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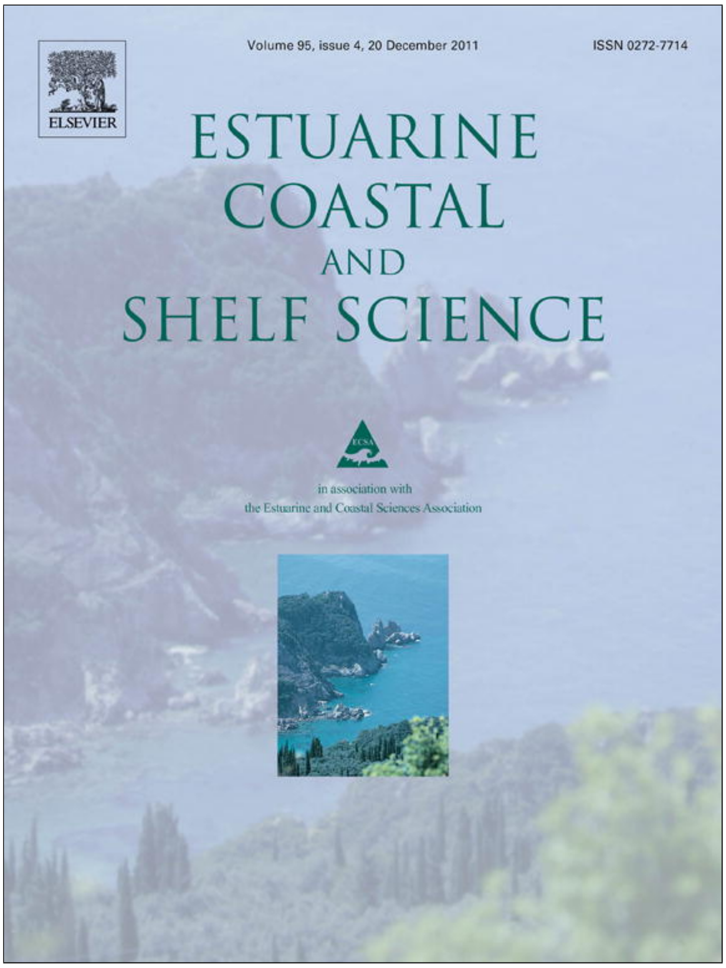
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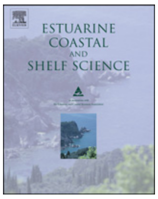

There's more to the picture than meets the eye: Sampling microphytobenthos in a heterogeneous environment

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ABSTRACT

Distributions of microphytobenthos are highly heterogeneous at scales as small as a few centimetres. However, the sampling protocols currently used for the absolute determination of microphytobenthos biomass through chlorophyll *a* concentration measurements in surface sediments are too limited to take this variability into account, typically relying on 3-5 samples taken within a randomly located 1 m^2 quadrat in a given environment. We address this issue by objectively and quantitatively inferring the minimum number of samples required to obtain reliable estimates of microphytobenthos biomass on the basis of high-resolution sub-sampling (225 regularly spaced samples) within each of nine 1 m^2 quadrats at an unvegetated sheltered intertidal sandbank of the Gold Coast (Queensland, Australia). The results were generalised using data obtained in previous studies on an exposed sandy shore and on sheltered estuarine sandy muds of the Eastern English Channel. Estimates of chlorophyll *a* concentration exhibited a high degree of heterogeneity, both between and within quadrats. The number of samples needed to estimate the average chlorophyll *a* concentration, and hence mean microphytobenthos biomass with 95% confidence intervals, ranged from 15 to 115, and mainly depended on the presence of global and local gradients within the quadrats. These results have major implications for intertidal ecology by implying a possible systematic bias in the measurement of both microphytobenthos biomass and production of up to 40%. Finally, we emphasise that this issue can be circumvented using field spectrometry or PAM fluorescence measurements coupled with traditional sediment sampling techniques, and urge for unified protocols to be adopted for the routine use of these combined methods.

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1. Introduction

The microphytobenthos is an important component of coastal marine ecosystems where it may account for up to 50% of the overall primary production (Perissinoto et al., 2002; Montani et al., 2003). These organisms are particularly abundant and productive in intertidal mudflats (e.g. Spilmont et al., 2006; Davoult et al., 2009; Migné et al., 2009) but also contribute to the stabilization of sediment, and nutrient and trophic fluxes in all intertidal soft sediments, including sandflats (Spilmont et al., 2005, 2009a; Anschutz et al., 2009; Stal, 2010). Hence, information on their productivity and biomass are necessary to strengthen our understanding of intertidal systems structure and trophodynamics and to allow the modelling of e.g. their role in the global carbon cycle.

Microphytobenthos biomass is typically quantified through the measurement of chlorophyll *a* concentration in surface sediment, which is considered as a basic environmental descriptor in most benthic studies (Bale and Kenny, 2005). Indirect methods such as field spectrometry and PAM fluorometry are used as proxies for chlorophyll *a* concentration, and are useful for mapping large areas and for high resolution temporal measurements (e.g. Honeywill et al., 2002; Carrère et al., 2004; Jesus et al., 2005, 2006b; Forster and Jesus, 2006; Murphy et al., 2009). These techniques are, however, still debated since there is no methodological consensus (Jesus et al., 2006a, 2006b; Serodio et al., 2009). The determination of microphytobenthos biomass hence still requires the collection of sediment cores from which pigments are extracted (Brotas et al.,

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2007). The sampling and extraction methods have also long been debated (see Grinham et al., 2007; and references therein). However, the rationale behind the choice of a sampling strategy (i.e. the number and the spatial repartition of sediment cores) has still not been thoroughly and objectively assessed, even though it fundamentally controls the accuracy of biomass, and ultimately production estimates (Seuront and Spilmont, 2002). Typically, chlorophyll *a* concentrations are estimated from 3 to 5 replicate samples taken within randomly located 1 m^2 quadrats in a given environment (see reviews in Seuront and Spilmont, 2002; and Underwood, 2010). Microphytobenthos distributions have, however, long been known to exhibit centimetre-scale patchiness (Varela and Penas, 1985; Blanchard, 1990; Pinckney and Sandulli, 1990) which therefore should be taken into account in the design of sampling strategies. Most studies that have examined the spatial distribution of cholorophyll *a* at scales below 1 m^2 (referred as the microscale hereafter) aimed to describe the spatial structure and eventually the dynamics of the microphytobenthic biomass (Guarini et al., 1998; Sandulli and Pinckney, 1999; Azovsky et al., 2000, 2004; Seuront and Spilmont, 2002; Moreno and Niell, 2004; Jesus et al., 2005; Seuront and Leterme, 2006; Brito et al., 2009; Murphy et al., 2009). To our knowledge, only two studies specifically discussed their results in terms of the sampling protocol (Grinham et al., 2007; Chapman et al., 2010). However, their microscale results were relatively limited in terms of replication, because they were integrated in broader studies examining variations over multiple spatial scales.

In this context, the present study examined the spatial heterogeneity of cholorophyll *a* concentration within the classical metre-square benthic sampling unit. Specifically, 225 sediment samples (resolution 6.7 cm) were taken within each of nine 1 m^2 quadrats during a single low tide on an intertidal sandbank. These data, together with data obtained on an exposed sandflat and on estuarine sandy muds of the Eastern English Channel, are used to assess the impact of microscale patchiness on estimates of microphytobenthos biomass and to propose an objective lower limit for the number of samples that need to be taken in order to obtain reliable estimates of this biomass.

2. Material and methods

2.1. Sampling

Sediment samples were collected at low tide from an unvegetated sheltered intertidal sandbank of the Broadwater, Southport (Gold Coast, Queensland, Australia) (Spilmont et al., 2009a; and; Jordan et al., 2009 describe the sampling site). The tidal range at the sampling site was ca. 1.3 m, the sediment was composed of coarse sand (mean grain size 180–250 μm: Jordan et al., 2009). Nine 1 m^2 quadrats were arranged in a square of 15 m side length (Fig. 1). Within each quadrat, 225 equidistant sediment cores were collected using 1.9 cm^2 (1.6 cm inner diameter) plastic tubes inserted to a depth of 1 cm. Labelled samples were immediately stored on ice in the dark. Three quadrats were sampled concurrently, all nine quadrats were sampled within a 4 h period. During the sampling, a few cores were lost, and coring was impossible in some places (e.g. presence of shells or debris). Thus, a total of 1995 sediment cores were analysed. In the laboratory, 8 mL 95% acetone were added and samples were extracted in the dark at 4 °C for at least 24 h. Chlorophyll *a* (Chl*a*) concentrations (mg m^{-2}) were

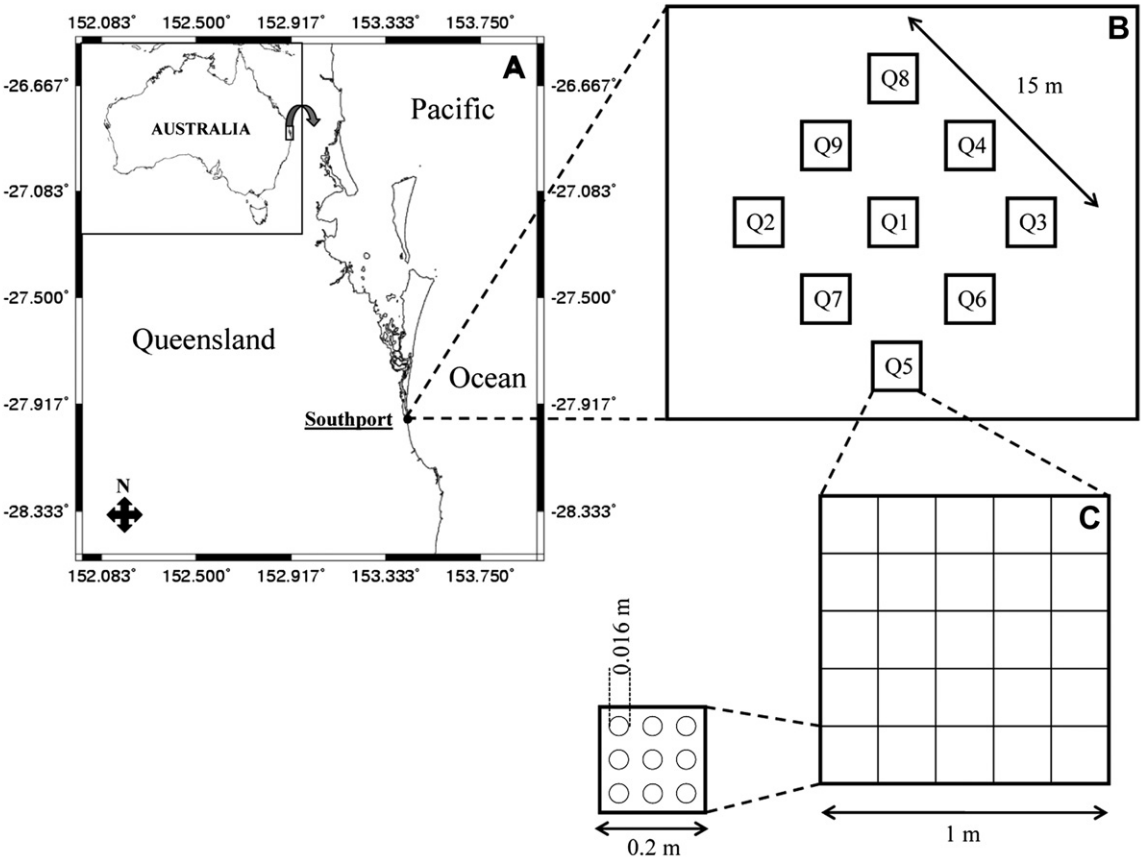

Fig. 1. Location of the study area on the Queensland coast, Australia (A), and schematic representations of the distributions of the quadrats at the sampling location (B) and of the sampling units within the quadrats (C).

determined spectrophotometrically and calculated taking into account the sampling area and the solvent volume as described in Seuront and Spilmont (2002).

To generalise the results to other systems and ensure the relevance and generality of our approach, data previously obtained on the coast of the Eastern English Channel were used. The exposed sandy beach of Wimereux was characterised by medium size sand (200–250 μm, modal size) whereas Le Crotoy (Bay of Somme) was characterised by sandy muds (125–250 μm modal size). On each of these study sites, a single 1 m^2 quadrat (Fig. 1C) was sampled at each sampling occasion, *i.e.* in autumn at Wimereux (Seuront and Spilmont, 2002), in autumn and spring in the Bay of Somme (Seuront and Leterme, 2006 and Seuront, 2010; respectively).

2.2. Data analysis

The distribution of chlorophyll *a* within each quadrat was quantified using basic descriptive statistical analyses including skewness, coefficient of variation and coefficient of dispersion (Table 1). More specifically, the ratio between maximum and minimum biomass r_b was use as a normalized measure of maximal variability (Seuront and Spilmont, 2002). To determine the number of samples needed for an accurate estimation of the average chlorophyll *a* concentration in each quadrat, we proposed a procedure based on Bartoli et al. (2003). A bootstrap technique was used to randomly resample 1000 sets of n subsamples, with n ranging from 3 to, at least, 100; for each value of n, the output consisted of 1000 values of the mean. The distance between the bootstrap-generated means and the best estimate of the true average concentration (BEA) was calculated for each value of n as the error d (Bartoli et al., 2003):

$$d = |BEA - A_n|/BEA \qquad (1)$$

where BEA is the best estimate of the average concentration (calculated on all the samples collected in the same quadrat), and A_n the average concentration of the n bootstrap-generated subsamples. One thousand d values were thus calculated for each value of n; then the 95th percentile values of d were plotted against n. The number of samples needed to estimate the average concentration with a confidence of 95% and accuracy of 5% were then determined graphically, *i.e.* finding n for $d \leq 0.05$.

Data from each quadrat were further examined for global gradients and local trends. Global gradients were inferred following the simple procedure proposed by Webster and Oliver (2001): for each quadrat, data were arranged in a two-way table and the means of both rows and columns were then computed and plotted. A significant decrease or increase (Pearson correlation test, $\alpha = 0.05$) in the row and/or column means would then characterise a trend in the direction of one or both axes. A summed cumulated function (Ibanez et al., 1993) was used for local trends detection; the mean of each series (columns or rows) was subtracted from the data and the residuals were cumulated (residuals were standardised, *i.e.* divided by the mean, to allow comparisons between quadrats from different sediment types). Successive values higher and lower than the mean respectively produce successive positive and negative residuals, which produce an increasing and a decreasing slope (note that values not very different from the mean show no slope). Local trends were then inferred graphically as slope changes (i.e. successive values lower or higher than the mean), with the steepness of the slope characterising the intensity of the gradient.

3. Results

Basic statistical analyses computed on the 9 quadrats from the Gold Coast (Table 1) indicated a high variability in chlorophyll *a* concentrations both between quadrats (Kruskal–Wallis test, $p < 0.05$), with average concentrations ranging from 15.25 to 28.15 mg m^{-2}, and within quadrats (Fig. 2; CV and r_b ranging respectively from 10.3 to 30.1% and 2.1–10.6). Chlorophyll *a* concentrations were normally distributed in 4 out of the 9 sampled quadrats. The frequency distributions of the 5 remaining quadrats were characterised by a significantly positive skewness (Table 1), corresponding to distributions characterised by a wide range of low density patches and a few dense patches; see e.g. quadrats Q2, Q3 and Q6 (Fig. 2). The same observations apply to the additional data from Wimereux (QW) and the Bay of Somme (QSa in autumn and QSs in spring; Table 1). Note that the average concentrations measured in the Bay of Somme were significantly higher than those estimated on both sandy sites, with the lowest concentrations (42.54 and 30.14 mg m^{-2} for QSa and QSs, respectively) being of the same order of magnitude than the highest concentrations in other quadrats.

The number of samples needed to estimate the average chlorophyll *a* concentration with a confidence of 95% ranged from 15 to 115 (Table 1). The quadrats that required the most samples to obtain an accurate concentration estimate were also the most skewed and/or were consistently over-dispersed (coefficient of dispersion >>1) and characterised by CV higher than 25%. The

Table 1
Basic statistical analyses (Sd: standard deviation; CV: coefficient of variation; CD: coefficient of dispersion) calculated for the nine quadrats sampled on the Gold Coast (Q1–Q9), at Wimereux (QW; Seuront and Spilmont, 2002) and in the Bay of Somme in autumn and spring (QSa and QSs, respectively; Seuront and Leterme, 2006; Seuront, 2010). N represented the number of samples analysed in each quadrat. Concentrations are expressed as mgChl*a* m^{-2}. Normality was tested using the Kolmogorov–Smirnov test ($\alpha = 0.05$), r_b is the ratio between the maximal and minimal measured concentrations within the quadrat, and n the minimum sample size required for an accurate estimation of the mean concentration with 95% confidence. The error d in the estimation of the average concentration for $n = 3$ and $n = 5$ are presented as $d_{(n=3)}$ and $d_{(n=5)}$, respectively.

Quadrat	Normality	Skewness	N	Range	r_b	Mean	Sd (CV)	CD	Minimum sampling (n)	$d_{(n=3)}$	$d_{(n=5)}$
Q1	Yes	−0.01	224	11.99–32.25	2.7	21.36	3.90 (18.3)	0.71	49	0.21	0.17
Q2	No	0.15	225	14.70–30.27	2.1	21.41	2.20 (10.3)	0.23	15	0.12	0.09
Q3	No	0.76	216	4.64–49.38	10.6	19.01	5.72 (30.1)	1.72	85	0.33	0.26
Q4	Yes	0.09	223	9.88–26.60	2.7	18.12	3.26 (18.0)	0.59	42	0.21	0.15
Q5	Yes	−0.01	224	13.96–26.27	1.9	20.38	2.39 (11.7)	0.28	20	0.13	0.10
Q6	No	0.60	220	6.27–31.39	5.0	15.25	4.98 (32.7)	1.63	96	0.39	0.29
Q7	Yes	0.07	218	9.64–30.09	3.1	17.79	3.41 (19.2)	0.65	46	0.20	0.16
Q8	No	0.46	225	13.15–43.78	3.3	28.15	4.30 (15.3)	0.66	32	0.18	0.13
Q9	No	0.36	220	11.10–29.58	2.7	17.94	3.48 (19.4)	0.68	48	0.20	0.17
QW	No	0.55	225	1.90–27.96	14.7	10.78	4.12 (38.2)	1.6	115	0.40	0.34
QSa	No	0.48	225	42.54–113.98	2.7	77.83	10.17 (13.1)	1.3	24	0.16	0.12
QSs	Yes	−0.02	225	30.14–170.33	5.7	103.8	29.11 (28.1)	8.2	81	0.30	0.24

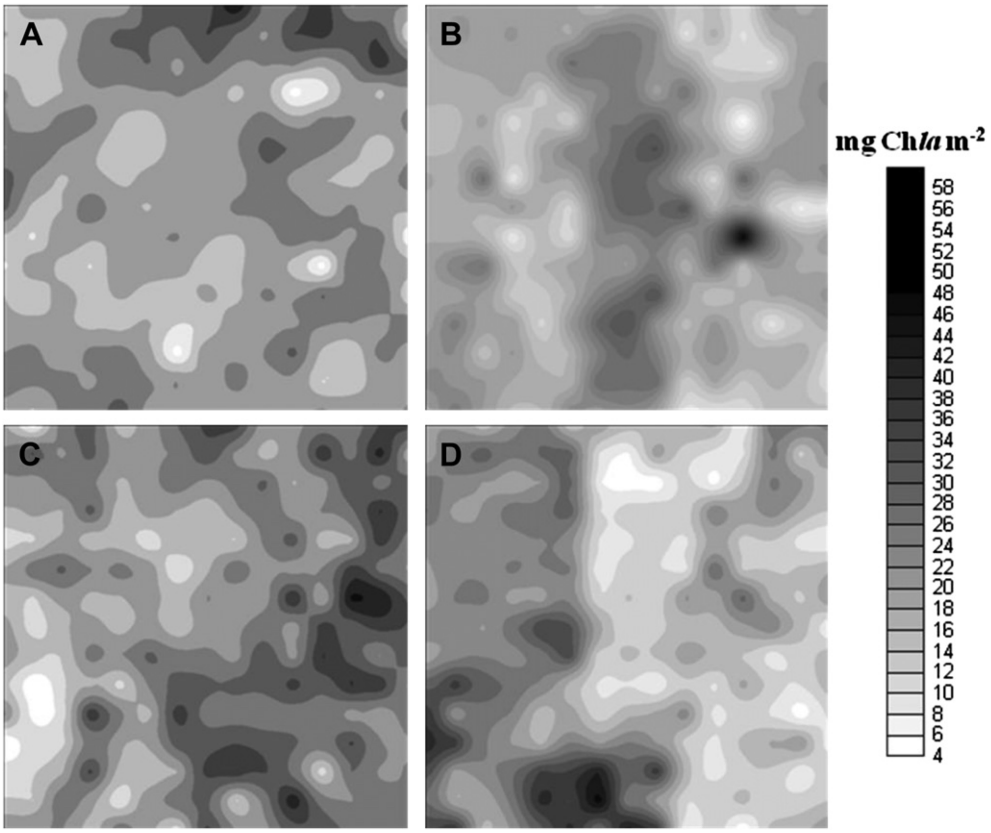

Fig. 2. Examples of the spatial heterogeneity in chlorophyll *a* concentrations (mg Chl*a*.m^{-2}) at the square meter scale for quadrats Q2 (A), Q3 (B), Q4 (C) and Q6 (D), using the kriging interpolation method.

sampling effort was also related to the presence of trends within the quadrats, i.e. the more trends that were detected, the larger the number of samples that were needed (Tables 1 and 2). More specifically, both the presence and the intensity of local gradients led to higher *n*. For instance, the quadrat Q2 did not exhibit any local gradient (Table 2, Figs. 2 and 3) and returned the lowest value of *n* ($n = 15$; Fig. 4). No trend, neither general nor local, was detected in quadrat QSa which was also characterised by a low value of *n* (Tables 1 and 2). In contrast, the quadrat Q6 was characterised by the presence of all possible gradients and trends (Table 2), with the local ones being abrupt (Figs. 2 and 3) and returned the second highest value of *n* ($n = 96$; Fig. 4). The quadrat Q3 ($n = 85$; Fig. 4) was singular, since only one local, but very strong gradient was detected (Table 2, Figs. 2 and 3), leading to a higher value of *n* than the one obtained with 2 global trends (cf. Q2). The other quadrats, including QSs, represented intermediate situations where at least one general gradient and one local trend, as shown as an example for quadrat Q4 in Figs. 2 and 3. Note that even in the quadrats where the chlorophyll *a* concentration is normally distributed, 20 to 81 samples are needed to estimate the average chlorophyll *a* concentration with a confidence of 95% (Table 1).

The error *d* (Eq. (1)) in the estimation of the average chlorophyll *a* concentration resulting from a limited sampling effort ranged from 12 to 40% with 3 replicates and from 9 to 34% with 5 replicates, when estimated using data from quadrats Q2 and QW which represented the two most extreme situations in sandy sediments (Table 1, Fig. 4). In muddy sediments, *d* range from 16 to 30% and from 12 to 24% with 3 and 5 replicates, respectively (QSa and QSs; Table 1). On average, considering all the sampled square metres, the mean error was 24% with $n = 3$ and 19% with $n = 5$ (Table 1).

4. Discussion

In most intertidal benthic studies, the estimation of chlorophyll *a* concentrations for habitat description purposes is typically based on 3 to 5 replicates (e.g. see reviews in Seuront and Spilmont, 2002 and Underwood, 2010). This classical sampling strategy is, however, intrinsically based on the hypothesis of a homogeneous distribution of chlorophyll *a* at microscales. The present work explicitly shows that limited replication can lead to errors in the estimate of

Table 2
Results of the global gradients and local trends detection both in the vertical (X-axis) and horizontal (Y-axis) directions, for each of the nine 1 m^2 quadrats sampled on the Gold Coast (Q1–Q9) and for data acquired at Wimereux (QW; Seuront and Spilmont, 2002) and in the Bay of Somme (QSa and QSs; Seuront and Leterme, 2006; Seuront, 2010). The global gradient detection returned “yes” in the table for a significant decrease or increase (Pearson correlation test, $\alpha = 0.05$) of the chlorophyll *a* concentration along the axis. The local trend detection returned “yes” when slopes changes were seen in the summed cumulated function analysis.

Quadrat	General X-axis	General Y-axis	Local X-axis	Local Y-axis
Q1	Yes	No	Yes	Yes
Q2	Yes	Yes	No	No
Q3	No	No	Yes	No
Q4	Yes	No	Yes	Yes
Q5	Yes	No	Yes	No
Q6	Yes	Yes	Yes	Yes
Q7	Yes	Yes	Yes	No
Q8	Yes	No	No	Yes
Q9	Yes	No	Yes	No
QW	No	Yes	Yes	Yes
QSa	No	No	No	No
QSs	No	No	Yes	Yes

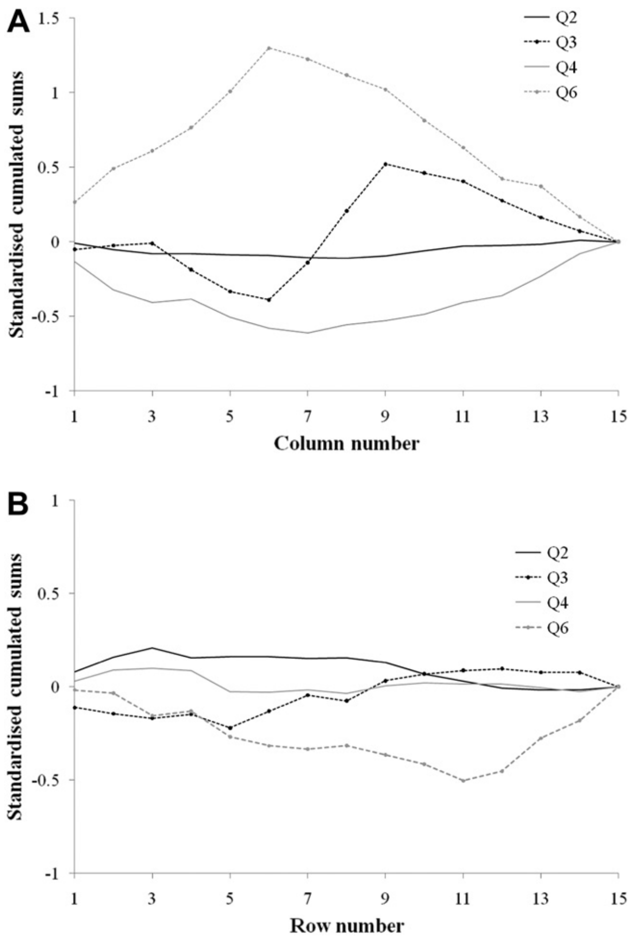

Fig. 3. Plots of standardized cumulated sums, calculated for the horizontal (A) and vertical (B) axes of quadrats Q2 (dark line), Q3 (dark circles and dashed line), Q4 (grey line) and Q6 (grey circles and dashed line), used for the detection of local trends.

mean chlorophyll *a* concentration as high as 40% ($n = 3$) and 34% ($n = 5$) in sandy sediments, and 30% ($n = 3$) and 24% ($n = 5$) in estuarine sandy muds. Thus, most of the results published in the literature (which are mostly related to muddy sediments) are potentially flawed because of chronic under-sampling. This could be particularly problematic when chlorophyll *a* concentration data are used to draw conclusions about microphytobenthos standing stocks and the functioning of intertidal sediments. For example, the calculation of the assimilation number (ratio of gross community production to chlorophyll *a* biomass), used as an indicator of deposited active pelagic cells contributing to the benthic community metabolism (Migné et al., 2004; Spilmont et al., 2005; Denis and Desreumaux, 2009), could be uncertain when based on a limited number of sediment samples and should thus not be used alone, but only with complementary microscopic cell observations (Spilmont et al., 2005, 2009b). Our results also generalise at the microscale previous observations conducted at larger scales concluding that the characterisation of a habitat based on few replicates in a limited area is biased, since it erroneously depends on an underlying hypothesis of homogeneity (Chapman et al., 2010).

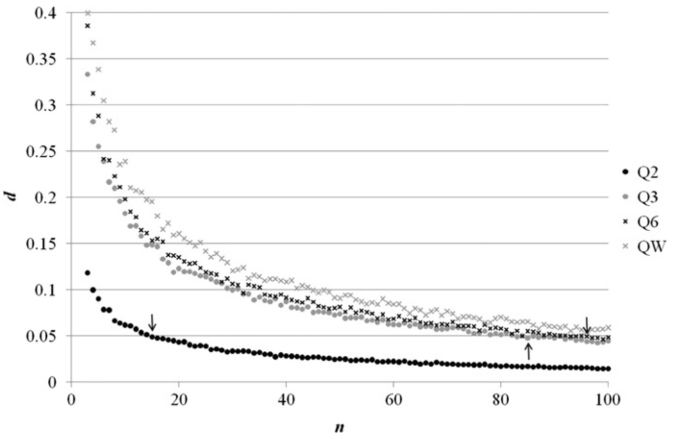

Fig. 4. Distance *d* between the bootstrap-generated mean chlorophyll *a* concentrations calculated after extracting 1000 replicates of *n* samples (with $3 \leq n \leq 100$) and the actual average concentration, calculated for all the samples collected in the same quadrat for quadrats Q2 (dark circles), Q3 (grey circles) and Q6 (dark crosses) and QW (grey crosses). The arrows show *n* for $d < 0.05$, i.e. the number of samples needed to estimate the average concentration with a confidence of 95%.

Studies of temporal patterns of microphytobenthos biomass, based on only a few replicates per time are also common, and for the above-mentioned reasons, should be carefully interpreted. Indeed, the calculated error in biomass estimations found in this study and the ratio between minimal and maximal biomasses r_b (Table 1; see also Seuront and Spilmont (2002) for a review) are of the same order of magnitude as the recorded biomass variation in a range of temporal and mesoscale spatial studies (e.g. Guarini et al., 1998; Montani et al., 2003; Spilmont et al., 2006; Brito et al., 2009). As a consequence, the reported variation in chlorophyll *a* concentrations at seasonal and annual scales may be, at least partially, much more related to the limited number of replicates collected per sampling event than to actual natural variations. In this context, the use of PAM fluorometry or field spectrometry, which appear more amenable to studies requiring a high degree of replication, constitutes a real improvement and a necessary step forward. However, both methods still suffer from severe drawbacks that limit their use in the field. Indeed, the use of PAM fluorometry to estimate surface biomass is based on the measurement of F_o (minimum fluorescence yield) that requires dark adaptation or low light treatment of the samples (e.g. Jesus et al., 2006b), which is not accomplished easily in the field and do not allow measurements on large surface areas. Furthermore, fluorescence measurements are not free from artefacts, both of migratory and physiological origin (e.g. Honeywill et al., 2002; Jesus et al., 2006a). Spectral reflectance measurements require optimal meteorological conditions, since the reflectance signal is obviously very sensitive to irradiance change and data acquisition should be performed under clear sky around solar noon (e.g. Forster and Jesus, 2006). Despite this, regarding spectral reflectance, there is the possibility to cover wide areas. Finally, these methods should always be used together with sediment sampling as reference and calibration to allow for the reliable conversion of the PAM/reflectance measurements into chlorophyll *a* concentrations (Carrère et al., 2004).

Since benthic primary production locally correlates with microphytobenthos biomass (e.g. Migné et al., 2004), it is likely that, at the microscale, microphytobenthic primary production and, as a consequence, associated rates of dissolved nutrients vary as greatly as chlorophyll *a* concentrations. The microscale heterogeneity addressed in the present work is therefore particularly relevant to methods used to assess primary production and related solute fluxes, such as microelectrodes. The sediment area investigated by microelectrodes is small, 0.1–0.2 cm^2 (Rabouille et al., 2003), and replication in studies using microelectrodes is typically poor (i.e. $n = 3$ to 12: *e.g.* Gebersdorf et al., 2005; Denis and Desreumaux, 2009; First and Hollibaugh, 2010). Therefore, although this method is particularly useful for the determination of oxygen penetration or maximal production depths and for high frequency monitoring (Rabouille et al., 2003), the related production estimates are likely to be strongly biased, as they implicitly ignore the heterogeneity of the environment. In addition, a direct consequence of the present observations is that data extrapolation may be erroneous, though still currently in use (e.g. Meyer et al., 2008; Denis and Desreumaux, 2009; Hochard et al., 2010), unless the number of electrodes deployed is very high, as already stressed more than a decade ago (MacIntyre et al., 1996). Other techniques that spatially integrate wide areas should hence be preferred, such as benthic chambers (Migné et al., 2002).

We show using an intensive sampling strategy based on 2670 samples that the number of sediment samples needed to accurately estimate average microphytobenthos biomass ranged from 15 to 115, which is 3- to 10-fold greater than the usual sample size typically reported in the literature. Only Grinham et al. (2007) have previously tried to estimate a minimum replication number for microphytobenthos biomass estimates and they concluded that 8 cores were sufficient. However, their results are based on the analysis on 30 samples randomly taken within a single 6 by 3 m quadrat. Whilst this apparent discrepancy can only be resolved by further studies covering a wider range of environmental conditions, it may be related to the observed increase in spatial heterogeneity in microphytobenthos biomass distributions with increased sampling resolution (Seuront and Spilmont, 2002). In our study, the quadrats that required the higher number of replicates to attain an accurate biomass estimate were those characterised by over-dispersion and sharp gradients. These patterns of dispersion cannot be observed prior to sampling and therefore applying the precautionary principle, the worst case scenario should be considered, i.e. 115 samples should be collected. However, this would be very time-consuming and beyond the scope of most studies using direct measures of chlorophyll *a* concentration as a descriptor. A possible alternative would be to collect larger cores; sediment samples for pigment analysis are traditionally collected using syringe coring (Joint et al., 1982) which lead to core diameters ranging from 13 to 29 mm (Grinham et al., 2007). If the number of samples determined in the present study is converted into a surface area, 115 samples would lead to 218.5 cm^2, i.e. between 34 and 165 samples (with 29 and 13 mm inner diameter cores, respectively). The use of larger cores thus decreases the sampling effort as obviously as would the acceptance of lower accuracy and confidence levels, although we do not advise the latter option. The trade-off between the core diameter and the number of samples mainly depends on how many samples can be realistically collected and analysed in a given environment and scientific framework. From the previous development, it is however unambiguous that, even with the largest traditional cores, the sampling effort has to be much more intensive than the typical 3 to 5.

We therefore emphasise that, in order to routinely obtain reliable assessments of microphytobenthos biomass, a combined sampling strategy of highly replicated field spectrometry or PAM fluorometry measurements and, for calibration and validation of these measurements, traditional sediment sampling for a direct determination of chlorophyll *a* concentrations is required. However, even these traditional methods are still debated, especially regarding the sampling depth. In the present study, the 1 cm depth was chosen for several reasons: (i) it is well known that the bulk of the active biomass is located in the top centimetre of sandy sediments (e.g. Underwood, 2010), (ii) it is a convenient depth for a quick and repeatable sampling when numerous cores have to be collected, (iii) it avoided any biomass change due to vertical migrations in the uppermost millemetres during the sampling period (although they are not expected in sandy sediments) and (iii) our results are comparable with those in the literature which often refer to samples collected down to 10 mm (Seuront and Spilmont, 2002). Thus, the Chl*a* concentrations presented here do not correspond to the productive biomass that is located in the sediment's photic zone and which determination requires alternative techniques such as contact coring (Ford and Honeywill, 2002) or cryolanding (Wiltshire et al., 1997), which are unfortunately not compatible with an intensive sampling. It is also emphasised that informations on the actual productive biomass could have been obtained by the determination of degradation products (i.e. phaeopigments). However, this implies an acidification step that has many disadvantages and should be applied with care (Jeffrey et al., 1997) which is not compatible with processing ca. 2000 samples in a time period compatible with good preservation. However, the same sampling procedure with phaeopigment analysis would indicate whether the active biomass has the same properties as the biomass investigated here, hence this would allow us to draw conclusions on the potential direct implications on production rates.

5. Conclusions

Microphytobenthos distributions exhibit a high degree of spatial variability. Whilst this phenomenon has long been recognised (MacIntyre et al., 1996), it still is not routinely taken into account in the design of sampling strategies for the study of the intertidal and shallow water soft sediments, where microphytobenthos proliferates. This variability can only be assessed with adequate sampling design that would require a considerable number of samples to be taken for conventional analyses of chlorophyll *a*a concentrations or, more conveniently, by utilising the complementary information that can be obtained using field spectrometry or PAM fluorometry. Both techniques, however, need to follow an appropriate and standardised protocol that still needs to be clearly established. Our study focused on intertidal sediments. However, similar ranges of microscale heterogeneity in microphytobenthos biomass can be expected to occur in other soft sediments environments, as has already been observed in shallow subtidal areas (Ní Longphuirt et al., 2007).

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