover: direct effects of nutrients (blue arrow), effects of nutrients via water clarity and effects of salinity (direct and in interaction) (green arrows).



Material and methods

Compiled data sets

This study expands the compilation of data on total cover of erect macroalgae (TC) and associated physico-chemical information already gathered through previous projects in Scandinavia (Moy et al. 2010, Norderhaug et al. 2011) and in Danish coastal waters (Carstensen et al. 2008) and stone reefs (Dahl and Carstensen 2008). The expanded compilation includes additional monitoring data sets from Scandinavia as well as from other countries facing the Baltic Sea. The full data set represents 7 countries: Norway, Sweden, Finland, Estonia, Lithuania, Germany and Denmark with a total of 163 stations covering the depth range from shallow waters and down to 7-30 m and time series of 2-21 years depending on area (Table 1, Fig. 2).

TC is typically assessed visually by a diver as a percentage of the sea bottom within either a frame or a larger area (up to about 25 m^2). TC is not a mandatory variable in all national monitoring programs of the Nordic- and Baltic Sea countries, and sampling methods vary between countries. To achieve a common TC data set across Nordic and Baltic seas some assumptions and estimation procedures had to be applied. Some monitoring/survey programs assess TC as a percentage of the stable hard sea bottom while others assess it as a percentage of the entire sea bottom, and the latter type of data was normalised to represent cover on stable hard sea bottom using information on the fraction of the bottom composed by stable hard substratum. Moreover, we only included data sets from stations with at least 10% hard bottom. Further details regarding the national data are described below.

The Norwegian Coastal Monitoring Program initiated sampling of TC on stable hard bottom in 2007 (Moy et al. 2010, Norderhaug et al. 2011). Locations severely affected by sea-urchin (Norderhaug & Christie 2009) were omitted. The Danish monitoring program has measured TC on stable hard sea bottom since 1993 (Table 1), and also includes information on cover of sea-urchins, drifting alga mats and blue mussel (*Mytilus edulis)*.

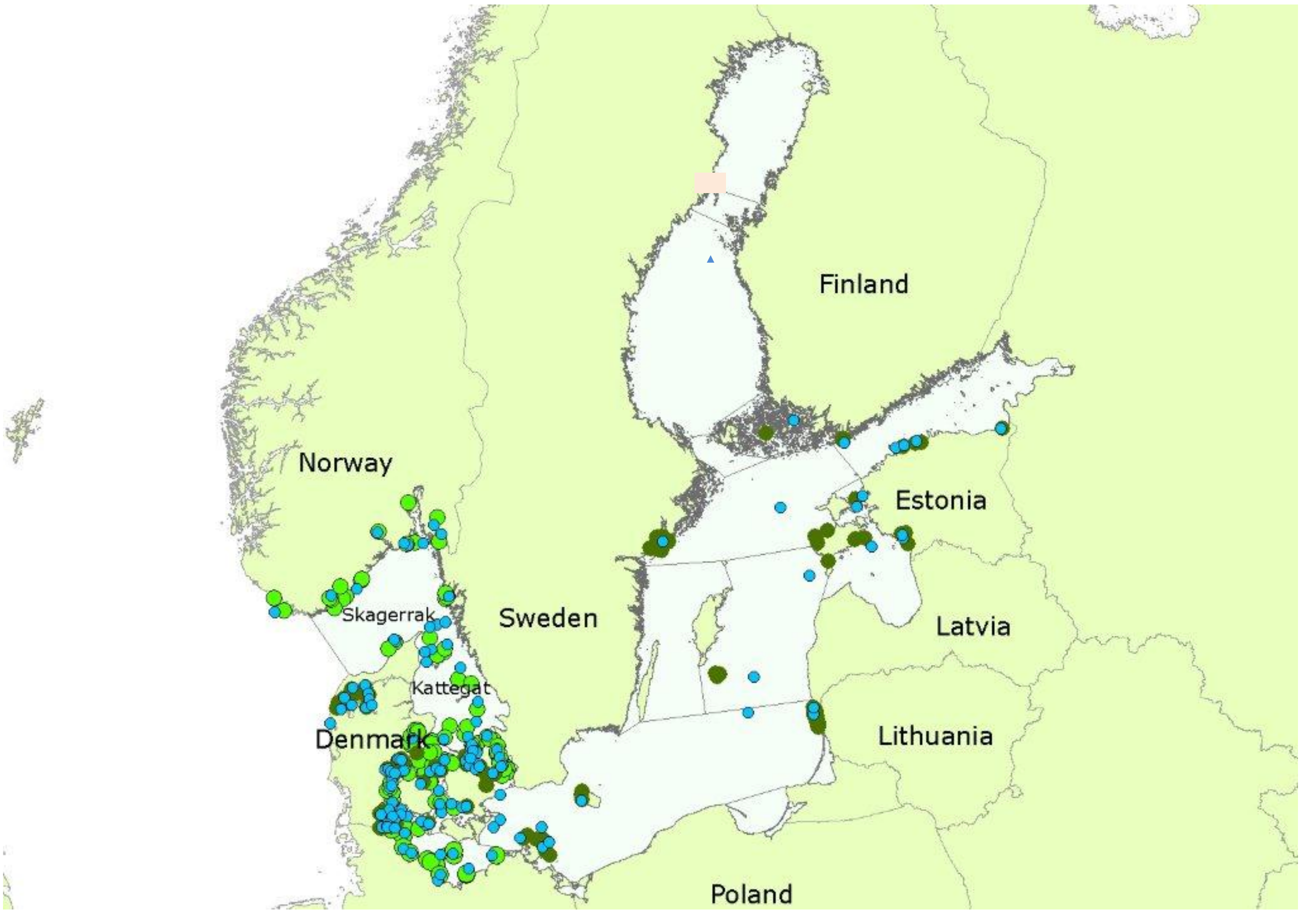


Fig.2. Map of macroalgal stations (light green), aggregated macroalgal stations (dark green) and physico-chemical stations (Blue). ‘Aggregated macroalgal stations’ refer to cases when a number of sites in e.g. a fjord were aggregated to one fjord station or, in the case of Lithuanian data, when one station was composed of a number of sites forming a grid.

Table 1. Sampling stations (St.) for macroalgae included in the analyses. Some stations were aggregates of sites, i.e. a number of sites in a fjord aggregated to one fjord station or, in the case of Lithuania, one station was composed of a number (stated in parenthesis) of sites forming a grid. The depth range was truncated towards the coast (at 1 m, 3 m, 5 m or 6 m, depending on exposure) in order to omit depth ranges markedly affected by exposure and focus the analysis on water depths prone to light limitation (Truncation of Danish inner fjords: 1 m, outer fjords: 3 m, open coasts: 5 m; marked by '**').

Nation ***-Data provider***	**Area**	**St. (No.)**	**Depth range (m)**	**Data period**	**Years (No.)**	**Main sampling month**
Norway *-Norwegian Inst. of Water Research, NIVA*	Skagerrak	14	5-30	2007-2009	3	Jun., Aug.
	North Sea	2	5-28	2007-2008	2	June
Denmark *-National Env. Research Inst., NERI, Aarhus Univ.* *- Environmental Centres*	Skagerrak/ Kattegat and Belt Sea area	30	6-24	1993-2009	17	Jun., Aug.
	Adjacent fjords/ Western Baltic	18	1**-14	2001-2009	9	Jun., Aug.
Sweden *-Univ. of Gothenburg*	Skagerrak	6	5-20	2007-2008	2	Aug.
-Univ. of Stockholm	Baltic Proper	34	3-26	1974-2008	21	Jun.– Sep.
Germany	W. of Gedser-Darß	10	5-7	2007-2009	3	Jul.-Sep.
- MariLim contracted by XX	E. of Gedser-Darß	12	5-13	2006-2009	4	Jul.-Sep.
Lithuania *- Klaipeda Univ.*	Baltic Proper	16 (*127)	5-19	2003-2006	3	May-Oct.
Estonia- *Estonian Marine Inst., Univ. of Tartu*	Baltic Proper	18	3-15	1995-2008	13	Jul.-Sep.
Finland – *Finnish Env. Inst. & Univ. of Helsinki*	Baltic Proper / Gulf of Finland	3	3-16	1998-2009	8	Jun.-Aug.

Monitoring along the Swedish west coast in Kattegat-Skagerrak has included estimates of TC since 2007, assessed as a percentage of the hard sea bottom based on image analysis of replicate photos each representing 0.25 m^2 of hard sea bottom. In the Askö area in the Baltic Sea, TC has not been monitored, but was estimated as the cumulative cover of individual macroalgal species based on the experience that the vegetation is only slightly multi-layered from water depths below 3 m (Hans Kautsky, pers com.). Cover was assessed relative to the entire bottom, but converted to cover of hard bottom based on information on substratum types and expert knowledge on how cover varied between substratum types (Kautsky pers com.). The Finnish program has included TC since 2007 only; for earlier years TC was estimated using the same procedure and assumptions as for the Askö data (approved by Ari Ruuskanen). TC was assessed relative to the entire sea bottom until 1999 and then relative to the stable hard sea bottom, therefore data from the early period were recalculated using information on the percentage of hard sea bottom within the sampling frames. The Estonian monitoring program assesses the cover of all vegetation, higher plants as well as macroalgae, as a percentage of the entire sea bottom. TC on hard stable sea bottom was estimated using the assumption that the summed cover of stones, boulders, rock and limestone made up the stable hard sea bottom:

*TC = (Total cover – higher plant/Chara cover)*100/(stone% + boulder%+ rock/lime stone)*

The Lithuanian monitoring program does not include TC but this variable could be estimated using the same procedure and assumptions as for the Askö and the Finnish data set (approved by Martynas Bučas) and considering cobbles and boulders to constitute the hard stable sea bottom (Bučas et al. 2007).The German monitoring program, like the Estonian, assesses cover of the entire vegetation, higher plants as well as macroalgae, as a percentage of the entire sea bottom. TC was estimated using the assumption that the summed cover of boulders, cobbles, pebbles, gravel and clay reefs made up the suitable hard sea bottom (at stations with high mussel cover, mussels were also considered hard sea bottom):

*TC= algal cover*100/(percentage cover of boulders, cobbles, pebbles, gravel and clay reefs)*

We focused the analysis exclusively on datasets from a depth range sufficiently deep to the physical disturbance was considered to no longer be a major controlling factor for TC. The coastward end of this depth range was estimated on Danish datasets (Carstensen et al. 2008, Dahl and Carstensen, 2008) as the water depth with highest cumulative algal cover using non-parametric adjustment based upon a fitted loess function (Cleveland 1979).This adjustment was made separately for a large number of areas and resulted in categorisation of the datasets in four exposure groups: weakly exposed areas where maximum cover was located at water depths of ~1 m (inner, protected fjords, bays), moderately exposed areas with maximum cover at water depths of ~3 m (e.g. outer fjords, bays) and more exposed coastal areas with maximum cover at water depths of ~5 m (Carstensen et al. 2008) and open reef areas with maximum cover at ~6 m (Dahl and Carstensen 2008, see Table 1). Fitting non-parametric spline functions describing TC as a function of depth indicated that the exposure classes and depth ranges developed on Danish data were applicable to the larger dataset.

Physico-chemical variables

Total macroalgal cover was related to the physico-chemical variables salinity, nutrient concentration, chlorophyll concentration and Secchi depth. The physico-chemical stations were matched with nearby macroalgal stations based on expert judgement. In some cases several macroalgal stations were matched with the same physico-chemical station, while in other cases several physico-chemical stations were used to characterize the water quality at one macroalgal station.

Norwegian physio-chemical data were provided by the Norwegian Coastal Monitoring Program (Norderhaug et al. 2011) and generally represented a sampling frequency of every second week conducted according to standard guidelines. Danish physico-chemical data were retrieved from NERI's database and represent monitoring data provided by local Environmental Centres, NERI as well as SMHI, according to common guidelines (Andersen et al. 2004). Sampling frequency typically ranged between weekly and monthly but was occasionally lower e.g. for Secchi depth in open waters. Swedish data originated from the national and the SMHI off-shore monitoring programs and were retrieved from the SHARK database hosted by the Swedish Meteorological and Hydrological Institute. Sampling was conducted monthly according to common guidelines: http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/hav/vegbotva.pdf. Finnish physico-chemical data, obtained from SYKEs database, represent permanent sampling stations near macroalgal stations with monthly-by monthly sampling during January-March (if no ice occurred), and weakly-bimonthly sampling in April-August. Estonian data represent monthly samples though with some variation in sampling month between years and stations. Data are part of the national monitoring program and provided by the Estonian Marine Institute. Lithuanian data also make part of the national monitoring program with sampling four times per year according to standard guidelines. German data were obtained from the monitoring program of the state agencies for environment of Schleswig-Holstein and Mecklenburg-Vorpommern with 50 stations along the German Baltic coastline with a monthly sampling following standard guidelines.

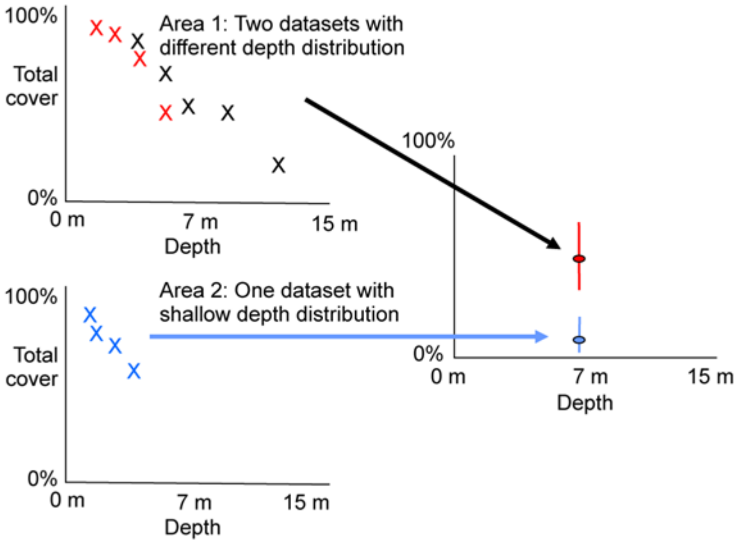
Data handling, harmonization and analysis

The first step in the data analysis was to compute comparable mean values for macroalgal cover and physico-chemical variables for each station. This data harmonisation was conducted on the basis of generalized linear models (Nelder and Wedderburn 1972), using the PROC GENMOD in the statistical software package SAS/STAT 9.2 (SAS Institute Inc, 2009). For macroalgal cover we computed marginal mean values for each station based on a model which took into account variability in algal cover between stations, water depths, levels of sea-urchin cover and years (Fig. 2):

$E(X_{ijkl}) = \beta_0 + \beta_1(station_i) + \beta_2(depth_j) + \beta_3(sea\text{-}urchin_k) + \beta_4(year_l) + e_{ijkl} (1)$

Marginal means of total macroalgal cover were estimated for a given station at a water depth of 15 m for open water stations and 7 m for coastal stations in order to adjust for differences in depth between stations and thereby facilitate a direct comparison. The model also took into account differences in sea-urchin cover between stations, TC was estimated a sea-urchin occurrence of near zero. When several sites constituted one station, marginal mean values were also estimated at the level of the station; e.g. in the Lithuanian data set where each grid of data (containing several sites) was considered one station, and in the Danish data set where each fjord or subfjord (containing several sites) was considered one station. As total cover values have fixed boundaries at 0 and 100, data were transformed by an arc-sin function prior to analysis.

Figure 2. Example of the data harmonization procedure for estimating marginal means of total algal cover at a water depth of 7 m in two different areas. From Carstensen et al. (2008).



For physico-chemical data we applied a similar generic model which took into account variation between stations, between years and between months (January-June) of sampling. Skagerrak, Kattegat and the Danish Straits had long time series which were used to obtain better estimates of the seasonal variation (months) and variation between stations. The model employed was:

$$E(X_{ijkl}) = \beta_0 + \beta_1(station_i) + \beta_2(year_j) + \beta_3(month_k) + e_{ijkl} \qquad (2)$$

From this model marginal mean values of physico-chemical variables were estimated for each sampling station each year for the period January-June in order to reduce the effect of phytoplankton growth on the estimated nutrient levels. Data on nutrient and chlorophyll-*a* concentration were ln-transformed before analysis in order to linearize the relationship to Secchi depth and algal cover. For open waters, nutrient concentrations were estimated for 10 – 20 m depth and salinity for 10 – 20 m depth, for inner waters the upper depth limit was relaxed to span from the surface. In some cases insufficient data from the winter period prevented estimation of marginal means..

The outcome was a data set linking annual area-specific total cover estimated for two chosen standard depths, 7 and 15 m, with mean values of water quality variables in the 6 months period (January-June) prior to macroalgal monitoring in summer (typically between May and September, Table 1). Data on vegetation for which it was not possible to estimate average values of salinity and total nitrogen or Secchi depth were discarded. Data were separated into three groups:

I. North Sea to the Gedser-Darß sill in the western Baltic: This group represent surface water salinity >12.2 psu, reduced presence of blue mussel (*Mytilus edulis*) and relatively high water transparency. For this group of data the relationship between the algal vegetation and the physico-chemical variables was examined using the estimated TC at the standard depth of 15 m.

II. Norwegian, Swedish and Danish open and coastal waters: Subset of the above data set excluding German data as these were found to behave differently from the rest (see below).

III. Danish fjords and the majority of the Baltic Sea east of the Gedser-Darß sill. This group of data was characterized by generally reduced water transparency, salinities <12.2psu except in some Danish fjord and often a high frequency of blue mussel in association with the vegetation. For this group of data the relationship between the algal vegetation and the physico-chemical variables was examined using the estimated TC at the standard depth of 7 m.

The next step was to fit a suite of generalized linear models mixed effect models, using the PROC MIXED of the statistical software package SAS/STAT 9.2 (SAS Institute Inc, 2009) to test the hypotheses (Fig. 1, Table 2). First, we introduced all the potential independent variables in the regression, and then excluded variables one by one until only the variables significant contributing ti the ability of the model to describe data remained.

Table 2. Suite of regression models used to test the hypothesized relationships between algal cover TC and physico-chemical variables (Fig. 1). Possible effects of country (C) and station (St) are considered random.

Secchi depth(S) vs.nutrients (total: TN, TP and inorganic: DIN, DIP)and salinity (s)

$E(S) = b_0 + b_1\ln(TN) + b_2\ln(TP) + b_3(s) + b_4(\ln TN)(s) + b_5(\ln TP)(s) + B_1(St) + B_2(C) + e$

$E(S) = b_0 + b_1\ln(DIN) + b_2\ln(DIP) + b_3(s) + b_4(\ln DIN)(s) + b_5(\ln DIP)(s) + B_1(St) + B_2(C) + e$

Total algal cover (TC) as function of Secchi depth (S) and salinity (s)

$E(\mathrm{arsin}(TC)) = b_0 + b_1(S) + b_2(s) + b_3(S)(s) + B_1(St) + B_2(C) + e$

Total algal cover (TC) vs. of nutrients(total: TN, TP and inorganic: DIN, DIP) and salinity (s)

$E(\mathrm{arsin}(TC)) = b_0 + b_1\ln(TN) + b_2\ln(TP) + b_3(s) + b_4(\ln(TN)(s) + b_5(\ln(TP)(s) + B_1(St) + B_2(C) + e$

$E(\mathrm{arsin}(TC)) = b_0 + b_1\ln(DIN) + b_2\ln(DIP) + b_3(s) + b_4(\ln(DIN)(s) + b_5(\ln(DIN)(s) + B_1(St) + B_2(C) + e$

Total algal cover vs. the nutrients found to control Secchi depth

A special case of either model 4 or 5

Results

Vegetation cover in relation to physico-chemical factors: The North Sea to the Gedser-Darß sill in the western Baltic Sea

Vegetation cover, nutrient concentration and Secchi depth all showed distinct year to year changes as well as variation between stations within each country (Fig. 3). Secchi depth correlated negatively with TN (Table 3, model 1). Secchi depth also showed a negative correlation with inorganic nutrients combined with a positive correlation with salinity and interactions between salinity and inorganic nutrient concentrations (Table 3, model 2).

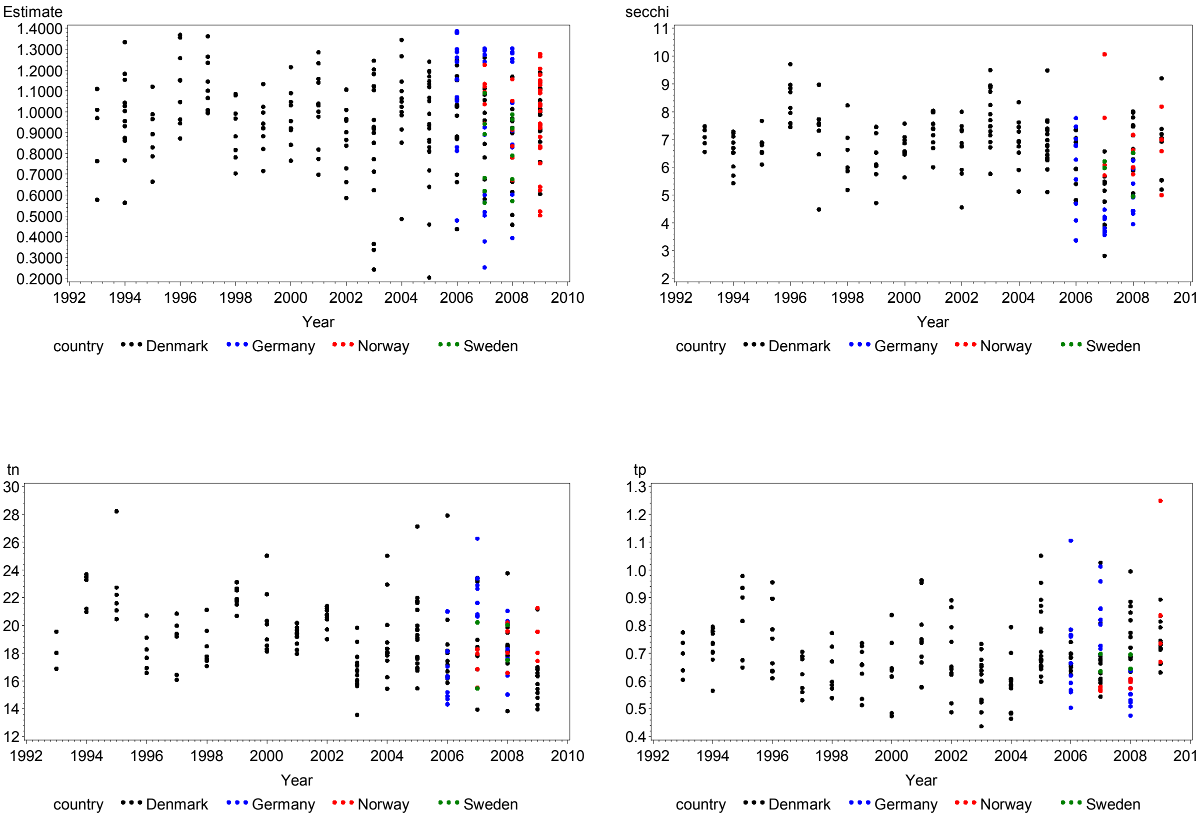


Figure 3. Temporal and spatial variation in total algal cover (TC, arc sin transformed, upper left panel), Secchi depth (upper right panel), total nitrogen concentration (TN, µmol/l) and total phosphorus concentration (TP,µmol/l) from 1993 to 2009 in the area from the North Sea to the Gedser-Darß sill in the western Baltic Sea.

In both cases country and station also played a significant role. Total algal cover (TC) normalized to 15 m water depth showed a strong positive correlation with both Secchi depth and salinity, again with country and station also playing a significant role (Table 3, model 3).

TC also showed negative correlation with TN and salinity and positive correlation with the interaction of the two (Table 3, model 4) and, eventually, TC correlated negatively with DIN and positively with salinity (Table 3, model 5). Although relationships between TC and the main regulating factors, Secchi depth, TN concentration and salinity were significant, they exhibited substantial scatter (Fig. 4).

Table 3. Suite of regression models describing the response of Secchi depth and total algal cover to nutrient concentrations and salinity in the North Sea to the Gedser-Darß sill in the western Baltic Sea.

Secchi depth (S, m) vs. nutrients (total: TN, TP; inorganic: DIN, DIP; µmol/l) and salinity (s, psu)

$S = 14.3307 - 2.7994(\ln TN) + B_1(\text{Station}) + B_2(\text{Country}) + e$

$P<0.0001$

$S = 1.3366 - 2.1955(\ln DIP) + 0.09546(\ln DIP)(s) + 0.7222(\ln DIN) - 0.04581(\ln DIN)(s) + 0.2011(s)+ B_1(\text{Station}) + B_2(\text{Country}) + e$

$p=0.1843$ (DIN), $p=0.0354$ (DIN*s), $p=0.0417$ (DIP), $p=0.0127$ (DIP*s), $p=0.0160$ (s)

Total algal cover (TC, Arc sin %) vs.Secchi depth (S, m) and salinity (s, psu)

$TC(\text{Arcsin}) = 0.3835 + 0.03962(S) + 0.01192(s) + B_1(\text{Station}) + B_2(\text{Country}) + e$

$P=0.0004$ (S), $p=0.0059$ (s)

Total cover (TC, %) vs. nutrients(total: TN, TP; inorganic: DIN, DIP; µmol/l)and salinity (s, psu)

$TC(\text{Arcsin})= 3.8741 - 1.0935(\ln TN)+ 0.03247(\ln TN)(s) - 0.08463(s) + B_1(\text{Station}) + B_2(\text{Country}) + e$

$P=0.0022$ (TN), $p= 0.0417$ (s), $p=0.0232$ (s*TN)

$TC(\text{Arcsin}) = 0.6423 - 0.03968(\ln DIN) + 0.01110(s) + B_1(\text{Station}) + e$

$P=0.0011$ (DIN), $p= 0.0048$(s)

Total algal cover vs.the nutrients found to control Secchi depth

Similar to 4 as the same nutrients regulated Secchi depth and TC

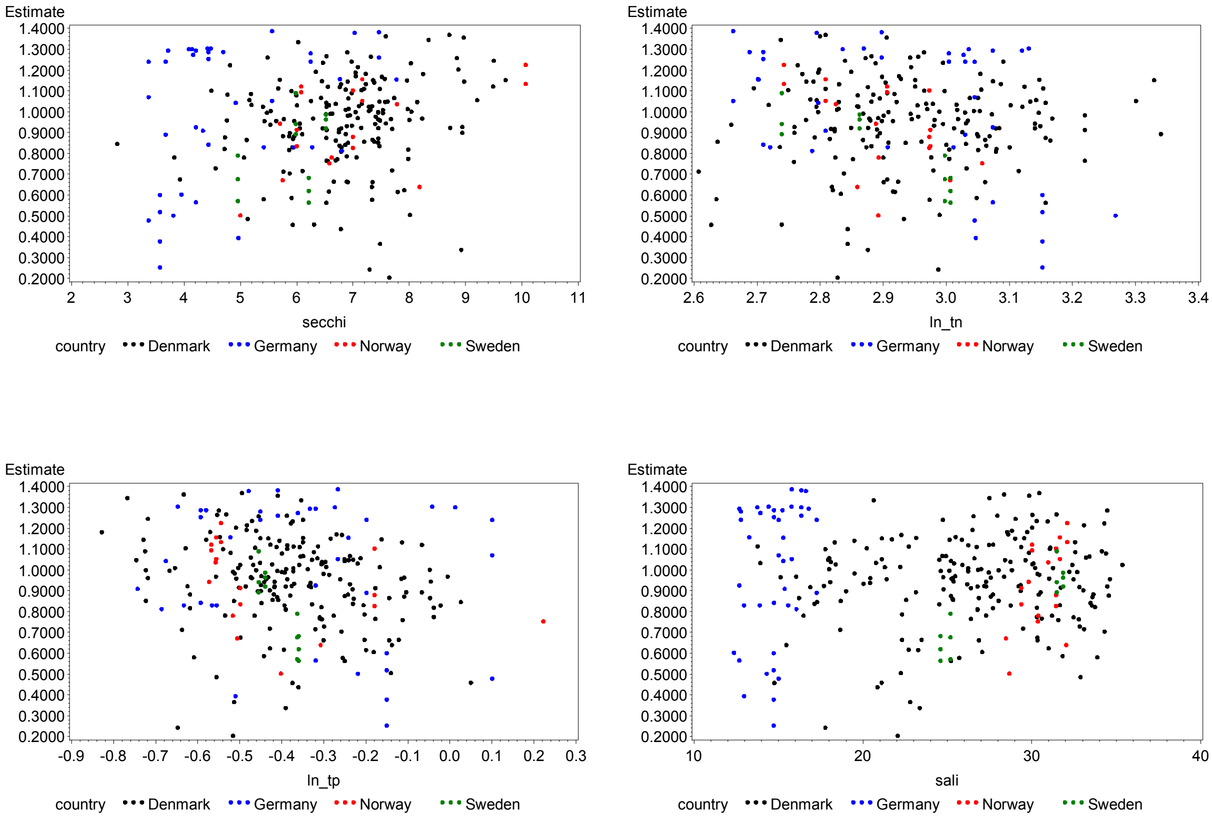


Figure 4. Total macroalgal cover (arc-sin transformed, %) estimated at 15 m water depth as a function of Secchi depth (upper left panel), TN (upper right panel), TP (lower left panel) and salinity (lower right panel) in the North Sea to the Gedser-Darß sill in the western Baltic Sea.

Vegetation cover in relation to physico-chemical factors: Norwegian, Swedish and Danish open and coastal waters

In the data set from the North Sea to the Gedser-Darß sill in the western Baltic, the German data created the random effect of country and we therefore repeated the analysis without these data. Secchi depth still showed a significantly negative correlation with TN (Table 4, model 1) and was also negatively correlated with DIN and DIP, and positively correlated with salinity and a combined effect of salinity and inorganic phosphorus (Table 4, model 2). Total cover correlated positively with Secchi depth and salinity (Table 4, model 3), negatively with TP and positively with salinity (Table 4, model 4) but showed no correlation to inorganic nutrients (Table 4, model 5). Although we identified significant correlations between TN and Secchi depth on the one hand and between Secchi depth and vegetation cover on the other, TN did not correlate significantly with total cover (Table 4, model 6, P=0.2946). None of the models showed any effect of country. All relationships between TC and the main regulating factors, Secchi depth, TP concentration and salinity showed substantial scatter (Fig. 5).

Table 4. Suite of regression models describing the response of Secchi depth and total algal cover to nutrient concentrations and salinity in Norwegian, Swedish and Danish open and coastal waters (as in Table 3 but without German data).

Secchi depth (S, m) vs. nutrients (total: TN, TP; inorganic: DIN, DIP; µmol/l) and salinity (s, psu)

$S = 12.7087 - 2.0406(\ln TN) + B_1(\text{Station}) + e$

$P<0.0038$ (TN)

$S = -1.1696 - 0.5323(\ln DIN) – 4.9082(\ln DIP) +0.3147(s) + 0.1857(\ln DIP)(s) + B_1(\text{Station}) + e$

$P=0.0009$(DIN), $p=0.0012$ (s), $p=0.0015$ (DIP), $p=0.0009$ (DIP*s)

Total algal cover (TC, Arc sin %) vs. Secchi depth (S, m) and salinity (s, psu)

$TC(\text{Arcsin}) = 0.3860 + 0.03257S + 0.01112s + B_1(\text{Station}) + e$

$P=0.0017$ (S), $p=0.0036$ (s)

Total cover (TC, %) vs. nutrients (total: TN, TP; inorganic: DIN, DIP; µmol/l)and salinity (s, psu)

$TC(\text{Arcsin})= 0.6006– 0.2242(\ln TP) + 0.008125(s) + B_1(\text{Station}) + e$

$P=0.0029$ (TP), $p= 0.0379$(s)

$TC(\text{Arcsin}) = 0.5766 +0.01194s + B_1(\text{Station}) + e$

$P = 0.0017$ (s)

Total algal cover vs. the nutrients found to control Secchi depth

Non significant

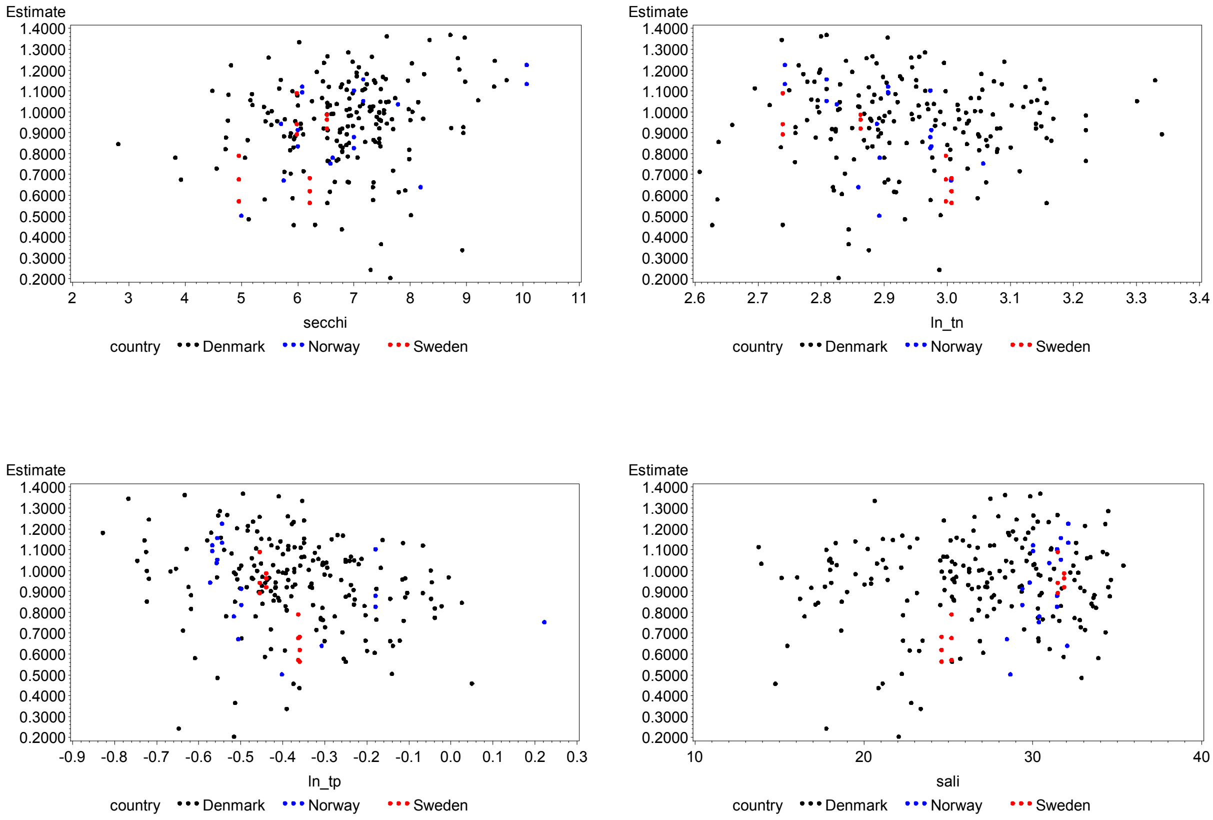


Figure 5. Total macroalgal cover (arc-sin transformed, %) estimated at 15 m water depth as a function of Secchi depth (upper left panel), TN (upper right panel), TP (lower left panel) and salinity (lower right panel) in Norwegian, Swedish and Danish open and coastal waters

Vegetation cover in relation to physico-chemical factors: Danish fjords and the Baltic Sea east of the Gedser-Darß sill

The data set from this area represented 6 countries: Denmark, Estonia, Finland, Germany, Lithuania and Sweden. A few observations of algal cover were available from Swedish waters in the 1970s and 1980s but the majority of observations represented the period after 1990 and particularly after 2000. Physicochemical data associated with the algal observations were also most abundant after 2000. Algal cover as well as nutrient concentrations and Secchi depth showed considerable variability between years and areas (Fig. 6).

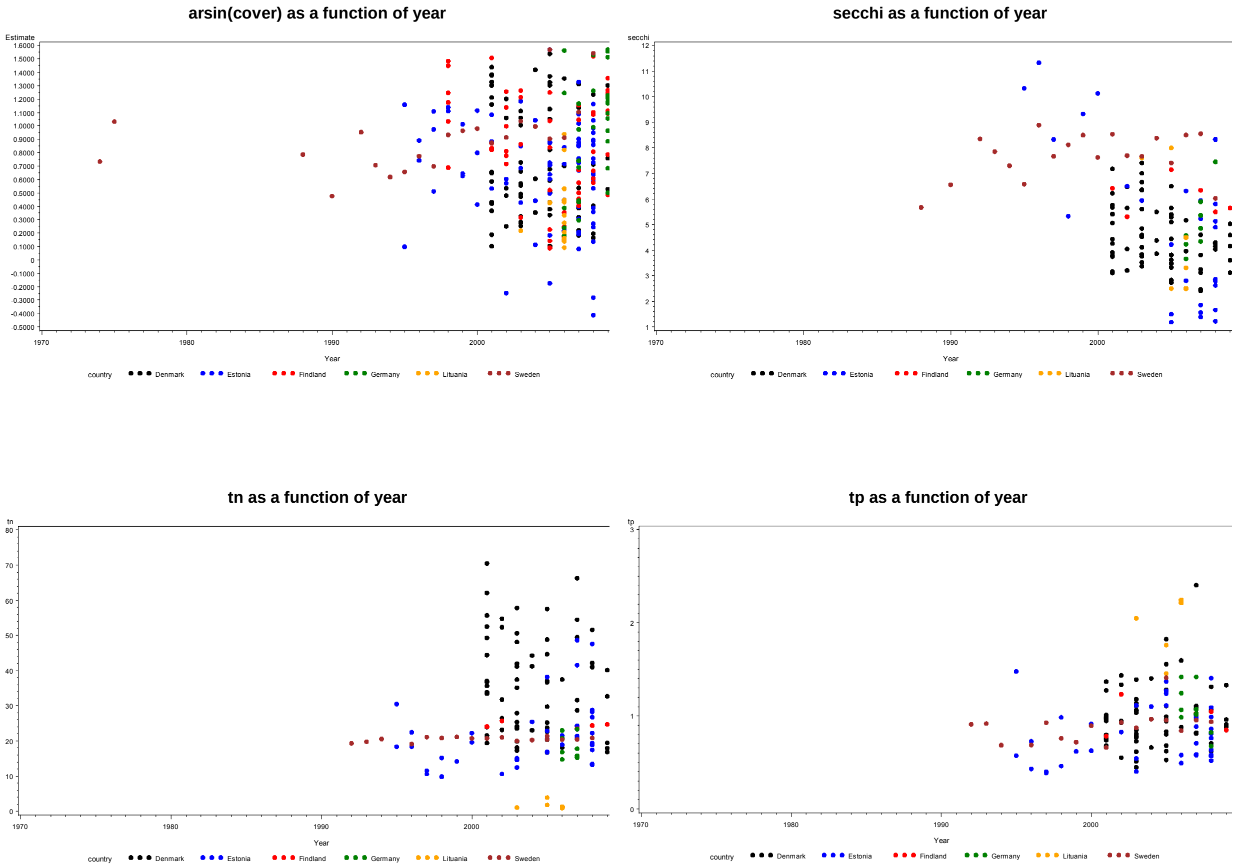


Figure 6. Temporal and spatial variation in total algal cover (TC, arc sin transformed, upper left panel), Secchi depth (upper right panel), total nitrogen concentration (TN, µmol/l) and total phosphorus concentration (TP,µmol/l) from the 1970s to 2009 in Danish fjords and the Baltic Sea east of the Gedser-Darß sill.

In this area Secchi depth correlated negatively with TN, TP and salinity and positively with the interaction between TN and salinity without any effect of country (Table 5, model 1). Secchi depth also correlated negatively with DIN and showed an effect of country (Table 5, model 2). TC normalized to 7 m water depth correlated positively with Secchi depth without any effect of country (Table 5, model 3). TC correlated negatively with TN and salinity but positively with the interaction between TN and salinity and also showed an effect of country (Table 5, model 4). TC showed no correlation with inorganic nutrients (Table 5, model 5). As TN was the main factor correlating with both Secchi depth and total cover, model 6 corresponds to model 4. Although relationships between TC and the main regulating factors, Secchi depth, TN concentration and salinity were significant, they showed substantial scatter (Fig. 7).

Table 5. Suite of regression models describing the response of Secchi depth and total algal cover to nutrient concentrations and salinity in Danish fjords and the Baltic Sea east of the Gedser-Darß sill.

Secchi depth (S, m) vs. nutrients (total: TN, TP; inorganic: DIN, DIP; µmol/l) and salinity (s, psu)

$S = 38.7514 – 11.7725 (\ln TN) – 1.7444 (\ln TP) – 4.6545(s) + 1.6341(\ln TN)(s) + B_1(Station) + e$

$p = 0.0005$(TN), $p = 0.0116$ (TP), $p = 0.0016$ (s), $p = 0.0006$ (TN*s)

$S = 5.4232 - 0.5574(\ln DIN) + B_1(Station) + B_2(Country) + e$,

$p = 0.0282$ (DIN)

Total algal cover (TC, Arcsin %) vs. Secchi depth (S, m) and salinity (s, psu)

$TC(Arcsin) = 0.2344 + 0.06628(S) + B_1(Station) + e$

$p = 0.0012$ (S)

Total cover (TC, %) vs. nutrients (total: TN, TP; inorganic: DIN, DIP; µmol/l)and salinity (s, psu)

$TC(Arcsin) = 5.7571 – 1.8677(\ln TN) - 0.7488(s) + 0.2766(\ln TN)(s) + B_1(St) + B_2(C) + e$

$p = 0.0029$ (TN), $p = 0.0062$(s), 0.0019 (TN*s)

Non significant

Total algal cover vs. the nutrients found to control Secchi depth

Not reported as TN was the main nutrient found to control both Secchi depth and total algal cover

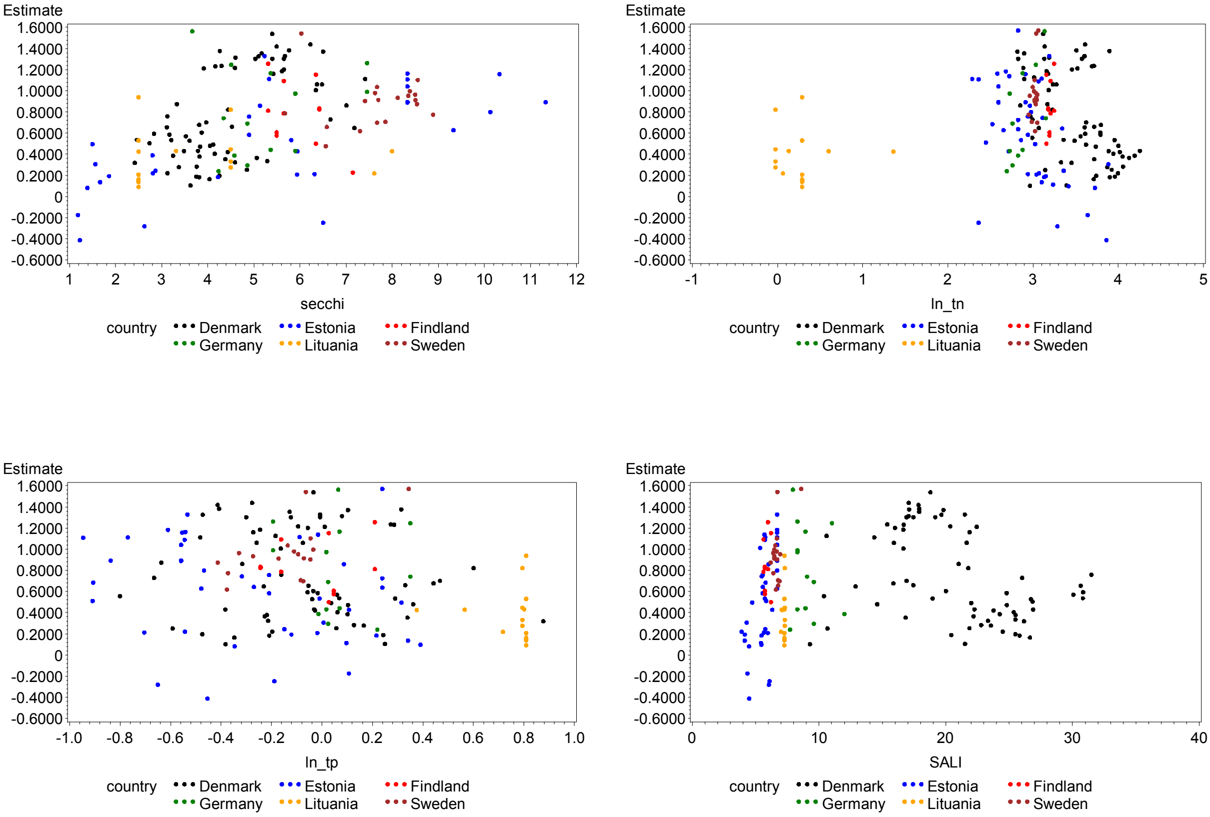


Figure 7. Total macroalgal cover (arc-sin transformed, %) estimated at 7 m water depth as a function of Secchi depth (upper left panel), TN (upper right panel), TP (lower left panel) and salinity (lower right panel) in Danish fjords and the Baltic Sea east of the Gedser-Darß sill.

Discussion

Our study documents significant negative effects of eutrophication on total macroalgal cover (TC) across the open Norwegian North Sea to the inner Baltic Sea and thereby confirmed our primary hypothesis. In the entire region, Secchi depth declined in response to increasing TN, and in more brackish areas also in response to increasing TP. The large data set with multiple combinations of e.g. levels of nutrients and salinity improves the possibility to distinguish between the various effects and in this case attribute the effect to nutrients. Moreover, Secchi depth generally declined in response to increasing DIN, DIP or both. Total macroalgal cover (TC) declined in response to declining Secchi depth and in response to increasing TN or TP and DIN or DIP, but the analyses were not conclusive regarding N versus P limitation. The combined role of N and P in regulating the algae was particularly apparent in the more saline region where TN caused reduced Secchi depth which again caused reduced TC, but where TP, rather than TN correlated directly with algal cover. This result differs from earlier studies of macroalgae in Danish open and coastal waters which have more unequivocally identified N as the most limiting nutrient (Krause-Jensen et al. 2007, Carstensen et al. 2008, Dahl and Carstensen 2008). Recent reviews highlight the importance of a balanced control of nitrogen and phosphorus even though nitrogen is generally the major limiting nutrient of coastal and marine waters (Conley et al 2009, Howarth et al. 2011).

Our hypotheses regarding a positive effect of salinity on TC and Secchi depth were only partly confirmed. In open waters with relatively high salinity, salinity did show a positive correlation with Secchi depth and TC whereas interaction effects of salinity and nutrients could be either positive or negative. However, in the more brackish coastal waters and fjords, salinity showed no direct correlation with Secchi depth, a negative correlation with TC and positive interactive effects with nutrients on both Secchi depth and TC. The positive effect of salinity on TC in the open, most saline waters is likely related to the high diversity of algal species in the most saline waters (Nielsen et al., 1995) which, by representing various life forms and forming a multi-layered community, should be able to exploit the incoming light more efficiently and thus be more productive and dense than a less diverse community (Spehn et al., 2000). A large diversity is not universally a prerequisite for high cover, however, as, for instance, *Fucus vesiculosus* as a monoculture may exhibit high cover.

In the more brackish areas, the negative effect of salinity on TC may be associated with reduced predation by sea star (*Asterias rubens*) on blue mussel. Blue mussels often occur in very high number closely associated with algal vegetation east of the sill at Gedser-Darß and Drogden and in some Danish fjords. The low salinity in this region reduces the occurrence of sea star and consequently reduces the capacity of sea star to exert grazing control of the blue mussel population as is the case on reefs in Kattegat and other saline waters. Blue mussels may affect TC through a combination of shading by newly settled shells and sedimenting faeces, competition with algae for substrate, and by constituting an unstable algal-substrate supporting less algal cover (Albrecht 1998). The negative effect of salinity on total algal cover in the areas of low salinity may also be related to the fact that green algae, which are not as firmly fixed to the substratum as brown and red algae, constitute an increasing fraction of the algal community as salinity declines (Nielsen et al. 1995). The special hydrographic characteristics of the study area may contribute to explaining the diverging effect of salinity between regions. In the study area, the traditional estuarine gradients of nutrient-rich freshwater from land mixing with less nutrient rich saline water towards the open coasts is thus overlaid by a large-scale gradient of brackish, less nutrient-rich Baltic mixing with saline, more nutrient-rich North Sea and Skagerrak waters.

All the identified relationships between macroalgal cover and physico-chemical factors showed considerable variability. We identify three factors as major contributors to this variation which we discuss below: 1) omission of important regulating factors in the analyses, 2) methodological differences in data acquisition between countries and 3) imperfect coupling between algal sites and physico-chemical sites.

Macroalgal communities are regulated by a complex of anthropogenic pressures and environmental factors which are not all included in the analysis. Eutrophication, taken into account in the analysis, is a major factor acting upon the attached macroalgal community through effects on e.g. water clarity, growth of epiphytic and drifting opportunistic algae, and oxygen conditions (e.g. Cloern et al. 2001, Kemp et al. 2005, present results). The large salinity gradients of the region, also taken into account in the analysis, equally have fundamental effect on the algal communities (Nielsen et al. 1995, Middelboe et al. 1997, present results). However, other pressures not considered in the analysis, also affect the macroalgal community and thereby contribute to the variability in the identified relationships between eutrophication, salinity and macroalgal cover. Fishing activity acting through top-down control is expected to exert a major regulating effect on the macroalgal community which may also interact with effects of eutrophication (e.g. Jackson 2001, Steneck et al. 2002, Baden et al. 2010). Physical disturbance of the sea bottom and coastal construction works may also play a regulating role, both directly through burial or destruction of the macroalgae and indirectly by reducing water clarity (e.g. HELCOM 2010). Water temperature is also a major regulator of metabolic activity, growth and geographical distribution of seaweeds (Lüning 1990) which is, unfortunately, not included in the present analysis. The geographical gradient covered by the present study indeed includes a significant variation in seawater temperature which is likely to explain some of the spatial variation in data. Projected temperature increases are also likely to affect large-scale patterns of distribution and abundance of macroalgal communities globally (Müller et al 2009) as well as in the study region. Increased temperatures may also accentuate the negative effects of eutrophication on marine vegetation as higher temperatures tend to stimulate respiration rates and thereby increase the light demand of the vegetation. The recent decline in the distribution of sugar kelp along the Skagerrak coast of Norway may be due to increased temperature in combination with increased run-off from land and interaction with eutrophication (e.g. Moy et al. 2008, Moy & Christie submitted). Inclusion of water temperature in future analyses of the large-scale dataset may, thus, reduce the unexplained variation. Physical exposure expressed as fetch or wave indices would also be relevant to include in future analyses as it is an important modulating factor in macroalgae communities (Wernberg and Connell 2008).

Methodological differences in data acquisition among the national monitoring programs cause major variation in the results since we had to apply many assumptions in order to make the various estimates of total cover comparable (see methods). The significant effect of country indicates that the data harmonization was not completely successful. The German data set, in particular, differed from the rest most likely due to the many estimation procedures needed to obtain data on total cover on hard substratum. Moreover, only few of the German samples represented water depths where the algal community was light limited and the 'algal cover versus depth model' was therefore weak. The exclusion of German data from the analysis eliminated the significant effect of country and thereby increased the comparability of the remaining data. The fact that we conducted separate analyses for the saline, open areas in the west and the more brackish waters of the Danish fjords and the Baltic Sea east of the Darß sill also helped increase the comparability among the data included in each set of analyses. The open more saline areas had deeper algal communities which were best described by mean values at 15 m water depth while the less saline and generally more protected stations of the fjords and the Baltic Sea had shallower algal belts which were better described by mean values at 7 m water depth. Natural variability in algal cover between subsamples, stations, divers, years and seasons also contributes to the uncertainty in the estimated mean values of algal cover; a variability which can be reduced by optimizing design and intensity of monitoring programs and conducting training and intercalibration exercises among divers.

Imperfect coupling between algal stations and physico-chemical stations is another major methodological reason for the considerable variability of the relationships between algal cover and environmental variables. In some cases physico-chemical stations were located at quite some distance from algal stations and were sampled at low frequency, implying that the actual physico-chemical conditions at the sampling sites were not well described. The estimation of average values of nutrients and Secchi depth could probably be improved substantially by increasing the sampling frequency of physico-chemical stations to better match the highly dynamic seasonal variation and by ensuring that the physico-chemical stations actually reflect the conditions in the near-score locations where the algae grow.

In spite of large variability in the identified relationships, our results demonstrate that it is possible to describe a key element for hard bottom habitats, in this case 'total algal cover' as a function of eutrophication pressure and salinity over wide geographical ranges. The study also highlights the advantage of applying models which allow harmonization and comparison of data sampled at different depths, years, seasons and by different divers and subsequently relating the data set to physico-chemical regulating factors. A future adjustment of national monitoring programs to include a direct measurement of total algal cover on hard stable substrate could easily be implemented and would reduce the random variability in data and thereby improve the predictive power of future models. Harmonised large-scale monitoring programs with coupled information on macroalgal cover and a range of governing environmental variables also have perspectives with regard to establishing maps of the macroalgal habitats and their potential cover. Such data would allow a better quantification of this important habitat and thereby also make it easier to protect it.

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Case study 4: Combined effects of eutrophication, salinity, and climate on colonization depth of eelgrass (*Zostera marina L.*) in the Baltic Sea

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Comment:

The statistical analyses in this case study are preliminary. They will be redone with a focus on main regression effects before submission of the manuscript to an international journal.

Abstract: Eelgrass (*Zostera marina*) is the most widely distributed marine angiosperm in the northern hemisphere and forms extended meadows providing important ecological services at sites were habitat requirements are fulfilled. These attributes in combination with a marked sensitivity to human pressure make eelgrass an often used indicator of ecological status. The depth extension of eelgrass meadows depends on water clarity and therefore responds to anthropological pressure such as eutrophication which increases light attenuation in the water column. This study aims to explore to what extent variations in climatic conditions and salinity affect the response of eelgrass depth limits to eutrophication. We compiled 2848 observations of eelgrass depth limits and associated information on nutrients, water clarity, salinity, water temperature and insolation across the wide gradients of the Baltic Sea region

spanning 13 degrees of latitude, and 12 degrees of longitude from Denmark in the south west to Finland in the north east. Eelgrass depth limits ranged from 0.5 m to 11.5 m, and analyses using mixed models demonstrated a general increase in depth limits as a function of declining nutrient concentration and increasing water clarity at the occurring summer temperatures across the region. A significant interaction between water temperatures and both water clarity and nutrient concentration showed that increasing water temperatures increased the positive effect of water clarity and the negative effect of nutrients on depth limits. This pattern was further influenced by a negative effect of salinity probably referring especially to the high salinity-range. Further interaction effects of the various variables as well as additional climatic and geographical influence were also apparent in the data as was an effect of country indicating national differences in methodology and possibly also a difference in the distance between eelgrass transects and water chemistry stations. All in all, the fixed effect parameters in the models could explain about 30 % of the variation in eelgrass depth limits in the Baltic Sea region based on variations in water quality, salinity, climatic variables and additional geographical effects.

Introduction

In the coastal zone, sunlight penetrates to the seafloor where it fuels photosynthesis by benthic primary producers such as seagrasses, macroalgae, microphytobenthos and corals. These benthic organisms can contribute markedly to total primary production in areas where light conditions allow (Sand-Jensen and Borum 1991, Borum and Sand-Jensen 1996, Gattuso et al 2006, Krause-Jensen et al. in press) and where other habitat requirements such as proper sediment conditions are also fulfilled (Koch 2001, Kemp et al. 2005).

Seagrasses, marine flowering plants, are the most conspicuous primary producers on illuminated sandy and soft sea floors on the globe. They are widely distributed and form extensive, highly productive meadows which furnish the coastal ecosystem with important ecological services. Theyform habitat and nursery area for many species, stabilize the sediments, protect the coasts against erosion and function as a filter retaining nutrients and carbon (Hemminga and Duarte 2000, Orth et al. 2006). Seagrasses can form meadows from the shallow waters and down to water depths ultimately set by light penetration. The maximum colonization depth demarks the deep edge of the seagrassmeadow where about 11-35% of surface light reaches the bottom(Dennison 1987, Duarte 1991, Nielsen et al. 2002, Duarte et al. 2007).

Eelgrass (*Zostera marina L*) is the most widely distributed marine angiosperm in the northern hemisphere where it thrives across large ranges in temperature and salinity, in Europe extending as far south as the Portuguese Atlantic coast and the N. Mediterranean coast to as far north as northern Norway, Iceland and Greenland (den Hartog 1970, Green and Short 2003). It also extends from fully marine conditions (35 psu) along open marine coasts to brackish water of salinities down to 5 psu in inner parts of estuaries and e.g. to inner parts of the Baltic Sea (den Hartog 1970).

The colonization depth of seagrasses mainly regulated by water clarity, ranging across species and regions from > 40 m in clear waters to < 1 m in unclear waters (Duarte 1991, Duarte et al. 2007). The relationship between colonization depth and water clarity shifts at a threshold of light attenuation of 0.27 m^2 with the seagrasses growing in turbid waters showing higher light demands than those growing in clear waters. This finding implies that separate equations are needed to predict colonization depths for seagrasses such as *Posidoniaoceanica*growing in clear waters of the Mediterranean Sea as opposed to e.g. *Z. marina* growing in more turbid waters further north (Duarte et al. 2007).

Relationships between seagrass colonization depth and water clarity have also been documented for *Z. marina* (Dennison 1987, Nielsen et al. 2002). And as the colonization depth reflects water clarity, it also correlates with anthropological pressure such as eutrophication, which causes increased light attenuation e.g. through stimulation of phytoplankton growth and ensuing increased chlorophyll concentration (Nielsen et al. 2002). Eelgrass colonization depth, and probably also colonization depths of other species, do, however, show considerable variation at a given water clarity (Duarte 1989, Nielsen et al. 2002). The reason for this variation has to some extent been explored and the anomaly in depth limit has been found, in e.g. Danish coastal waters, to correlate with differences in sediment conditions, nutrient- and salinity levels (e.g. Greve and Krause-Jensen 2005, Krause-Jensen et al. 2011).

However, no studies have yet analysed whether the same relationships between seagrass colonization depth and water clarity are applicable across large geographical scales that represent marked variations in climatic conditions and salinity and explored the probable influence of these variables on the relationship between eelgrass and light attenuation.

The climatic conditions of the north may exert negative as well as positive effects on eelgrass growth and depth colonization. The shorter growth season and the longer dark period imply that eelgrass has fewer days with sufficient light to support positive net photosynthesis. On the other hand, lower temperatures help keep respiration rates low relative to photosynthesis rates and thereby should increase the possibility to maintain a positive net photosynthesis at the low light levels available at the colonization depth. It has been demonstrated that the growth of *Z. marina* at high light intensity increases with temperature whereas growth in low light environments decreases as temperatures increase (Bulthuis 1987; Stæhr and Borum 2011).

Even though *Z. marina* is a euhaline species tolerating a wide range of salinities, it is also likely that the plant requires more energy to balance losses at the edge of the salinity range and that this would be reflected in higher light demands and, thus, relatively lower colonization depth at low salinities.

This study aims to explore to what extent variations in climatic conditions and salinity affect the response of eelgrass colonization depth to water clarity and eutrophication in the North Sea/Baltic Sea region. We hypothesize 1) that colonization depths correlate with light attenuation in the water column across the entire region with the deepest colonization in the clearest waters but that 2) colonization depths are relatively shallower furthest north where the growth season is shorter, and 3) also shallower furthest west in the Baltic Sea where eelgrass grows at the edge of its distribution range with respect to salinity.

Methods

Data

Data on *Z. marina* colonization depths were compiled along S-N and W-E gradients in the Baltic Sea region. The compilation extended from the southern Danish coasts to the Gulf of Finland in the north and from and the Swedish Skagerrak coasts in the west to the Gulf of Finland in the inner part of the Baltic Sea in the east (Table 1). The colonization depth was defined as the maximum depth of individual eelgrass shoots. Danish eelgrass data originated from the national Danish database on marine data (MADS), compiled as part of the Danish National Monitoring and Assessment Program in marine areas.Local departments of the Nature Agency perform the monitoring using common guidelines (Krause-Jensen et al. 2001) and report the results to the national database maintained by the National Environmental Research Institute (NERI). Swedish data originated fromHafok AB and the Swedish database of marine monitoring data maintained by Sverigesmeteorologiskaochhydrologiskainstitute (SMHI), Finnish data were supplied by Christoffer Boström, ÅboAkademi and data on depth limits of *Z. marina* in German waters were supplied by Karin Fürhaupter and Rolf Karez.

The data on *Z. marina* depth limit was associated with physico-chemical variables in the water column: concentrations of total nitrogen (TN), total phosphorus (TP), Secchi depth, salinity, and water temperature. Data on water chemistry were provided along with the *Z. marina* data from each partner. However, additional data sourcing was needed, and water chemistry data (TN, TP and salinity) were also obtained from the distributed marine database DAS hosted by The Baltic Nest Institute in Stockholm, while Secchi depth data were obtained from ICES. Climatic data in form of satellite data for daily solar radiation and water surface temperature was obtained from National Oceanic and Atmospheric Administration, United States Department of Commerce. Prior to analysis, nutrients (TN and TP concentrations) and winter water temperature were aggregated into yearly estimates covering the first six month of the year, while salinity, Secchi depth and summer temperature were aggregated into yearly summer estimates covering the period May to October. The aggregation was conducted station-wise by fitting a generalized linear model accounting for variation in sampling times and intensity using the statistical software package SAS/STAT 9.2 (SAS Institute Inc, 2009).

Statistical analyses

Data were analysed by fitting mixed models describing the observed depth limits of *Z. marina* as a function of physico-chemical and geographical variables, using the PROC MIXED in the statistical software package SAS/STAT 9.2 (SAS Institute Inc, 2009). A strong correlation between nutrients and Secchi depths led to the formulation of two sets of mixed effect models describing the depth limit of *Z. marina* as either a function of nutrient concentration (TN, TP) or Secchi depth combined with salinity and climatic variables (winter and summer temperature, solar radiation). Each of these models was further fitted with the inclusion of geographical positioning order to evaluate the importance of potential systematic geographical variation not accounted for by the available physio-chemical parameters. Physico-chemical and geographical parameters were included as fixed effects,while effect of 'Country' was included as random to account for unexplained local effects such as variation in sampling methodology. The approach resulted in four initial models:

1a. The full initial model including Secchi depth, salinity and climatic variables but excluding geographical position:

$$E(\text{depth limit of } Z.\ marina) = \beta_0 + \beta_1{*}Secchi + \beta_2{*}Salinity + \beta_3{*}Solar\ radiation + \beta_4{*}Temperature\ (winter) + \beta_5{*}Temperature\ (summer) + \beta_6{*}Secchi\ {*}Solar\ radiation + \beta_7{*}Secchi\ {*}\ Temperature\ (winter) + \beta_8{*}Secchi\ {*}Temperature\ (summer) + \beta_9{*}Secchi\ {*}\ Salinity + B(Country_i) + \varepsilon,$$

where the effect of country ($B(Country_i)$ $\sim N(0,\sigma^2_{country})$) is random.

2a.The full initial model including Secchi depth, salinity, climatic variables, and geographical position:

$$E(\text{depth limit of } Z.\ marina) = \beta_0 + \beta_1{*}Secchi + \beta_2{*}Salinity + \beta_3{*}Solar\ radiation + \beta_4{*}Temperature\ (winter) + \beta_5{*}Temperature\ (summer) + \beta_6{*}Longitude\ (UTM) + \beta_7{*}Latatitude\ (UTM) + \beta_8{*}Secchi\ {*}Solar\ radiation + \beta_9{*}Secchi\ {*}$$

$$\text{Temperature (winter)} + \beta_{10}{*}\text{Secchi}\ {*}\text{Temperature (summer)} + \beta_{11}{*}\text{Secchi}\ {*}\ \text{Salinity} + B(\text{Country}_i) + \varepsilon,$$

where the effect of country ($B(Country_i)$ ~N$(0,\sigma^2_{\text{country}})$) is random.

3a.The full initial model including nutrients, salinity, climatic variables but excluding geographical position:

$$E(\text{depth limit of Z. marina}) = \beta_0 + \beta_1{*}\ln(\text{totN}) + \beta_2{*}\ln(\text{totP}) + \beta_3{*}\text{Salinity} + \beta_4{*}\text{Solar radiation} + \beta_5{*}\text{Temperature (winter)} + \beta_6{*}\text{Temperature (summer)} + \beta_7{*}\ln(\text{TN})\ {*}\text{Solar radiation} + \beta_8{*}\ln(\text{TP})\ {*}\text{Solar radiation} + \beta_9{*}\ln(\text{TN})\ {*}\text{Temperature (winter)} + \beta_{10}{*}\ln(\text{TP})\ {*}\text{Temperature (winter)} + \beta_{11}{*}\ln(\text{TN})\ {*}\text{Temperature (summer)} + \beta_{12}{*}\ln(\text{TP})\ {*}\text{Temperature (summer)} + \beta_{13}{*}\ln(\text{TN})\ {*}\text{Salinity} + \beta_{14}{*}\ln(\text{TP})\ {*}\text{Salinity} + B(\text{Country}_i) + \varepsilon,$$

where the effect of country ($B(Country_i)$ ~N$(0,\sigma^2_{\text{country}})$) is random.

4a.The full initialmodel including nutrients, salinity, climatic variables and geographical position:

$$E(\text{depth limit of Z. marina}) = \beta_0 + \beta_1{*}\ln(\text{totN}) + \beta_2{*}\ln(\text{totP}) + \beta_3{*}\text{Salinity} + \beta_4{*}\text{Solar radiation} + \beta_5{*}\text{Temperature (winter)} + \beta_6{*}\text{Temperature (summer)} + \beta_7{*}\text{Longitude (UTM)} + \beta_8{*}\text{Latatitude (UTM)} + \beta_9{*}\ln(\text{TN})\ {*}\text{Solar radiation} + \beta_{10}{*}\ln(\text{TP})\ {*}\text{Solar radiation} + \beta_{11}\ln(\text{TN})\ {*}\text{Temperature (winter)} + \beta_{12}{*}\ln(\text{TP})\ {*}\text{Temperature (winter)} + \beta_{13}\ln(\text{TN})\ {*}\text{Temperature (summer)} + \beta_{14}{*}\ln(\text{TP})\ {*}\text{Temperature (summer)} + \beta_{15}{*}\ln(\text{TN})\ {*}\text{Salinity} + \beta_{16}{*}\ln(\text{TP})\ {*}\text{Salinity} + B(\text{Country}_i) + \varepsilon,$$

where the effect of country ($B(Country_i)$ ~N$(0,\sigma^2_{\text{country}})$) is random.

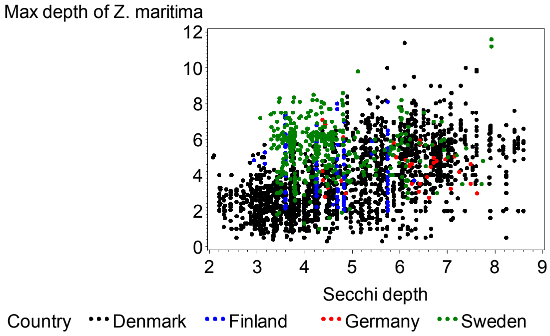
Nutrient concentrations (TN and TP) were log transformed prior to the analysis in order to linearize the relationships between the parameters and the depth limit of *Z. marina*. In order to be able to compare the final models using model selection criteria like corrected Akaike Information Criterion (AICC) and Bayesian Information Criterion (BIC), a subset of 2016 observations without missing values for any of the parameters included in the models was selected.

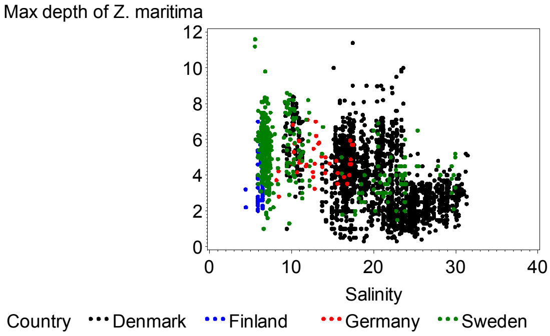
Results

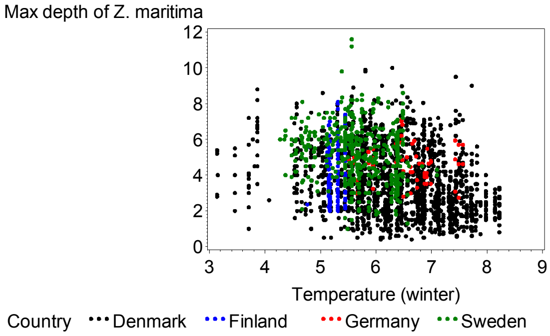
We compiled 2848 observations of *Z. marina* depth limits and associated information on physico-chemistry and climatic variables from the Baltic Sea spanning 13 degrees of latitude, from Denmark in south (53ºN) to Finland in north (66ºN) and 12 degrees of longitude from Denmark in west (9ºE) to Finland in east (21 ºE, Table 1, Fig. 1). Colonization depths ranged from 0.5 m to 11.5 m while the associated physico-chemical and climatic variables showed the following ranges: nutrients (Jan-Jun): 2-115 µM TN and 0.38-5.3 µM TP, Secchi depths (May-Sep): 2.1-8.6 m, Salinity (May-Oct): 4-31 psu, water temperature in winter (Oct-Mar) 3.1-8.2ºC, water temperature in summer (May-Oct) 10.6-15.3 ºC and annual cumulated solar radiation 240-271 rad (Figure 1). The correlation between nutrient levels and Secchi depth (Fig. 2) resulted in two sets of mixed models explaining the variation in depth limits based on the above variables.

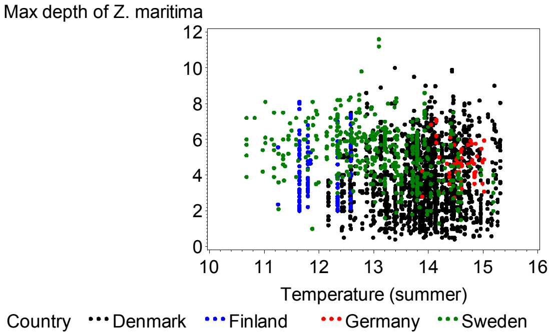
Table 1. Data on depth limit of *Zostera marina* included in the analysis.

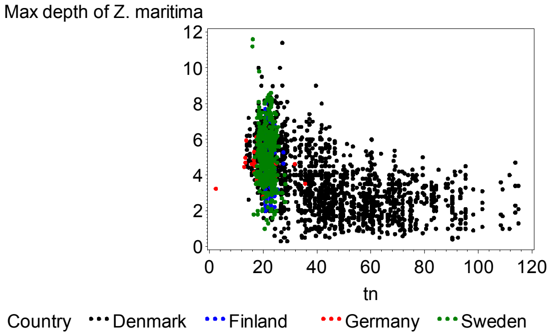
Country	Area	Number of stations	Time span	Number of observations
Denmark	Kattegat	311	1986 - 2009	2158
	BeltSea			
	Fjords			
	Western Baltic			
Finland	Gulf of Finland	111	2005 - 2009	111
	Achipelago			
Germany	Western Baltic	20	2006 - 2009	56
Sweden	Skagerrak	325	1974 - 2009	523
	Kattegat			
	The Sound			

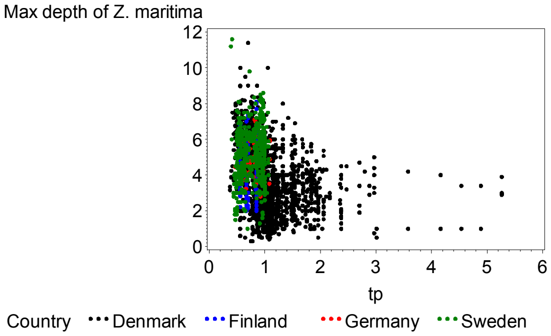

Max depth of Z. maritima
12
10
8
6
4
2
0
2
3
4
5
6
7
8
9
Secchi depth
Country
Denmark
Finland
Germany
Sweden

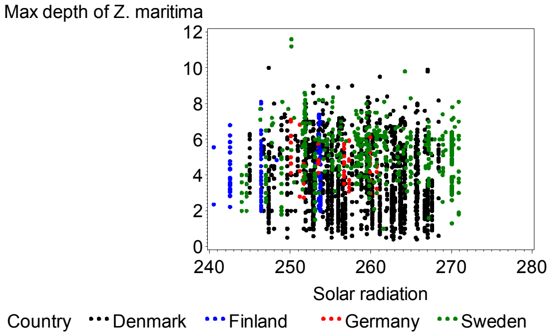

Max depth of Z. maritima
12
10
8
6
4
2
0
0
10
20
30
40
Salinity
Country
Denmark
Finland
Germany
Sweden

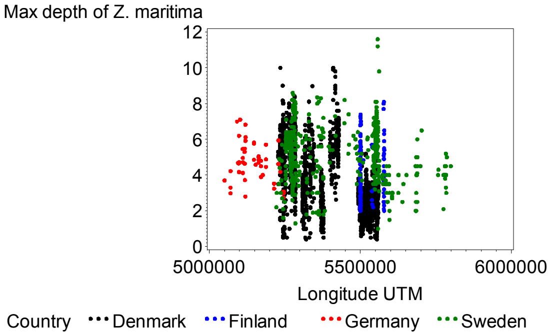

Max depth of Z. maritima
12
10
8
6
4
2
0
3
4
5
6
7
8
9
Temperature (winter)
Country
Denmark
Finland
Germany
Sweden


Max depth of Z. maritima
12
10
8
6
4
2
0
10
11
12
13
14
15
16
Temperature (summer)
Country
Denmark
Finland
Germany
Sweden


Max depth of Z. maritima
12
10
8
6
4
2
0
0
20
40
60
80
100
120
tn
Country
Denmark
Finland
Germany
Sweden


Max depth of Z. maritima
12
10
8
6
4
2
0
0
1
2
3
4
5
6
tp
Country
Denmark
Finland
Germany
Sweden


Max depth of Z. maritima
12
10
8
6
4
2
0
240
250
260
270
280
Solar radiation
Country
Denmark
Finland
Germany
Sweden


Max depth of Z. maritima
12
10
8
6
4
2
0
5000000
5500000
6000000
Longitude UTM
Country
Denmark
Finland
Germany
Sweden

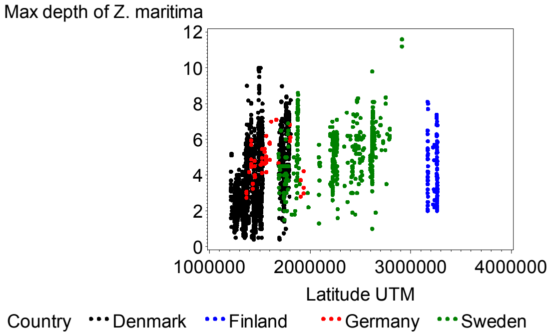


Figure 1. Scatter plots of depth limit (m) of *Zostera marina*v.s. each of the physico-chemical variables and geographical position: concentrations of total nitrogen (TP, µM) and total phosphorus (TP, µM). Secchi depth (m), Salinity (psu), Water temperature (ºC) in winter and summer, respectively, solar radiation (rad), longitude and latitude (UTM).

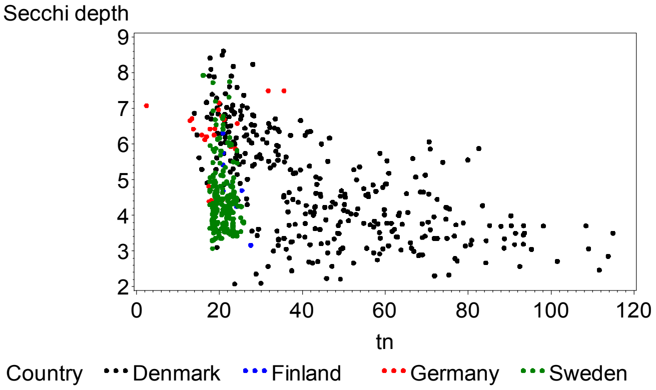


Figure 2.Relationship between total nitrogen (TN, µM) concentration and Secchi depth.

Stepwise reductions of the initial full mixed effect models based upon analysis of variance of the fixed effect parameters and likelihood-ratio test of the random effect parameters showed that they, without significantly affecting their abilities to describe the depth limits of *Z. marina*, could be reduced to:

1b.Including Secchi depth, salinity and climatic variables but excluding geographical position:

$$E(\text{depth limit of Z. marina}) = \beta_0 + \beta_1{*}\text{Secchi} + \beta_2{*}\text{Salinity} + \beta_3{*}\text{Solar radiation} + \beta_4{*}\text{Temperature (summer)} + \beta_5{*}(\text{Secchi}{*}\text{Temperature (summer)}) + B(\text{Country}_i) + \varepsilon,$$

where the effect of country $(B(\text{Country}_i) \sim N(0,\sigma^2_{\text{country}})$ is random.

2b.Including Secchi depth and geographical parameters:

$$E(\text{depth limit of } Z.\ marina) = \beta_0 + \beta_1 * Secchi + \beta_2 * \text{Solar radiation} + \beta_3 * \text{Temperature (summer)} + \beta_4 * \text{Longitude (UTM)} + \beta_5 * \text{Latitude (UTM)} + \beta_6 * Secchi * \text{Temperature (summer)} + B(Country_i) + \varepsilon,$$

where the effect of country ($B(Country_i)$ ~N(0,$\sigma^2_{\text{country}}$)) is random.

3b.Including nutrients, salinity and climatic variables but excluding geographical position:

$$E(\text{depth limit of } Z.\ marina) = \beta_0 + \beta_1 * \ln(totN) + \beta_2 * \ln(totP) + \beta_3 * Salinity + \beta_4 * \text{Solar radiation} + \beta_5 * \text{Temperature (summer)} + \beta_6 * \ln(TN) * \text{Solar radiation} + \beta_7 * \ln(TP) * \text{Temperature (summer)} + \beta_8 * \ln(TN) * \text{Temperature (summer)} + B(Country_i) + \varepsilon,$$

where the effect of country ($B(Country_i)$ ~N(0,$\sigma^2_{\text{country}}$)) is random.

4b.Including nutrients, salinity, climatic variables and geographical position:

$$E(\text{depth limit of } Z.\ marina) = \beta_0 + \beta_1 * \ln(totN) + \beta_2 \ln *(totP) + \beta_3 \text{Solar radiation} + + \beta_4 * \text{Temperature (summer)} + \beta_5 * \text{Longitude (UTM)} + \beta_6 * \text{Latitude (UTM)} + \beta_7 * \ln(TN) * \text{Solar radiation} + \beta_8 * \ln(TP) * \text{Temperature (summer)} + \beta_9 * \ln(TN) * \text{Temperature (summer)} + \varepsilon,$$

where the effect of country ($B(Country_i)$ ~N(0,$\sigma^2_{\text{country}}$)) is random.

All four mixed models were complex and included several significant fixed effects and interaction effects influencing the models' abilities to describe the depth limit of *Z. marina* (see significant parameter estimates in Table 2 for models including Secchi depth and in Table 3 for models including nutrient concentrations) as well as random effects of country (see Table 4 for all models). Estimated depth limit of *Z. marina* for combinations of parameters found to interact are shown in Figures 3-4, and for the estimates in these figures other factors in the models were kept constant at the mean. In spite of this complexity, the analyses showed some clear patterns: There was a positive effect of increasing Secchi depth on the depth limit of *Z. marina*, which was found to interact with temperature so the positive effect of Secchi depth increased with increasing temperature (Figure 3). Likewise the effects of nutrients, TN as well as TP, was found to be temperature dependant, so increasing nutrient concentrations appeared to have more negative effect at high temperatures. The main effect of light and water clarity were both negative, butas water clarity showed a significant interaction with water temperature,these main effects only represented a situation with summer water temperature of 0°C. When the interaction with water temperature was taken into account, the models described a positive effect of water clarity at water temperatures above 12°C (which represented 98% ofthe data), and this positive effect increased with increasing water temperature (Figures 3-4).Similar considerations but with opposite sign apply to the direct and interactive effects of nutrient levels and water temperature. The apparent negative effect of light was likely due to a strong positive correlation between light and temperature ($p<0.0001$).

Depth limits also declined as a function of increasing salinity in models that did not take geographical position into account,while salinity was not a significant variable in the models including geographical position, suggesting that the effect of salinity in these models was part of the geographical variation accounted for. Depth limits generally increased towards north and west in the Baltic Sea when effects of gradients in the physico-chemical and climatic variables were already taken into account by the model, suggesting that additional parameters not available for the present study but showing a systematic variation through the Baltic Sea, also influenced the depth limit of *Z. marina*. Moreover, all models showed a significant random effect of country ($p<0.001$ for all four models) indicating systematic differences in e.g. sampling procedures and possibly also of the varying distance between eelgrass transects and water chemistry stations between countries.

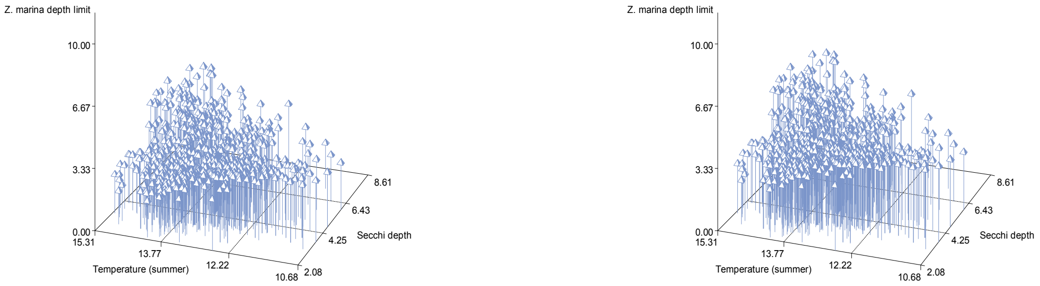


Figure 3. Upper left: Depth limit of *Z. marina* as a function of Secchi depth and temperature (summer) estimated from the model including Secchi depth, salinity, climatic variables and geographic position. Upper right: Depth limit of *Z. marina* as a function of Secchi depth and temperature (summer) estimated from the model including Secchi depth, salinity and climatic variables.

Table 2. Parameter estimates for fixed effects in the two models describing the depth limit of *Z. marina* as a function of physico-chemical variables (incl. Secchi depth), climatic variables with/without geographical position.

Effect	**Estimate**	**STD**	**DF**	**t Value**	**Pr> |t|**
Model including Secchi depth, salinity and climatic variables					
Intercept	26.5984	2.6121	2	10.18	0.0095
Secchi depth	-2.9084	0.4190	1980	-6.94	<0.0001
Salinity	-0.1020	0.008372	1980	-12.19	<0.0001
Solar radiation	-0.02899	0.005665	1980	-5.12	<0.0001
Temperature (summer)	-1.1105	0.1592	1980	-6.97	<0.0001
Secchi * Temperature (summer)	0.2372	0.03024	1980	7.84	<0.0001
Model including Secchi depth, salinity climatic variables and geographic position					
Intercept	32.5122	3.4278	3	9.48	0.0025
Secchi depth	-2.5338	0.4094	2006	-6.19	<0.0001
Solar radiation	-0.03031	0.005515	2006	-5.50	<0.0001
Temperature (summer)	-0.9732	0.1572	2006	-6.19	<0.0001
Longitude (UTM)	-2.59E-6	0	2006	-Infinity	<0.0001
Latitude (UTM)	2.413E-6	0	2006	Infinity	<0.0001
Secchi * Temperature (summer)	0.2094	0.02950	2006	7.10	<0.0001

Table 3. Parameter estimates for fixed effects in the two models describing the depth limit of *Zostera marina* as a function of physico-chemical (including nutrient concentrations) variables with/without geographical position.

Effect	**Estimate**	**STD**	**DF**	**t Value**	**Pr> |t|**
Model including nutrients, salinity and climatic variables					
Intercept	24.5456	11.2801	3	2.18	0.1178
ln(tp)	1.7445	0.7954	2004	2.19	0.0284
ln(tn)	-2.7555	3.2193	2004	-0.86	0.3921
Salinity	-0.09861	0.008498	2004	-11.60	<.0001
Solar radiation	-0.1613	0.04152	2004	-3.89	0.0001
Temperature (summer)	1.9827	0.3353	2004	5.91	<.0001
ln(tp)* Temperature (winter)	-0.1466	0.06115	2004	-2.40	0.0166
ln(tn)* Temperature (summer)	-0.5720	0.09428	2004	-6.07	<.0001
ln(tn) *Solar radiation	0.03566	0.01169	2004	3.05	0.0023
Model including nutrients, salinity, climatic variables and geographic position					
Intercept	37.0101	11.3682	3	3.26	0.0473
ln(tp)	2.1747	0.8115	2003	2.68	0.0074
ln(tn)	-4.3352	3.1639	2003	-1.37	0.1708
Solar radiation	-0.2026	0.04094	2003	-4.95	<0.0001
Temperature (summer)	2.2487	0.3353	2003	6.71	<0.0001
Longitude (UTM)	-2.37E-6	0	2003	-Infinity	<0.0001
Latitude (UTM)	2.476E-6	0	2003	Infinity	<0.0001
ln(tn)* Temperature (summer)	-0.6416	0.09403	2003	-6.82	<.0001
ln(tp)* Temperature (summer)	-0.1869	0.06205	2003	-3.01	0.0026
ln(tn)* Solar radiation	0.04633	0.01152	2003	4.02	<.0001

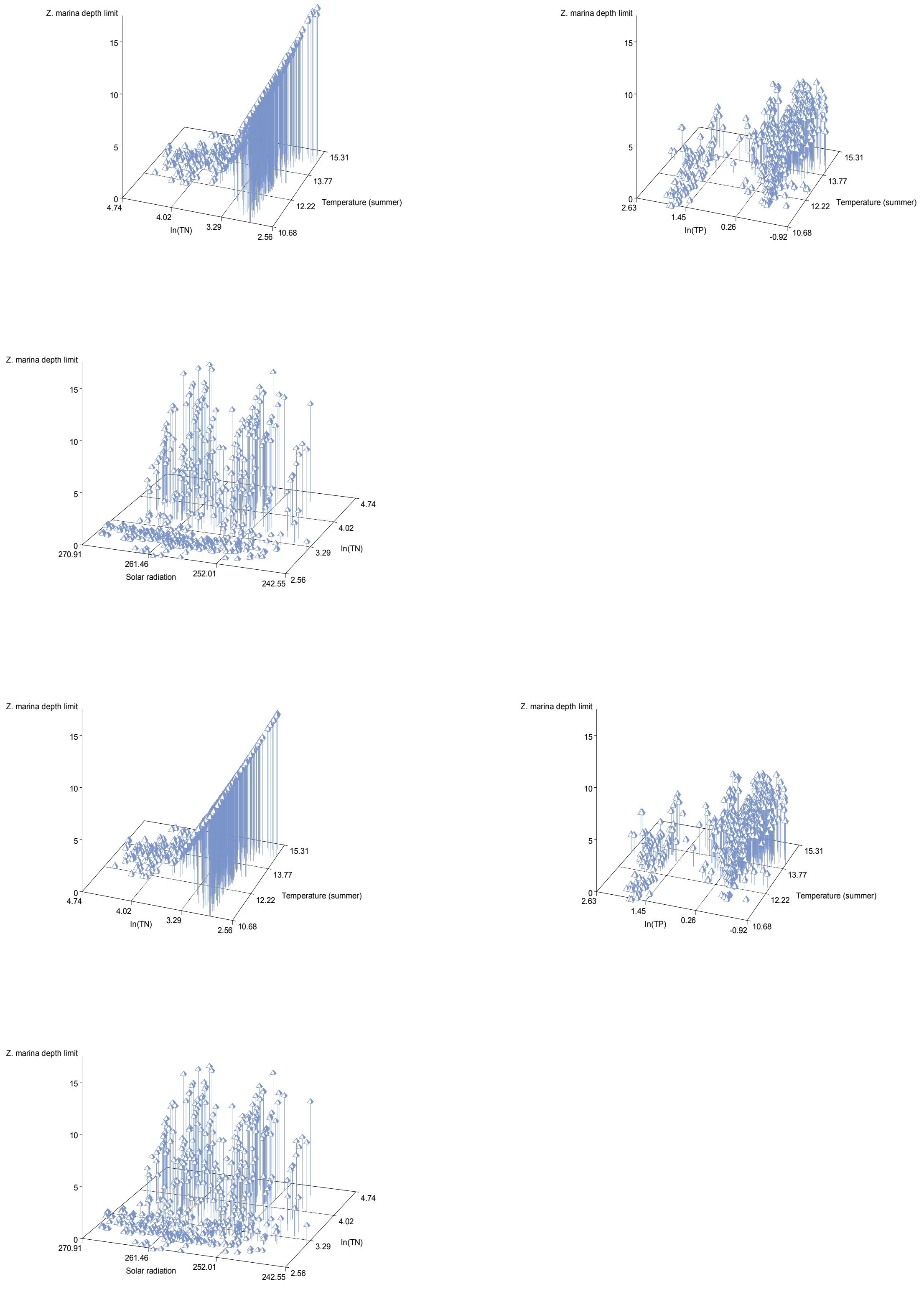


Figure 4. Top row left: Depth limit of *Z. marina* (DL) as a function of ln(TN) and temperature (summer); top towright: DL as a function of ln(TP) and temperature (summer); Second row left: DL as a function of ln(TN) and solar radiation; estimated from the model including nutrients, salinity, climatic variables and geographic position. Third row left: DL *Z. marina* as a function of ln(TN) and temperature (summer); Third rowright:DL of *Z. marina* as a function of ln(TP) and temperature (summer); Bottom row left: DL of *Z. marina* as a function of ln(TN) and solar radiation; estimated from the model including nutrients, salinity and climatic variables.

Table 4. Parameter estimates for the random factor 'Country'.

Effect	Estimate	STD	DF	t Value	Pr> |t|
Model including Secchi depth, salinity and climatic variables					
Denmark	-0.07930	0.5542	1980	-0.14	0.8862
Finland	-0.8858	0.5599	1980	-1.58	0.1138
Germany	0.1821	0.4231	1980	0.43	0.6669
Sweden	0.9651	0.5495	1980	1.76	0.0792
Model including Secchi depth, salinity, climatic variables and geographic position					
Denmark	0.8679	0.8757	2006	0.99	0.3218
Finland	-2.5510	0.8979	2006	-2.84	0.0045
Germany	0.6504	0.8946	2006	0.73	0.4672
Sweden	1.0327	0.8687	2006	1.19	0.2347
Model including nutrients, salinity and climatic variables					
Denmark	0.5383	0.4577	2004	1.18	0.2397
Finland	-1.0165	0.4789	2004	-2.12	0.0339
Germany	-0.4052	0.4932	2004	-0.82	0.4113
Sweden	0.8835	0.4597	2004	1.92	0.0548
Model including nutrients, salinity, climatic variables and geographic position					
Denmark	1.4142	0.9933	2003	1.42	0.1547
Finland	-2.8510	1.0221	2003	-2.79	0.0053
Germany	0.4149	1.0113	2003	0.41	0.6816
Sweden	1.0220	0.9871	2003	1.04	0.3006

A further comparison of the two models based upon Secchi depth using the two model selection criteria, corrected Akaike Information Criterion (AICC) and Bayesian Information Criterion (BIC), listed in Table 4, shows however, that the simpler model not including longitude and latitude is to be preferred despite the significant contributions of the geographical position.

Table 5. Model selection criteria, corrected Akaike Information Criterion (AICC) and Bayesian Information Criterion (BIC) for models describing the depth limit of *Zostera marina* as a function of physico-chemical and geographical position.

Model	Number of parameters	AICC	BIC
Models including Secchi depth and additional variables			
Secchi depth, climatic variables	7	7199.8	7198.0
Secchi depth, climatic variables, latitude, longitude	8	7256.7	7255.4
Models including nutrient concentrations and additional variables			
Nutrient concentrations, climatic variables	10	7177.2	7176.0
Nutrients concentrations, climatic variables, latitude, longitude	11	7169.6	7168.3

A comparison of predicted and observed depth limits of *Z. marina* generally show a fine match except for the fact that all four models tend to underestimate the highest observed *Z. marina* depth limit (Figure 5). The models based upon nutrients in combination were able to explain slightly more of the variation in the data than the two based upon Secchi depth. For the two models based upon Secchi depth it was found that fixed effect environmental parameters in the model not including geographical variables explained 27% of the variation in the data, while the environmental and geographical variables in the model including longitude and latitude explained 31%. For the two models based upon nutrients it was found that fixed effect environmental parameters in the model not including geographical variables explained 31% of the variation in the data, while the combination of environmental and geographical variables explained 33%.

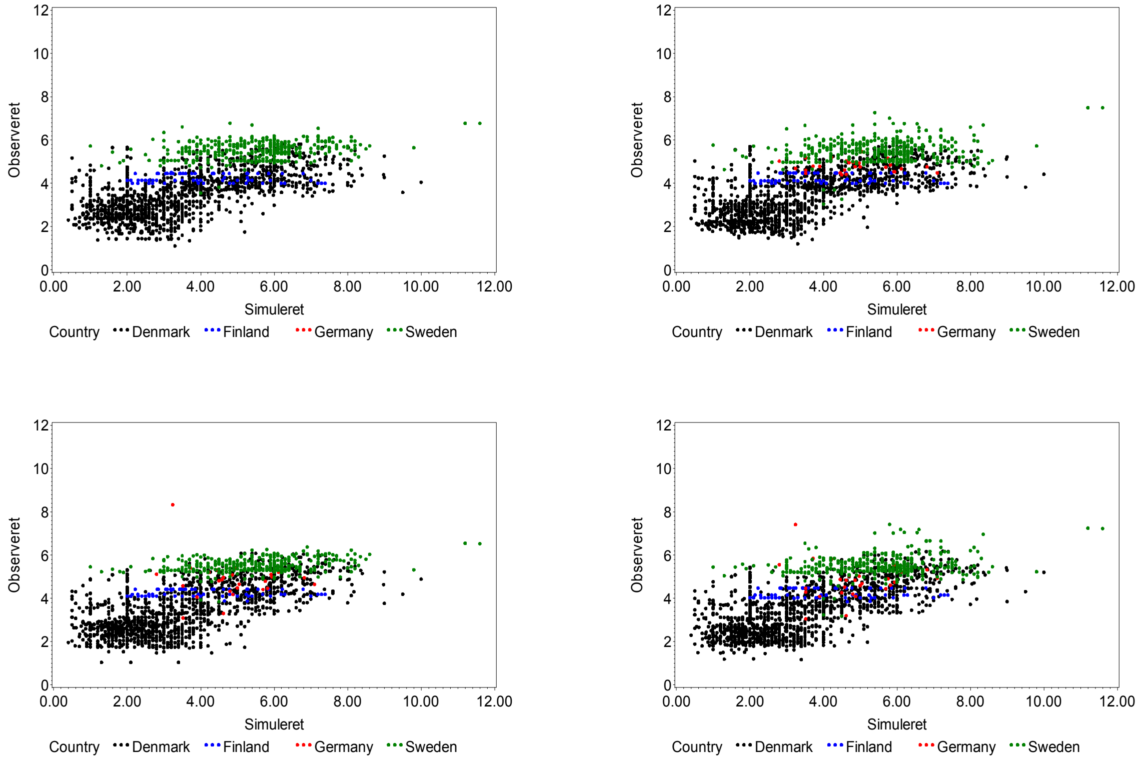


Figure 5. Scatter plots of observed versus predicted depth limit (m) of *Zostera marina*. Top left: predicted values from model including Secchi depth, salinity and climatic variables. Top right: predicted values from model including Secchi depth, salinity, climatic and geographical position. Bottom left: predicted values from model including nutrient concentrations, salinity and climatic variables. Bottom right: predicted values from model including nutrient concentrations, salinity, climatic variables and geographical position.

Discussion

Our study represents the first compilation of *Z. marina* colonization depths for the entire Baltic Sea and the first combined analysis of colonization depth in relation to environmental gradients in the region. The results show that the scale-regulation of eelgrass depth limits in the Baltic Sea is complex, being significantly influenced by marked gradients in eutrophication, salinity and climatic conditions across the region, which can be satisfactorily described through large scale mixed models.

The results confirmed our hypothesis that *Z. marina* colonization depth reflects the marked gradients in water quality across the Baltic Sea region. Eelgrass depth limits thus showed a general and significant increase as a function of increased water clarity and declining levels of TN and TP within the temperature range of the data. The interaction terms between temperature and both nutrients and water clarity lead to the effect of these factors being reinforced by high temperatures, , across the entire region (Figure 3-4), where the gradient in water clarity spanned a factor of about 4 while the gradients of TN and TP concentrations spanned factors of about 14 and 55, respectively! Similar relationships between colonization depth and TN concentration have earlier been identified for more restricted regions, e.g. Danish coastal waters and (Nielsen et al. 2002, Sagert et al. 2005). Water clarity and nutrient levels are correlated as nutrient-rich waters tend to contain larger amounts of light attenuating components. Light attenuation is caused by absorption and scattering by organic particles such as phytoplankton, inorganic particles, dissolved organic matter and water itself. Eutrophic conditions stimulate phytoplankton growth leading to higher chlorophyll concentrations, but may also increase the amount of suspended inorganic particles e.g. through increased resuspension of bottom material as the benthic vegetation is shaded and the seafloor exposed to waves (e.g. Duarte 1995, Carr et al. 2010). Moreover, concentrations of dissolved organic matter may also increase in more eutrophic areas. The negative effect of higher nutrient concentrations on eelgrass depth limits may also act through increased occurrence of algal mats further shading the rooted vegetation (Cloern et al. 2001, Valiela et al. 2007). A considerable part of the phytoplankton and opportunistic macroalgae eventually sediments on the sea floor where it consumes oxygen as it decomposes. High nutrient concentrations may therefore also affect seagrasses negatively by creating unsuitable sediment conditions and increased risk of anoxia, which may kill eelgrass (Kemp et al. 2005, Pulido and Borum 2010, Krause-Jensen et al. 2011). While nitrogen tends to be the major limiting nutrient in marine areas, phosphorus is often the primary limiting nutrient in fresh waters and brackish areas (Conley et al. 2009, Howarth et al. 2011), and it is therefore likely that there is a change in the relative importance of N and P in regulating eelgrass depth limits across the salinity gradient of the Baltic Sea.

The large salinity gradient of the Baltic Sea also influenced eelgrass depth limits significantly. The data set spanned a salinity gradient of 4-31 psu, covering a primary gradient of declining salinity from the west to the inner eastern part of the Baltic Sea as well as secondary estuarine salinity gradients. Depth limits declined as a function of increasing salinity in models that did not take geographical position into account (models # 1 and 3, Tables 2 and 3) but was not a significant variable in the other models (models # 2 and 4, Tables 2 and 3), suggesting that the effect of salinity in the latter models was part of the geographical variation accounted for. The decline in depth limits at high salinitiescould probably relate to the fact that the high salinities are typically found in more open areas outside the fjords and estuaries where conditions in terms of exposure and sediment composition may be less suitable and thus more energy-demanding for eelgrass.

In addition to the effects of water quality and salinity, our study demonstrated climatic effects on eelgrass depth limits. All models demonstrated that higher temperatures reinforced the negative effects of high nutrient concentrations and the positive effect of high water clarity. Higher temperatures increase respiration costs relative to photosynthetic gains and thereby reduce rates of netphotosynthesis (Stæhr and Borum 2011). This mechanism leads to an increase in the light compensation point of eelgrass which again leads to reduced depth penetration. At high temperature eelgrass therefore needs clearer waters in order to obtain a given depth distribution.As nutrient levels and Secchi depths are inversely correlated, higher temperatures also increase the negative effect of high nutrient levels on eelgrass depth limits. A future warmer climate is therefore likely to interact negatively with effects of eutrophication and worsen the eutrophication effects on eelgrass depth limits. Negative effects of warming also relate to increased risk of anoxic events and a reduced tolerance of eelgrass for anoxia at higher temperature (Pulido and Borum 2010).

Solar radiation was also included as climate variable in the model. However, the negative effect of solar radiation on eelgrass depth limit, as indicated by the models, is counter-intuitive and probably due to strong correlation between solar radiation and water temperature in the data set, blurring the possible effect of solar radiation. Solar radiation was assessed as the mean daily solar energy during the summer month at each site. When assessed in this manner, the range in solar radiation among sites was quite low, i.e. from 240 to 271 rad, Probably an analysis of the number of hours with solar energy above a certain level would better reflect the reduction in the length of the growth season and the corresponding longer dark period towards north where production may be insufficient to balance respiration costs.

The model selection criteria indicated that the inclusion of geographical position in the analysis did not increase the performance of the model based upon Secchi depth, salinity and climatic parameters, even though these variable was significant. As the additional effect of position was only minor we will not discuss it further but assume that the major latitudinal and longitudinal gradients in the data were captured by the environmental variables,

For the model based upon nutrients, the climatic data available for the present study were insufficient to explain the entire systematic geographical variation in the data, indicated by both the significant contributions of latitude and longitude to the models' ability to describe the observed depth limits and that by comparison of AICC and BIC of the two models also indicating a better description of the data by the model including geographical variables..

Some of the scatter in the data may be due to the fact that water chemistry sites were not fully overlapping with eelgrass sites,and for some stations mainly originated from more open water stations and therefore did not reflect the exact conditions the *Z. marina* was exposed to. Local variation in monitoring techniques across the region may also contribute to explaining the variation in data, as indicated by the significant contribution of the random parameter describing the effect of the country.

The present compilation and large scale analysis of eelgrass colonization depth in the North sea/Baltic Sea demonstrates that it is possible to describe a key sea grassindicator of ecological status and relate it to physico-chemistry over wide geographical ranges. In European monitoring programs on seagrasses, colonization depths range among the top 3 most commonly used seagrassmetrics (Marbà et al. in prep), and could potentially candidate as a common indicator if standardized methods were applied across large regions. Harmonised monitoring programs operating across large spatial scales allowing coupling of biological indicators with water quality also have perspectives with regard to establishing habitat maps and planning large-scale management measures for seagrass ecosystems.

Acknowledgement

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Case study 5: Intertidal seagrass (*Z. noltii*) along anthropogenic pressure gradients in Atlantic estuaries – the Mondego's case study, Portugal.

By Joao M Neto, Dimitri V. Barroso, Pablo Barría, Joao Carlos Marques

2. Materials and methods

2.1. Study site

The study area is a southern Europe Atlantic estuary located at the western coast of Portugal (Figure 1). The Mondego estuary (40º08'N, 8º50'W) is a shallow Transitional Water (TW) classified as a mesotidal well-mixed estuary with irregular river discharges and included in the Portuguese A2 type (Bettencourt et al., 2004), and as NEA 11 in the WFD (2000/60/EC). The southern arm of the estuary, where seagrass meadows can be found, constitutes a subsystem with 7 km length, 0.5 km width, 2 to 4 m depth and 2.57 km2 in area. The marine influence is strong, and the average tidal amplitude of 1 to 3 m allows up to 75 % of this subsystem's area to be air exposed during low tide. (Neto et al., 2010)

Due to its regional economic value, all the basin was subjected to several physical modifications over the years (Neto et al., 2010). In this sense, the estuary has been continuously receiving high nutrient loads from the Mondego River catchment area, particularly those caused by the direct runoff from the 15,000 ha of cultivated land in the lower river valley (Neto et al., 2008). The estuary supports industrial activities, salt works, mercantile and fishing harbours, as well as the urban pressures from Figueira da Foz, a centre of seasonal tourism activity

Two distinct time intervals could be observed throughout the study period from 1986 to 2009. A first period goes until 1997 and is characterised by a general degradation process occurring in the south arm of the Mondego. A second period, from that date until 2009, is characterised by the implementation of several mitigation measures that resulted in the beginning of an ecological recovery process in the south arm.

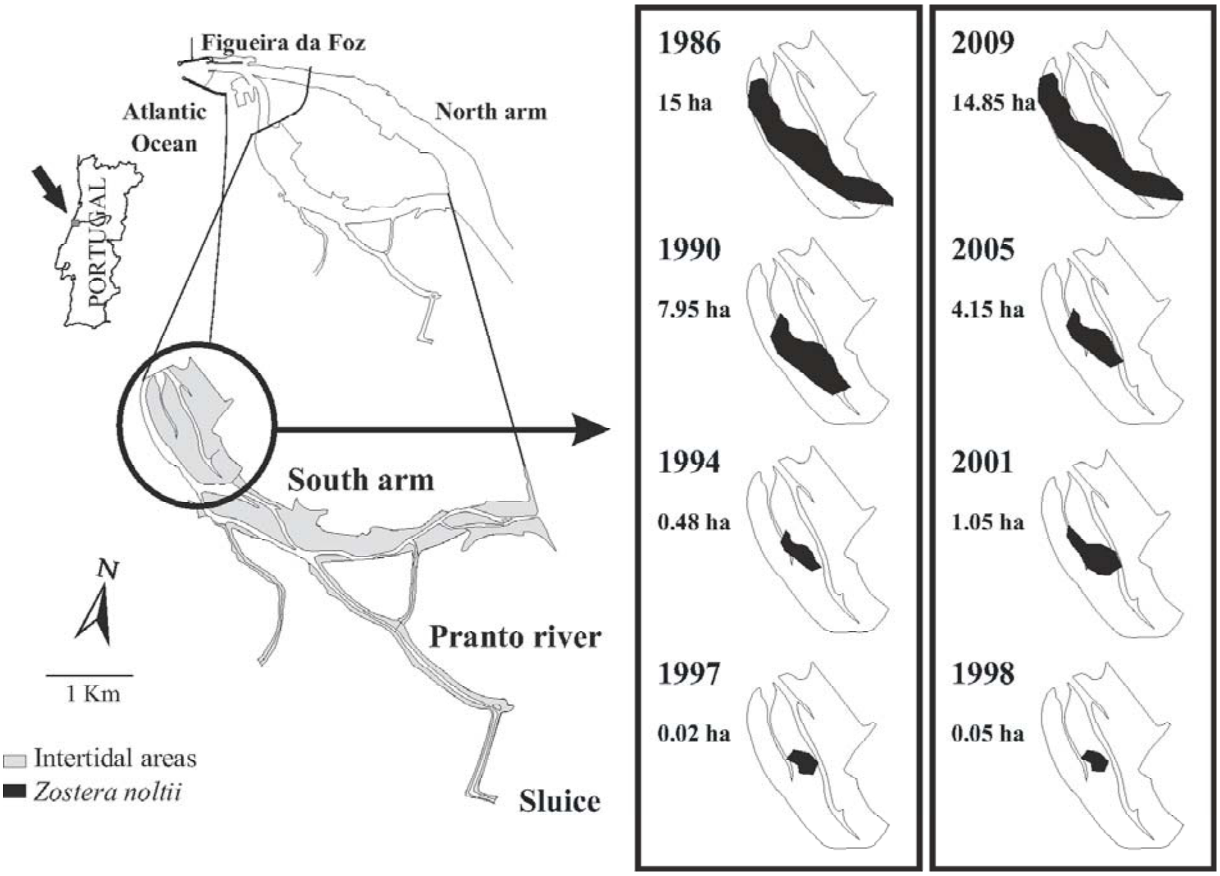


Figure 1. The Mondego estuary. Sampling area in the south arm (circle) and the *Zostera noltii* bed extent along the study period.

The last years of the first period were characterized by an intense anthropogenic disturbance (e.g., margins' regularization in 1990 and 1992, in the north arm), which culminated in a complete interruption of the communication between north and south arms of the river. Eutrophication symptoms were then visible in the south arm, mainly as proliferations of opportunistic green macroalgae (Martins et al. 2001, Marques et al. 2003) and the reduction of the seagrass cover area, possibly due to the decrease in dissolved oxygen and the increase in nitrite and ammonia concentration.

The second period started with the implementation of experimental mitigation measures (1997 and 1998) into the south arm. The communication between the two arms was re-established and the water discharge was partially diverted from the Pranto sluice to an alternative sluice placed upstream in the north arm. The connection between north and south arms was re-opened through a 1 m2 section which allowed the water to periodically flow from 1.5 to 2 hours before and after each high tide peak (Neto et al., 2010). After the positive results obtained with the experimental reestablishment of the upstream communication between the two estuarine arms, this connection was widened in 2006.

The land use inside the estuary (e.g., channel navigability, harbour facilities, food production, urban development), and the interventions materialised to make them possible (e.g., margins regularization, embankments, dredges) have deeply interfered with the estuarine hydromorphology. Although the strong physical changes have been located in the northern arm, side effects coming from the hydrological modifications were felt in the southern arm.

2.2. Assessment of anthropogenic pressures

Following the proposal of Aubry and Elliott (2006), three categories of indicators were considered to assess the anthropogenic pressures in the sampling site: a) hydromorphological changes (represented by the ‘land claim’ and the ‘shore line re-enforcement’); b) resource use change (represented by the ‘maintenance dredging area and volume’, ‘maintenance disposal area and volume’, ‘other fisheries near shore disturbance’, ‘marina development’ and ‘tourism and recreation’; and c) environmental quality and its perception (represented by ‘nutrients concentration’ and ‘natural turbidity’). The selected pressure indicators (Table I) were the ones considered as potentially significant on influencing the quality of the seagrass meadows.

Pressure			Scores					
Category	Indicator	Criteria	No change (0)	Very low (1)	Low (3)	Medium (5)	High (7)	Very high (9)
Hydromorphological changes	Land claim (ha)	Consider both: mudflats and tidal marshes. This indicator includes both anthropogenically induced changes (land claim) and natural variations, since the 1900-1950s or before big morphological changes occurred (since when trustful maps are available).	No change	<0.5 % lost	<1% lost	<5% lost	<10%lost	≥ 10% lost
	Shoreline re-enforcement (%)	Percentage of the shoreline or estuarine margin that suffered re-enforcement work.	No change	<5%	<30%	<60%	<90%	≥ 90%
Resource use change	Maintenance dredging area (ha)	The annually subtidal dredged area in relation to total area of estuaries (or WB).	No dredging	<1%	<10%	<30%	<50%	≥ 50%
	Maintenance dredging volume (tons)	The amount of material dredged annually from estuaries (1 m3 of sand dredged is equivalent to 2 tons).	No dredging	< 5000 tons	<100,000 tons	< 1 million tons	< 4 million tons	≥ 4 million tons
	Maintenance disposal area (ha)	The area designated for disposal in estuaries (or WB) or length affected by disposal (for tidal rivers) as suggested within the Water Framework Directive for the designation of Heavily Modified Water Bodies (HMWB).	No disposal	<1%	<10%	<30%	<50%	≥ 50%
	Maintenance disposal volume (tons)	Represented by the total tonnage annually disposed in estuaries.	No disposal	< 5000 tons	<100,000 tons	< 1 million tons	< 4 million tons	≥ 4 million tons
	Other fisheries nearshore disturbance	Percentage of the length of coast or estuarine (or WB) area affected by fishery.	No fishery activities	< 10%	<30%	<60%	<90%	≥ 90%
	Marina Development	The intensity of marina development is measured by the number berths / km2 of the WB.	No marina	< 100 berths / km2 WB	<150 berths / km2 WB	<300 berths / km2 WB	<500 berths / km2 WB	≥ 500 berths / km2 WB
	Tourism and recreation	Percentage of the length of coast (riverbank) or estuarine (or WB) area affected by tourism and recreation activity.	None	< 10%	<30%	<60%	<90%	≥ 90%
Environmental quality and its perception	Nutrients (μmol/L)	Quantified as the DIN winter median concentration (μmol/L)	< 6.5	< 10	< 30	< 60	< 90	≥ 90
	Natural turbidity	Measured as the mean secchi disk transparency (m) during growing season (May to September).	< 0.5	< 1	< 1.5	< 2	< 2.5	≥ 2.5

Table I. Categories, indicators and criteria used to assess anthropogenic pressures in the Mondego.

2.3. Biological data

A long-term data series from the Mondego estuary (1986 to 2009) was used to provide information on the basic structural parameters 'bed extent', 'biomass' and 'shoot density' of *Zostera noltii* meadows. Sampling was performed at the intertidal area of the south arm of the Mondego estuary, during low tide and using a manual corer (13.5 cm Ø). Samples were randomly collected inside the *Zostera* meadow to provide data on biomass and shoot density. The bed extent mapping was based on field observations (GPS to register the meadows perimeter), vertical photographs and GIS methodology (ArcView GIS version 8.3). Samples were collected with different periodicities along the study period. Depending on the purpose they were collected from twice a month during several year to a lower frequency of only one to three sampling events concentrated in the growing season. Samples were sorted in the laboratory, the shoots counted and the biomass determined as dry weight (g DW after weight stabilisation at 70 ºC).

2.4. Metrics and quality assessment method (SQI)

The Seagrass Quality Index (SQI) includes three different metrics: 1) species richness, as the number of taxa, 2) the bed extent, as the areal cover of the meadows, and 3) the shoots density, as the number of shoots per m2 (Table II).

Table II. Metrics used in the Seagrass Quality Index (SQI), reference condition for each metric, and the weight it has into the final EQR result. The available intertidal is considered as the area that is suitable for seagrass to grow and does not include occupations of several orders or saltmarsh area.

Metric	Reference Condition	Weight
Number of taxa	1	0.2
Bed extent	5 % of the available intertidal	0.3
Shoot density	12000	0.5

The deviation from the reference condition is calculated for each metric. The number of taxa, since only one species is naturally present in the system, scores 1 when *Z. noltii* is not present or 5 when it is present. The bed extent is converted in a scale 0 – 1 by dividing the measured areal cover (ha) by the reference condition bed extent area. The shoots density follows the same process as the bed extent, and a result comprised between zero and one is then obtained. Except for the no. of taxa, the other metrics are scored in a continuos way inside the range 0 – 1. After this first round of calculations, the EQR is obtained through the use of the combination rule expressed in equation 1.

$$EQR = (T/5)*0.2 + BE*0.3 + SD*0.5 \qquad \text{(Equation 1)}$$

where T is the no. of taxa, BE is the bed extent / bed extent reference condition, and the SD is the shoot density / shoot density reference condition.
An equidistant scale translates the EQR obtained into the EQS classes (Table III).

Table III. Boundaries for the EQR and correspondent EQS used in the Seagrass Quality Index (SQI).

EQR	EQS
0 – 0.2	Bad
0.21 – 0.39	Poor
0.40 – 0.59	Moderate
0.60 – 0.79	Good
0.80 – 1	High

2.5. Data analysis

Data on the structural parameters of the seagrass were analysed along the study period. The response of the bed extent and biomass structural parameters to the different levels of pressure was also analysed, both towards degradation and after the implementation of the first (experimental) mitigation measures.

The response of the SQI method, which comprises the metrics 'bed extent', 'shoot density', and the no. of taxa, was also tested against the different pressure levels. The ability of the SQI in reporting into the five ecological quality classes (bad, poor, moderate, good and high) (WFD, 2000/60/EC) was also examined and compared to the pressure level acting at the moment.

Different combinations of categories of pressures (hydromorphological changes, resource use change, and the environmental quality and its perception) were tested to compare the response of the biological parameters and the EQRs against the pressure levels.

The correlation between biological data (metrics and the SQI EQRs) and the anthropogenic pressures (total pressure and the sum of resources change plus environmental quality) was tested through the Pearson product moment correlation coefficient, with StatSoft, Inc. (2004) STATISTICA (data analysis software system), version 7.

3. Results

The evolution of the bed extent and the biomass in the estuary can be seen in figures 1 and 2. For both parameters, the slope of the lines (Figure 2) are different for the periods before and after 1997. This year corresponds to the moment when the mitigation measure were implemented in the south arm of the Mondego.

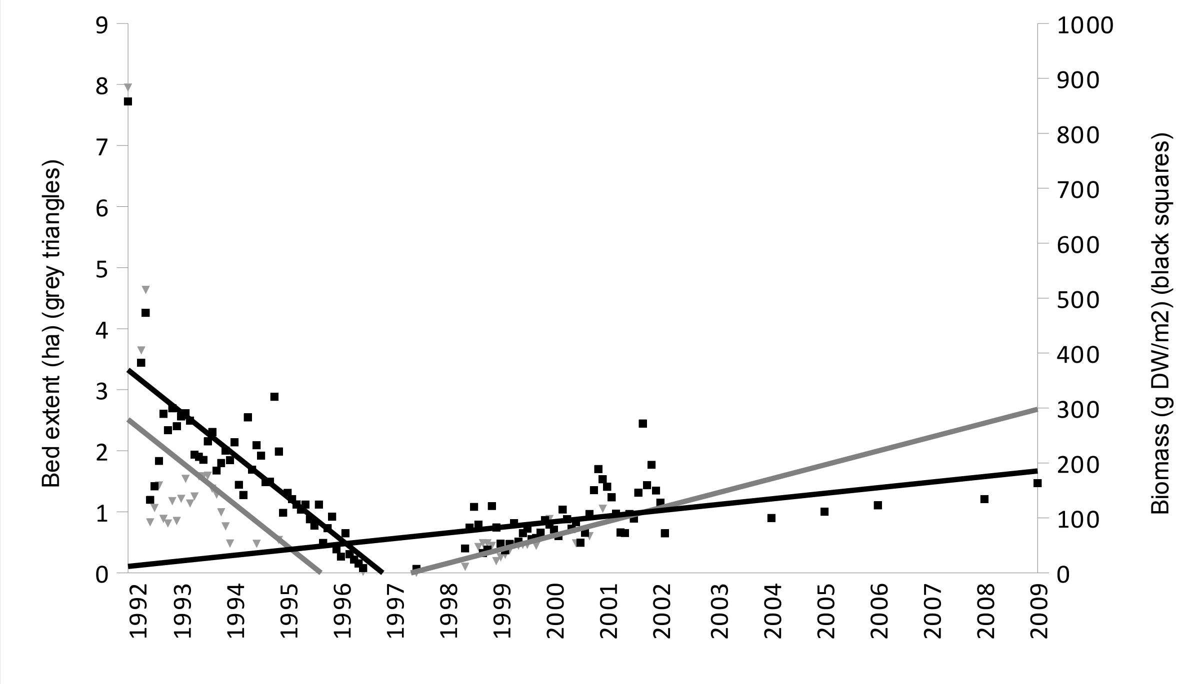
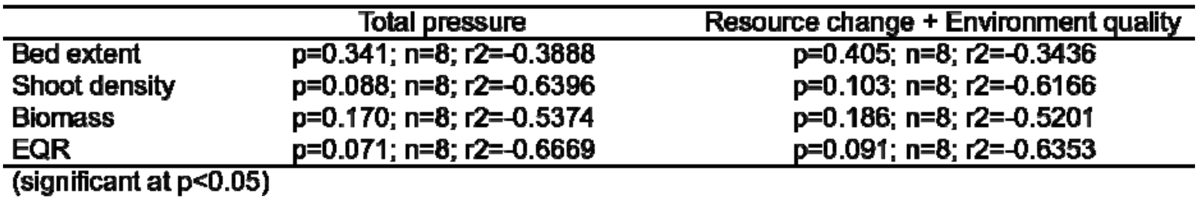


Figure 2. Plot of the structural parameters bed extent and biomass along the study period. Trend lines for both periods, towards degradation and after implementation of mitigation measures, are also shown.

The correlation between the response of the metrics bed extent, shoot density and biomass, and the SQI methodology against the pressure varied (Table IV). The total pressure included also the hydromorphological changes pressure. The correlations presented by the metrics and the SQI method were higher when compared to the pressure quantified as the resource change use + environmental quality and its perception than for the total pressure, but followed the same trend, bed extent < biomass < shoot density < SQI.

The coefficient of determination for the correlations, calculated through the Pearson product moment correlation coefficient analysis, between the biological data (metrics and EQR) and the pressure data (total pressure and the sum of resource change plus the environmental quality) is shown in Table IV. No significant differences were found between any pair of variables compared.

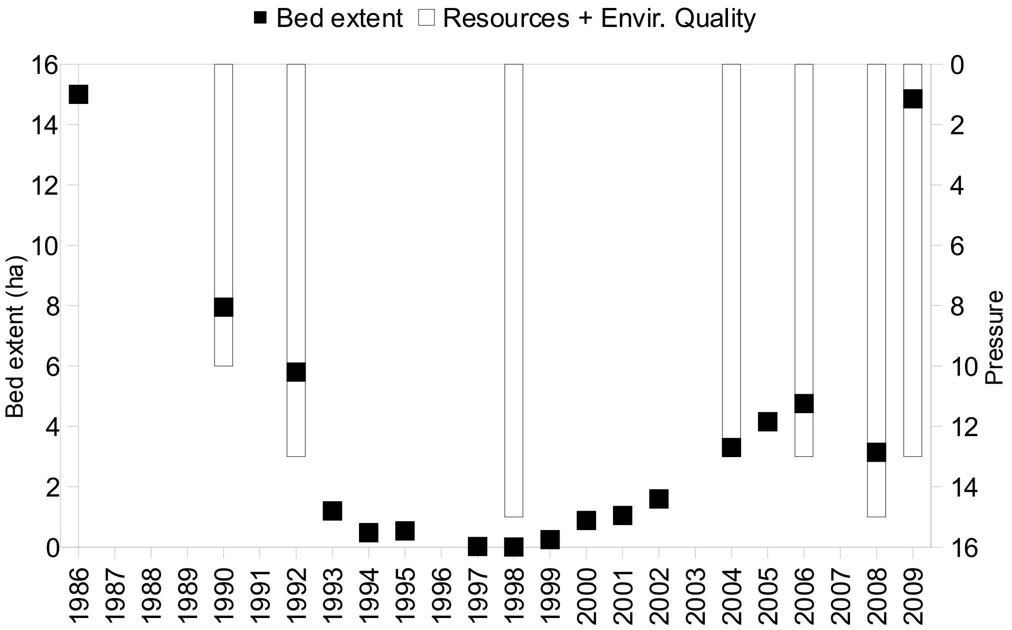
Table IV. The Pearson product moment correlation results.



	Total pressure	Resource change + Environment quality
Bed extent	p=0.341; n=8; r2=-0.3888	p=0.405; n=8; r2=-0.3436
Shoot density	p=0.088; n=8; r2=-0.6396	p=0.103; n=8; r2=-0.6166
Biomass	p=0.170; n=8; r2=-0.5374	p=0.186; n=8; r2=-0.5201
EQR	p=0.071; n=8; r2=-0.6669	p=0.091; n=8; r2=-0.6353

(significant at p<0.05)

The response of each metric and SQI method results against the different pressure levels can be seen in Figure 3. Although in different scales, all the variables present a parallel relation to the pressure level.


■ Bed extent
□ Resources + Envir. Quality
Bed extent (ha)
Pressure
0
2
4
6
8
10
12
14
16
1986
1987
1988
1989
1990
1991
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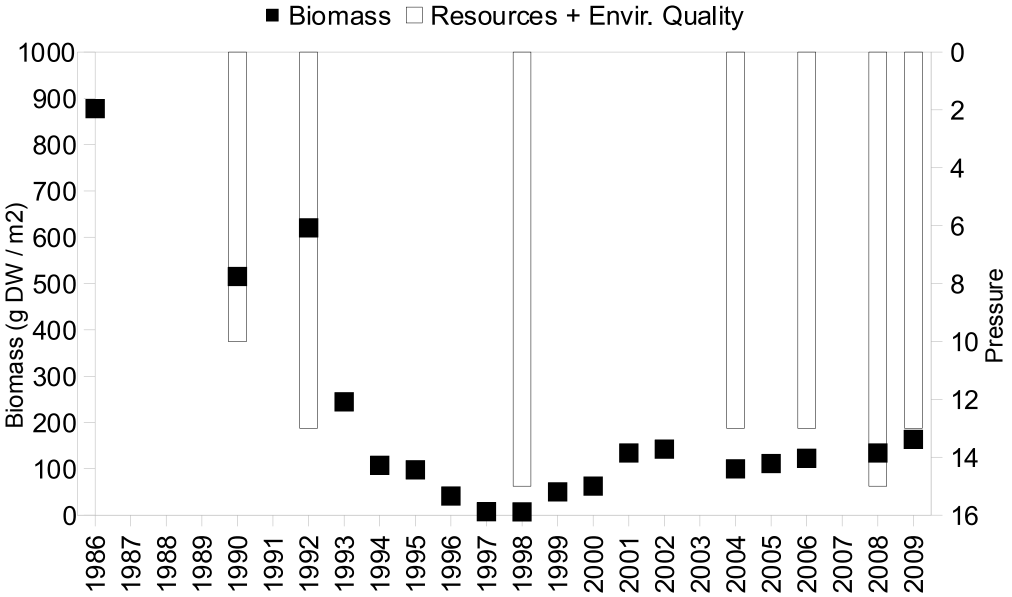
2004
2005
2006
2007
2008
2009


■ Biomass
□ Resources + Envir. Quality
Biomass (g DW / m2)
Pressure
0
100
200
300
400
500
600
700
800
900
1000
0
2
4
6
8
10
12
14
16
1986
1987
1988
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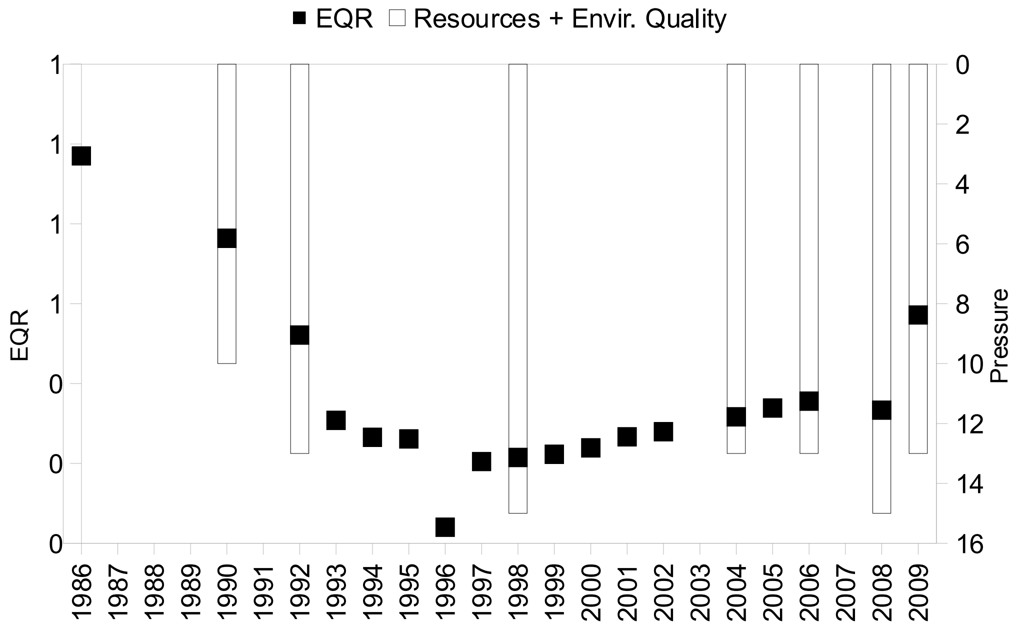
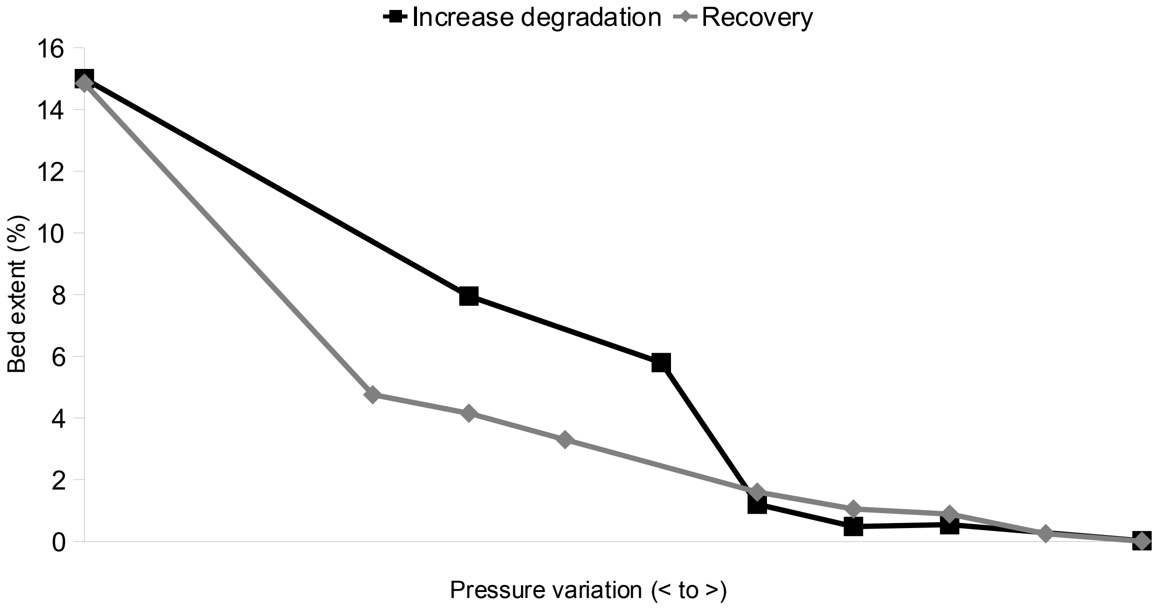


Figure 3. Plot of the bed extent, shoot density, biomass and SQI (EQR), and the pressure values of the sum of the resources use change plus the environmental quality categories for the study period in the system.

With the intention to track the response of *Zostera* structural metrics along the degradation and the recovery pathways, the time series were 'folded' at the moment of the implementation of the experimental mitigation measures (1997-1998) and then overlapped (Figure 4). The resulting pathways were different for degradation and for recovery processes. The recovery of biomass was slower than the for the bed extent metric.



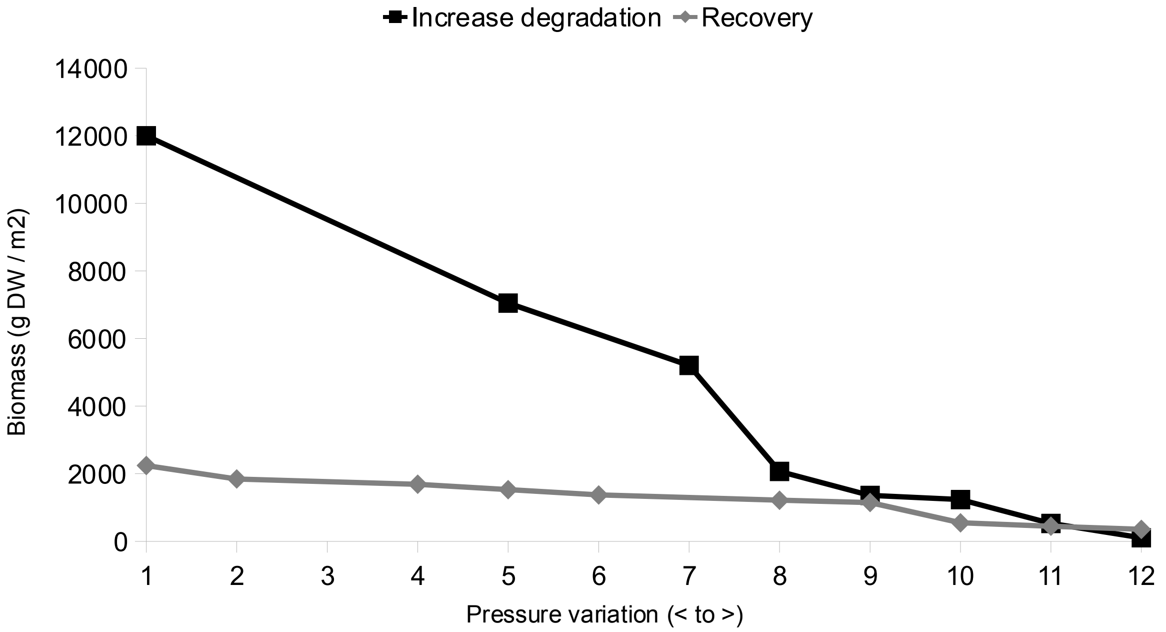


Figure 4. Values of the bed extent and biomass towards degradation and the recovery track followed after the implementation of mitigation measure.

4. Discussion

Data covering a first period where the seagrass meadows were under degradation and a second one where its recovery process was ongoing, allowed to compare the response of the structural metrics of *Zostera* for both situations. During the first period eutrophication symptoms were observed in the system and *Zostera* presented a severe reduction in the bed extent and biomass (Figures 1, 2 and 3). The shoots density and the EQR that resulted from the SQI application also showed the same decreasing tendency (Figure 3). For all the structural parameters the recovery was not as fast as the degradation, which is a possible process to occur when restoration is tried to aquatic systems.

The EQR results show that the SQI performs well when used to assess the quality of seagrass meadows in estuarine systems. The SQI reported EQR values in all quality classes, and showed a good correlation with the pressure values. Although the good relation observed for the EQR and the pressure level, is too much evident that the recovery of the system didn't follow exactly the same track as the degradation process. This way is apparently slower and the stability of the basic parameters in a minimum level is important for recovery to proceed.

The experimental mitigation measures (1998) produced positive results and were used to support the decision to enlarge the communication between the north and south arms (2006). Although the time after this enlargement could be considered as not sufficient to produce solid results on the recovery of seagrass meadows in the Mondego, it is possible to observe a solid re-establishment of the seagrass population in the south arm. Bed extent (Figures 3 and 4) showed an impressive increase in 2009 and, although weaker, the SQI also increase its EQR value for this year (Figure 3). The Zostera meadows achieved cover areas close to the ones from the 1980s, 150 000 m2, away from the 200 m2 registered in 1997 (before the experimental mitigation).

5. Conclusions

The response of bed extent, biomass and shoot density against pressure levels registered in the estuary were significantly correlated. The SQI produced EQR values also significantly correlated with the anthropogenic pressure affecting the system.

The pathways followed in degradation and in recovery were different, with much slower variations observed for the recovery phase. Different structural parameters presented different responses to the release of pressure observed after the mitigation. Even though we are in presence of a fast response seagrass species, the response for some parameters (e.g., biomass) registered a considerable delay. The bed extent is registering similar occupations as the ones registered for the seagrass in the decade of the 1980s.

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Case study 6: Multimetric indices along anthropogenic pressure gradients in the Atlantic coast. Application of the Marine Macroalgae Assessment Tool (MarMAT) in Portuguese intertidal rocky shores.

By Joao M Net, Rui Gaspar, Leonel Pereira

2. Materials and methods

2.1. Study site

The study area is located along the western coast of Portugal (Figure 1), inside the EU North-East Atlantic (NEA) region, typology NEA 1 (A5 PT type). This region of the coast is an open and exposed euhaline and mesotidal (1 to 3 m amplitude) coastal area that is frequently turbid and nutrient-enriched due to coastal upwelling (Ambar & Dias, 2008).

During the summer, the Canary Current, which has a strong southward flow (12 cm s^{-1}) originating from the north, and the Azores Current, which enters the region from the south and has a west-to-east circulation, affect the Portuguese coast. During the winter, the Azores Current has twice the velocity it has in the summer, and there is little circulation of seawater in the region. During this season the circulation of seawater along the Iberian Coast flows predominantly south to north with a velocity of approximately 1.6 cm s^{-1} (Ambar & Dias, 2008).

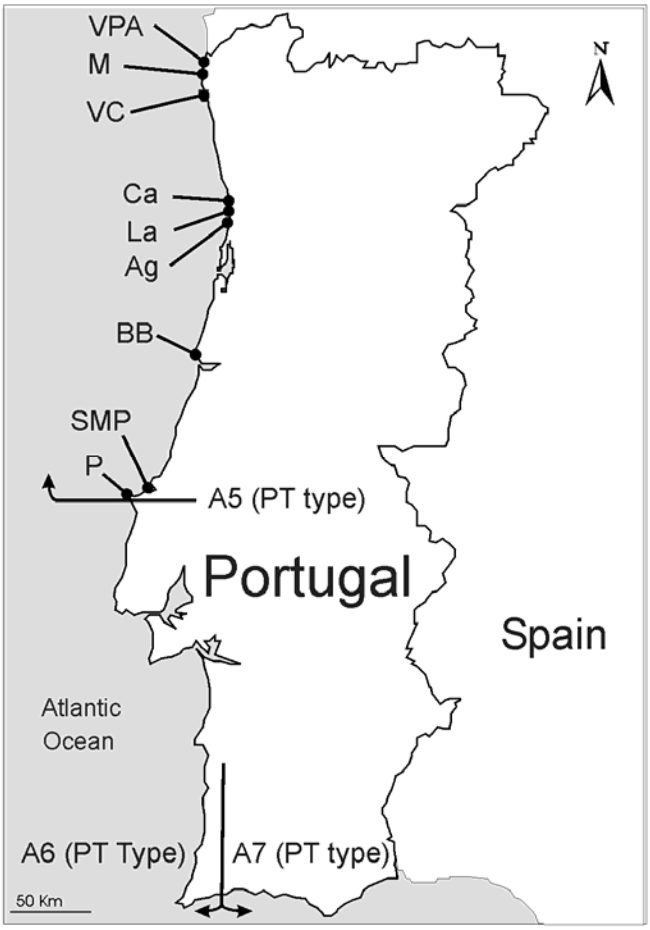


Figure 1. Portuguese coastal waters' typology: A5 (exposed = European NEA1), A6 (moderately exposed), and A7 (sheltered). Study sites: VPA = Vila Praia de Âncora; M = Montedor; VC = Viana do Castelo; Ca = Cabedelo; La = Lavadores; Ag = Aguda; BB = Buarcos Bay; SMP = São Martinho; P = Peniche.

Sampling was conducted at nine intertidal rocky shore sites located along the study area: the Vila Praia de Âncora (VPA), Montedor (M), Viana do Castelo (VC), Cabedelo (Ca), Lavadores (La), Aguda (Ag), Buarcos Bay (BB), São Martinho do Porto (SMP) and Peniche (P) shores (Figure 1). These sites experience different levels of anthropogenic pressure; eight of these sites (Table I) were selected to test the response of the MarMAT to different levels of environmental stress (tool validation).

Table I. Sampling sites names, codes, and dates. Information on which sites were assessed for anthropogenic pressures and the location (city) from which the pressure information is thought to initiate.

Sampling sites	Site code	Sampling date	Assessed against pressure	Pressures related to location
Vila Praia Âncora	VPA-3	July 2010		
Montedor	M-3	July 2010	Yes	Caminha
Viana Castelo	VC-5	August 2007	Yes	Viana do Castelo
Viana Castelo	VC-6	July 2010		
Cabedelo	Ca-2	July 2007		
Cabedelo	Ca-3	September 2007	Yes	Oporto
Lavadores	La-2	July 2007		
Lavadores	La-3	September 2007	Yes	Vila Nova de Gaia
Aguda	Ag-2	July 2007		
Aguda	Ag-3	September 2007	Yes	Espinho
Buarcos Bay	BB-9	October 2007		
Buarcos Bay	BB-10	June 2008		
Buarcos Bay	BB-11	June 2009		
Buarcos Bay	BB-12	September 2009	Yes	Figueira da Foz
Buarcos Bay	BB-13	July 2010		
São Martinho do Porto	SMP-2	August 2009	Yes	Alcobaça
Peniche	P-3	September 2009	Yes	Peniche

2.2. Assessment of anthropogenic pressures of study sites

Three indicators were considered to be proxies of anthropogenic pressures influencing the sampling sites: a) urban land use (represented by the number of inhabitants); b) industrial land use; and c) agricultural, forest and fishing areas (*sensu* the Land Uses Simplified Index (LUSI); Royo et al., 2009). The anthropogenic pressures identified from land, which may be related to impacts (e.g., macroalgal communities degradation) observed in coastal zones. Are on the basis of this method.

Pressures were calculated from information available from the National Institute of Statistics (http://www.ine.pt) for the study period and were scored from 1 to 4 following the criteria shown in Table II. Eight sites were analysed; the locations (cities) considered to have a significant influence on the anthropogenic pressure level for each site are shown in Table I.

Table II – Criteria used to assess anthropogenic pressures. Indicators of anthropogenic pressure and years considered for the assessment.

	SCORES			
	1	2	3	4
No. Inhabitants x 1000 (2008)	<350	<700	<1050	<1400
Industrial Land Use (ha) (2008)	<1250	<2500	<3750	<5000
Agriculture/Forest/Fishing Surface Area (ha) (1999)	<4500	<9000	<13500	<18000

The total pressures were compared with the EQR from sampling sites to validate the response of the MarMAT against anthropogenic pressures.

2.3. Biological data

Sampling was performed during low tide on the intertidal rocky substrates, primarily during the summer and spring (Table I). All data resulted from non-destructive quantitative assessments and were restricted to a shore sample collected from a single low tide event. A 10- to 15-metre band located perpendicular to the water line was chosen to represent the macroalgal populations at each site. Within that sampling band, all macroalgal taxa observed were recorded (to the species level or closest relative) to estimate the taxonomic composition. A transect was defined within the banded area and perpendicularly to the water line to simultaneously record the site's macroalgal abundance (coverage of opportunistic taxa). Seven samples were collected at each site; each sample was collected at a different intertidal depth level (one representative level for each main intertidal zone: lower-, mid- and upper-littoral; one intermediate level above and one intermediate level below those zones). This was accomplished by photographing a 0.2 x 0.2 m wire quadrat (sub-divided into 16 sub-quadrats) positioned along the transect and located over the rocky substratum covered by macroalgae. A sample was considered to be three replicates (three photographed quadrats) placed perpendicular to the transect line. Twenty-one replicates per transect were analysed.

2.4. Metrics and quality assessment method (MarMAT)

The MarMAT includes seven different metrics: 1) species richness, 2) proportion of Chlorophyta, 3) number of Rhodophyta, 4) number of opportunists / ESG I (ratio), 5) proportion of opportunists, 6) shore description, and 7) coverage of opportunists (%) (Neto et al., in press). Similar to other assessment tools (e.g., the RSL, the CFR), the MarMAT is based on a Reduced Taxa List (RTL) (Table III) adapted to the variety of shore typology. The RTL considered in this study was developed by Gaspar et al. (in press) for the same study area.

Table III – Reduced Taxa List (RTL) for the A5 Portuguese Coastal Water (CW) typology (NEA 1 for Portuguese northern coast). ESG = Ecological Status Groups.

Reduced Taxa List: (CW A5 PT type)	ESG	Opportunistic
Chlorophyta:		
Bryopsis spp.	II	Yes
Other Filamentous Chlorophyta (1)	II	Yes
Cladophora spp.	II	Yes
Codium spp.	II	
Ulva spp. ('Sheet-type')/*Ulvaria obscura/Prasiola stipitata* (2)	II	Yes
Ulva spp. ('Tubular-type')/*Blidingia* spp. (3)	II	Yes
Phaeophyceae (Heterokontophyta):		
Bifurcaria bifurcata	I	
Cladostephus spongiosus	I	
Colpomenia spp./*Leathesia marina*	II	
Cystoseira spp.	I	
Desmarestia ligulata	II	
Dictyopteris polypodioides	II	
Dictyota spp.	II	
Filamentous Phaeophyceae (4)	II	Yes
Fucus spp.	I	
Halopteris filicina/H. scoparia	II	
Himanthalia elongata	I	
Laminaria spp.	I	
Pelvetia canaliculata	I	
Ralfsia verrucosa	I	
Saccorhiza polyschides	I	
Rhodophyta:		
Acrosorium ciliolatum/Callophyllis laciniata/Cryptopleura ramosa	II	
Ahnfeltia plicata	I	
Ahnfeltiopsis spp./*Gymnogongrus* spp.	II	
Apoglossum ruscifolium/Hypoglossum hypoglossoides	II	
Asparagopsis armata /Falkenbergia rufolanosa	II	
Bornetia spp./*Griffithsia* spp.	II	
Calliblepharis spp.	I	
Catenella caespitosa/Caulacanthus ustulatus	II	
Champiaceae (5)	II	
Chondracanthus acicularis	II	
Chondracanthus teedei	II	
Chondria spp.	II	
Chondrus crispus	I	
Calcareous encrusters (6)	I	
Calcareous erect (7)	I	
Dilsea carnosa/Schizymenia dubyi	II	
Gelidiales (8)	I	
Gigartina pistillata	II	
Gracilaria spp.	II	
Grateloupia filicina	II	
Halurus equisetifolius	II	
Hildenbrandia spp.	I	
Laurencia spp./*Osmundea* spp.	II	
Mastocarpus stellatus/Petrocelis cruenta	I	
Nitophyllum punctatum	II	
Other Filamentous Rhodophyta (9)	II	Yes
Phyllophora spp./Rhodymenia pseudopalmata	II	
Palmaria palmata	I	
Peyssonnelia spp.	I	
Plocamium cartilagineum/Sphaerococcus coronopifolius	I	
Porphyra spp.	II	Yes
Pterosiphonia complanata	II	
Scinaia furcellata	I	

1) *Chaetomorpha*, *Pseudendoclonium*, *Rhizoclonium*, Ulothricales. 2) *Ulva* spp. 'Sheet-type' in opposition to 3) 'Tubular-type' in the sense 'of 'ex- *Enteromorpha* spp.'. 4) Ectocarpales/*Sphacelaria* spp. 5) *Champia*, *Chylocladia*, *Gastroclonium*, *Lomentaria*. 6) *Lithophyllum*, *Melobesia*, *Mesophyllum*, *Phymatolithon*. 7) *Amphiroa*, *Corallina*, *Jania*. 8) *Gelidium*, *Pterocladiella*. 9) *Acrochaetium*, *Aglaothamnion*, *Antithamnion*,

Bangia, *Boergeseniella*, *Brongniartella*, *Colaconema*, *Callithamnion*, *Ceramium*, *Compsothamnion*, *Dasya*, *Erythrotrichiaceae*, *Herposiphonia*, *Heterosiphonia*, *Janczewskia*, *Leptosiphonia*, *Lophosiphonia*, *Ophidocladus*, *Pleonosporium*, *Plumaria*, *Polysiphonia*, *Pterosiphonia* (except *P. complanata*), *Pterothamnion*, *Ptilothamnion*, *Rhodothamniella, Streblocladia*, *Vertebrata*

Species richness, proportion of Chlorophyta, number of Rhodophyta, proportion of opportunists, and the ratio of the number of opportunists / ESG I are calculated based on taxa in the RTL. The coverage of opportunists was estimated from the photographed quadrats, having in mind the species considered as opportunists in the RTL.

In degraded habitats, an increase in the number of opportunist species and an extension of their coverage area was expected to occur (see Gaspar et al., in press). In fact, the competitive advantage of opportunists tends to lead to the elimination of sensitive species and to an increase in biomass and coverage area for r-selected species. The *coverage of opportunists* (CO), expressed as a percentage, is given by the area covered by these taxa (only opportunistic taxa included in the RTL) in relation to the whole area covered by the macroalgae (all species corresponded to 100% coverage). Each photographed replicate was analysed using the following formula (equation 1):

$$CO\ (\%) = QCO \times 100 / (16 - EQ) \qquad \text{(Eq. 1)}$$

where QCO is the number of sub-quadrats with opportunistic macroalgae and EQ is the number of empty sub-quadrats. A resolution of ¼ of a sub-quadrat was used for the calculations.

The CO was calculated as the arithmetic average of all of the replicates from a site.

The objective of including a *shore description* metric was to make shores with different substrata comparable and, consequently, make different environmental conditions for macroalgae growth comparable. Shore descriptions serve as a correction factor for species richness scores, which is a metric included in the MarMAT. The scoring of the shore description metric follows the field sampling form proposed by Wells et al. (2007) (Table IV).

Table IV – Field sampling form for the shore descriptions (adapted from Wells et al., 2007).

General information						
Shore name		**Date**				
Water body		**Tidal height**				
Latitude / Longitude		**Time of low tide**				
Shore descriptions						
Presence of turbidity	Yes =0	Sand scour	Yes =0	No =2		
(known to be non-anthropogenic)	No =2	Chalk shore	Yes =0	No =2		
Dominant shore type		**Subhabitats**				
Rock ridges/outcrops/platforms	=4	Wide shallow rock pools				
Irregular rock	=3	(>3 m wide and <50 cm deep)	=4			
Boulders large, medium and small	=3	Large rockpools (>6 m long)	=4			
Steep/vertical rock	=2	Deep rockpools (50% >100 cm deep)	=4			
Non-specific hard substrate	=2	Basic rockpools	=3			
Pebbles/stones/smallrocks	=1	Large crevices	=3			
Shingle/gravel	=0	Large overhangs and vertical rock	=2			
		Others habitats (please specify)	=2			
Dominant biota		Caves	=1			
Ascophyllum		None	=0			
Fucoid						
Rhodophyta mosaics		**Total number of sub-habitats**				
Chlorophyta		>4	3	2	1	0
Mussels						
Barnacles		**General comments**				
Limpets						
Periwinkles						
Sum of categories' scores		N/A	15 - 18	12 - 14	8 - 11	1 - 7
Shore description equivalent score		0	1	2	3	4

Scores from each of the categories are added together, and depending on their range, an equivalent score (Table IV) is used to calculate the final shore description classification. Only the highest score was used to estimate the sum of categories' scores for categories with more than one description recorded (e.g., shore type, habitat type) (Wells et al., 2007).

The value of each of the MarMAT metrics varied from 0 to 4; this range was divided into five intervals that corresponded to the five quality classes, in accordance with the Normative Definitions (annex V in WFD) (WFD, 2000/60/EC): bad - 0, poor - 1, moderate - 2, good - 3, and high - 4 (Table V).

Table V – Boundaries for each of the MarMAT metrics, sum of scores and EQR. Translation of the achieved EQR in the EQS (bad, poor, moderate, good or high) when assessing the ecological quality of rocky shores.

Metrics	Bad	Poor	Moderate	Good	High
Species richness (a)	0 - 6	7 - 13	14 - 20	21 - 27	28 - 54
Proportion of Cholophyta	0.32 - 1	0.27 - 0.31	0.21 - 0.26	0.15 - 0.20	0 - 0.14
Number of Rhodophyta	0 - 3	4 - 8	9 - 12	13 - 17	18 - 33
Number of opportunists / ESG I	≥1.23	1.01 - 1.22	0.80 - 1.00	0.58 - 0.79	<0.58
Proportion of opportunists	0.59 - 1	0.47 - 0.58	0.35 - 0.46	0.23 - 0.34	0 - 0.22
Coverage of oportunists (%) (a)	72 - 100	59 - 71	46 - 58	33 - 45	0 - 32
Shore description	-	15 - 18	12 - 14	8 - 11	1 - 7
Corresponding score to metrics class	0	1	2	3	4
Sum of scores	0 - 7	8 - 14	15 - 21	22 - 28	29 - 36
EQR	0 - 0.2	0.2 - 0.4	0.4 - 0.6	0.6 – 0.8	0.8 - 1
EQS	Bad	Poor	Moderate	Good	High

(a) factor of 2, counts twice in the metrics sum of scores calculation.

The scores of the different metrics were integrated to provide an overall classification of the shore. For example, if 15 taxa were found on a given shore, the shore received a score of 2 for species richness (moderate); if the proportion of Chlorophyta on a given shore was 0.15, the shore received a score of 3 (good) (Table V). The sum of the scores obtained for the different individual metrics was integrated in the 'sum of scores' (0 to 36).

The EQR (equation 2) converts the 'sum of scores' values to a scale from 0 to 1, in accordance with the definition provided in the WFD (WFD, 2000/60/EC).

$$\text{EQR} = \text{Sum of Scores} / 36 \qquad \text{(Eq. 2)}$$

EQR values close to 1 correspond to high quality ecological status, while EQR values close to 0 correspond to low quality ecological status. The 0 to 1 EQR interval is subsequently translated into the EQS classes (bad, poor, moderate, good and high) using the boundaries provided in Table V.

2.5. Data analysis

The total pressure values, the value of each metric and the EQR values (MarMAT) were calculated for each sampling site.

The response of several macroalgae metrics (species richness, no. and proportion of Chlorophyta, no. and proportion of Phaeophyceae, no. and proportion of Rhodophyta, no. and proportion of opportunists, ESG I, ESG II, ESG I/ESG II, no. of opportunists / ESG I, no. of opportunists / ESG II, % coverage of opportunists) were analysed against different pressure levels.

To validate the MarMAT, more specifically to test its response against the anthropogenic pressure, the total pressure values were compared with the EQR obtained for the sampling sites. A set of eight sampling sites was used in this analysis.

The correlations between the pressure values and the metrics and EQR values were statistically validated through a Pearson product moment correlation test, with StatSoft, Inc. (2004) STATISTICA (data analysis software system), version 7.

In order to check the MarMAT compliance with the WFD, its performance was analysed and verified its ability to report in all of the five quality classes.

3. Results

The assessment of the anthropogenic pressures affecting the sampling sites resulted in values ranging from 3 to 12 (Figure 2). Higher anthropogenic pressure values were obtained for Cabedelo (Ca), a site under the influence of Oporto city (see details in Gaspar et al., in press). Somewhat lower anthropogenic pressure values were obtained for Lavadores (La) and for São Martinho do Porto (values ranged from 5 to 6). Low pressures values were obtained for three sites, the Montedor (M), Aguda (Ag) and Peniche (P) (value of 3).

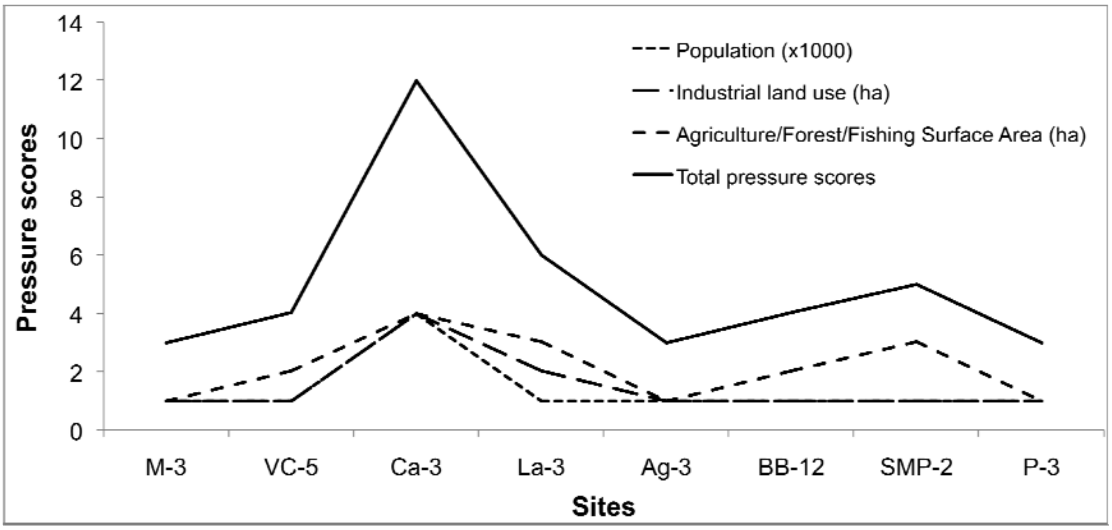


Figure 2. Quantification of anthropogenic pressures observed along the study area. Number of inhabitants; industrial land use (ha); agriculture/forest/fishing surface area (ha). Study sites: Montedor (M); Viana do Castelo (VC); Cabedelo (Ca); Lavadores (La); Aguda (Ag); Buarcos Bay (BB); São Martinho do Porto (SMP); Peniche (P).

From the analysis of the result of the macroalgae metrics against the pressures registered in each sampling site, it can be seen that the majority of the selected macroalgae metrics responded well to the increasing pressure. An increase of the pressure value was always captured by the selected metrics (Figure 4). Metrics showing a positive correlations were the proportions of Chlorophyta, Rhodophyta and opportunists, the ratios ESG I/ESG II, no. of opportunists/ESG I and no. of opportunists/ESG II, and the coverage of opportunists (%) (Figure 3f, 3h, 3i, 3l, 3m, 3n and 3o). An inverse signal was shown by metrics such as the species richness, the nos. of Chlorophyta, Phaeophyceae, Rhodophyta and of opportunists, the proportion of Phaeophyceae, and the ESGs I and II, which presented a negative correlations with the pressure values (Figure 3a, 3b, 3c, 3d, 3e, 3g, 3j and 3k).

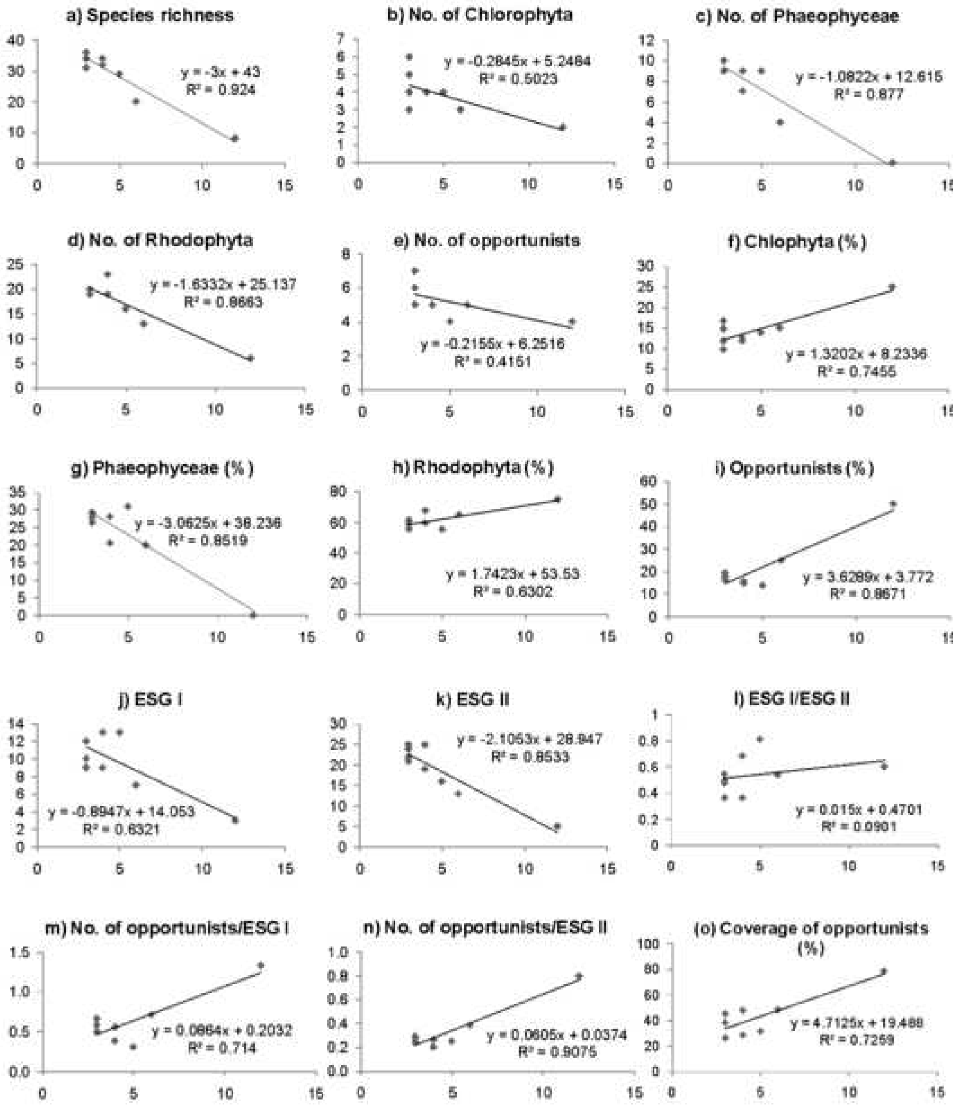


Figure 3. Correlation between each macroalgae metric (vertical axes) and the total pressure scores (horizontal axes) obtained for each sampling site. (Gaspar et al., in press)

The response of the ecological assessment performed by the MarMAT against the anthropogenic pressure level is shown in Figure 4. The total anthropogenic pressure and the EQR values quantified for sampling sites had a significant inverse correlation. The coefficient of determination for the correlation, calculated through the square of the Pearson product moment correlation coefficient (*r*), between both data series (MarMAT EQR vs. anthropogenic pressures), is also shown in Figure 4 ($p<0.001$; n=8; $r^2=0.91$).

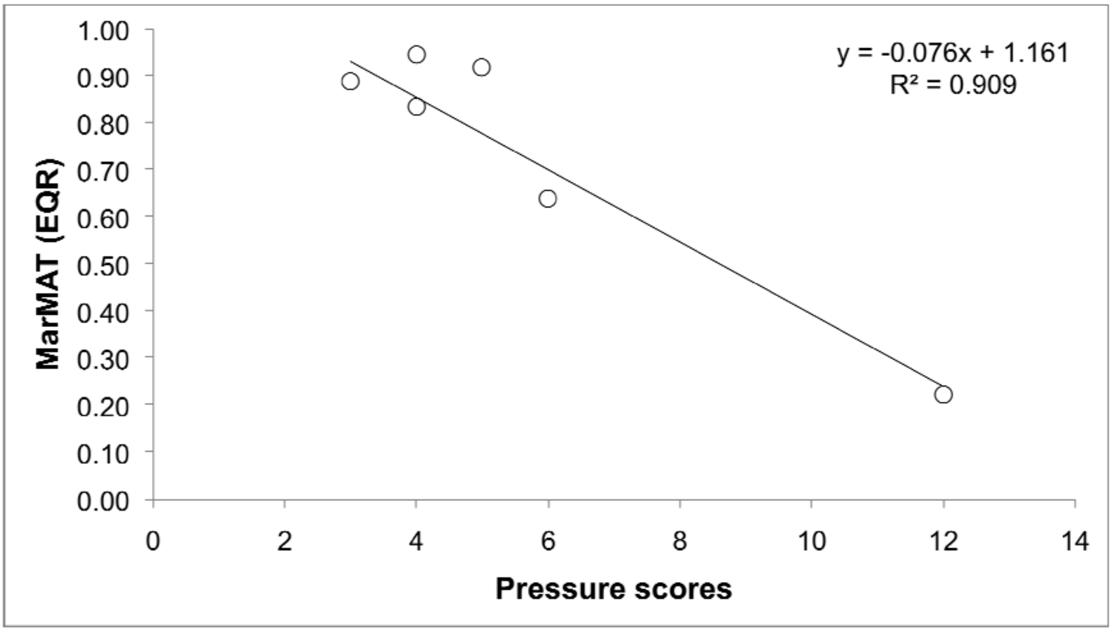
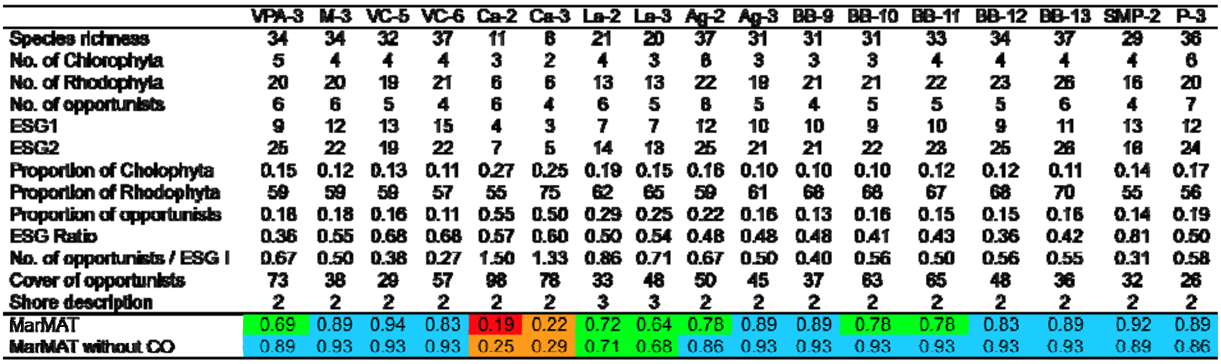


Figure 4. Plot of EQRs reported by the MarMAT versus the total anthropogenic pressures for sites M-3, VC-5, Ca-3 La-3, Ag-3, BB-12, SMP-2, and P-3 (see table I).

The EQRs resulted from the application of MarMAT to the sampling sites are shown in Table VI. MarMAT reported to four of the five quality classes used in the assessment scheme followed in the WFD (bad, poor, moderate, good and high). All quality classes except for 'moderate' were obtained by the MarMAT.

Concerning the application of MarMAT without the metric coverage of opportunists, although a good correlation could be found with MarMAT ($p<0.001$; n=17; $r^2=0.92$) the achieved results were narrower in terms of quality classes reported (Table VI). The MarMAT without CO was less flexible, and was not able to identify the decrease in quality observed for some sampling sites (e.g., BB-10 and BB-11).

Table VI – Assessment results for the MarMAT and the MarMAT without coverage of opportunists. Values for metrics used in the MarMAT calculation, for the EQRs and colours for EQS classes (see Table VI).



	VPA-3	M-3	VC-5	VC-6	Ca-2	Ca-3	La-2	La-3	Ag-2	Ag-3	BB-9	BB-10	BB-11	BB-12	BB-13	SMP-2	P-3
Species richness	34	34	32	37	11	8	21	20	37	31	31	31	33	34	37	29	36
No. of Chlorophyta	5	4	4	4	3	2	4	3	6	3	3	3	4	4	4	4	6
No. of Rhodophyta	20	20	19	21	6	6	13	13	22	19	21	21	22	23	26	16	20
No. of opportunists	6	6	5	4	6	4	6	5	8	5	4	5	5	5	6	4	7
ESG1	9	12	13	15	4	3	7	7	12	10	10	9	10	9	11	13	12
ESG2	25	22	19	22	7	5	14	13	25	21	21	22	23	25	26	16	24
Proportion of Cholophyta	0.15	0.12	0.13	0.11	0.27	0.25	0.19	0.15	0.16	0.10	0.10	0.10	0.12	0.12	0.11	0.14	0.17
Proportion of Rhodophyta	59	59	58	57	55	75	62	65	59	61	68	68	67	68	70	55	56
Proportion of opportunists	0.18	0.18	0.16	0.11	0.55	0.50	0.29	0.25	0.22	0.16	0.13	0.16	0.15	0.15	0.16	0.14	0.19
ESG Ratio	0.36	0.55	0.68	0.68	0.57	0.60	0.50	0.54	0.48	0.48	0.48	0.41	0.43	0.36	0.42	0.81	0.50
No. of opportunists / ESG I	0.67	0.50	0.38	0.27	1.50	1.33	0.86	0.71	0.67	0.50	0.40	0.56	0.50	0.56	0.55	0.31	0.58
Cover of opportunists	73	38	29	57	98	78	33	48	50	45	37	63	65	48	36	32	26
Shore description	2	2	2	2	2	2	3	3	2	2	2	2	2	2	2	2	2
MarMAT	0.69	0.89	0.94	0.83	0.19	0.22	0.72	0.64	0.78	0.89	0.89	0.78	0.78	0.83	0.89	0.92	0.89
MarMAT without CO	0.89	0.93	0.93	0.93	0.25	0.29	0.71	0.68	0.86	0.93	0.93	0.93	0.93	0.93	0.93	0.89	0.86

4. Discussion

Most of the metrics included in the MarMAT were previously considered in tools developed in other EU MSs for the same purpose, such as the RSL (Wells et al., 2007) in the UK, the CFR (Juanes et al., 2008) and the CARLIT (Ballesteros et al., 2007) in Spain, and the EEI (Orfanidis et al., 2003) in Greece. The metrics included in the MarMAT methodology showed a good response on the detection of environmental degradation (Gaspar et al., in press). These metrics were not very different from the ones initially proposed by Wells et al. (2007) for the RSL method, but due to progress made in the scientific field concerning the classification of macroalgal taxa (ESG and opportunistic species) (Orfanidis et al., 2011), some metrics were replaced. The number of Rhodophyta showed a stronger correlation with anthropogenic pressure values than its proportion. Therefore, the former is now included in the MarMAT. After the ESG's reclassification (made by Orfanidis et al. (2011) for macroalgal taxa) the ratio no. of opportunists / ESG I was more efficient metric in detecting degradation of macroalgal communities than the ESG I / ESG II metric. Therefore, the former replaced the latter in the MarMAT.

The use of a large dataset (including historical and monitoring data) allowed Gaspar et al. (in press) to improve the robustness of the RTL for the coastal study area (A5 Portuguese CW type) as well as improve the reference conditions for several macroalgae metrics. For this reason, the RTL and the reference conditions determined by Gaspar et al. (in press) were used in the present study. To verify that the MarMAT will perform well when different CWs are assessed, the RTL must be correctly adapted, and it must include the most representative taxa of each CW typology.

In the present study, the pollution gradient (anthropogenic pressure) affecting the sampling sites was assessed through the quantification of anthropogenic pressures known to disturb coastal areas. These are considered to be proxies of disturbances impacting coastal communities; this concept is based on anthropogenic land use (Table II). A quantification of anthropogenic pressures that considers the magnitude of human presence around coastal sites, such as the concept outlined in the LUSI (Royo et al., 2009), can be used when it is difficult to directly quantify anthropogenic pressures. The results reported in this study (Figure 2) support this notion and suggest its applicability to similar situations.

In general, the MarMAT provided EQS classifications that met expectations. As suggested by the WFD, the data used in this study consisted of primarily summer samples so that a higher homogeneity of sampling conditions was guaranteed and both the seasonal episodic explosion of transient species, which occurs in April–May, and poor weather conditions, which make sampling more difficult during the autumn and winter, were avoided. Such conventions may need to be adapted in other geographical areas (Ballesteros et al., 2007).

The MarMAT successfully captured the total anthropogenic pressure calculated for the sites (Figure 3). This validates the MarMAT methodology in terms of the requirements listed in the WFD for the behaviour of assessment tools. Another important feature that assessment tools must incorporate is the ability to report the five quality classes (bad to high). The MarMAT results indicated that the Cabedelo site (Ca-2) had a bad EQS (EQR = 0.19) and that the Montedor, Viana do Castelo, and Aguda sites had high EQS (e.g., EQR = 0.94) (Table VI). In addition, a classification of high EQS was obtained whenever the total anthropogenic pressure was low along the length of the study area. This indicates that the list of taxa, the RTL, is balanced and does not restrict the outputs of the tool.

The results achieved by the single metrics against the different pressure levels also suggest that the metrics included in MarMAT were able to capture the different intensities of the environmental degradation signal.

In addition to using a list of species that are easy to identify in the field and a set of other metrics estimated from taxa belonging to this list (e.g., the proportion of Chlorophyta, the proportion of opportunists), the results from the MarMAT tests are improved by including a coverage value, as first mentioned in the CFR (Juanes et al., 2008). This last metric was included to fulfil the abundance parameter required by the WFD, and a factor of 2 was applied to emphasise its importance. This improvement was considered advisable according to Guinda et al. (2008), who compared the performances of the CFR (which includes a coverage metric) and the RSL (which does not include a coverage metric) and concluded that the CFR responded more accurately to the analysed pollution gradients.

MarMAT successfully obtained the complete range of quality classes. The MarMAT without CO was also efficient, but it did not obtain the bad EQS class (Ca-2) and did not lower the classification from high to good in BB-10, BB-11 and BB-12. In general, the classification provided by this version was higher than the classification provided by the MarMAT. Apparently, the inclusion of the CO metric resulted in a higher accuracy of the assessment. Boundaries adopted here were equidistant (0.20) but they may be improved or adjusted through comparison and intercalibration of the MarMAT with other methodologies (e.g., the RSL, the CFR). This procedure will ensure compliance regarding EQS assessments in contiguous coastal areas and the WFD.

5. Conclusions

The results of the present study illustrate that macroalgae can efficiently integrate the effects of different environmental conditions and are therefore good ecological indicators of water quality.

The Marine Macroalgae Assessment Tool (MarMAT) is compliant with the WFD recommendations regarding the need to evaluate parameters such as abundance and taxonomic composition. Moreover, the macroalgae Reduced Taxa List (RTL) was robust in representing the natural variability of macroalgal taxa in the northern Portuguese coastal waters (CW). The response of the MarMAT against the anthropogenic pressures was in accordance with expectations, consistently providing the worst EQS classifications at sites reporting higher total anthropogenic pressure values. In addition, inclusion of the geographical adaptation of the reference conditions and the boundaries is important to improve the reporting accuracy of assessment methods. The MarMAT constitutes an efficient assessment tool for macroalgal communities of the Atlantic coast.

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Discussion

The 6 case studies all documented that seagrass- and macroalgal indicators responded significantly to anthropogenic pressures. This finding can be attributed to the sensitivity of the vegetation to changes in environmental conditions in combination with its relatively long life span and attached growth form, allowing the vegetation to exhibit a time-integrated response to pressure. The potential of the vegetation as indicator of coastal environmental status is further reinforced by the large ecological importance of the vegetation for the coastal ecosystem (Steneck et al. 2002, Hemminga and Duarte 2000).

A less comforting finding was that many of the pressure-response relationships showed considerable variability, and the analysed pressures often explained only a limited part of the total variance of the indicator. The relationships between the *P. oceanica* metrics and environmental pressures explained, in the best of the cases, less than 50% and typically only 5-20% of the variance within the metrics. The full POMI index only responded significantly to pressures in Catalonian coastal waters, not in the Balearic Island coastal zone, or across the entire region. In North European seas, large-scale relationships between total macroalgal cover and eutrophication pressure as well as between eelgrass depth limits and eutrophication pressure also demonstrated considerable unexplained variation even though additional environmental variables such as salinity, temperature, and geographical position were included in the analyses. However, the Ecological Evaluation Index (EEI) showed a significant decline in response to the total set of pressures, which in the best case explained up to about 90% of the variation in EEI in selected coastal waters. Similarly strong relationships to pressure were found for intertidal macroalgae based on the MarMAR index. In the Mondego Estuary, vegetation metrics also responsed clearly to changes in pressures and explained in the best case 67% of the variation in pressures, although the correlation was only border-significant.

Case studies demonstrating a lack of strong relationships indicate that the variance of vegetation indicators is markedly influenced by other non-tested environmental parameters, and/or that the intricate interactions of ecological processes and anthropogenic pressures affecting the metrics are too complex for the indicator to distinctly reflect pressures. Such variability can be reduced by acknowledging the complex regulation pattern of natural ecosystems and including not only selected pressures in the analyses, but a broader array of pressures and environmental variables known to affect the vegetation. This was exemplified by the analyses of macroalgal cover in north European seas, which demonstrated a marked influence of salinity. The analyses of eelgrass depth limits in north European seas also demonstrated a significant influence of salinity as well as an interaction between water quality and water temperature across the large scale gradient included in the analyses. A similar need for including anthropogenic pressures as well as natural environmental variables in pressure-response relationships has been highlighted for benthic fauna indicators, which also show markedly different response patterns among water bodies (Neto et al. 2010). Still, it is probably naïve to expect highly predictive pressure-response relationships (see further discussion below).

Variations in sampling methods across regions and imperfect coupling between vegetation sites and pressures may also explain part of the variability. Such sampling variability can be reduced through stream-lining of methods across regions.

Even though we base our conclusions on ‘just’ 6 field studies and supporting information from the scientific literature, we believe that the selected studies are well-representative for the responses of macroalgal and seagrass indicators to drivers of deterioration on a European scale as they represent a wide diversity in types of vegetation, indicators, habitats, regions and pressures. The case studies represent pressure-response relationships for macrophyte types ranging from angiosperms represented by seagrasses and brackish water angiosperms to macroalgae of various functional groups. The seagrass studies concern the long-lived, slow-growing *Posidonia oceanica*, endemic to the Mediterranean Sea, the more wide-spread and faster-growing *Zostera marina* and the even faster growing and smaller *Zostera noltii*, while the brackish water angiosperms are represented by species of the genera *Ruppia* and *Potamogeton*. Communities characterised by fast-growing species are more likely than slow-growing species to revert to their original status upon the release of a pressure, as the fast-growing species are typically pioneers that are adapted to disturbances, whereas slow-growing species tend to be climax species that demand long colonisation time.

The case-studies also represent a wide diversity of indicators, some composed of a single metric others of a combination of metrics into indices. The indicators described an array of vegetation characteristics that belong to different categories (see deliverable 4.2.1): 'distribution' (e.g. seagrass depth limits and bed extent), 'abundance' (e.g. cover, biomass, shoot density), 'composition' (species richness, abundance and relative abundance of functional groups – e.g. the Ecological evaluation index, EEI) as well as 'shoot characteristics', 'processes' and 'chemical constituents' (included in the *Posidonia oceanica* monitoring index (POMI)). The seagrass quality index includes metrics on 'distribution' and 'abundance' while the MarMAT index includes metrics on composition.

The case-studies further represented a broad range in habitats and regions, covering the intertidal as well as the subtidal zone, soft bottom habitats as well as hard bottom habitats and all European Seas from the warmer Mediterranean and Adriatic Seas in the south over the Black Sea to the colder North Sea and the Baltic Sea in the north. The studies analyse gradients in pressure on water-body-scale, on larger scale across several water bodies in a region or between regions and across a temporal gradient in pressure in a single area.

Eutrophication pressure was considered in all case studies, but other important anthropogenic pressures such as physical modification of the coasts and tourism were considered in some of the case studies. Pressure-response relationships are central for the Water Framework Directive, which relies on the assumption that a pressure-induced change in ecosystem status is reversed when the pressure is released. The best way to test this fundamental assumption is by analyzing time-series of data from specific areas covering periods of increase as well as decrease in the strength of a pressure and the ensuing response of the ecosystem. Pressure-response relationships based on such time series of data are still relatively few as many monitoring programs have only recently been initiated. Spatial data sets covering the response of an indicator across areas exposed to differences in pressure, are often used as a substitute for temporal data sets. This is also true for most case-studies analyzed in this deliverable, which except for one are based on spatial data sets. Temporal aspects do make part of the spatial data sets since a given state is the result of past influences, but the various ecosystems included may have widely different history and represent different stages of e.g. eutrophication-oligotrophication suites.

The analyses of changes in the seagrass quality index along Portugal's coast (case study 5) showed a decline in the index in response to increasing pressure followed by a decline in the index in response to released pressure. The study thereby demonstrated reversibility even though the recovery phase was slower than the degradation phase. Though some studies provide positive perspectives regarding recovery of ecosystems from pressures (Jones and Schmitz 2009), several long temporal data sets from coastal areas do not give strong support to the concept of reversibility of ecosystems to their pre-pressure state, upon release of the pressure (Duarte et al. 2009, Kemp et al. 2009, Carstensen et al. in press). Pressures may trigger positive feed backs that amplify their effects on the ecosystem and, upon passing a threshold level, may push the ecosystem to an alternative state that is not easily reversed, even upon release of the main pressure. The pathways of responses to an increase in pressure such as eutrophication may therefore differ from those of a release of pressure, as was the case for the seagrasses in the Mondego Estuary (case study 5). Moreover, other environmental conditions and pressures may differ from those of the past and thereby create an alternative baseline. For instance, global warming, over-fishery or coastal constructions may imply that even if eutrophication pressure is released, the ecosystem will not return to the reference condition but rather to a new state defined by the new combination of environmental conditions. This interaction between organisms and their surroundings makes ecosystems dynamic units that do not necessarily respond in a predictive, smooth manner to perturbations but may show abrupt changes, alternative stable states, hysteresis effects and/or changing baselines (e.g. Scheffer et al. 2003, Duarte et al. 2009, Kemp et al. 2009).

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