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Microscale techniques to measure photosynthesis: A mini-review

Abstract

The benthic primary production of subtidal coastal sediments is grossly under sampled – especially in relation to its potential importance. This is partly due to lack of appropriate measuring techniques. Microsensor approaches represent a strong tool for describing and quantifying microscale benthic photosynthesis and related processes, however, spatial and temporal extrapolation from single point measurements in heterogeneous subtidal sediments is complicated. Complementary use of chambers and ^{14}C incubations can be helpful but these approaches have their own caveats and limitations. New approaches such as in situ PAM measurements and in situ PAM imagine may turn out to be very helpful in extrapolating microsensor data to larger scales – and robust intercalibrations between the techniques have been presented. Planar O_2 optodes and O_2 eddy-correlation measurements represent other very promising in situ approaches that overcome the limitations of microscale techniques for accessing benthic primary production. The present manuscript gives a brief review on available microscale techniques for quantifying subtidal benthic primary production

Introduction

The potential importance of subtidal benthic microphytes for local, regional and global carbon cycling is becoming increasingly recognized. In 2003, there existed a little more than 100 studies quantifying the benthic primary production in marine areas but most of these most have been performed in the intertidal zone (Cahoon 1999). Subtidal studies amount to less than 40 and only around 10 studies have been performed at water depth >5 m. Only 4 studies have been conducted in the Arctic area, hosting the relatively largest area of shallow-water, shelf sediment. Our current database on benthic microphytic activity in subtidal areas is very limited.

Apart from the relative few studies on benthic microphytic activity, the use of various techniques; ^{14}C-incubations, chamber incubations and microsensor approaches hamper the assessment of benthic microphytes activity. The ^{14}C incubation in principle measures the gross photosynthesis, however, inaccurate determination of the specific activity or slurry procedures at the best allow only a crude

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estimate of the in situ activity. Chamber or core incubations resolve a net-activity but typically average the activity of microphythic patches with that of bare sediment and the faunal respiration. Further, the approach is essentially a 'black box' and gives a very poor insight in the interstitial activity distribution and the photosynthesis related respiration. In contrast microsensor measurements can both resolve the net and the gross activity at a very high spatial and temporal resolution and thereby provide detailed insight in the microphytic activity. However, due to significant heterogeneity in natural systems extrapolation to larger areas might be very difficult, and detailed mapping of larger heterogeneous areas is sometimes an impossible task. Below the traditional microsensor technique is described in more detail and potential area extrapolation of subtidal microsensor data by the aid of other newly developed techniques is discussed.

Measuring benthic activity by microelectrodes.

Since the introduction of the Clark type oxygen microelectrode to aquatic biology our knowledge on the microscale oxygen dynamics at benthic interphases as increased tremendously (e.g. Revsbech *et al*. 1980; Revsbech and Jørgensen 1986; Jørgensen and Des Marais 1990). Due to their minute size (tip diameter < 2 μm) fast response time (< 1s), and low stirring sensitivity (<1 %), the oxygen concentration at a given point and at a give time can be accurately measured (Gundersen *et al*. 1998, Glud *et al*. 2000). Vertical concentration profiles can easily be obtained and provide detailed insight in the microbial activity (Figure 1)

For dark incubated sediments the community dark respiration ($J_{dark,up}$) can be calculated from the linear concentration gradient obtained within the DBL as $J_{dark,up} = - D_0\ dC/dz$ (Sten-Knudsen 2002), where D_0 is the temperature and salinity corrected molecular diffusion coefficient for O_2 and C is the O_2 concentration at the depth z. Knowing the O_2 penetration depth (O_{pen}) it is a simple matter to calculate the average volume specific respiration rate. The net respiration at the respective depths can also be modelled from the curvature of the concentration profile within the sediment (Nielsen *et al*. 1990; Berg *et al*. 1998). This, however require a tortuosity corrected diffusion coefficient for the sediment, which typically is estimated from semi empirical relations between porosity and tortuosity (e.g. Ullman and Aller 1982; Iversen and Jørgensen 1993). However, in cases of high meiofaunal activity or high intercellular water content (e.g. microbial mats) such simplified approaches may underestimate the in situ transport coefficients and other alternative approaches must be applied (Aller and Aller 1992; Glud and Fenchel 1999; Berg *et al*. 2001; Wieland *et al* 2001).

In order to estimate the net-photosynthesis from microprofiles obtained in light the oxygen flux out of the photic zone can be calculated as: $J_{light,up} + J_{light,down} = D_0\ dC_{DBL}/dz_{DBL} + D_e\ dC_{SED}/dz_{SED}$, where D_e is the tortuosity corrected diffusion coefficient and dC/dZ refer to the gradients within the DBL and at the lower boundary of the photic zone, respectively (Fig 1). The lower boundary of the photic zone is typically indicated by the turning tangent of the concentration profile, but can also be defined from

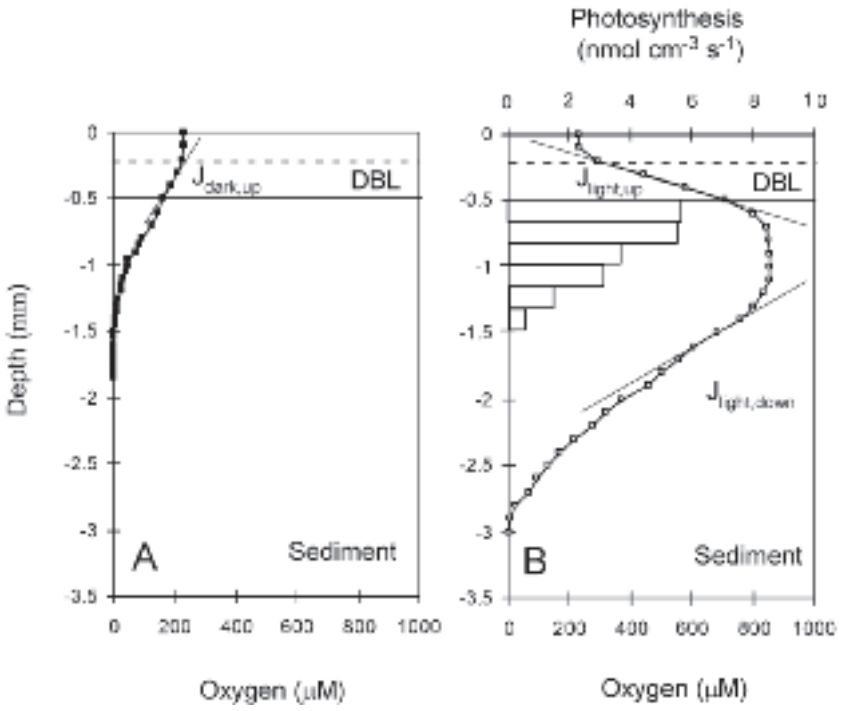

Figure 1. Two microprofiles measured in sediment cores recovered from a water depth of 1.2 m in Helsingør Habor, Denmark. The profiles were measured at the exact same spot in darkness and by a down-welling irradiance of 600 µE m^{-2} s^{-1}, respectively. The white blocks indicate the gross photosynthetic rates measured by the light-dark-shift approach at each depth. The temperature was 15 °C and the salinity 12 $^{0}/_{00}$

microprofiles of light or the gross photosynthetic rates (see below). In the present example the oxic zone in darkness is identical to the photic zone at the applied irradiance allowing a direct comparison between the activity in the two situations. In case the two zones cover different depth-spans this has to be corrected for (e.g. Kühl *et al*. 1996). Using the profiles above (and a measured porosity of 0.8) the community dark respiration amounted to 39.5 mmol m^{-2} d^{-1} and the net photosynthesis at the applied irradiance was 252.8 mmol m^{-2} d^{-1}. The downwards flux accounted for 25% of the net photosynthesis. This is on the high end of typical encountered values which usually are in the range of 10-20% (e.g. Kühl *et al* 1996; Epping and Jørgensen 1999; Wenzhöfer *et al* 2000; Fenchel and Glud 2000; Glud *et al*. 2002; Christensen *et al*. 2003). The relative fraction of the produced O_2 that diffuse downward primarily depends on the O_2 penetration depths and the thickness of the diffusive boundary layer (DBL) (Glud *et al*. 1992; Kühl *et al*. 1996).

The gross photosynthetic rates of microphytic communities can be measured at each depth by the so-called light-dark-shift technique (Revsbech and Jørgensen 1983). The approach is based on the fact that at steady state the O_2 production at a give point equals the respiration and the O_2 export away from that point. Assuming

that the respiration and the O_2 export remain constant just after light eclipse it follows that the O_2 decrease in darkness equals the O_2 production rate in light (Revsbech and Jørgensen 1983). The two assumptions behind the technique have been carefully evaluated and it has been documented that the community respiration remains constant within the first 5 s after light eclipse (Glud *et al*. 1992). However, due to changes in the concentration gradient at the onset of darkness the export rate from a given point change within that period it is therefore essential to obtain the absolutely initial rate of decrease at each depth to resolve the correct activity distribution (Glud *et al*. 1992; Lassen *et al*. 1998). If a dark incubation longer than 1-2 s is applied the activity distribution will be smeared and high rates are underestimated while low rates are overestimated, the depth integrated activity does, however, remain constant (Glud *et al*. 1992). To obtain the initial O_2 decrease is usually now problem applying high quality microelectrodes and fast measuring equipment, but simple diffusion based modeling allow for correction if longer dark incubations have been used (Glud *et al*. 1992; Lassen *et al*. 1998). In Figure 1 the gross photosynthetic rate decreased almost exponentially with depth reflecting the biomass distribution and the light extinction. The depth integrated photosynthesis amounted to 338.8 mmol O_2 m^{-2} d^{-1} and by subtracting the net photosynthesis it followed that the respiration within the photic zone was 86 mmol m^{-2} d^{-1} in light or more than twice the community dark respiration. It has generally been found that the light induced enhancement of the aerobic 'respiration' of the photic zone is in the order of 35-80% (e.g. Canfield and Des Marais 1993; Glud *et al* 1999; Kühl *et al* 1996). Reasons for this is ascribed to a combination of photorespiration, stimulation of heterotrophic respiration following leakage of photsynthate to the carbon limited bacterial community and reoxidation of anaerobic metabolites (e.g. H_2S, FeS) accumulating during the dark period. To what extent the various O_2 consuming processes matter depends on the microbial community and a number of microenvironmental controls (e.g. irradiance, temperature, organic loading, salinity, pH). Simplified approaches comparing light and dark incubated communities to access the 'dark respiration' in light thus grossly underestimate the actual activity.

A detailed study performed at the site where the cores for Figure 1 were recovered showed that during a 12 h light period about 60% of the O_2 produced within the photic zone was consumed by respiration (or photorespiration). 12% was used for 'repaying' an oxygen-debt in the form of anaerobic metabolites (presumably FeS) accumulating during the previous dark period and the remaining 25% was released from the sediment (Fenchel and Glud 2000).

As demonstrated above O_2 microsensor studies can provide a very detailed insight in the benthic photosynthesis, respiration and the associated processes. However, to extrapolate findings to a lager sediment area is difficult when dealing with natural, heterogeneous sediments. To make an O_2 concentration microprofile and measuring the associated gross photosynthetic activity takes in the order of 20-40 min, consequently there is a limit to how many measurements that can be performed at given location, especially in situ where environmental controls might change during the measurment. Automated profiling systems may to some extent overcome this problem and have been successfully deployed; the approach does, however, require relatively

sophisticated measuring equipment (e.g. Gundersen and Jørgensen 1990; Wenzhöfer *et al*. 2000; Glud *et al*. 2002). Intercalibration between microsensor data and other techniques being faster and/or covering larger areas represent an approach for microsensor based investigations to be extracted to the ecosystem level.

Newly developed pulse amplitude modulated (PAM) fluorometers – especially the so-called diving PAM – represent a very fast and simple way of measuring proxies for biomass and photosynthesis for benthic communities. Several studies have documented strong correlations between chl *a* content and the minimal fluorescence yield (F_0) of sediments and between the relative electron transport rate (ETR) between photosystem I and II and photosynthetic activity measured as ^{14}C incubation approaches, chamber incubations and microprofile data (e.g. Barranguet and Kromkamp 2000; Glud *et al* 2002;). However, at the physiological level the relations are poorly defined and far from universal and studies have documented that the relations are confounded by migration patterns, light acclimation or environmental stress (e.g. Perkins *et al*. 2001). Nevertheless extrapolation of microprofile data to ecosystem level has been successfully performed by the aid of PAM-measurements (Glud *et al*. 2002). Other contributions in the present volume provide a more detailed insight and discussion on the potential of PAM fluorometer measurements both on microscale and for imaging (Kromkamp and Forster, this volume; Oxborough *et al*.2000).

Measuring benthic activity by optodes

Another recent development applicable for accessing the activity of benthic communities are planar optodes (Glud *et al*. 1996). The technique is based on dynamic fluorescence quenching of oxygen acting on an immobilized fluorophore (Kautsky 1939). In the absence of O_2 the fluorophore absorbs light and emits the absorbed energy as fluorescence of a defined intensity and life time. In the presence of O_2, quenching decreases both the intensity and the lifetime of the fluorescent signal (Klimant *et al*. 1995; Hartmann *et al*. 1997).

The principle has been used to construct so-called microoptodes for traditional microprofile measurements and large scale sensors for hydrographic measurements (Klimant *et al*. 1995; Klimant *et al*. 1997; Tengberg *et al*. 2003). For planar optodes the oxygen quenchable fluorophore is immobilzed in plasticized PVC (or another immobilization agent like sol-gels) at the surface of a transparent support foil. For most planar optodes applied so far a ruthenium based complex has been applied, but other alternatives exists (Klimant *et al*. 1997; Precht *et al*. 2004, Oguri *et al*. 2006). In order to avoid scattering effects and saturation the planar optode is typically covered by an optical insulation provided by a 20 µm layer of black silicone. As the sensing layer has a similar thickness and the support foil is about 175 µm thick, the total thickness of planar optodes are around 200 µm. For laboratory work the sensors can be fixed at the inside of a flume channel and excitation light can be provided from the outside (Figure 2). Irrespective of using fluorescent intensity or fluorescent lifetime as the O_2 sensitive parameter the signal is collected by a 12 bit digital camera after passing an emission filter (Glud *et al*. 1996; Holst *et al*. 1998).

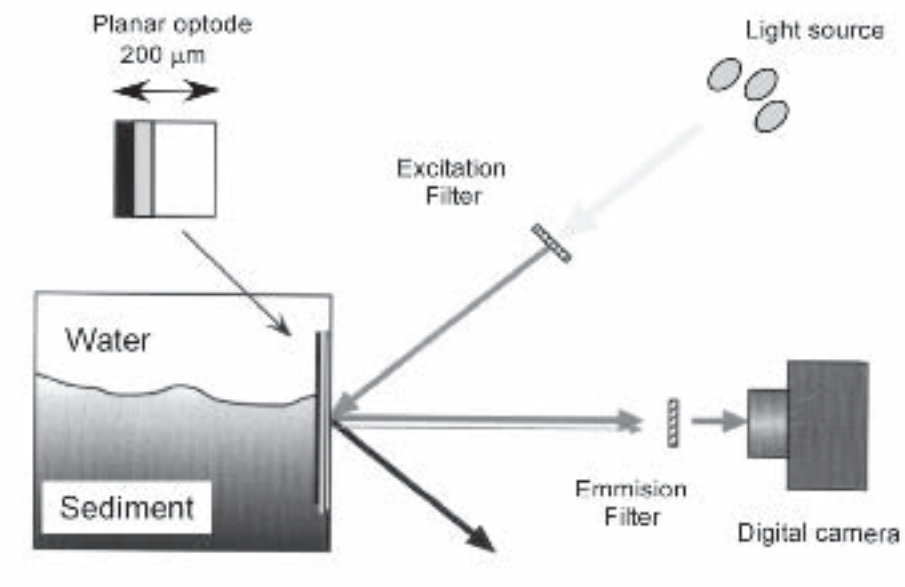

Figure 2. The basic measuring set-up for laboratory based planar optode measurements. As light source newly developed blue or bluegreen Light emitting diodes (LED) are now applied. More details are given in Glud *et al*. 1996, Holst *et al*. 1998, Glud *et al*. 2000)

In contrast to the Clark type microelectrodes the relationship between the O_2 concentration and the sensing signal is nonlinear, but can be expressed by a modified Stern-Volmer equation.

$$\frac{I}{I_0} = \frac{\tau}{\tau_0} = \alpha + \frac{1}{(1 = K_{SV}\, C)}\,(1 - \alpha)$$

where I_0 and I are the fluorescent intensities at anoxia and in the presence of O_2 at a concentration C, respectively, and τ and τ_0 are the equivalent fluorescent lifetimes. The α value represent the fraction of nonquenchable signal, while K_{sv} is the quenching coefficient (Klimant *et al*. 1995; Holst *et al*. 1998). In case of a well defined α (typically around 0.15) a two point calibration routine following the outline in Figure 3 can be applied, otherwise a three-point calibration routine is required. Calibrations can either be performed pixel by pixel or by applying universal constants for the image. Lifetime based sensing allow the use of transperent optodes facilitating alignment between O_2 distribution and structures in the sample (Holst and Grünwald 2001, Frederiksen and Glud 2006). A more detailed discussion on various approaches for quantifying fluorescent lifetime is given elsewhere (Holst *et al*. 1998; Glud *et al*. 2000).

The spatial resolution of obtained O_2 images depends on the optics in front of the camera, but a lower limit is given by the thickness of the layers of optical insulation layer and sensing chemistry allowing a horizontal diffusion within the planar sensor.

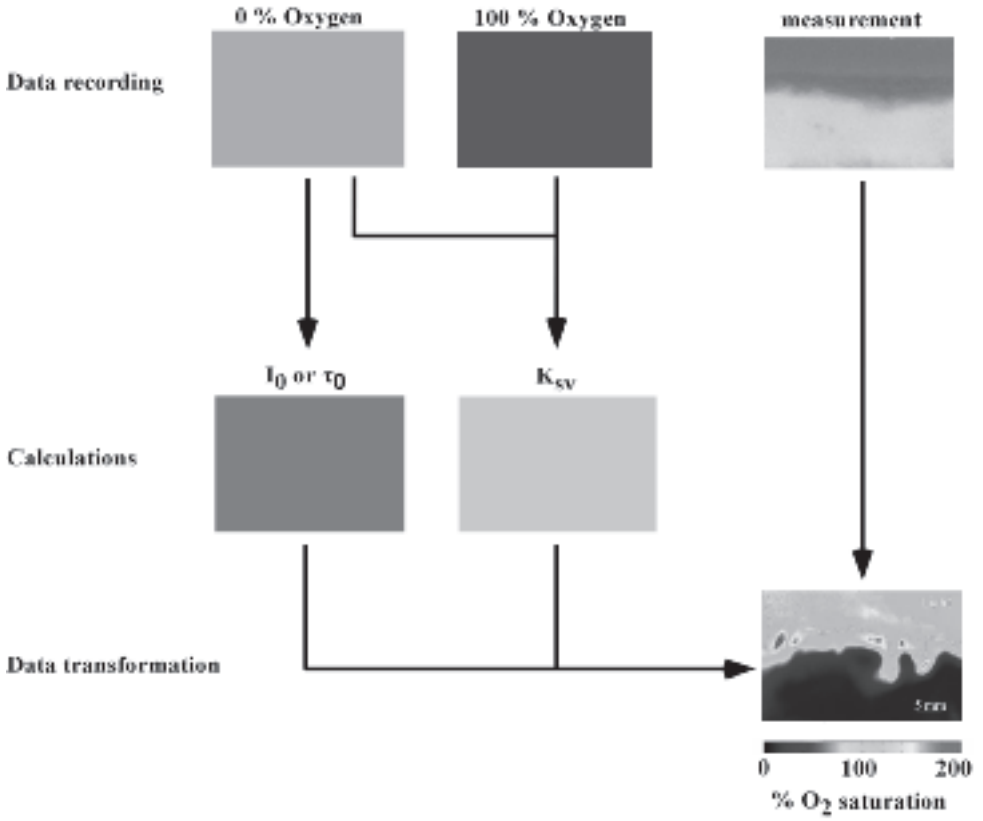

Figure 3. Knowing the α-value, images of the two constants required by the modified Stern-Volmer equation (I_0 (or τ_0) and K_{sv}) can be derived by a two-point calibration and measuring data can be transformed into O_2 images. The presented image was obtained at a diatom covered sediment and the horizontal heterogeneity is apparent, scale bars and sediment surface is included. For further discussion on the image please refer to Fenchel and Glud 2000.

This might be further confounded by light scattering. In our standard configuration images cover an area of 5 x 7 cm and as the camera chip has 640 x 480 pixels the spatial resolution is in the order of 100 x 100 µm. The temporal resolution is limited by the sensor response time but is usually in the order of 5-20 seconds. A single O_2 image by our system in principle contains 640 vertical microprofiles and images obtained in laminated photosynthetic active microbial mats have documented a relatively homogenous horizontal structure (Figure 4). Despite of this, sites only separated by a few mm still express different light responses (Figure 4). In principle all the simple calculations performed on traditionally obtained microprofiles (Figure 1) can be performed on such O_2 images. Thereby very detailed insights in the benthic O_2 dynamics i.e. net photosynthesis, respiration, distribution of hotspots and autotrophic-heterotrophic coupling can be provided (Glud *et al*. 1999). Applying the light-dark shift

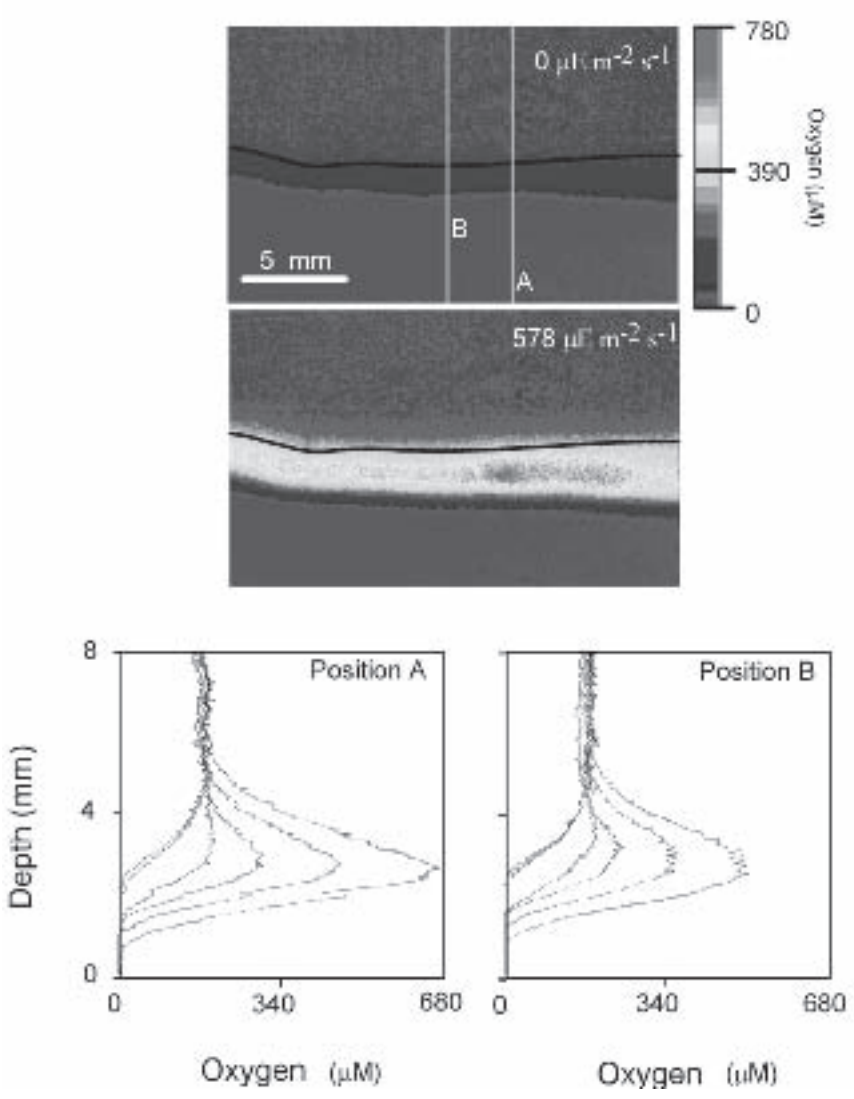

Figure 4. Two O_2 images obtained in a cyanobacteria dominated mat in darkness and at a down welling irradiance of 578 µE m^{-2} s^{-1}. Profiles extracted along the two lines indicated in the upper panel are shown in the lowest panel as they appear when the mat was exposed to 6 different light levels. (Redrawn from Glud *et al*. 1999)

technique also allow images of the gross photosynthetic activity to be resolved, but due to the relatively long response time of planar optodes the activity images are smeared – see above (Glud *et al*. 1999).

In contrast, as also outlined in Figure 3, measurements in subtidal diatom covered sediments have resolved a very high degree of horizontal heterogeneity (Figure 5). Hotspots of activity can be identified and by obtaining frequent images the temporal dynamic of the sediment-water interface can be documented (Wenzhöfer and Glud 2004). However, in extreme cases as shown in Figure 5 it becomes difficult to perform simple vertical calculations as the exchange rather has to be approached as radial diffusion between hotspots and the surroundings. To complicate matters even further this sediment was affected by intensive irrigation by *hediste diversicolor*. Software handling entire images and extracting exchange rates (or net activities) in such extreme cases still have to be developed. Simple 1D diffusion based calculations can of course still be performed on selected spots of the image. Another complication is that planar optodes operates along a wall. Hydrodynamically seen that can be a problem and extracting information on e.g. DBL dynamics should be done with caution.

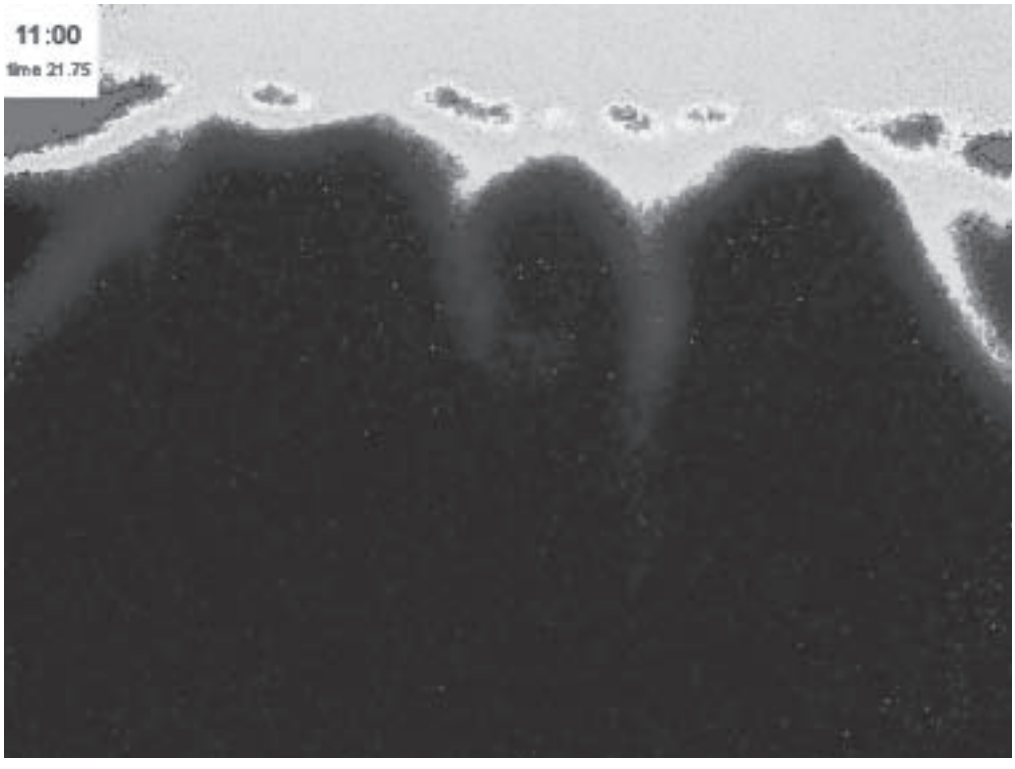

Figure 5. In situ images of the oxygen distribution measured in a diatom covered sediment inhabited by *hediste diversicolo*r. The extensive horizontal variation is apparent along actively ventilated infauna burrows. (Data from Wenzhöfer and Glud 2004)

Despite the problems, which in many cases can be dealt with, planar O_2 optodes offers a possibility to obtain a more realistic representation of O_2 distribution in benthic microphytic communities. Images at a given location can be obtained within minutes and large areas can be scanned relatively fast. Thereby planar optodes complement traditional microelectrodes approaches and combined measurements can provide a very detailed insight in the benthic microphytic activity. Planar optodes can be adopted to inverted periscopes for on line in situ measurements (Glud *et al*. 2001). A new in situ approach based on eddy-correlation-techniques may also provide a very power full future tool to quantify benthic O_2 exchange rates (i.e. net photosynthesis) over large sediment areas non-invasively (Berg *et al*. 2003).

References

ALLER, R.C., and J.Y. AILER 1992. Meiofauna and solute transport in marine muds. Limnol. Oceanogr. **37**: 1018-1033.

BARRANGUET, C. and J. KROMKAMP 2000. Estimating primary production rates from photosynthetic electron transport in estuarine microphytobenthos. Mar. Ecol. Progr. Ser. **204**: 39-52.

BERG, P., N. RISGAARD-PETERSEN, and S. RYSGAARD. 1998. Interpretations of measured concentrations profiles in sediment porewater. Limnol. Oceanogr. **81**: 289-303.

BERG, P., H. RØY, F. JANSSEN, V. MAYER, B.B. JØRGENSEN, M. HUETTEL, D. DE BEER. 2003. Oxygen uptake by aquatic sediments measured with a novel non-invasive eddy-correlation technique. Mar. Ecol. Prog. Ser. **261**: 75-83.

BERG, P., S. RYSGAARD, P. FUNCH, and M.K. SEJR. 2001. Effects of bioturbation on solutes and solids in marine sediments. Aquat. Microb Ecol **26**: 81-94.

CAHOON, L.B. 1999. The role of benthic microalgae in neritic ecosystems. In: Oceanography and Marine Biology: An Annual Review (Eds Ansell, A.D., Gibson R.N., Barnes M.). **37**: 47-86

CANFIELD, D.E., and D.J. DES MARAIS. 1993. Biogeochemical cycles of carbon, sulfur and free oxygen in a microbial mat. Geochim. Cosmochim. Acta **57**: 3971-3984.

CHRISTENSEN, P.B., R.N. GLUD, T. DALSGAARD, and P. GILLESPIE. 2003. Implications of long line mussel farming on benthos, oxygen, and nitrogen dynamics in coastal sediments, Aquaculture **218**: 567-588.

EPPING, E.H.G., and B.B. JØRGENSEN. 1996 Light-enhanced oxygen respiration in benthic photrphic communities. Mar. Ecol. Progr. Ser. **139**: 193-203

FENCHEL, T., and R.N. GLUD. 2000. Benthic Primary production and O_2-CO_2 dynamics in a shallow water sediment: Spatial and temporal heterogeneity. Ophelia **53**: 159-171.

FREDERIKSEN, M.S. and R.N. GLUD. 2006 Oxygen dynamics in the rhizosphere of zostera marix: A two-dimensional planar optode study Limnol. Oceanogra **51**: 1072-1083.

GLUD R.N., N.B. RAMSING, and N.P. REVSBECH. 1992. Photosynthesis and photosynthesis coupled respiration in natural biofilms quantified with oxygen microelectrodes. J. Phycol. **28**: 51-60

GLUD, R.N., N.B. RAMSING, J.K. GUNDERSEN, and I. KLIMANT. 1996. Planar optrodes, a new tool for fine scale measurements of two-dimensional O_2 distribution in benthic communities. Mar. Ecol. Prog. Ser.**140**: 217-226.

GLUD, R.N., M. KÜHL, O. KOHLS, and N.B. RAMSING. 1999. Heterogeneity of oxygen production and consumption in a photosynthetic microbial mat as studied by planar optodes. J. Phycol. **35**: 270-279.

GLUD, R.N., and T. FENCHEL. 1999. The importance of ciliates for interstitial solute transport in benthic communities. Mar. Ecol. Prog. Ser. **186**: 87-93.

GLUD, R.N., J.K. GUNDERSEN, and N.B. RAMSING. 2000. Electrochemical and optical oxygen microsensors for in situ measurements. In: *In situ* Analytical Techniques for Water and Sediment. (eds.. J. Buffle & G Horvai). John Wiley & Sons, pp. 19-73.

GLUD, R.N., M. KÜHL, F. WENZHÖFER, and S. RYSGAARD. 2002. Benthic diatoms of a high Arctic fjord (Young Sound, NE Greenland): Importance for ecosystem primary production. Mar. Ecol. Prog. Ser. **238**: 15-29

GLUD, R.N., A. TENGBERG, M. KÜHL, P.O.J. HALL, I. KLIMANT, and G. HOST 2001. An in situ instrument for planar O_2 optode measurements at benthic interfaces. Limnol. Oceanogr. **46**: 2073-2080.

GUNDERSEN, J.K., AND B.B. JØRGENSEN. 1990. Microstructure of diffusive boundary layers and the oxygen uptake of the sea floor. Nature **345**: 604-607.
GUNDERSEN, J.K., N.B. RAMSING, and R.N. GLUD, 1998. Predicting the signal of oxygen microsensors from physical dimensions, temperature, salinity, and oxygen concentrations. Linmol. Oceanogr. **43**: 1932-1937
HARTMANN, P., W. ZIEGLER, G. HOLST, and D.W. LUBBERS. 1997. Oxygen flux fluorescence lifetime imaging. Sensors and Actuators B **38-39**: 110-115.
HOLST, G., O. KOHLS, I. KLIMANT, B. KÖNIG, M. KÜHL, and T. RICHTER. 1998. A modular luminescence lifetime imaging system for mapping oxygen distribution in biological samples. Sensors and Actuators B **51**: 163-170.
HOLST, G., and B. GRUMWALD. 2001 Luminescence lifetime imaging with transparent oxygen optodes. Sensors and Actuators B **74**: 78-90.
IVERSEN, N., and B.B. JØRGENSEN. 1993. Diffusion coefficients of sulfate and methane in marine sediments: Influence of porosity. Geochim. Cosmochim. Acta **57**: 571-578.
JØRGENSEN, B.B., and D.J. DES MARAIS. 1990. The diffusive boundary layer of sediments: Oxygen microgradients over a microbial mat. Limnol. Oceanogr. **35**: 1343-1355.
KAUTSKY, H. 1939. Quenching of luminescence by oxygen. Trans. Faraday Soc. **35**: 216-219.
KLIMANT, I., V. MEYER, and M. KÜHL. 1995. Fiber-optic oxygen microsensors, a new tool in aquatic biology. Limnol. Oceanogr. **40**: 1159-165.
KLIMANT, I., M. KÜHL, R.N. GLUD, and G. HOLST. 1997. Optical measurements of oxygen and other environmental parameters in microscale: Strategies and biological applications. Sensors and Actuators B **38-39**: 29-37.
KÜHL, M., R.N. GLUD, H. PLOUG, and N.B. RAMSING. 1996. Microenvironmental control of photosynthesis and photosynthesis-coupled respiration in an epilithic cyanobacterial biofilm. J. Phycol. **32**: 799-812.
LASSEN C., R.N. GLUD, N.B. RAMSING, and N.P. REVSBECH. 1998. A method to improve the spatial resolution of photosynthetic rates obtained by oxygen microsensors. J. Phycol. **34**: 89-93.
NIELSEN, L.P., P.C. CHRISTENSEN, N.P. REVSBECH, and J. SØRENSEN. 1990. Denitrification and oxygen respiration in biofilms studied with a microsensor for nitrous oxide and oxygen. Microb. Ecol. **19**: 63-72**.**
OGURI, K., H. KITAZATO, and R.N. GLUD. (2006) Planar oxygen optode used with platinum Octaetylporphyrin UV-LED excitation light source for high-resolution imaging in low oxygen environments. Mar. Chem. **100**: 95-107**.**
OXBOROUGH, K., A.R.M. HANLON, G.J.C. UNDERWOOD, and N.R. BAKER. 2000. In vivo estimation of the photosystem II photochemical efficiency of individual microphytobenthic cells using high-resolution imaging of chlorophyll a fluorescence. Limnol. Oceanogr. **45**: 1420-1425
PERKINS. R.G., G.J.C. UNDERWOOD, V. BROTAS, G.C. SNOW, B. JESUS, and L. RIBEIRO. 2001. Responses of microphytobenthos to light: Primary production and carbonhydrate allocation over an emersion period. Mar. Ecol. Prog. Ser. **223**: 101-112.
PRECHT, E., U. FRANKE, L POLERECKY, and M. HUETTEL. 2003. Oxygen dynamics in permeable sediments with wave driven pore water exchange. Limnol. Oceanogr. **49**: 693-705.
REVSBECH, N.P., J. SØRENSEN, T.H. BLACKBURN, and J.P. LOMHOLT. 1980. distribution of oxygen in marine sediments measured with microelectrodes. Limnol. Oceanogr. **25**: 403-411.
REVSBECH, N.P., and B.B. JØRGENSEN. 1983. Photosynthesis of benthic microflora measured with high spatial resolution by the oxygen microprofile method: Capabilities and limitation of the method. Limnol. Oceanogr. **28**: 749-756.
REVSBECH, N.P., and B.B. JØRGENSEN. 1986. Microelectrodes and their use in microbial ecology. Advances in Microbial Ecology, 9, Marshall K.C., editor, Plenum, New York, pp. 293-352.
STEN-KNUDSEN. 2002. Biological membranes: Theory of transport, potentials and electrical impulses, pp 671. Cambridge University Press, Cambridge.
TENGBERG A., J. HOVDENES, D. BARRANGER, O. BROCANDEL, R DIAZ, J. SARKKULA, C. HUBER, and A. SHANGELMAYER. 2003. Optodes to measure oxygen in the aquatic environment. Sea Tech. **44**: 2-8
ULLMAN, W.J., and R.C. ALLER. 1982. Diffusion coefficients in nearshore marine sediments. Limnol. Oceanogr. **27**: 552-556
WENZHÖFER, F., O. HOLBY, R.N. GLUD, H.K. NIELSEN, and J.K. GUNDERSEN. 2000. In situ microsensor studies of a hydrothermal vent at Milos (Greece). Mar. Chem. **69**: 43-54.
WENZHÖFER, F. and R.N. GLUD 2004. Small-scale spatial and temporal variability in coastal benthic O_2 dynamics: Effects of fauna activity. Limnol. Oceanogra. **49**: 1471-1481.
WIELAND, A., D. DE BEER, L.R. DAMGAARD, M KÜHL, D. VAN DUSSCHOTEN, and H. VAN AS. 2001. Fine scale measurement of diffusivity in a microbial mat with nuclear magnetic resonance imaging. Limnol. Oceanogr. **46**: 248-259.