

## Estimating benthic primary production: scaling up from point measurements to the whole estuary

### Abstract

Good predictions of benthic primary production are needed in order to guide management plans for the estuarine environment, and to understand more about estuarine ecology. This article discusses the advantages and disadvantages of three different methods for estimating net and gross rates of microphytobenthic primary production. Calculations using different modeled profiles of microalgal chlorophyll in the sediment were applied to data from a mid-intertidal site in the Westerschelde estuary. Gross primary production was closely correlated to the daily photon dose received during emersion periods, but there were considerable differences between the three modeling approaches. A model in which chlorophyll was evenly distributed throughout the upper 2 mm of the sediment surface resulted in lower gross production than models in which vertical migration of cells to the sediment surface was simulated. Net production was shown to be highly dependent on the selected value for dark respiratory losses. Respiration was important because much of the benthic chlorophyll is not situated in the photic zone, and because periods of darkness at night and during tidal immersion were longer than the periods of light. Biomass-normalised gross primary production was estimated for a range of different high and mid-shore sites throughout the Westerschelde estuary, and was linearly related to the daily photon dose. Following this, a method is proposed for the mapping of primary production at the whole estuary scale.

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### Introduction

Estuaries and wetlands are judged to be valuable to mankind because of the high rates of useful biogeochemical activity that they support (Turner *et al.* 2000; Woodward and Wui 2001). However, in many developed countries the total area of estuarine habitat, including intertidal sediments and salt marshes are diminishing, due to land reclamation and port development. For example, in the British Isles, a national loss of mudflats of 25 % has been calculated, with some estuaries showing 80 % to 100 % losses in area (UK Biodiversity Action Plan 2002). Consequently, there may have been major detrimental changes of ecosystem function in the form of lower

capacity for nutrient cycling and food production. There is a clear need from a management point of view to be able to better quantify the biological processes occurring in estuaries, in order to guide development plans and if necessary to design replacement habitats of equal value. From a scientific standpoint, better characterization of the key ecosystem biochemical pathways – of which net primary production and denitrification could be considered the most important – is a step towards understanding the ecology and evolutionary adaptations of marine intertidal organisms. The aim of this article is to consider some existing methods for estimating primary production of intertidal sediments, and in particular to identify areas where information is lacking.

Microphytobenthic photosynthesis takes place in a thin layer called the photic zone at the sediment surface. Rates of photosynthesis are affected by many factors, varying from the position of the cells with respect to the gradient of light, the sediment surface temperature, and the availability of sufficient nutrients to build enzymes. Researchers cannot measure all of these factors accurately, and the financial limitations of many monitoring programs may mean that often none can be routinely measured!

Photosynthetic rate measurements usually involve the incubation or measurement of a small amount of sample material, selected from a low number of sites, at a limited number of times in the year. The distribution of MPB can be variable across space and time, and obtaining a representative number of samples can be difficult. Interpolation and kriging techniques are necessary to scale up from point samples to larger scales (Guarini *et al.* 1998). In this respect, estimation of MPB biomass from remote sensing can be very useful.

So what are the options for routine estimation of primary production of mudflats? The tendency in the scientific literature has been for increasing layers of complexity to be added to models, which requires more and more parameters to be measured. Coastal zone managers have taken an alternative approach, for example by using annual averages of MPB biomass at a site as an indicator of the potential level of primary production (de Jong and de Jonge 1995). A similar approach, in which the photosynthetic biomass, the daily dose of light, and the optical depth are combined, seems to work well for phytoplankton in estuaries (Cole and Cloern 1987). Here, we test the results of different models using a detailed series of measurements in the Westerschelde estuary.

## Methods and modelling

### *Experimental dataset*

Environmental conditions, microphytobenthic biomass and photosynthetic activity were measured throughout the Westerschelde estuary during 2002 and 2003. For this work, a subset of data was selected for the period May 2003, from an intertidal site, Appelzak, located close to the Belgian-Netherlands border. The site was located at the mid-tide level, with mud and fine sands dominating the sediment. Emersion times were calculated for each day by using measured water heights within the estuary and

were checked on two occasions by direct observation of the moment when the site was inundated by the flood and exposed again by the ebb. The measured emersion times were found to agree with the predicted times. Due to the strong attenuation of irradiance by the water column of this part of the estuary, photosynthesis was considered to be zero during tidal immersion.

Daily values for air temperature, wind, rainfall and sunshine hours were collated from records at a local meteorological station. Maximum air temperature was used as a proxy for sediment surface temperature in the primary production model (for a more advanced method see Guarini *et al.* 1997). The mean irradiance (PAR;  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) was recorded at hourly intervals by a Licor Li-192 sensor located at the Netherlands Institute of Ecology in Yerseke. Hourly PAR values at the sediment surface were calculated using look-up tables for all periods of emersion during the study month.

Algal biomass (chlorophyll *a*) was measured in the top 2 mm of the sediment by using the contact coring technique (Ford and Honeywill 2002). All pigment determinations were made using 90% acetone extraction and HPLC separation. Photosynthesis-irradiance curves were measured on suspensions of microphytobenthos in filtered estuary water using radiocarbon uptake. Carbon fixation rates were normalized to the algal biomass of the suspension, and simultaneous least-squares fitting was used to fit the cardinal parameters of photosynthesis-irradiance curves ( $P_{\text{max}}$  and  $\alpha$ , Figure 1). The effect of temperature on microphytobenthic photosynthesis was not

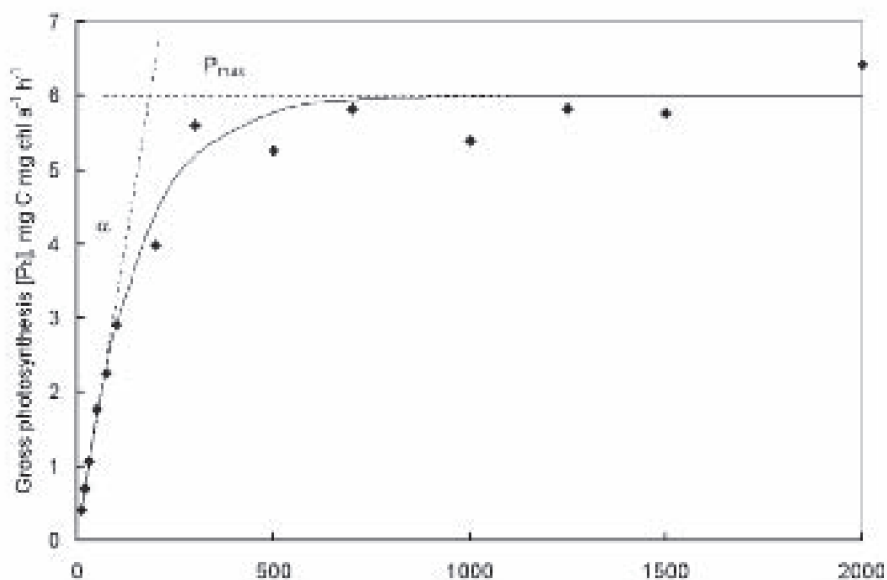


Figure 1. The relationship between gross photosynthetic carbon fixation and irradiance for microphytobenthic cells suspended in seawater.  $P_{\text{max}}$  is the maximum rate of photosynthesis at light saturation, and  $\alpha$  is the efficiency of light use at subsaturating irradiance.

measured in this study, but has been examined in detail by Blanchard *et al.* (1996, 1997) and by Morris and Kromkamp (2003). These results showed clearly that  $\alpha$  is independent of temperature, and that  $P_{\text{MAX}}$  has a unimodal relationship with temperature. The temperature response equations and coefficients of Blanchard *et al.* (1997) were used to convert  $P_{\text{max}}$  from the measurement temperature (20°C) to values that would occur at the SST of the site.

As no published data is available on the respiratory loss rates of MPB, we assumed that this would vary in proportion to metabolic activity. Accordingly,  $R$  was set to a fixed percentage of the temperature-adjusted  $P_{\text{max}}$ , which should be related to the growth rate and activity of the MPB cells (Geider 1992). Excretion of 'EPS' and other loss processes were beyond the scope of this modelling exercise.

### *Approaches to modeling primary production*

A number of different production models have been described in the more recent literature. All of these models feature a functional separation between the total MPB biomass, and a subfraction of the biomass that is exposed to light at the sediment surface. This fraction is usually termed the photosynthetically-active biomass, or PAB (e.g. Serôdio *et al.* 1997). There is widespread agreement that the ratio between total biomass and PAB is determined by the migratory behaviour of diatom cells, and that this ratio is to some extent predictable (Pinkney and Zingmark 1991). Probably the most thoroughly tested of the current generation of benthic production models is that of Blanchard and colleagues. Their model has been tested against real observations of MPB populations in mesocosm experiments and field data, and its development can be seen in these papers: Blanchard *et al.* (2001), Guarini *et al.* (2000a,b), and elsewhere in this volume. The basis of this model is shown in Figure 2 – when the conditions are right (e.g. during daylight, during low tide) there is a movement of MPB to the surface. The surface of the sediment is seen as a box, or pool, which can be filled up with cells. Once full, there is no room for any further increases in PAB. Only the cells in this box can photosynthesise and add to that day's primary production. Photosynthetic rates in the PAB box are calculated from photosynthetic rate parameters measured on suspensions of MPB (Figure 1). This model corresponds very well to the latest electron microscope images of sediments surfaces (Herlory *et al.* 2004), in which a layer of cells is clearly seen lying on top of the sediment. The amount of chlorophyll in the box is fixed at 25 mg m<sup>-2</sup>, and any chlorophyll in excess of this amount is deemed non-photosynthetically active. However, the total chlorophyll content in the upper layers of the sediment will often greatly exceed this value, for example we have recorded values in Westerschelde sediments of up to 400 mg chl a m<sup>-2</sup> in the top 2 mm of the sediment.

Macintyre *et al.* (1995) and Barranguet *et al.* (1998) used a different approach to model daily primary production at their intertidal sites. These authors used essentially the same techniques for modelling sediments as others have used for measuring primary production in a mixed water column. In these sediment-optical models, an attenuation coefficient determines the penetration of irradiance into the sediment. Algal cells are uniformly distributed with depth, with rates of photosynthesis per unit

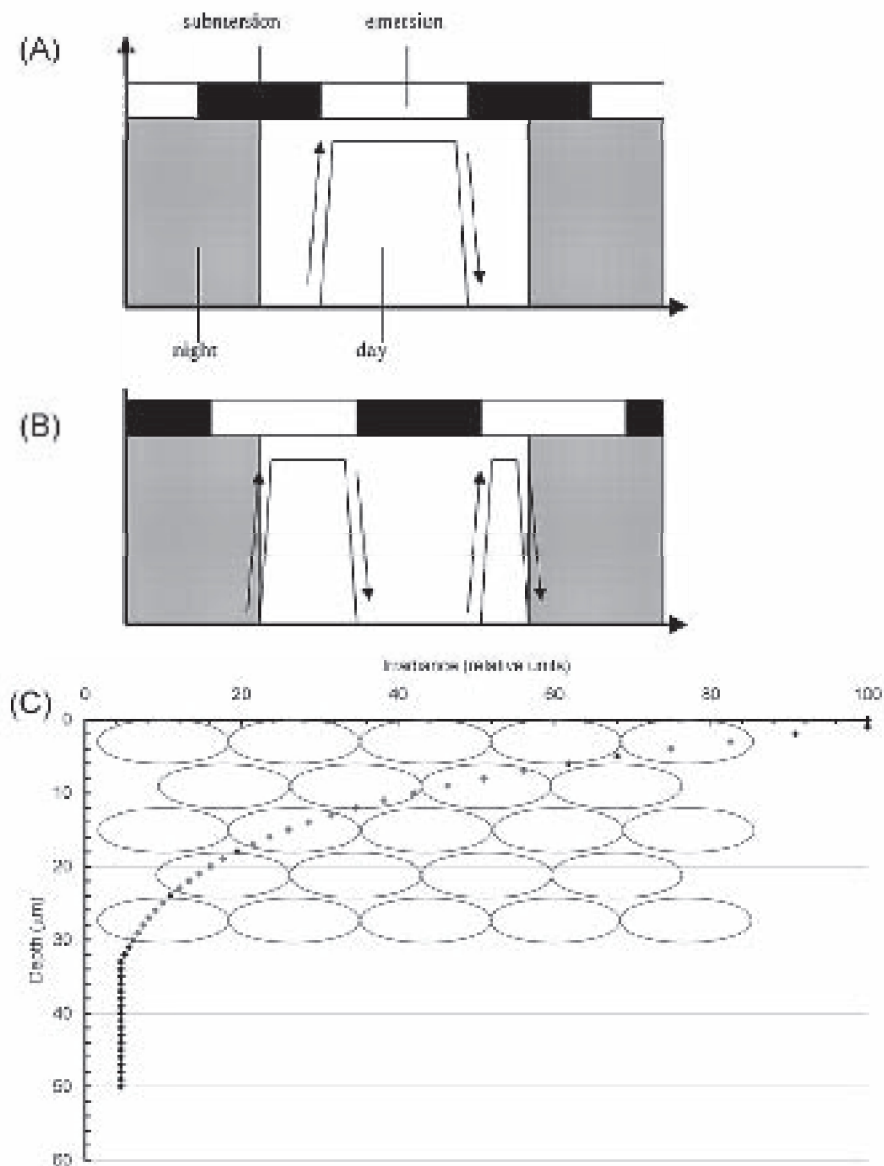


Figure 2. Conceptual model of the changes in photosynthetically-active biomass (PAB) in microphytobenthos. (A) Cells migrate to the photic zone at the surface of the sediment during an emersion period centred around noon (B) Upward and downward migration of cells during two short daylight emersion periods in early morning and late afternoon (modified after Guarini *et al.* 2000a) (C) Close packing of diatom cells at the sediment surface and the associated decrease in irradiance within the sediment.

chlorophyll again defined by laboratory measurements on dilute suspensions. The interaction of irradiance and photosynthetic parameters, together with the amount of chlorophyll allows the rate of photosynthesis to be calculated at each depth. Integration over depth and time gives the daily gross primary carbon fixation.

However, there are other data that argue against the use of the ‘surface pool’ or ‘uniform profile’ models. If the surface of the sediment is frozen, then sectioned with a very fine technique, called cryo-slicing, which gives layers of sediment only 0.1 mm thick, then a quantitative distribution of chlorophyll with depth can be made. Details of this method can be found in papers by de Brouwer and Stal (2001), Kelly *et al.* (2001) and Honeywill *et al.* (2002). The results showed that not all of the chlorophyll was at the surface, nor was there a uniform distribution with depth (except at night, after downward migration). In fact, the most common result of cryo-slicing was an exponentially-decreasing gradient of chlorophyll with depth.

### *Gross and net primary production calculations*

Which of these descriptions of the microphytobenthic environment is correct, and what are the implications for modelling primary production? Forster and Kromkamp (2004) gave a description of irradiance conditions within cohesive, muddy sediments in relation to different profiles of chlorophyll. Primary production values for three of the possible chlorophyll profiles given in that paper are calculated here – these are the UNI profile (corresponding to a uniform distribution of chlorophyll with depth as in Macintyre *et al.* 1995, Barranguet *et al.* 1998), the SML profile (a surface monolayer of cells, Guarini *et al.* 2000a), and the KY profile (an exponentially decreasing gradient of chlorophyll with depth, Kelly *et al.* 2001).

Daily gross primary production was calculated for the Appelzak field site during May 2003 for each of the profiles, using a depth interval of 10  $\mu\text{m}$  and a time step of 10 min. Additional calculations were made for the integral net primary production by subtraction of respiratory losses, and the effect of using different values for respiration was tested.

## **Results**

The daily photon dose at the sediment surface during emersion periods varied from 6 to 32  $\text{mol photon m}^{-2}$ , driven by meteorological and tidal conditions. Changes in irradiance appeared to be the main cause of variability in daily gross primary production ( $P_g$ ) (Figure 3). Day-to-day variability in  $P_g$  was high, for example see the large differences between the 5<sup>th</sup> and 6<sup>th</sup> of May 2003. Production rates were highest at the beginning and end of the month, corresponding to periods when low water was centred on the local solar noon. Mean  $P_g$  at the mid-intertidal site varied from 270  $\text{mg C m}^{-2} \text{d}^{-1}$  to 490  $\text{mg C m}^{-2} \text{d}^{-1}$  according to the type of model used (Table 1). The UNI and SML models give similar, lower values of  $P_g$ , whereas KY gave consistently higher values.

The net daily primary production ( $P_n$ ) models were run with a dark respiration rate that was equivalent to 5% of the maximum photosynthetic rate. These runs also

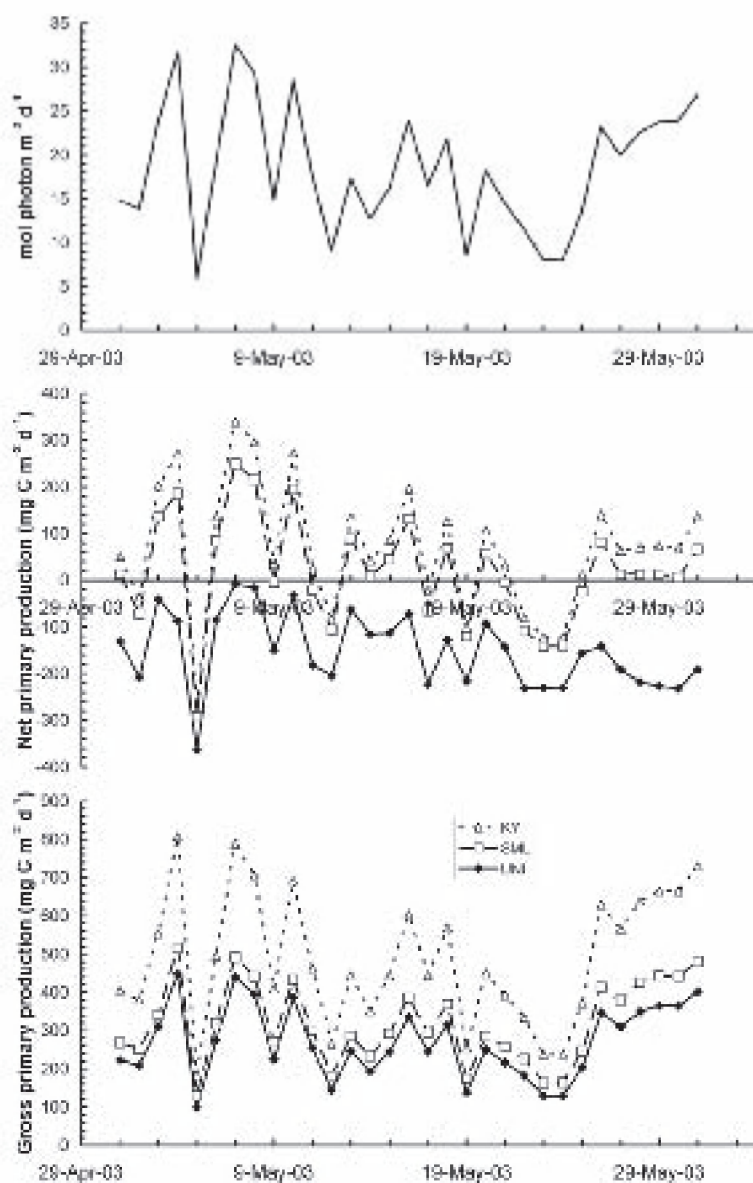


Figure 3. Calculations of primary production at Appelzek in May 2003 using different modeling approaches. (A) Daily photon dose received at the intertidal site (B) Net primary production using the three different chlorophyll profiles (C) Gross primary production.

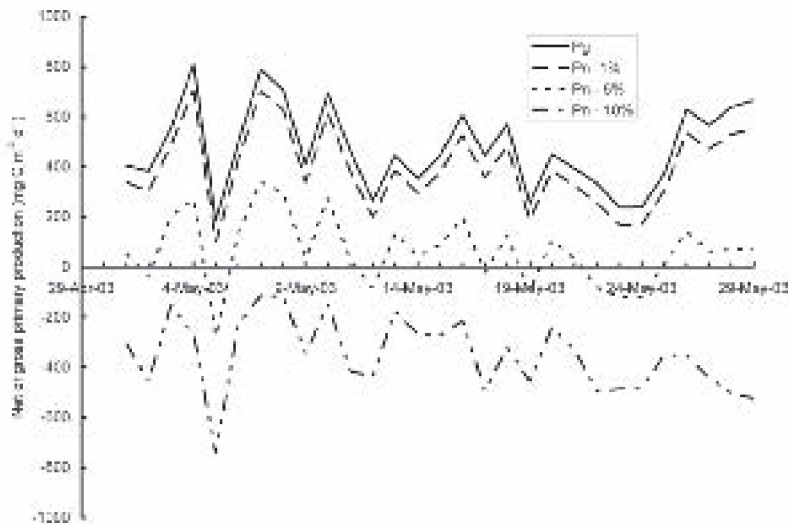


Figure 4. The effect of dark respiration rate on calculated daily net primary production at Appelzak during May 2003. Net primary production at respiration rates of 1%, 5% and 10% of the maximum photosynthetic capacity are shown in comparison to the gross primary production.

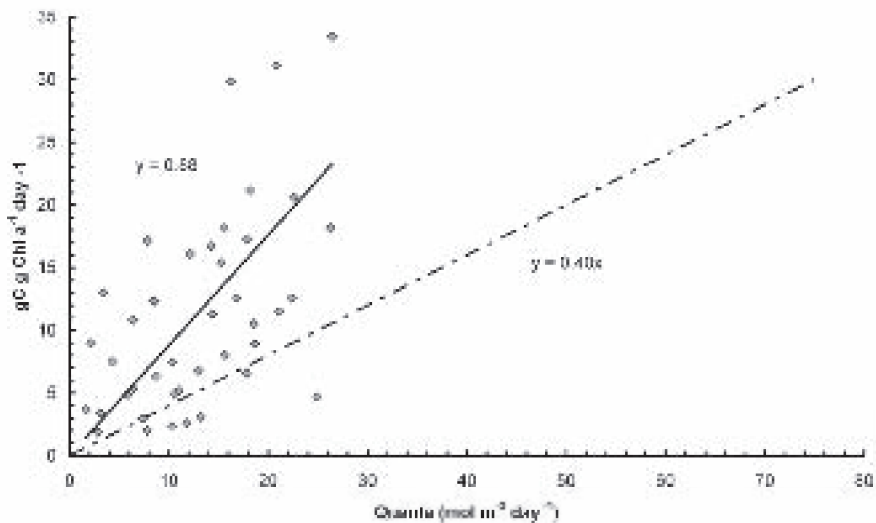


Figure 5. The relationship between biomass-normalised gross daily production and irradiance for microphytobenthic samples from the Westerschelde estuary collected during 2002 and 2003. The linear regression for MPB ( $y=0.88x$ ) is compared with a similar estimate of light use efficiency for phytoplankton ( $y=0.40x$ ) made by Falkowski *et al* (1994).

showed the same ordering of values as for  $P_g$ , with  $\text{UNI} < \text{SML} < \text{KY}$ , but now SML and KY showed the best agreement. The UNI model predicted negative rates of  $P_n$  for each day of May 2003, whereas negative production occurred only on days with low incident irradiance in the other two models. The biweekly periodicity caused by the spring-neap tidal cycle was less obvious in the graph of  $P_n$ . Net primary production was only weakly correlated with photon dose, whereas gross production was more closely related to the variability in incident irradiance.

The calculation of  $P_n$  was highly sensitive to the respiration rate, as shown in Figure 4 where the KY model calculations are shown for three different respiratory loss rates. Increasing the respiration rate successively from 1% to 5% then 10% greatly decreased the net production, and dampened the sensitivity of production to irradiance.

The previous examples were calculated for a site with a constant algal biomass, and constant photosynthetic parameters during the month of observations. Calculations of  $P_g$  were made for samples from other areas in the Westerschelde estuary, where a range of chlorophyll concentrations and photosynthetic parameters were encountered. The KY chlorophyll profile was used to model gross production at each site. After normalisation to the amount of biomass, there was a linear dependence of  $P_g$  on the daily photon dose (Figure 5). The slope of the relationship, which describes the light use efficiency of an intact microphytobenthic assemblage, was higher than the efficiency previously calculated for phytoplankton in various water columns (Falkowski *et al.* 1994).

## Discussion

A model can only be as accurate as its underlying data and algorithms, and it is important to remain aware of the potential for multiplication of errors as the complexity of the model increases. In the case of microphytobenthic production estimates, whilst there are some factors that can be accurately quantified (e.g. incident irradiance), there are others for which little information is available (e.g. respiration rates). The input parameters that can be most accurately measured or predicted are the irradiance and temperature at the sediment surface during emersion, the total microalgal biomass, and the photosynthetic activity of a sample in suspension. The latter can be estimated using a variety of techniques such as uptake of radiocarbon label, oxygen evolution, or PAM fluorescence (see chapters by Glud, and Kromkamp and Forster elsewhere in this volume).

The more difficult to measure parameters are those that define the actual exposure of benthic microalgal pigments to light. A quantitative description of the chlorophyll-depth profile in the sediment can be used if available, together with a description of the attenuation of irradiance in the sediment. Alternatively, the detection of chlorophyll fluorescence from cells at the sediment surface can be used as a proxy for the level of PAB; this method has been pioneered and validated in the work of Serôdio and colleagues (Serôdio *et al.* 1997, 2001; Serôdio 2003). All three model scenarios tested here had equal amounts of microalgal chlorophyll in the upper 2 mm

of sediment – the only difference was in the shape of the chlorophyll-depth profiles. The exponentially-decreasing chlorophyll profile (KY) gave the highest gross and net production. In this profile, more of the algal chlorophyll is exposed to the light than in the uniform profile (UNI), where the deeper strata of chlorophyll receive no light. The SML profile also had chlorophyll placed in the optimum position to capture photons, but the pool size of PAB was in this case smaller than for the KY profile, hence the lower production rates per unit area of sediment.

A further uncertainty in the calculation of primary production occurs when models such as the ones used here are unconstrained by the supply of dissolved inorganic carbon, the substrate for photosynthesis. Situations can arise when the DIC demand from a photosynthesizing biofilm will greatly exceed the pool of DIC in the porewater and overlying surface water. This is likely to occur when dense surface biofilms are exposed to prolonged periods of high light. The relatively weak isotopic fraction observed between  $^{13}\text{-C}$  and  $^{12}\text{-C}$  which has been observed in virtually all studies of MPB to date suggests that DIC limitation may be widespread. Inhibition of photosynthesis by high porewater concentrations of oxygen may also occur. In both case, the observed photosynthetic rate would be lower than the maximum rate determined on a dilute suspension. Preventing this overestimation of primary production will require the additional parameterization of concentration gradients and diffusion coefficients in the sediment photic zone (shown previously by Ludden *et al.* 1985). It is likely that the differences between the different models will be reduced if DIC limitation is taken into account, as this will affect the KY and SML models more than the UNI model.

Net primary production is perhaps the most relevant variable to consider when working on the ecology of tidal flats.  $P_n$  can be closely related to the growth rate of MPB if the conversion factor from units of carbon to chlorophyll is known. However, the calculation of  $P_n$  is highly dependent upon the dark respiration value used. Respiration rates are difficult to measure, and are known to be both irradiance- and temperature-dependent. Therefore, quantification of respiration rates as well as other loss or organic carbon such as exudation, should be a priority area for research. From the modeling of  $P_n$  made here, for a mid-tidal site with equal emersion and immersion periods, and also from studies in which we have modeled the growth of biofilms in mesocosms experiments, we suggest that respiration (and other losses) are not likely to be more than 5 % of the maximum photosynthetic rate. A respiration rate as low as 1.5 % of  $P_{\text{max}}$  was required in order to balance a growth model of phytoplankton in the Westerschelde estuary (Kromkamp and Peene 1995). It is feasible that respiration rates similar to, or lower than this could occur in MPB due to oxygen limitation in the deeper sediment layers during immersion.

The good correlations observed between biomass-normalised gross production and daily photon dose across a range of intertidal sites show that general rules can be applied to benthic production models. The higher light use efficiency observed for MPB than for phytoplankton is a direct result of diatom vertical migration, which maximizes photon capture by placing cells at the surface of the sediment. Phytoplankton in a mixed water column must compete with water, cDOM and other attenuating compounds, thus water columns have a lower photosynthetic efficiency compared to

Table 1. A summary of the modelling results for net and gross primary production at Appelzak during May 2003. For each unit of production, the daily mean and monthly sum are shown. The three chlorophyll profiles, KY, SML and UNI are described in the text.

		$P_n$		$P_g$	
		Sum	Mean	Sum	Mean
		mg C m <sup>-2</sup>	mg C m <sup>-2</sup> d <sup>-1</sup>	mg C m <sup>-2</sup>	mg C m <sup>-2</sup> d <sup>-1</sup>
Model	KY	2076	67	15182	490
	SML	570	18	9906	320
	UNI	-4723	-152	8370	270

cohesive sediments. Given data on the photon dose and biomass for a given site, it should be possible to predict  $P_g$  with reasonable accuracy. The photon dose can be calculated for any site if a digital elevation model of the estuary together with surface irradiance measurements and tidal curves are available. Synoptic maps of microphytobenthic biomass can be obtained from remote sensors such as Landsat, so it should be possible in the near future to routinely map primary production at the estuary scale.

## Acknowledgements

This work was funded by the European Union 5<sup>th</sup> Framework project 'EVK3-CT-2001-00052 A system of hierarchical monitoring methods for assessing changes in the biological and physical state of intertidal areas (HIMOM)'. This is NIOO publication number 3544.

## References

- BARRANGUET, C., J.C. KROMKAMP, and J. PEENE 1998. Factors controlling primary production and photosynthetic characteristics of intertidal microphytobenthos. *Mar. Ecol. Prog. Ser.* **173**: 117-126.
- BLANCHARD, G.F., J.M. GUARINI, P. GROS, and P. RICHARD 1997. Seasonal effect on the relationship between the photosynthetic capacity of intertidal microphytobenthos and temperature. *J. Phycol.* **33**: 723-728.
- BLANCHARD, G.F., J.M. GUARINI, F. ORVAIN, and P.G. SAURIAU 2001. Dynamic behaviour of benthic microalgal biomass in intertidal mudflats. *Journal Of Experimental Marine Biology And Ecology* **264**: 85-100.
- BLANCHARD, G.F., J.M. GUARINI, P. RICHARD, P. GROS, and F. MORNET 1996. Quantifying the short-term temperature effect on light-saturated photosynthesis of intertidal microphytobenthos. *Mar. Ecol. Prog. Ser.* **134**: 309-313.
- COLE, B.E., and J.E. CLOERN 1987. An empirical model for estimating phytoplankton productivity in estuaries. *Mar. Ecol. Prog. Ser.* **36**: 299-305.
- DE BROUWER, J.F. C., and L.J. STAL 2001. Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. *Mar. Ecol. Prog. Ser.* **218**: 33-44.
- DE JONG, D.J., and V.N. DE JONGE 1995. Dynamics and distribution of microphytobenthic chlorophyll-a in the western Scheldt estuary (SW Netherlands). *Hydrobiologia* **311**: 21-30.

- FALKOWSKI, P.G., R. GREEN, and Z. KOLBER. 1994. Light utilization and photoinhibition of photosynthesis in marine phytoplankton, p. 409–434. *In* [eds.] N.R. Baker and J.R. Bowyer, *Photoinhibition of photosynthesis from molecular mechanisms to the field*. BIOS.
- FORD, R.B., and C. HONEYWILL 2002. Grazing on intertidal microphytobenthos by macrofauna: is pheophorbide a useful marker? *Mar. Ecol. Prog. Ser.* **229**: 33–42.
- FORSTER, R.M., and J.C. KROMKAMP 2004. Modelling the effects of chlorophyll fluorescence from sub-surface layers on photosynthetic efficiency measurements in microphytobenthic algae. *Mar. Ecol. Prog. Ser.* **284**: 9–22.
- GEIDER, R.J. 1992. Respiration: taxation without representation, p. 333–360. *In* [eds.], P.G. Falkowski and A.D. Woodhead *Primary Productivity and Biogeochemical Cycles in the Sea*. Plenum.
- GUARINI, J.M., G.F. BLANCHARD, C. BACHER, P. GROS, P. RIERA, P. RICHARD, D. GOULEAU, R. GALOIS, J. PROU, and P.-G. SAURIAU 1998. Dynamics of spatial patterns of microphytobenthic biomass: inferences from a geostatistical analysis of two comprehensive surveys in Marennes-Oleron bay (France). *Mar. Ecol. Prog. Ser.* **166**: 131–141.
- GUARINI, J.M., G.F. BLANCHARD, and P. GROS 2000a. Quantification of the microphytobenthic primary production in European intertidal mudflats – a modelling approach. *Cont. Shelf Res.* **20**: 1771–1788.
- GUARINI, J.M., G.F. BLANCHARD, P. GROS, D. GOULEAU, and C. BACHER 2000b. Dynamic model of the short-term variability of microphytobenthic biomass on temperate intertidal mudflats. *Mar. Ecol. Prog. Ser.* **195**: 291–303.
- GUARINI, J.M., G.F. BLANCHARD, P. GROS, and S. J. HARRISON 1997. Modelling the mud surface temperature on intertidal flats to investigate the spatio-temporal dynamics of the benthic microalgal photosynthetic capacity. *Mar. Ecol. Prog. Ser.* **153**: 25–36.
- HERLORY, O., J.M. GUARINI, P. RICHARD, and G.F. BLANCHARD 2004. Microstructure of microphytobenthic biofilm and its spatio-temporal dynamics in an intertidal mudflat (Aiguillon Bay, France). *Mar. Ecol. Prog. Ser.* **282**: 33–44.
- HONEYWILL, C., D. M. PATERSON, and S. E. HAGERTHEY 2002. Determination of microphytobenthic biomass using pulse-amplitude modulated minimum fluorescence. *Eur. J. Phycol.* **37**: 485–492.
- KELLY, J.A., C. HONEYWILL, and D.M. PATERSON 2001. Microscale analysis of chlorophyll-a in cohesive, intertidal sediments: the implications of microphytobenthos distribution. *J. Mar. Biol. Ass. U K* **81**: 151–162.
- KROMKAMP, J., and J. PEENE 1995. Possibility of net phytoplankton primary production in the turbid Schelde estuary (SW Netherlands). *Mar. Ecol. Prog. Ser.* **121**: 249–259.
- LUDDEN, E., W. ADMIRAAL, and F. COLIJN 1985. Cycling of carbon and oxygen in layers of marine microphytes; a simulation model and its eco-physiological implications. *Oecologia* **66**: 50–59.
- MACINTYRE, H.L., and J.J. CULLEN 1995. Fine-scale vertical resolution of chlorophyll and photosynthetic parameters in shallow-water benthos. *Mar. Ecol. Prog. Ser.* **122**: 227–237.
- MORRIS, E.P., and J.C. KROMKAMP 2003. Influence of temperature on the relationship between oxygen and fluorescence-based estimates of photosynthetic parameters in a marine benthic diatom (*Cylindrotheca closterium*). *Eur. J. Phycol.* **38**: 133–142.
- PINCKNEY, J., and R. ZINGMARK 1991. Effects of tidal stage and sun angles on intertidal benthic microalgal productivity. *Mar. Ecol. Prog. Ser.* **76**: 81–89.
- SERÔDIO, J. 2003. A chlorophyll fluorescence index to estimate short-term rates of photosynthesis by intertidal microphytobenthos. *J. Phycol.* **39**: 33–46.
- SERÔDIO, J., J.M. DA SILVA, and F. CATARINO 1997. Nondestructive tracing of migratory rhythms of intertidal benthic microalgae using in vivo chlorophyll a fluorescence. *J. Phycol.* **33**: 542–553.
- SERÔDIO, J., J.M. DA SILVA, and F. CATARINO 2001. Use of in vivo chlorophyll a fluorescence to quantify short-term variations in the productive biomass of intertidal microphytobenthos. *Mar. Ecol. Prog. Ser.* **218**: 45–61.
- TURNER, R.K., J.C.J.M. VAN DEN BERGH, T. SODERQVIST, A. BARENDREGT, J. VAN DER STRAATEN, E. MALTBY, and E.C. VAN IERLAND 2000. Ecological-economic analysis of wetlands: scientific integration for management and policy. *Ecological Economics* **35**: 7–23.
- WOODWARD, R.T., and Y.S. WUI 2001. The economic value of wetland services: a meta-analysis. *Ecological Economics* **37**: 257–270.
- UK BIODIVERSITY ACTION PLAN (2002) online at [www.ukbap.org.uk/reporting](http://www.ukbap.org.uk/reporting).