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Spatial variation of chlorophyll on estuarine mudflats determined by field-based remote sensing

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ABSTRACT: We present an approach to field-based remote sensing of the spatial variation of chlorophyll (as an index of microphytobenthic biomass) in intertidal soft sediments using digital colour-infrared imagery. Variation in amounts of chlorophyll *a* was quantified from small (cm) to large (100 m) scales within a single tidal cycle, using levels of replication and resolution that would be difficult to achieve using conventional sampling strategies. Eight scales of interest were selected: 100, 50, 2 and 1 m, and 40, 8, 4 and <2 cm. These scales have been associated with variability in chlorophyll in previous studies. The fully hierarchical design allowed spatial variance to be estimated independently at each chosen spatial scale. Data were collected along 2 replicate transects at 2 locations within each of 2 different estuaries. A strong linear relationship was found between measured chlorophyll and image estimates of chlorophyll. Replicate transects in 2 of the 4 locations showed similar patterns of variability across spatial scales. Relatively large amounts of variability were found at the smallest spatial scales (4 and <2 cm) and at the 40 cm scale, but the 8 cm scale was an unimportant source of variation. At some scales, transects gave similar results, providing generality to the conclusions reached. At other scales, transects gave quite different results, drawing attention to problems of inferring ecological processes when there are only single sets of data. Large differences in variability were also found from location to location. These patterns suggest that spatial variability is governed by complex interactions between fauna, microflora and sediment.

KEY WORDS: Chlorophyll · Intertidal mudflat · Remote sensing · Spatial scale · Variation · Microphytobenthos

INTRODUCTION

Microphytobenthos (MPB) are important in the ecology of intertidal soft sedimentary habitats (Pinckney & Zingmark 1993, MacIntyre et al. 1996, Decho 2000). They are food for some species of fish (Yang et al. 2003), some species of birds (Meininger & Snoek 1992), benthic macrofauna (Herman et al. 2000) and a large variety of meiofauna, including copepods (Decho 1988), and nematodes (Riera et al. 1996). MPB play an important role in biochemistry of sediments by regulating the exchange of nutrients with the water column (Rizzo et al. 1992) and stabilizing the sediment surface — for example, by the release of exopolymers (Underwood & Paterson 1993). Unlike subtidal MPB (Cahoon et al. 1993), intertidal MPB are, in some areas, more productive than phytoplankton (Cadee & Hegeman 1974). The distribution of MPB is, however, extremely patchy over space (Admiraal 1984, Saburova et al. 1995, Seuront & Spilmont 2002) and time (MacIntyre & Cullen 1995, Serodio et al. 1997). Variability in distribution is governed by many diverse biotic and abiotic processes, including diel rhythms of vertical migration (Consalvey et al. 2004), hydrodynamic factors (Safi 2003), size of sediment grains (Colijn & Dijkema 1981), salinity and nutrient gradients (Underwood et al. 1998), small-scale variations in topography (Plante et al. 1986), and shading (Stutes et al. 2006).

Description of patterns is a prerequisite to developing explanatory models and testable hypotheses about ecological processes (Underwood et al. 2000). Where important variation has been identified at some spatial

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scale, conceptual models can be developed about the processes causing variation. Knowledge of patterns of abundances of organisms across a range of spatial scales is therefore essential to understanding ecological processes in intertidal habitats and for targeting sampling effort (Legendre & Fortin 1989).

Non-random distribution or spatial patchiness is a well-recognised property of assemblages in intertidal soft sedimentary habitats (Fleeger & Decho 1987, Sun & Fleeger 1991, Azovsky et al. 2000). Small-scale patchiness in distribution of MPB has often been reported in the literature (e.g. Faure-Fremiet 1951, MacIntyre et al. 1996). Surveys of the spatial distributions of MPB among estuaries have shown that their small-scale distributions are also very variable from one location to another (Guarini et al. 1998, Defew et al. 2002), i.e. small-scale patterns differ at larger scales. The few studies that have investigated explicitly the range of spatial scales over which MPB (as indicated by chlorophyll or other pigments) vary (Table 1), have provided valuable information about variability in patterns in intertidal and other marine habitats.

Small-scale (cm) patchiness of MPB has been analysed using variograms (Jesus et al. 2005), but most studies have used spatial autocorrelation (e.g. Decho & Fleeger 1988, Blanchard 1990, Sandulli & Pinckney 1999). Spatial autocorrelation is calculated as a function of distance (lag), usually between regularly spaced points (Cliff & Ord 1973). Lag distances at which there is negative or no correlation correspond to the average radii of different-sized patches (Sokal & Oden 1978). It is, however, difficult to use spatial autocorrelation where spatial variation is to be investigated from small (cm) to large (100s m) spatial scales because of the large number of samples required (Underwood & Chapman 1996). Spatial distribution has also been investigated in terms of 'patch size' by quantifying the degree of aggregation or homogeneity in samples of sediment (Saburova et al. 1995, Azovsky et al. 2000). None of the above approaches provides the information necessary to determine the amount of variation contributed independently by each spatial scale, unconfounded by variation at other scales.

Although several studies have investigated small-scale (from cm to 10s cm) variability in MPB (e.g. Plante et al. 1986, Azovsky et al. 2000), it is difficult to compare studies because they used samples of different sizes (1 cm^2 to 10 cm^2), collected samples from plots of different sizes, and used distances between replicates that either were different or cannot be ascertained. The size of core used to sample the sediment sets the lowest limit of resolution of sampling and integrates variability over the core. In addition, many data used in analyses of scale have been collected on different days and, in some cases, in different years, so they are temporally confounded (see 'Discussion' in Tolhurst & Chapman 2005). Such data indicate little about patterns that may emerge during a single tidal cycle. Other studies have tended to focus upon variability at larger (10s m to km) scales (e.g. Light & Beardall 1998, Safi 2003).

Many studies used destructive sampling of the mud surface for measures of MPB (but see Jesus et al. 2005, Chapman & Tolhurst 2007). The extraction, identification and counting of MPB cells from sediment are rarely done, because they are technically difficult, time-consuming and costly (but see Nilsson et al. 1991). Instead, amounts of chlorophyll *a* (chl *a*) and other pigments are widely used as a surrogate of biomass of MPB. However, collection of these biochemical data also requires destructive sampling and costly, time-consuming laboratory techniques, thereby limiting the amount of replication that is feasible. Sampling in intertidal environments is limited to low tide and extensive sampling by large numbers of people can disrupt the surface of the sediment. Large changes in the amount of MPB at the surface can occur within a single tidal cycle due to vertical migration (Consalvey et al. 2004) and de-watering (Perkins et al. 2003), making it difficult to make measurements of changes in space which are independent of changes over time.

To investigate spatial distributions of MPB in intertidal soft sediments, it is therefore preferable to obtain observations over the shortest possible interval of time. Remote sensing from field-based platforms has been used to measure surface chlorophyll at great spatial resolution (Murphy et al. 2004, 2006). Remote sensing from satellites and aircraft is increasingly being used to gather information from soft-sedimentary intertidal areas in a snapshot of time (e.g. Deronde et al. 2006). Large amounts of data can be acquired within a short interval of time, thus minimizing effects due to temporal changes. Because these data are contiguous, spatial variance can be estimated at any chosen hierarchy of scales. The term 'scale' is often used in ecology without proper definition (Denny et al. 2004). Scales, in the context used here, are defined as the distances over which variability in amounts of MPB is estimated.

This paper quantifies variability of chlorophyll (as a surrogate measure of biomass of MPB) in intertidal soft sediments at a hierarchy of spatial scales, using a digital colour-infrared (CIR) camera (Murphy et al. 2004). Variation in chl *a* was measured over a range of small (cm) to large (100 m) scales, within a single tidal cycle, using levels of replication and resolution that would be difficult or impossible to achieve using conventional sampling strategies.

Eight scales of interest were selected, based on previously published data on variations in MPB, sediments or benthos (Table 1): 100, 50, 2, 1 m, and 40 cm,

Table 1. Summary of studies that have explicitly examined spatial variation in MPB in soft sediments as a function of scale. FI = Fisher's index of dispersion; G = Geary's C; M = Moran's I, na.: not applicable

Habitat	Spatial scales examined	Size of individual plot	Number of replicates	Size of sample (diameter or area)	Method	Source
Subtidal sand	3 cm to 1 km	Contiguous samples: 3 m transect	Unknown	2.6 cm	Spectral analysis	Plante et al. (1986)
		Sampling grid: 2, 3 or 5 m square (15–25 sampling points)	6 per gridpoint	4.4 cm	ANOVA	
Intertidal mud	cm	na	na	0.9 cm	Spatial autocorrelation (G,M,FI)	Decho & Fleeger (1988)
Subtidal	10 cm to 10 m	Unknown	10	5 cm^2	ANOVA	Delgado (1989)
Oyster pond	cm	na	na	0.9 cm	Spatial autocorrelation (G,M)	Blanchard (1990)
Intertidal sand	Micro-, meso-, macroscale	Numerous	Numerous different samples	1, 3, 4, 5, 6.25 9, 10 cm^2	Cassie spatial distribution index	Saburova et al. (1995)
Subtidal	10 m–10s km	2 m^2	4	22.5 mm	ANOVA	Light & Beardall (1998)
Intertidal sand	cm	na	na	1.55 cm	Spatial autocorrelation (G,M)	Sandulli & Pinckney (1999)
Intertidal silt-sand	25 cm to 5.5 km	1 m^2	8 per plot	10 cm^2	Spatial homogeneity index	Azovsky et al. (2000)
Intertidal sand	cm	1 m^2	na	1.9 cm^2	Fractal statistics	Seuront & Spilmont (2002)
Shallow water	10s of m to 100s of km	Unknown	3	2 cm	Various multivariate analyses	Safi (2003)
Intertidal sand-mud	cm	25 cm^2	na	0.4 cm	Variogram analysis	Jesus et al. (2005)

[a]Micro: 10 to 1000 cm^2; meso: 1000 cm^2 to 100 m^2; macro: 100 to 10 000 m^2

8, 4 and <2 cm. Small (cm) spatial scales were selected because several studies have shown that MPB are variable at these scales (Sandulli & Pinckney 1999, Jesus et al. 2005). Spatial patchiness of MPB at cm scales has been correlated with distributions of meiofauna (Decho & Fleeger 1988, Blanchard 1990) and microtopography of sediment (Plante et al. 1986). Although variation is theoretically expected to increase with spatial scale (Brown 1984, Palmer 1992), many studies have shown that most variation in marine invertebrates occurs at very small spatial scales (Morrisey et al. 1992, Underwood & Chapman 1996). Variation at larger spatial scales (10s to 100s of m) may be influenced by physical factors such as gradients of nutrients (Underwood et al. 1998) and differences in types of sediment (Cadee & Hegeman 1977). The 50 m scale was included in our analysis because it has been shown to be an important source of variability for subtidal MPB (Light & Beardall 1998) and chlorophyll has been shown to vary between sites ~50 m apart in different intertidal habitats in Sydney Harbour (Chapman & Tolhurst 2007, Tolhurst & Chapman 2007). Processes that influence scales of variation in MPB are likely to differ among different locations, or along different transects in a single location. Our hypothesis is that patterns of variability across scales would be similar within locations, but different among locations.

To test the hypothesis that variability across scales would show similar patterns within locations, data were collected along replicate transects in each location sampled. To evaluate relative patterns of scales of variation in different places, data were collected from 2 locations within each of 2 different estuaries (10s of km apart). The scales at which patterns of variation are general may indicate that widespread processes are contributing to those patterns, whereas scales which differ between replicate transects or locations indicate where more local processes are important.

MATERIALS AND METHODS

Study areas. Two intertidal mudflats were selected in the upper reaches of Sydney Harbour, Brays Bay (BB) and Fig Tree Bridge (FTB), and 2 in the Georges River, the east and west sides of Kogarah Bay (KBE and KBW, respectively), south of Sydney (Fig. 1). The locations were selected because they were accessible, had similar tidal ranges and the sediments appeared similar. Sampling during a pilot study to test methods and calibration was done at KBE and FTB in October 2005. At this time, most images were acquired under variable amounts of cloud-cover. All locations were sampled on 4 consecutive days, starting 24 September 2007 (Austral springtime). These images were collected in direct sunlight.

The benthic algal assemblages in all locations have been observed to be periodically dominated by green, filamentous macro-algae, which were intimately distributed amongst the sediment grains in the upper few mm of sediment. Diatoms, euglenids and other unicellular algae are generally present, but these do not form the dense mats observed in some European estuaries. At the time of sampling, there were, in fact, no visible filamentous algae on the surface of the sediment at 3 of the 4 locations (FTB, KBE, KBW).

Fig. 1. Field locations sampled for this study in New South Wales (NSW), Australia: 1 = Fig Tree Bridge; 2 = Brays Bay; 3 = Kogarah Bay East; 4 = Kogarah Bay West

Sampling methods. ***Colour-infrared imagery*:** A charge-coupled device digital colour-infrared (CIR) camera (Geospatial Systems) was used to acquire separate images at green (525 to 575 nm), red (645 to 689 nm) and near-infrared (758 to 833 nm) wavelengths; images were digitized to 8-bits per band (i.e. each image pixel in each band has a dynamic range of 0 to 255). The camera was fitted with a Sigma 14 mm f/2.8 ES/HMS super-wide-angled lens and mounted on a black metal stand 1.5 m above and normal to the surface of the mud. For calibration, a 15%-reflective calibration panel with near-Lambertian reflectance characteristics (Spectralon®) was placed in one corner of the image. The integration (exposure) time was optimized to give the greatest range of image values for the prevailing light, without saturating pixels over the brightest areas of the images.

Each CIR image was 1392×1039 pixels in size. Using the above configuration, the spatial resolution (i.e. the area of ground imaged by a single pixel) was 0.47×0.47 mm and the area of ground covered by each image was 65×48 cm.

The spatial scales of interest were classified into 2 groups: 'within-image' scales (i.e. smaller than the image dimensions; <2, 4, 8, and 40 cm) and 'between-image' scales (1, 2, 50 and 100 m). Data for the pilot study were acquired at one location from Kogarah Bay (KBE) and one location at FTB.

Replicate images, separated by a distance of 1 m, were acquired along a transect of sediment, parallel to and about 3 m away from low water, at distances of 0, 2, 50, 52, 100, 102, 150 and 152 m along the transect. For the pilot study, a single transect was imaged at each location, but for the main study, 2 replicate transects were imaged at each location (i.e. 32 images per location). Replicate transects ran parallel to each other and their starting points offset by 15 m.

Although the CIR camera had previously been calibrated against laboratory measures of chlorophyll (Murphy et al. 2004), samples of sediment were collected during the pilot study, using a small contact corer (Honeywill et al. 2002). This was done because the calibration panel used by Murphy et al. (2004) was a 17%-reflective Kodak grey card, while in this study, a superior reflectance standard was used (15%-reflective Spectralon). This may have changed the

relationship between measured chlorophyll and estimates derived from the image, requiring a new calibration.

Sediment: The amount of chlorophyll in sediment samples was compared with estimates from images. Between 2 and 4 samples were collected from within the field of view of some of the camera images (10 images from KBE and 8 from FTB).

A stainless-steel contact corer (48.8 mm diameter, 2 mm deep) was placed randomly on the surface of the sediment. Liquid nitrogen was poured into the corer. After about 30 s, the frozen mud was lifted from its position and pared level with the base of the corer to ensure a uniform depth (2 mm) over the entire core. A numbered paper disk, the same size as the internal diameter of the core, was then placed inside of the resulting hole in the sediment. The core was stored in liquid nitrogen for transport to the laboratory. A second 'reference' CIR image was then taken to record the locations from where the samples were taken.

The sediment in each core was freeze-dried, homogenised and a known amount (about 0.2 g) was sub-sampled. Chlorophyll was extracted from the sample using dimethyl formamide (DMF). The amount of chlorophyll was calculated spectrophotometrically, using the equation of Porra et al. (1989) and expressed per unit area of sediment (µg cm^{-2}; Murphy et al. 2005b).

Estimates of chlorophyll. ***CIR imagery:*** CIR images were acquired under a range of solar illumination conditions and camera integration times. To enable comparison of data across images, it was necessary to calibrate the data to relative reflectance, by standardizing the brightness of the pixels in each image to those over the calibration panel. Image values (digital number, DN) over the calibration panel were extracted and averaged. Relative reflectance (ρ) for each camera band (λ) was calculated using:

$$\rho_\lambda \text{image} = \frac{DN_\lambda \text{image}\ \delta_\lambda \text{panel}}{DN_\lambda \text{panel}} \quad (1)$$

Where ρ_λ image = relative reflectance of image at band λ, DN_λ image = DN of individual pixels in image at band λ, δ_λ panel = reflectance factor of the calibration panel for the wavelength range of band λ and DN_λ panel = average DN of pixels over the calibration panel for band λ.

The amount of chlorophyll in each pixel from each image was calculated by dividing the reflectance in the infrared (IR) band (where chlorophyll does not absorb) by reflectance in the red (where chlorophyll is highly absorptive; see Murphy et al. 2005a). The resulting IR:red ratio image had pixel values which were proportional to the amount of chlorophyll as an indicator of algal biomass (Murphy et al. 2004).

Extraction of IR:red ratio data from image: The IR:red ratio provided an accurate index of the amount of chlorophyll on the surface of the mud, although accuracy was significantly reduced in areas of the image where there was standing water, deep shade (caused by deep pits or animal burrows) and where specular reflectance (sun glint) was dominant. Areas with deep pits and areas with standing water were identified in the original (unprocessed) CIR images using interactive image analysis. IR:red ratio data were not extracted from these areas.

Two sets of data were extracted from each of the IR:red ratio images: (1) Calibration of estimates. To establish a calibration, spectrophotometric laboratory estimates of chlorophyll were matched with areas in the image from which they were sampled. The IR:red ratio values from pixels located within each area sampled for chlorophyll were averaged. Image and laboratory estimates of chlorophyll were compared using linear regression of measured chlorophyll on the average IR:red ratio. (2) Measures of spatial scales. Typically, the surface area of mud sampled for chlorophyll is about 2 to 5 cm^2 (e.g. Delgado 1989, Seuront & Spilmont 2002). Each pixel value in the IR:red ratio image represents the amount of chlorophyll in a much smaller area of mud, 0.47 × 0.47 mm. It was decided to extract areas in the image that were large enough to be relevant to benthic grazers, but smaller than those typically sampled by corers. A 10 × 10 pixel area (i.e. an area representing ~22 mm^2 of the surface) was selected as the size of individual replicates. Two replicate measures were taken in an area measuring 2 × 2 cm and separated by the distances specified by the within-image scales (4, 8 and 40 cm) in a fully nested design, using a template overlaid on the image. Thus, there were 2 replicates (each 10 × 10 pixels) in a 2 × 2 cm square, with 2 such squares with centres 4 cm apart. There were 2 of these separated by 8 cm and 2 sets of these scales with centres 40 cm apart (i.e. a total of 16 replicated areas sampled per image).

The template was then moved to another location in the image and the process repeated. Using images acquired during the pilot study from KBE, 10 different sets of replicates were sampled for all scales within each image. Analyses of these data indicated that 5 independent sets of data were adequate for analysis of the data for the main study.

RESULTS

Calibration of estimates

The amount of chlorophyll in samples acquired during the pilot study from each site was relatively small (mean ± SE, KBE: 3.31 ± 0.31 µg cm^{-2}; FTB: 3.11 ±

0.42 µg cm^{-2}). Despite the small range of values, there was a strong linear relationship between chlorophyll (µg cm^{-2}) and IR:red ratio (Fig. 2). There was no evidence of errors in calibration, which would be identified as points from individual images being offset by the same amount on the horizontal axis. Ineffective calibration of an image would cause samples from the same image to have different reflectances and, therefore different ratio values, relative to other images.

Scales of variation

A large number of independent sets of hierarchical data could be taken from an image. It was not known how well sampling at small scales within an image would represent the whole image. To test the hypothesis that different sets would show similar patterns in amounts of chlorophyll at increasing spatial scales (from <2 to 40 cm) within an image, 10 independent, randomly chosen sets were initially taken from each of 5 randomly selected images from the pilot study at KBE. Each image was analysed separately using ANOVA, using these 10 sets of data as 1 factor, orthogonal to the hierarchy of nested spatial scales (40, 8, 4 cm, with replicates <2 cm apart). In each image, there were significant interactions between the sets of data and more than one spatial scale, indicating that different extractions showed different patterns of variation in chlorophyll within the same image. Tests with different numbers of sets indicated that 5 sets adequately represented variability within a single image (as did 10 sets), while not becoming too time-consuming to extract. Thus, 5 sets were used for each image in the following analyses of the main study.

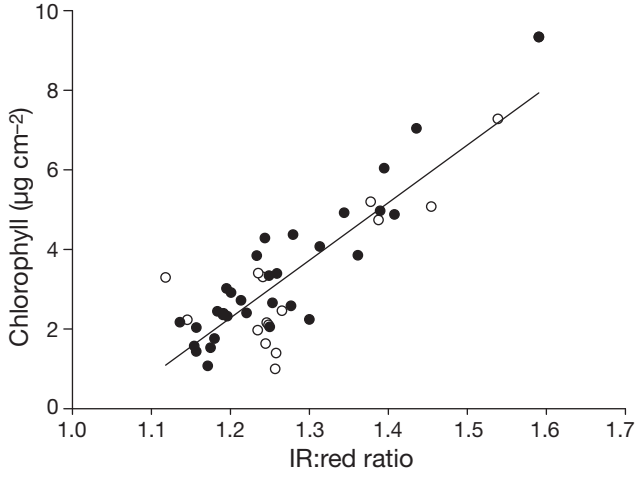

Fig. 2. Relationship between IR:red ratio and chlorophyll (µg cm^{-2}). Data from pilot study: Kogarah (●), r = 0.78; Fig Tree Bridge (○) r = 0.91; Combined r = 0.86; SE = 0.88; $p < 0.001$; n = 48

We analysed the data from the main study for all spatial scales (from <2 cm to 100 m) in a fully nested design for each of the 5 sets of data from each of the 2 transects in each of the 4 locations (FTB, BB, KBE, KBW). The components of variation were then extracted from the MS estimates in each analysis using the method described in Underwood (1997) to provide independent measures of the amount of variability attributable to each spatial scale. Components of variation were then averaged over the 5 sets of data. If this average was negative for a particular scale, it was set to zero in all sets of data in that analysis and the other components recalculated as per Fletcher & Underwood (2002).

Components of variation indicate the amount of variability contributed, independently, by each spatial scale. The component of variation at a particular scale is the amount of variation uniquely contributed by that scale over and above cumulative variation contributed by all smaller scales in the hierarchy. There were large amounts of variability among scales within a single location and between locations (Fig. 3a–d). Our original hypothesis was that variability across scales would show similar patterns between replicate transects within a location, but would differ among locations. Relative patterns of variability across scales were similar for replicate transects at FTB and BB (Fig. 3a,b). However, large amounts of variability were found between transects at BB and KBW (Fig. 3b,d). Differences in amounts of variability between transects at KBE were similar to those at FTB (cf. Fig. 3a,c; note that transects at KBE appear more dissimilar than do transects at FTB, simply because the vertical scale is different). At all locations, the smallest spatial scales (<2 cm and 4 cm) showed relatively large amounts of variation. The greatest amounts of variation across scales were found at BB and the smallest at KBE; these locations also had, respectively, the greatest and smallest average amounts of chlorophyll. KBW showed the greatest differences in variability between transects. At all locations, the scale of 40 cm was a major source of variability in at least 1 transect, but relatively small amounts of variation were contributed by the 8 cm scale.

To illustrate the amounts of chlorophyll being measured at each scale, the minimal and maximal values of mean amounts of chlorophyll (to indicate the range of values) are shown for 1 transect in each location (Table 2). Although BB had per-transect average amounts of chlorophyll similar to those of FTB (cf. Fig. 3a,b), the per-scale range over which chlorophyll varied was much larger for BB than FTB. KBE had the smallest range of chlorophyll of any location and also the least amount of variation at most spatial scales.

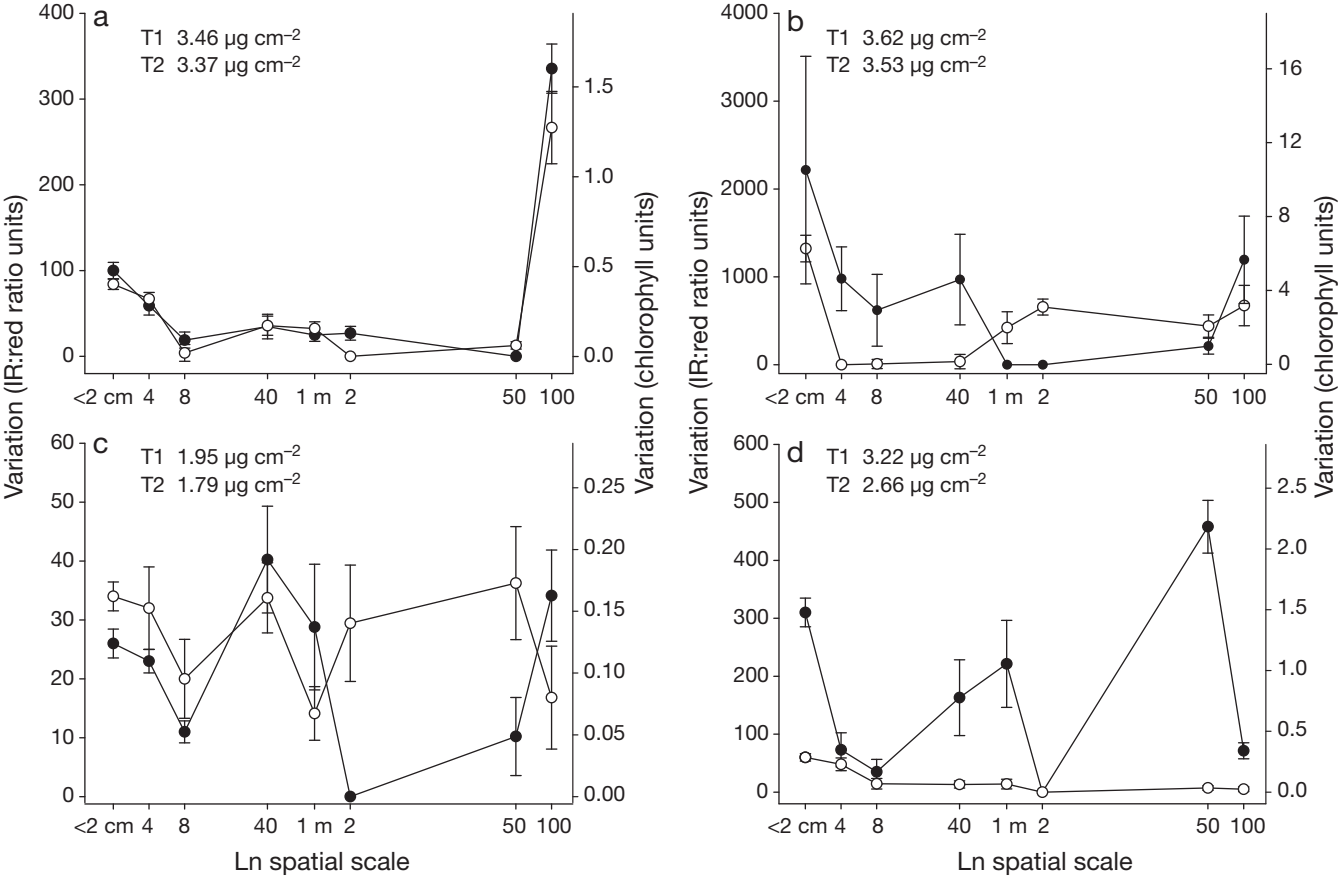

Fig. 3. Average components of variation (±SE) at each spatial scale for 5 independent sets of data extracted from the IR:red ratio images: Transect 1 (●); Transect 2 (○). Average amounts of chlorophyll for each transect derived from the IR:red ratios are shown at the top of the graph (n = 1280). (a) Fig Tree Bridge; (b) Brays Bay; (c) Kogarah Bay East; (d) Kogarah Bay West

Table 2. To illustrate the variability measured by components of variation (CV), minimal (Min.) and maximal (Max.) values of mean chlorophyll (µg cm^{-2}) at each scale are shown for Transect 1 in each of the 4 bays sampled: Fig Tree Bridge (FTB), Brays Bay (BB), Kogarah Bay East (KBE) and Kogarah Bay West (KBW). Minima and maxima are averaged from the 5 data sets extracted from each image. Boldface indicates components of variation at that scale have been set to zero (see 'Scales of variation' in 'Results'). Note that minimal values decrease and maximal values increase (i.e. range increases) towards smaller scales, as would be expected due to decreasing sample sizes and precision at the smaller scales

	FTB			BB			KBE			KBW		
	CV	Min.	Max.	CV	Min.	Max.	CV	Min.	Max.	CV	Min.	Max.
100 m	336	2.9	4.1	1195	2.5	4.7	34	1.5	2.0	71	2.5	2.8
50 m	**0**	2.8	4.3	212	2.4	5.7	10	1.5	2.4	485	2.4	2.9
2 m	27	2.6	4.4	**0**	2.3	5.8	**0**	1.3	2.5	**0**	2.3	2.9
1 m	25	2.6	4.5	**0**	2.2	7.7	29	1.1	2.5	221	2.2	3.1
40 cm	35	2.4	4.7	970	1.8	9.2	40	0.9	2.9	163	2.0	3.3
8 cm	19	2.0	5.0	621	1.6	10.7	11	0.8	2.9	35	2.0	3.5
4 cm	59	2.0	5.1	979	1.5	12.7	23	0.7	3.1	73	1.9	3.6

DISCUSSION

Many factors are known to influence spatial patterns of soft-bottom assemblages (Gray 1974, Ólafsson et al. 1994, Woodin et al. 1995). To unravel some of the complex processes which may influence these patterns, it is important to understand spatial scales of variability of MPB, which are a primary source of food for many species. Intertidal soft sediments are highly dynamic and surface MPB biomass and other properties of sed-

iments can change within a tidal cycle (Perkins et al. 2003, Consalvey et al. 2004). Existing techniques for quantifying MPB are expensive, costly and time-consuming, making it difficult to make measurements across space that are independent of time and vice versa. This is a particular problem in situations where MPB must be quantified over a range of spatial scales (Saburova et al. 1995), or in systems, like some European estuaries, where rapid vertical migration of diatoms causes large changes in surface biomass (Consalvey et al. 2004). Thus, any technique which improves these methods enables better understanding of spatial and temporal dynamics of MPB. The remote-sensing technique used here provides image estimates of chlorophyll (as an index of biomass of MPB) acquired over a short period of time, thus reducing problems due to changes in amounts of MPB during the tidal cycle. This allows greater replication and more scales of variation than can be achieved by conventional field sampling.

Most previous studies using conventional sampling strategies removed relatively large (> 3 cm^2) areas of mud to extract chlorophyll (e.g. Plante et al. 1986, Delgado 1989, Azovsky et al. 2000). Spatial structure of the chlorophyll within the sampled area of mud was thus homogenized into a single value. Analysis of small (≤ 1 cm) spatial scales requires precise characterization of scales being sampled. Our approach enabled precise (to within 1 mm) determination of positions and distances between sample units within images (<2 to 40 cm), essentially improving small-scale spatial analysis in such studies.

Many methods are used to measure spatial scales of variation. The limitation of hierarchical designs, such as nested ANOVAs, is that the number of degrees of freedom decreases with increasing spatial scale in the hierarchy. Hierarchical designs also require that the spatial scales of interest be defined prior to acquisition of data, especially for conventional destructive sampling. Where prior definition of relevant or realistic scales is not possible, hierarchical designs will not necessarily be effective (see Underwood & Chapman 1996, Denny et al. 2004). Using continuously sampled spatial data (e.g. for fractal analysis or spatial autocorrelation) will solve this problem, but will not allow simultaneous estimation of variability at very small and relatively large spatial scales. A major advantage of field-based remote sensing is that scales smaller than the dimensions of the image can be assembled into any hierarchy of spatial scales and the number of, and the area sampled by, replicates can be modified as required. Using replicated sets of data from the same image, we could examine in our pilot study how reliably any one set of data represented the spatial pattern in chlorophyll. With conventional destructive sampling, such analyses are generally impossible or prohibitively expensive. Estimation of chlorophyll from very small areas of sediment enabled us to quantify patchiness in the MPB at scales at which many meio- and macrofauna might be responding.

The present study has shown that amounts of variation were generally similar among some locations (e.g. scales of <2 cm to 50 m at FTB and KBE), but for other locations, large differences were found (e.g. BB had much greater variation than any other location). Several large-scale properties or processes could be influencing differences among locations, including variation in sediment characteristics (Brotas et al. 1995), hydrodynamic conditions (Colijn & Dijkema 1981, Safi 2003), tidal regime or wind causing suspension of MPB into the water-column (de Jonge 1995), salinity and nutrient gradients (Underwood et al. 1998), the intensity of urban development, and history of land-use. KBE and KBW are on opposite sides of a Bay (Fig. 1), yet exhibited large differences in spatial distributions in chlorophyll. KBE is bordered by dwellings located relatively close to each other. KBW also has dwellings, but in addition, it has areas of parkland in 2 locations. All locations are in urbanised catchments, but BB is close to an industrialised area that was occupied for many years by a large abattoir and paint factory. BB is also further away from the seaward entrance to the estuary than any other site. These factors may explain why BB is different from other locations in terms of its overall amounts of variability in chlorophyll. Any combination of the above factors may be causing variability among locations and the possible processes need to be distinguished by further sampling and experiments.

Spatial patterns between replicate transects within locations were not always similar. Variability of biogeochemical variables, including chlorophyll, has been found by previous studies between locations (20 to 40 m apart) within bays in Sydney Harbour (Chapman & Tolhurst 2007, Tolhurst & Chapman 2007). The present study showed that transects only 15 m apart exhibited differences in amounts of variability of chlorophyll from scale to scale. Although relative patterns of variability across scales were similar for replicate transects at some locations, (e.g. FTB, BB and KBE; Fig. 3a–c), large differences in variability were evident at some scales (see particularly <2 cm to 2 m scales at BB and <2 m, 1 m, and 50 m scales at KBW). Clearly, if a single transect had been used to characterize spatial variability at BB or KBW, quite different conclusions would have been reached, depending upon the position of the transect.

Different properties and processes may cause variability at different scales. At very small (cm to 10s cm) scales, grazing is often though to be a factor regulating patchiness of MPB. Some studies have shown that

meiofauna can occupy similar patch sizes and that their numbers are spatially autocorrelated with amounts of MPB (Decho & Fleeger 1988, Pinckney & Sandulli 1990). In contrast, other studies have shown that benthic grazers do not feed directly on MPB (e.g. Connolly et al. 2005). Defaecation by benthic animals (Thrush et al. 2006), burrowing by animals such as crabs (Warren & Underwood 1986) and variations associated with microtopography of the surface of the sediment (Plante et al. 1986), and indirect effects through shading may also affect distribution of MPB at small scales. Analyses showed that, as with many other taxa (Fraschetti et al. 2005), relatively large amounts of variation occur at the smallest scales; the smallest (<2 and 4 cm) scales showed the greatest variation. Therefore, patches of sediment ~22 mm^2 and less than 2 cm apart showed considerable variation in chlorophyll. These are similar scales to the patch sizes of MPB and meiofauna found by Pinckney & Sandulli (1990). Our data suggest that there are important ecological processes structuring MPB at very small spatial scales and that these may be general over larger scales. Interestingly, variation at the scale of 8 cm contributed little to the overall variation. This has implications for the sampling of chlorophyll in soft sediments, because sampling at 8 cm scales would permit more precise estimates of average amounts of chlorophyll to be made because of the small amount of variability between sampling units at this scale.

At the medium scale (1 to 2 m), variability in sediment grain size, recruitment of animals (Woodin et al. 1995), runoff by local drainage channels, and the activities of macrofauna, such as soldier crabs, are likely to influence variability. However, in the locations sampled here, these scales were relatively unimportant sources of variability. Variability at larger (50 to 100 m) spatial scales was large (compared with other scales) at FTB (100 m) and at KBW (50 m). Shoreline development, runoff from gardens and industrial outfalls, water movement, and variations in type of sediment can all cause variability at these scales. At FTB, a bridge at one end of the transect may be a source of variability at these scales by altering the local shading, thus reducing direct incident light and associated stress and improving survivorship of MPB.

A significant finding of this study is that large amounts of variability occur at the smallest scales and this is consistent with the findings of other studies (Plante et al. 1986, Sandulli & Pinckney 1999, but see Moreno & Niell 2004). Comparison of our data with other studies is difficult because data are collected in many different ways (e.g. area of mud sampled, distance between replicates, sites) using different designs. Definition of scale has not been consistent and different methods have been used to analyse data. The present study has demonstrated the utility of field-based remote sensing to gather independent data on chlorophyll across a broad range (cm to 100 m) of spatial scales. Independence of data in time and space is a fundamental requirement for testing of relevant models and hypotheses. These data focus on the need to replicate measures of patterns across many scales before reliable conclusions can be reached about patterns and sensible models proposed to explain them (Underwood & Chapman 1996). Field-based remote sensing has the potential for radical improvements in methods for understanding ecological processes in intertidal areas by providing new insights into spatial patterns of variability in MPB.

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