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Research papers

Seasonality of microphytobenthos revealed by remote-sensing in a South European estuary

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ABSTRACT

The spatio-temporal variation of microphytobenthos (MPB) at the scale of a large estuary (Tagus estuary, Portugal) was studied using a combination of field and satellite remote sensing data during 2003. This is the first attempt to use remote sensing to study MPB in an ecosystem with a Mediterranean-like climate. Satellite pour l'Observation de la Terre (SPOT) and Medium Resolution Imaging Spectrometer (MERIS) images were used to map benthic microalgae through the application of a Normalized Difference Vegetation index (NDVI). A significant relationship between in-situ benthic chlorophyll *a* measurements and SPOT NDVI values was used to derive a map for biomass spatial distribution.

At the scale of the whole intertidal area, NDVI time-series from 2003 revealed that MPB showed clear temporal variations, with lower values observed in summer compared to winter. This seasonal trend was found both in the SPOT and MERIS images and may be the result of extreme high temperatures that inhibit MPB growth. The main MPB biofilms were spatially stable through time at a large scale. Maximum NDVI values during the winter were found in the high shore with decreasing NDVI values towards the low shore. MPB light limitation at the lowest bathymetries is likely to occur in winter due to the high turbidity of Tagus estuary.

The biomass spatial distribution map, obtained for January 2003, indicated low values ranging from 0 to 20 mg Chl a m^{-2} for the lower shores, while in the upper shore biomass varied between 60 and 80 mg Chl a m^{-2}. This study suggests striking differences in MPB seasonal patterns between the northern and southern European estuaries and stresses the need for ecophysiological approaches to investigate the role of thermo- and photo-inhibition as structuring factors for MPB biomass distribution.

1. Introduction

Estuaries are among the most productive ecosystems of the world. Although they only represent less than 0.5% of the marine environment, estuaries may be responsible for up to 10% of the total net primary production (McLusky and Elliott, 2004). Intertidal flats, which are exposed either to the atmosphere or to the overlying water, are an important part of these systems. Their biological communities are influenced by several external factors, such as temperature and sunlight (Heip et al., 2005; McLusky and Elliott, 2004). Estuarine mudflats generally receive high nutrient inputs due to mainland run-off, riverine waters and anthropogenic-derived waters (Cabrita and Brotas, 2000; Elliott and McLusky, 2002; Gameiro et al., 2004; Brito et al., 2010a). Light and nutrient availability are likely to stimulate the growth of microalgae in these flats, which are inhabited by large populations of benthic unicellular photoautotrophs, globally referred as microphytobenthos, and typically composed of diatoms, dinoflagellates, euglenoids and/or cyanobacteria (MacIntyre et al., 1996).

Microphytobenthos (MPB) assemblages can contribute up to 50% of estuarine primary productivity (Underwood and Kromkamp, 1999), providing important environmental services in terms of carbon and nutrient fluxes (Richmond et al., 2007). They also have

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critical roles in the food web, given that they represent an important food source, especially for deposit- and suspension-feeding macrofauna (Miller et al., 1996), and in the stabilization of sediments (Paterson, 1989). MPB communities are highly variable in space, at scales ranging from centimeters to kilometers (Guarini et al., 1998; Seuront and Spilmont, 2002; Jesus et al., 2005). Moreover, these organisms also exhibit a high degree of temporal variability. Authors such as De Jong, de Jonge (1995) and Underwood and Kromkamp (1999) have identified a seasonal pattern with maximum chlorophyll *a* (Chl *a*) concentrations during the summer. However, other studies have found opposite patterns (e.g. Koh et al., 2007) or the lack of any clear seasonal trend (e.g. Brotas et al., 1995). Given the relevance of MPB in regional primary production estimates, as well as carbon and nutrient cycling, it is therefore critical to study the natural dynamics of these communities at the ecosystem-scale (Méléder et al., 2005; Brito et al., 2010a). In order to examine the distribution and temporal variations of MPB at the tidal flat scale, samples should be repetitively collected over wide areas and throughout a period of time. Such a sampling scenario is very unlikely to happen due to the inaccessible nature of the mudflats and the amount and cost of the work involved in subsequent field and laboratory work. Thus, there is a growing interest and need in the development of reliable remote sensing tools to provide a synoptic view of the MPB communities in intertidal flats (Paterson et al., 1998; Méléder et al., 2003; Murphy et al., 2005; Jesus et al., 2006; Kromkamp et al., 2006).

Remote sensing approaches to derive the intertidal biomass of MPB at the scale of a basin or estuary have been used only in few studies, such as Combe et al. (2005) and van der Wal et al. (2008, 2010). Most studies have used a multispectral reflectance index, the Normalized Difference Vegetation Index (NDVI), which combines information of red and near infrared wavelengths (Tucker, 1979), as a proxy for benthic chlorophyll *a* (Barillé et al., 2011). In the absence of macrophytes, NDVI has been considered as a good estimator of MPB biomass (Van der Wal et al., 2008) with a low sensitivity to sediment background influences compared to other vegetation indices (Barillé et al., 2011). It can be calculated with multispectral sensors of most Earth observation satellites, with high (e.g. SPOT-HRV; Méléder et al. 2003) or low (e.g. Aqua MODIS; van der Wal et al., 2010) spatial resolution. Thus, the retrieval of NDVI values from satellite data allows the synoptic evaluation of MPB biomass through time, which is critical for the understanding of ecosystem function and for the development of biogeochemical and ecosystem models (Blackford, 2001; Brito et al., 2011).

This study aims to investigate the spatio-temporal variation of MPB, at the scale of a large estuary, through the conjugation of field (MPB biomass) and satellite data (respectively SPOT-HRV and MERIS sensors). The seasonal, as well as the spatial dynamics of benthic microalgae were evaluated applying a NDVI range to discriminate MPB from intertidal macrophytes (macroalgae and angiosperms). A model to calibrate SPOT images into MPB biomass is proposed using *in-situ* chlorophyll *a* measurements.

2. Data and methods

2.1. Study site

This study was conducted in the Tagus estuary (Portugal), which is one of the largest estuarine systems on the western coast of Europe (38°44′N, 09°08′W; Fig. 1). The estuary covers an area of approximately 320 km^2 (Gameiro et al., 2007). A significant part of this area, representing up to 40% (i.e. 128 km^2), is intertidal and constituted by saltmarshes and intertidal flats (Catarino et al., 1985; Brotas et al. 1995); approximately 97 km^2 of sediment flats are exposed at low water during a spring tide (with a tidal height of 0.6 m; Granadeiro et al., 2007). The tidal regime is semi-diurnal and the mean tidal amplitude is 2.6 m. The intertidal flats are mainly dominated by muddy sediments covered by benthic microalgae (Brotas et al., 1995). Shellfish banks constituted by dead oysters and peppery furrow shells (*Scrobicularia plana*) are a major structure throughout the estuary, occupying ca 16 km^2 (Ferreira and Ramos, 1989). These banks were documented through the use of optics and SAR satellite data (more information in the website: http://www.brockmann-consult.de/vae-intertidal).

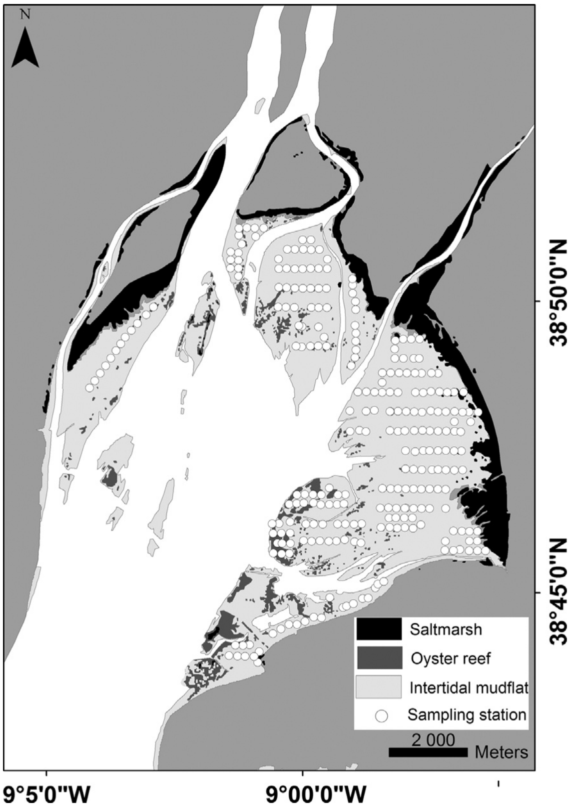

Fig. 1. Intertidal areas located in the northern part of the Tagus estuary. A measurement of microphytobenthos biomass was done at each sampling station (circles). Notice the presence of oyster reefs in the lower parts of the intertidal zone (dark gray).

2.2. Sediment collection and analysis

Twenty intensive field campaigns were carried out at low tide, from December 2002 to April 2003 at the north-eastern part of the Tagus estuary (Mendes, 2005). 217 stations were sampled in total, with a minimum distance of 250 m between them (Fig. 1). From these, 69 were sampled in January 2003. The sampling grid used was similar to the one presented by Granadeiro et al. (2007), covering part of the year of 2003. The year 2003 was characterized by an extremely warm summer in the Tagus estuary (Fig. 2). Monthly averages of air temperature from April to August exceeded the decadal average (2001–2011) by up to 5 °C. (data from SNIRH database, available at: http://snirh.pt/).

Microphytobenthos samples (area of ~1.8 cm^2) were taken by collecting the top 5 mm of sediment. Samples were then placed in appropriate recipients (e.g. plastic tubes) inside a cooler, protected against light and high temperatures and transported to the laboratory. At the laboratory, photosynthetic pigments were extracted from subsamples of freeze-dried sediment with 3 ml of 90% acetone. After 48 h of darkness at −20 °C, the samples were stirred in a vortex, centrifuged at 3500 rpm for 15 min, and extracts were analyzed with a Shimadzu UV-1603 spectrophotometer. Chlorophyll

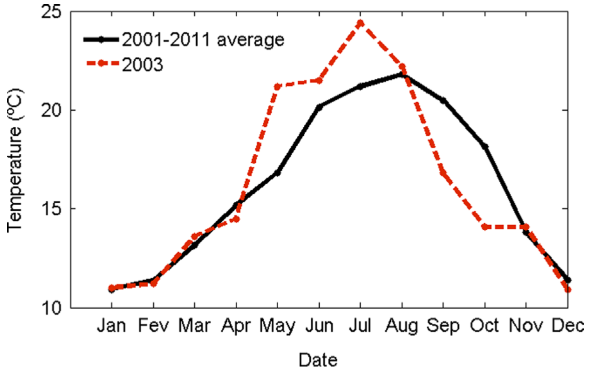

Fig. 2. Monthly average temperatures (°C) of the air in the Tagus estuary from the year of 2003 and the decennial average for the period between 2001 and 2011. Monthly averages were calculated from daily means.

Table 1
SPOT satellite imagery used to assess microphytobenthos spatial distribution in the Tagus estuary. U.T. = Universal Time.

Date	Satellite scene	Resolution (m)	Acquisition time (UT)	Water level(m)
24/01/2003	SPOT 5	10	11 h52	1.18
07/05/2003	SPOT 2	20	11 h22	1.37
17/07/2003	SPOT 4	20	11 h31	0.98
14/09/2003	SPOT 5	20	11 h22	0.93
24/12/2003	SPOT 5	10	11 h27	0.75

a and pheopigments values were obtained before and after acidification with 0.5 M HCl (12 μl of HCl to 1 ml of extract), respectively (Lorenzen, 1967). Microphytobenthic chlorophyll concentrations are presented as areal concentrations (i.e. mg m^{-2}).

2.3. SPOT-HRV data processing

Five SPOT multispectral satellite images obtained with the High Resolution Visible (HRV) sensor were selected in 2003 for this study (Table 1). They were chosen in order to meet the optimal imaging criteria for the acquisition of MPB distribution data: sun angle, tidal stage, and atmospheric conditions. These images were acquired during cloud free conditions ($<10\%$), low tide, and an almost zenith sun (Satellite off-zenith incidence angle varying from 5.6° to 18.6°). In addition, to study the seasonal variation, images were chosen to cover the whole annual cycle and the year of 2003 was retained due to the existence of a large amount of concomitant MPB biomass field data. SPOT images were calibrated to reflectance with FLAASH (Fast Line of sight Atmospheric Analysis of Spectral Hypercubes), available in ENVI® software and using MODTRAN4 transfer codes for the atmospheric corrections (Matthew et al., 2000). The FLAASH module retrieves sensor's gain and offset, as well as geometric information from SPOT metadata. For the Tagus estuary, the middle latitude winter and summer atmospheric models were used, depending on the period of the year, with a maritime aerosol model with an initial visibility of 80 km. The consistency of the atmospheric correction was checked by comparing spectral signatures of the main types of vegetation (micro and macroalgae, seagrass, perennial saltmarsh plants) with references from previous studies (Méléder et al., 2003; Barillé et al., 2010). The high spatial resolution images (10×10 m^2 pixel size) allowed unanbiguous identification of saltmarsh stands in the upper intertidal. The scenes were registered in the WGS 84 UTM 29 coordinates system.

The Normalized Difference Vegetation Index (NDVI; Tucker, 1979) was applied to the 5 images calibrated in reflectance (ρ), using red (XS2: 610–680 nm) and near-infrared (XS3: 780–890 nm) SPOT spectral bands. Thus

$$\mathrm{NDVI} = \frac{\rho(XS3) - P(XS2)}{\rho(XS3) + P(XS2)} \tag{1}$$

This index was chosen among various multispectral vegetation indices to identify benthic diatom biofilms because of its lower sensitivity to background influences compared to soil-corrected indices (Barillé et al. 2011). Matches between in-situ measurements, taken at least 250 m apart, and each pixel was then selected. In a preliminary study on MPB spatial distribution in Bourgneuf Bay (Méléder et al., 2003), a micro and macrophyte distinction was achieved using a NDVI range of 0–0.3, determined using spectrometric knowledge, image analysis and ground-truthing. This method was combined here with a Geographical Information System (GIS) overlay processing to exclude subtidal area using bathymetric data and macroalgae which frequently grow in the Tagus estuary attached to dead oyster shells. Oyster reefs maps produced previously, through the analysis of optical and SAR data within the framework of the Value Adding Element program of the European Space Agency (ESA), were used to remove theses pixels from the calculations.

2.4. MERIS data processing

The Medium Resolution Imaging Spectrometer (MERIS, Rast et al., 1999) was launched on-board the Envisat platform by ESA on March 2002 and provided global coverage of the biosphere until April 2012. With 15 spectral bands in the visible and near-infrared MERIS is a suitable instrument to monitor intertidal benthic microalgae, whose reflectance spectrum is characterized by a strong absorption peak in the 660–700 nm spectral range and a near-infrared plateau (Méléder et al., 2003). Data used in the present work were MERIS Level-2 surface reflectance reduced-resolution data (1040 m × 1160 m) resulting from the MERIS 3rd reprocessing (Goryl et al., 2010). Data were downloaded from the Optical Data processor (ODESA, http://www.odesa-info.eu/process_basic/basic.php) and processed using the Earth Observation Toolbox and Development Platform software (BEAM-VISAT 4.10, Brockmann consult Inc., http://www.brockmann-consult.de/cms/web/beam/). A total of 16 cloud-free MERIS scenes of the Tagus estuary intertidal zone were selected between late 2002 and early 2004 to study the seasonal variation of NDVI during the year 2003. Only scenes acquired at low-tide were selected and water heights at the time of MERIS data acquisition over the Tagus estuary ranged from 0.41 to 1.19 m (data courtesy of the French marine service for hydrography and oceanography, Service Hydrographique et Océanographique de la Marine, SHOM using Lisbon as water height reference). Valid intertidal pixels were determined using a combination of necessary conditions: (i) pixels flagged as cloud or water were rejected, (ii) macrophytes were distinguished from MPB using an empirical threshold of 0.15 for the reflectance at 890 nm, and (iii) pixels corresponding to subtidal area were rejected (see Section 2.3). An average of 17 valid pixels was selected for each scene (minimum 9, maximum 25), covering more than 170 ha of surface area. Reflectance data was extracted for all valid pixels at the following wavelengths: 412.5, 442.5, 490, 510, 560, 620, 665, 681.25, 705, 753.75, 775, 865, and 890 nm.

For the purpose of comparing the results with SPOT-derived NDVI, MERIS reflectance spectra were linearly interpolated and averaged between 610 and 680 nm and between 780 and 890 nm in order to simulate SPOT bands in the red (XS2: 610–680 nm) and near-infrared (XS3: 780–890 nm), and NDVI was derived as defined in Section 2.3. Average NDVI values were computed over the complete intertidal zone.

2.5. Statistical analysis

All statistical tests and numerical analyses were carried out using STATISTICA 10 (Statsoft Inc., 2011). Data were tested for normality and homoscedasticity of variance and parametric tests conducted. Temporal and spatial variations of MPB were compared with one-way analysis of variance (ANOVA). When significant, pairwise post-hoc comparisons were performed using the Tukey HSD test.

3. Results

3.1. Spatio-temporal dynamics of MPB

SPOT images calibrated to NDVI values were analyzed applying decision rules to map the intertidal flats. Negative values corresponded to water and bare sediments, MPB was identified within a 0–0.3 NDVI range, while values higher than 0.3 were associated to macrophytes. In the upper intertidal zone, around the +3 m isobath, the pioneer halophyte *Spartina maritima* forms conspicuous round-shaped structures surrounded by microphytobenthic biofilms. The lowest NDVI value observed for this angiosperm was 0.35, and the upper NDVI threshold of 0.3 efficiently discriminated MPB from saltmarsh species. This was not always the case for macroalgae and seagrasses in the lower intertidal since a confusion with pixels characterized by low macroalgal coverage could not be excluded below the upper threshold of 0.3. Patches of *Zostera* are present in a confined area located in the south margin of the Tagus River. This area was not considered in this analysis. Macroalgae whose distribution is closely related in the Tagus estuary to the presence of oyster shells were therefore subsequently removed applying oyster reefs distribution overlay. NDVI time-series revealed that MPB showed clear temporal variations, characterized by stable spatial patterns at a large scale (Fig. 3). The main assemblages were indeed always detected both in the upper intertidal around the +3 m isobath at the vicinity of the salt marsh fringe and in the lower intertidal areas.

Monthly mean NDVI ranging from 0 to 0.3 showed significant differences (Fig. 4; ANOVA, $P < 0.01$) with higher values observed in winter compared to spring and summer (Tukey-test, $P < 0.01$). Second order polynomial regressions (equations not shown) were fitted to the data to emphasize a clear macroscale seasonal cycle for MPB in the Tagus estuary ($P < 0.05$; Fig. 4). However, the higher coefficient of determination observed for the upper intertidal zone (Fig. 4B; $r^2 = 0.95$) compared to the whole system (Fig. 4A, $r^2 = 0.80$), suggested that seasonal variations were better detected for upper intertidal flat assemblages. This seasonal distribution of MPB was confirmed by the analysis of NDVI variations calculated from MERIS images (Fig. 5). In spite of MERIS lower spatial resolution (e.g. 1 km × 1 km), the general trend characterized by high winter and low summer values was also observed, although the higher image availability suggested a more complex pattern with two NDVI peaks in March and September.

Using the isobaths shown in Fig. 3, pixels were aggregated to calculate mean NDVI for each SPOT image according to bathymetry classes: 0–2, 2–3, 3–4 and 4–5 m (Fig. 6). The depth distribution of MPB was significantly different (ANOVA, $P < 0.01$), with a general pattern observed for each season indicating a decrease from the upper intertidal towards the low shore. This pattern was particularly visible in winter, with NDVI values significantly higher (Tukey-test, $P < 0.01$) in the high shore, i.e. 3–5 m (Fig. 6) followed by a steep decrease at 2–3 and 0–2 m. This trend was present but less discernable in spring due to weaker NDVI variations. In summer, NDVI values similarly decreased from upper to lower shore, as observed for winter, but an increase occurred near the water level, with significant higher NVDI for the 0–2 m region compared to 2–3 m (Tukey-test, $P < 0.01$).

3.2. NDVI vs. MPB biomass

A significant relationship was found between the NDVI values retrieved from the January 2003 SPOT image and *in-situ* benthic chlorophyll-*a* concentrations measured during winter field campaigns ($p < 0.05$; $n = 69$; $r^2 = 0.7$; Fig. 7). This relationship was described with an empirical model, as presented in Table 2. This equation was then used to calibrate the satellite image to a MPB biomass distribution map (Fig. 8). The highest chlorophyll *a* concentrations ranging from 60 to 80 mg Chl *a* m^{-2} were observed in the upper intertidal, near the fringe of the saltmarshes which had been previously excluded from the image with an NDVI threshold (Fig. 8, black arrows). In this saltmarsh area some high biomass concentration patches were observed near a freshwater stream (Ribeira das Enguias). The chlorophyll *a* concentrations estimated from SPOT data were consistent with previous winter field measurements obtained by Cabrita and Brotas (2000) in the top 0.5 cm of sediment for two sites of the intertidal zone (Fig. 8, white star), but these authors observed strong interannual variations, from 20 to 85 mg Chl *a* m^{-2} between two consecutive January datasets.

4. Discussion

A combination of *in-situ* and remote sensing data was used to evaluate the seasonal and spatial variations of MPB biomass at the regional scale of an estuary. Microphytobenthos is a community with a very variable spatial coverage at scales ranging from a few millimeters to kilometers (e.g. Seuront and Spilmont, 2002; Murphy et al., 2008; Davoult et al., 2009; Spilmont et al., 2011). It is therefore essential to have an overall evaluation of the community at the scale of the intertidal system since the description of its spatial structure is a prerequisite to understand processes (Murphy et al., 2008). Such data may have a variety of applications, from spatially distributed biogeochemical models, to management policies (Blackford, 2001; Brito et al., 2010a, 2011). Remote sensing imagery has been successfully used to describe the structure and dynamics of large strands of intertidal macrophytes (e.g. Phinn et al., 2008; Barillé et al., 2010), but this technique has not yet been fully explored for microphytes. In fact, only a small number of studies have explored the potential of remote sensing for studying MPB at macroscale (e.g. Méléder et al., 2003; Combe et al., 2005; van der Wal et al., 2008, 2010) and this is the first attempt to do so in an ecosystem with a Mediterranean-like climate.

Mean Normalized Difference Vegetation Index (NDVI) calculated from SPOT images showed the highest values during the winter months (January and December) and lowest values during spring/summer. This was valid both in the upper intertidal zone, from +2 to +5 m, as well as considering the whole intertidal area. The upper intertidal zone is a relatively large area, where the MPB cells seem to have high densities in the winter. Therefore, this zone has a significant role in the temporal dynamics of mean NDVI values. This seasonal cycle, with the highest values in the winter, is the opposite of the results obtained by van der Wal et al. (2010) for northern European estuaries. Using high frequency Aqua MODIS data, these authors observed MPB synchronous temporal patterns in several estuaries with winter minima and summer maxima. This pattern has been frequently reported for European estuaries,

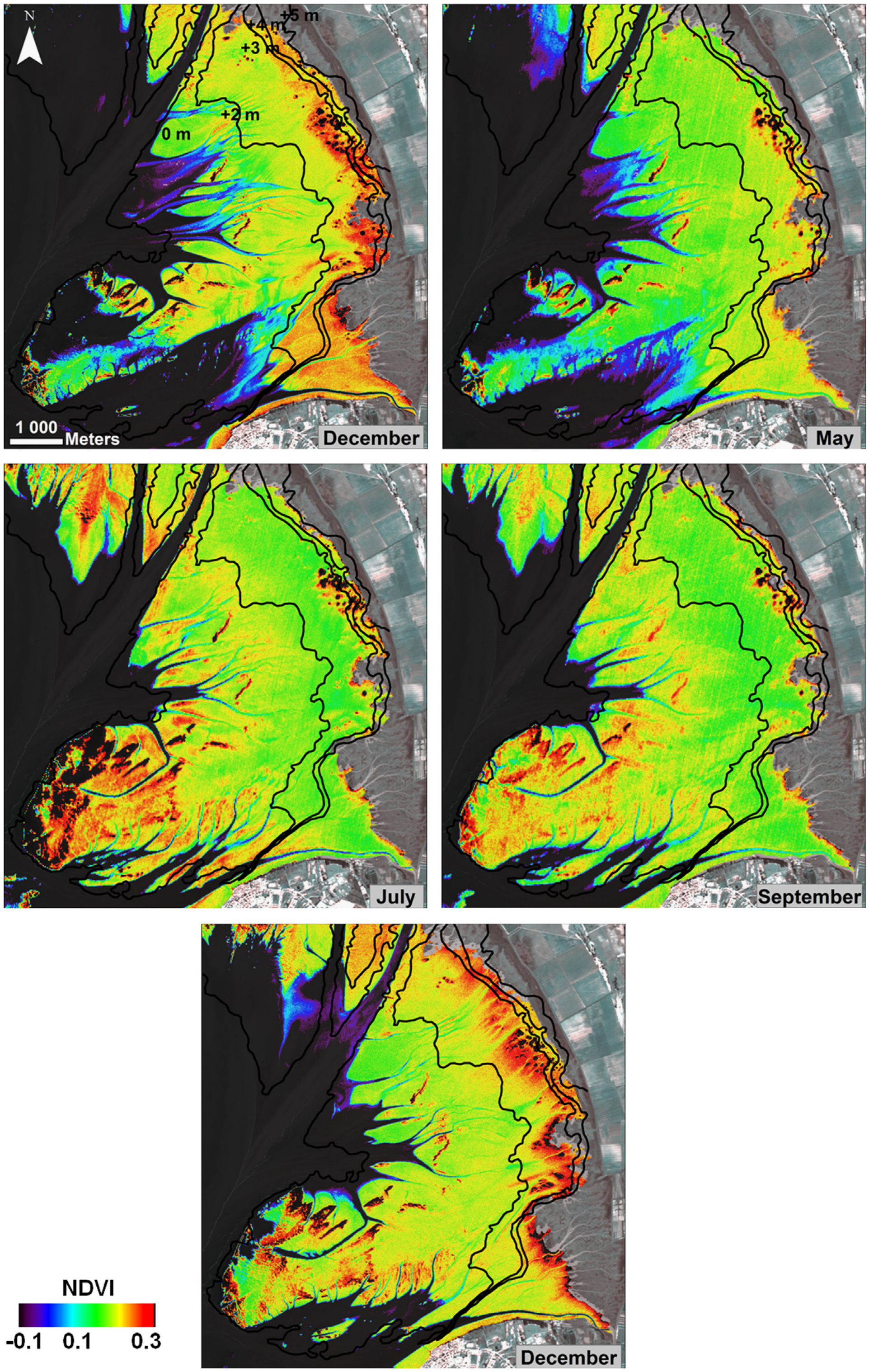

Fig. 3. Seasonal variations of the spatial distribution of microphytobenthos in the Tagus estuary. Benthic microalgae are identified by a Normalized Difference Vegetation Index (NDVI) from SPOT images ranging from 0 to 0.3. Isobaths (above Lowest Astronomical Tide) are indicated on each map.

i.e. higher MPB chlorophyll concentrations in spring and summer (e.g. Jong and de Jonge, 1995; Underwood and Kromkamp, 1999). The lower latitude of the Tagus estuary combined with the striking difference between the north and south seasonal dynamics suggest either the influence of different drivers structuring MPB distribution or the effect of different intensities of similar drivers, in particular irradiance and temperature. The trend detected in the southern Tagus estuary, with SPOT images, was also confirmed using MERIS data. However, given the higher frequency of MERIS images compared with SPOT, two additional peaks were observed in April and early September. With an orbital cycle of 26 days and lower image availability, SPOT could only capture the general trend. To our knowledge, this is the first time that MERIS data are used to map MPB. With 15 spectral bands in the visible and near infrared, the shape of MPB spectral signatures recorded with MERIS can be easily distinguished from that of intertidal

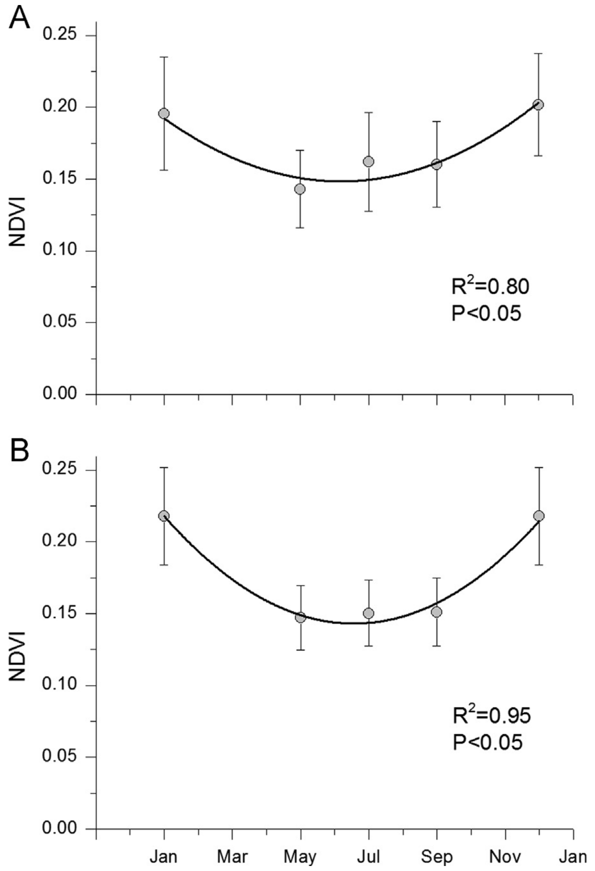

Fig. 4. Mean NDVI seasonal variations calculated from 2003 SPOT images in the intertidal area of the Tagus estuary. A. Entire intertidal area. B. Upper intertidal zone from +2 to +5 m. Means are indicated with their standard deviations. A second order polynomial model was fitted to the data to emphasize the seasonal cycle.

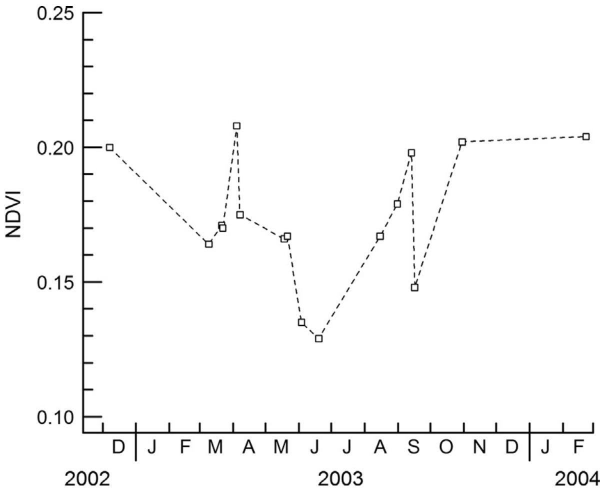

Fig. 5. Mean NDVI seasonal variation calculated from 2003 MERIS images in the whole intertidal area of the Tagus estuary.

macrophytes, being comparable with spectra obtained with the DAIS hyperspectral sensor (Combe et al., 2005). Even better results may be obtained with MERIS Full Resolution (FR) images (pixel size 300 m × 300 m) since a lower frequency of mixed-pixels situations is expected. The potential of MERIS FR images to map MPB is further increased by the quality, consistency and length of the MERIS archive (2002–2012). However, given that ENVISAT

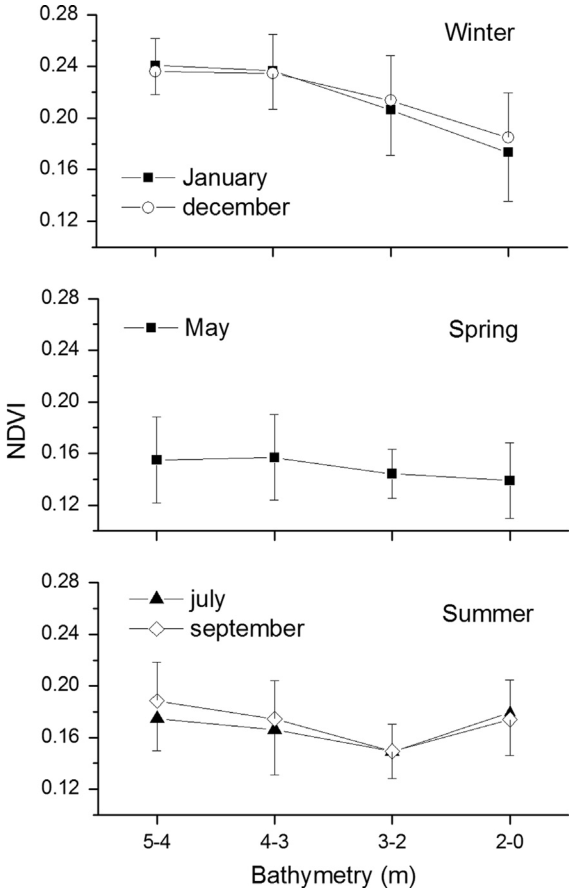

Fig. 6. Altitudinal distribution of microphytobenthos in the Tagus estuary intertidal flats. Mean NDVI ranging from 0 to 0.3 were calculated from SPOT images (Table 1) for different bathymetric levels (meters) for 3 seasons (winter, spring, and summer). Means are presented with their standard deviation (±SD for graphical convenience), since the confidence intervals were not visible due to the large number of pixel analyzed (*n* ranged from 3658 to 65533).

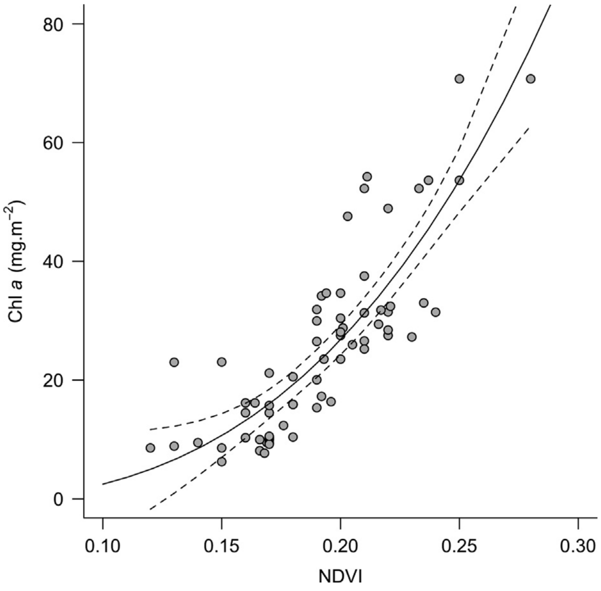

Fig. 7. Relationship between NDVI and benthic chlorophyll *a* concentration (mg chl *a* m^{-2}) measured as a proxy of microphytobenthos biomass during winter field sampling in the Tagus estuary intertidal flats. NDVI values were obtained from a January 2003 SPOT image. All available field data from December 2002 and January and February 2003 were used. 95% confidence intervals are also presented.

Table 2
Empirical model to describe the relationship between NDVI values retrieved from SPOT image and *in-situ* benthic chlorophyll-*a*.

Equation	$Chl\ a = a + b\ x\ NDVI^c$ mg m^{-2}		
Coefficients	*a*	*b*	*c*
Value	−0.904	3671.645	3.037
Standard error	6.90	3345.13	0.727

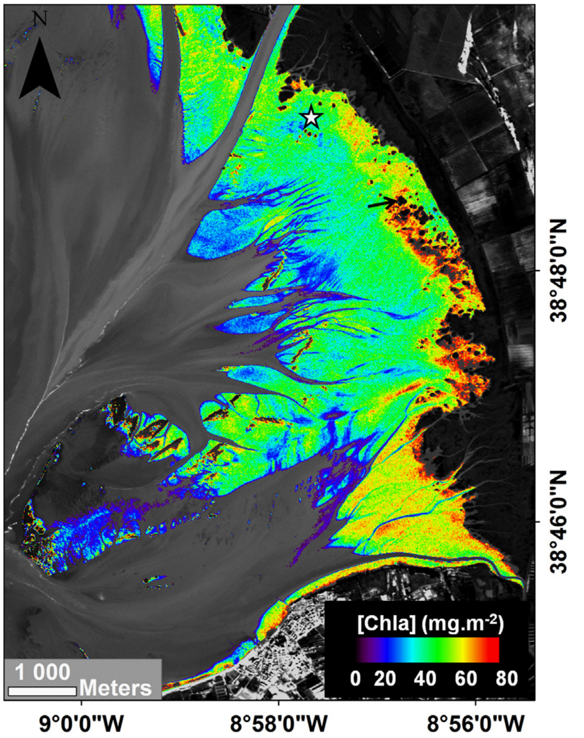

Fig. 8. Spatial distribution of benthic chlorophyll *a* (Chl *a*) concentration (mg chl *a* m^{-2}) used as a proxy of microphytobenthos biomass in the Tagus estuary in January 2003. A multispectral SPOT image with a 10 × 10 m spatial resolution was calibrated with the relationship shown in Fig. 7. Black arrows indicate an example of saltmarsh pionner vegetation (*Spartina maritima*) excluded with an NDVI threshold. The white star corresponds to the sampling sites considered by Cabrita and Brotas (2000).

satellite is no longer operational since April 2012, the Sentinel-2 and Sentinel-3 satellites are planned to be launched in a near future in the frame of the ESA Global Monitoring for Environment and Security (GMES) program. The Multi Spectral Instrument (MSI) on-board Sentinel-2, and the Ocean Land Color Imager (OLCI) on-board Sentinel-3, with 13 and 21 narrow spectral bands in the visible and near-infrared parts of the electromagnetic spectrum, respectively, should be suitable instruments for the remote-sensing of MPB.

Studies of MPB temporal patterns at a large scale based on satellite or aerial remote sensing avoid the flaw of sampling protocols based on a few replicates per time (Spilmont et al., 2011). This may explain the discrepancy with previous studies in the Tagus estuary, which suggested the non-existence of a defined temporal pattern for these benthic microalgae (Brotas et al., 1995; Brotas and Plante-Cuny 1998). Most assessments conducted worldwide rely on field data from a limited number of stations (e.g. Jesus et al., 2006; Brito et al., 2009). However, in the present study, remote sensing confirmed MPB dominance in the upper intertidal zone of the Tagus estuary, from +3 to +5 m (Brotas et al., 1995). This is also in agreement with the observation of van der Wal et al. (2010) showing that highest NDVI values are generally found in the upper intertidal flats.

Several environmental factors may be associated with the temporal and spatial variations found in the Tagus estuary. Microphytobenthos may be nutrient or light limited and may also be affected by other factors such as grazing and temperature conditions. According to the nutrient fluxes measured in the Tagus estuary by Cabrita and Brotas (2000), these are sufficient for an effective MPB growth during the summer. Brito et al. (2010b, 2011) have estimated the nutrient fluxes for a shallow lagoon in the south of Portugal and discovered that, although values were lower than in the Tagus estuary, they were also not limiting for MPB growth. In addition, Brito et al. (2011) found that for the Ria Formosa shallow lagoon light availability could be potentially limiting during the winter. This effect is likely to be more important in the Tagus given the high water turbidity during the winter, due to the river input. High values of water turbidity and light limitation are factors likely to justify the low NDVI values found at the lowest bathymetries during the winter months.

During the summer, the tidal flats are exposed to high air temperature and irradiance conditions, which contribute to the existence of extreme conditions at the sediment surface (Guarini et al., 1997). Sediment temperatures are likely to be higher than in the air and intense evaporation is likely to occur. In fact, Blanchard et al. (1997) and Morris and Kromkamp (2003) showed that MPB may suffer a reduction in the photosynthetic capacity beyond an optimum temperature of 25 °C, with strong thermo-inhibition after 30 °C. Daily mean air temperatures recorded in 2003 were up to 25 °C during the summer. From the available historical records from 1985 to 1994 (SNIRH; http://www.snirh.pt) it is reasonable to consider that maximum daily temperatures were similar or higher than 30 °C. 2003 was an extremely hot year, with daily means higher than the decennial average from May to September. In addition, Serôdio and Catarino (1999) and Brotas et al. (2003) have already recorded sediment temperatures that frequently reached maximum values of 30–31 °C during the summer. We thus hypothesized that the low NDVI values found in the whole intertidal area during the months of May to September may be the result of a thermo-inhibition (Blanchard et al., 1997). However, summer depression of MPB biomass is generally attributed to grazing (Cadée and Hegeman, 1974; Cariou-Le Gall and Blanchard, 1995). In order to investigate this, coupled studies considering both the benthic primary producers and grazers are yet to be performed. It is interesting to note that at high temperatures, evaporation of water is high and sediments should dry. However, according to our field data, MPB was significantly associated with water content (data not shown). Sediments at the highest bathymetries receive water inflow from saltmarshes and small water lines, and this is probably sufficient to maintain the MPB community in the upper intertidal zone. Experimental studies coupling remote-sensing and physiological measurements should be conducted to assess the photo- and/or thermo- inhibition of MPB communities in situ. The two approaches share common spectroradiometric methods and fruitful interactions with fluorometry are expected (Serôdio et al., 2012; Vieira et al., 2012).

Taking advantage of the existing large dataset, a significant relationship was found between NDVI and chlorophyll *a* concentrations, explaining approximately 70% of the variability, in spite of the mismatch between the surfaces sampled by sediment cores and the surface covered by a pixel. A lower coefficient of determination (50%) was obtained for the Westerschelde estuary, but with Aqua MODIS pixels of 250-m resolution (van der Wal et al. 2010). This suggests that this variability can be reduced with multispectral sensors of higher spatial resolution such as Quickbird (2.5-m pixel) or the sub-metric Pléiades-HR. The NDVI/Chl *a* relationship needs to be further developed in the future for multispectral applications, considering higher chlorophyll *a* concentrations. Currently, the dataset used covers concentrations from approximately 5 to 75 mg chl m^{-2}. The maximum concentrations found are considered low if compared with other studies such as Brotas et al. (2007) and Jesus et al. (2009). It should be emphasized that the biomass data used in the relationship strongly depend on the sampling depth of the cores. Given that MPB exhibit an heterogeneous vertical distribution in sediments and are strongly concentrated in the top 2 mm future campaigns should consider sampling depths smaller than 5 mm, in particular for epipelic assemblages colonizing cohesive sediment (Jesus et al., 2006; Barillé et al., 2007). Contrastingly, a lower biomass range of 0–35 mg chl m^{-2} has been proposed by Kazemipour et al. (2012) who mapped MPB with hyperspectral images calibrated with the optical properties of monospecific diatoms and euglenids cultures. However, these authors recognized that the sampling depth of ground-truth sediment cores remained an issue for remote-sensing images calibration.

5. Conclusions

In conclusion, MPB NDVI values for the whole estuarine area were found to be lower in the summer than during the winter. This trend was found both in the SPOT and MERIS images and may be the result of extreme high temperatures, recorded in the summer of 2003, which is likely to cause thermo-inhibition of MPB photosynthesis. The highest NDVI values during the winter were found at the highest bathymetries with decreasing NDVI values towards the lowest bathymetries. Light limitation of MPB growth is likely to occur during the winter, at the lowest shore heights, due to the high turbidity of the Tagus River. The relationship found between MPB chlorophyll concentrations and NDVI values should be tested further to study MPB temporal and spatial variability in other intertidal areas in southern Europe.

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