LIMNOLOGY
and
OCEANOGRAPHY: METHODS

Limnol. Oceanogr.: Methods 11, 2013, 1–15
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Precise continuous measurements of pelagic respiration in coastal waters with Oxygen Optodes

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Abstract

An analytical setup for respiration rate measurements was developed and evaluated in pelagic water samples using a commercially available optical oxygen sensor (Optode™). This setup required the development of a gas tight stopper to connect the sensors to a 1 dm^3 glass sample bottle, precise temperature control (± 0.05°C), and proper stirring of samples. The detection limit and precision of the method was 0.3 mmol O_2 m^{-3} d^{-1}. This was similar to the detection limit for the high-precision Winkler titration method reported in field studies. When compared with the Winkler method, the Optode sensor enabled operator-independent, high temporal resolution measurement of respiration, better coverage of plankton groups and detection of non-linear oxygen decline, without the need for wet chemistry. Respiration rates measured by the Optodes showed good accuracy when compared with measurements made with the Winkler titration method (3% deviation), followed the expected temperature response (Q_{10} = 3.0), were correlated with chlorophyll a and were congruent with earlier reported values in the literature. The main source of uncertainty was a necessary correction for system drift during the incubation period, due to oxygen release from the plastic components. Additionally, less stringent temperature control on board research vessels during rough seas reduced the precision. We conclude that the developed Optode system can be used to measure respiration in productive coastal waters. Samples from cold or deep waters were, however, often below the detection limit.

Respiration is one of the most important processes in the biosphere, from the level of metabolism in individual organisms to regulation of CO_2 and O_2 levels in the atmosphere. Respiration as a major removal process of oxygen is also fundamental to understand occurrence of hypoxic waters. The balance between auto- and heterotrophic processes in the sea is further measured by light and dark changes in oxygen, and of importance to study the downward transport of biomass through sedimentation (i.e., the marine biological pump) (Karl et al. 2003; Stramma et al. 2008; Reid et al. 2009). Thus, estimates of respiration are an important issue for natural science to understand mechanisms behind current societal concerns like the increasing levels of CO_2 in the atmosphere (i.e., the Greenhouse effect) and oxygen deficiency in coastal water bodies (hypoxia) (del Giorgio and Duarte 2002; Diaz and Rosenberg 2008). One reason for the insufficient knowledge about this process is that pelagic respiration studies currently are scarce, compared with CO_2 fixation measurements, partly due to methodological reasons.

Particularly, marine pelagic ecosystems requires accurate respiration rate measurements because the ambient oxygen pool may change as little as 0.01% d^{-1} (Robinson and Williams 2005). In absolute units, this demands that rates between 0.02 to 75 mmol O_2 m^{-3} d^{-1} have to be measured. For coastal waters, the observed rates are higher, ranging from 1.7 to 84 mmol O_2 m^{-3} d^{-1} (Hopkinson and Smith 2005).

One of the commonly applied techniques to measure respiration in water samples is to analyze changes in dissolved oxygen concentrations by end point titration with the high-precision Winkler titration technique comprising more than

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Acknowledgments

The technicians at Umeå University precision-tool workshop, UNIMEG, are acknowledged for constructing the stopper used for mounting the optode onto the glass bottle. The authors are grateful for the access to laboratory and ship time provided by Umeå Marine Sciences Centre and the Swedish Meteorological and Hydrological institute. The crews and technical staff of the research vessel, Argos, and coastguard vessel, KBV005, provided valuable support. The project was funded by the Kempe foundation to JW (SMK-2458).

DOI 10.4319/lom.2013.11.1

90% of the values reported in marine pelagic environments (Williams and Jenkinson 1982). The precision and detection limit of the Winkler technique are also adequate for pelagic measurements in the ocean. A theoretical detection limit of 0.07 mmol O_2 m^{-3} d^{-1} has been reported for automated Winkler titration, while practical precision reported from the field ranges between 0.1 and 2 mmol O_2 m^{-3} d^{-1} (Biddanda et al. 1994; Duarte et al. 2004; Williams et al. 2004; Maranger et al. 2005). Analysis of low respiration rates with this technique requires a relatively high level of sample replication per rate estimate, involving several titrations. The time-consuming wet chemistry and meticulous handling that are required hamper spatial and temporal coverage of respiration measurements and make this technique difficult for a layperson to use. Additionally, a linear decline in oxygen is typically assumed, and incubation times are relatively long. Furthermore, sample volumes are typically 60-120 mL, excluding sufficient numbers of larger zooplankton to determine their contribution to the measured respiration rate.

A sensor technique to measure oxygen is based on the Clark electrode. It has a reported precision of 0.1 µM when measuring oxygen concentration (Langdon et al. 1995). The inherent oxygen consumption of the electrode has been reduced and is often referenced to 4.7-47 × 10^{-7} µmol^{-3}h^{-1}, which is negligible (e.g., Briand et al. 2004). In the original reference to the method by Langdon (1984), a precision of 2.4 mmol $m^{-3}d^{-1}$ or higher is, however, reported for respiration measurement. This is a more than 10 times higher uncertainty than the results of the high precision Winkler technique.

Dynamic luminescence quenching (DLQ) has recently been introduced in marine sciences as an optical method to accurately determine oxygen concentrations with long-term stability (Körtzinger et al. 2004; Martini et al. 2007; Nicholson et al. 2008) and high precision (<1 µM) (Klimant et al. 1995; Tengberg et al. 2006). Oxygen measurements can be made with high frequency (i.e., seconds – minutes) and precision by a computer in an inert manner devoid of inherent oxygen consumption, suggesting that changes in oxygen over time can be measured with confidence. In addition, temporal changes in oxygen levels can be monitored without assuming linearity over longer time periods.

To our knowledge, the use of DLQ-based systems to directly measure plankton respiration without preconcentration of organisms has not been reported. This study thus investigates a novel application of the DLQ-technique and a new measurements system to more readily and precisely estimate respiration in aquatic environments than currently possible. However, in other applications, DLQ has recently been used to measure respiration in cultures, concentrated samples, and sediment-water interfaces. Warkentin et al. (2007) measured respiration of bacterial isolates and concentrated aquatic samples with the Presence Sensor Dish Reader (SDR) system. The same system was used to estimate copepod respiration in small glass vessels (Koster et al. 2008). Drazen et al. (2005) presented a novel technique with an Optode to measure respiration rates of deep sea fish, and Sommer et al. (2008) described an automatic system to regulate oxygen levels and measure sediment-water fluxes during in situ sediment incubation at vent sites. Additionally, Pakhomova et al. (2007), Almroth et al. (2009), and Almroth-Rosell et al. (2012) used the same type of Optodes with autonomous landers to perform sediment-water incubation studies.

In this study, we investigated whether the accuracy, detection limit, and precision of one type of commercially available Optode were sufficient to provide absolute respiration rates in unmanipulated marine pelagic samples. Samples were taken from coastal and offshore environments over a wide salinity range. An analytical unit was developed that allows the Optode to be mounted to a sample bottle that can be maintained under stable temperature conditions. Efforts were made to maximize precision and minimize handling while allowing measurements to be made on a routine basis. We also discuss the potential advantages and limitations of the present technique when performed in the field aboard a ship.

Materials and procedures

Study area

Field samples were collected from the Bothnian Sea and off the Swedish North Sea coast during the winter and spring of 2008 (Table 1). The samples encompassed salinities of 34.9-2.5 (g kg^{-1}) and temperatures of 1.7-17.7°C. Samples were taken from 5 and 60 m below sea level with 7 dm^3 Niskin bottles on a rosette water sampler. The samples thus covered a productivity gradient from oceanic to almost limnic conditions and different seasons. Winter samples were expected to have low respiration rates, approaching those of deep oceanic water and near the detection limit of previous investigations reported in the literature.

Table 1. Sampling sites in the Eastern North Sea coast and Baltic Sea.

Sea area	Station name	Latitude North	Longitude East	Sample depths (m)
Skagerrak	Å17	58° 16.5	010° 30.8	5 and 60
Bothnian Sea	C14	62° 06.0	018° 32.9	5 and 60
Bothnian Sea	B3	63° 30.0	019° 49.1	5
Bothnian Sea	B7	63° 31.5	019° 48.5	5

Experimental setup

Oxygen concentration in the respiration experiments were measured with four commercially available Optodes with an integrated temperature sensor (Optode 3835, Aanderaa Data Instruments AS, www.aadi.no). This type of sensor has been used in numerous earlier studies (*see* Introduction). The basic technique and evaluation of its function in aquatic environments was presented by Tengberg et al. (2006). As with most other oxygen sensors, Optodes measure the partial pressure of oxygen dissolved in water. By setting the internal sensor property to the salinity of the water sample, data were automatically converted to the absolute oxygen concentration ($[O_2]$, µmol dm^{-3}) using the formulas presented by Garcia and Gordon (1992). Sensor readings were plotted and logged in real-time using the software package Oxyview by Aanderaa Data Instruments AS (www.aadi.no).

Prior to the eight-month measurement period, a two-point calibration of the Optodes was performed according to the manufacturer's instructions using an air-saturated and temperature-equilibrated sample and an oxygen-depleted sample that was obtained by mixing 5 g of Na_2SO_3 into 500 mL water. The precision of the Optodes was routinely calculated from the 20 first quality assured values in each time series. During these measurements, samples were held at a stable temperature (± 0.05°C) with stirring on. The precision averaged ± 0.32 µmol dm^{-3} (SD, n = 96, Table 2). By performing our own two-point calibrations and regularly checking the sensor readings against those that had been obtained from Winkler titrations, we concluded that the sensor accuracy in this study was 4.2 µmol dm^{-3} (median, n = 96). No significant sensor drift was detected during the eight-month measurement period (see below). These values were within the manufacturers specifications.

Site specific values for salinity compensation of the oxygen readings were obtained from cabled CTD casts and entered into the Optode software to calculate the absolute $[O_2]$ values. The given accuracy and precision of the internal temperature sensor, used to compensate for temperature fluctuations, is ± 0.05°C and ± 0.01°C, respectively.

One portable computer was used to log data from 4 Optodes simultaneously. An 8-port serial hub with an external power supply (VScom, USB-8COM, www.vscom.de) was used to collect RS232 signals, and an 8-port hub (D-link, DUB-H7 7-PORT USB 2.0 HUB, www.dlink.com) provided electrical power (5V) from the computer to the four Optodes. If desired, the same system could run four additional Optodes, allowing further sample replication.

Analysis bottle and mounting of the Optode

A 1 dm^3 clear glass bottle (VWR) with a NS60 grounded neck, (inner ∅ 60 mm) was selected as the incubation vessel. This provides a sample volume about 10 times larger than is typically used in Winkler titrations. An important advantage of the present technique is that, at least in coastal areas, large zooplankton (5-10 individuals dm^{-3}) may also be sampled in a statistically acceptable number to be included in the respiration estimate.

Table 2. Accuracy and precision of the Optode sensors oxygen concentration measurements are shown. The oxygen concentrations and standard deviation for the Optodes were derived from the first 20 sensor readings (3-min interval) after stabilization of the incubation temperature. The accuracy was calculated by subtracting the oxygen concentration derived from the Winkler titration method from the Optode-derived value in samples taken at the same depth. The precision values show the standard deviation and coefficient of variation, respectively, for oxygen concentration measurements. The symbol n indicates the number of time series that were evaluated.

	Accuracy		Precision	
	(µmol dm^{-3})	(%)	(µmol dm^{-3})	(%)
Mean	3.6	4.6	0.45	0.16
SD	25.8	24.0	0.43	0.16
Median	–4.2	–1.2	0.32	0.10
Min.	–26.7	–8.0	0.07	0.03
Max.	93.6	160	2.27	0.88
n	96	96	98	98

A stopper was constructed from acrylic plastic (polymethylmethacrylate) to mount the Optode in, forming an air-tight seal by an O-ring (Nitrile 50.52 × 1.78 mm, Fig. 1). We took special care to ensure that the stoppers were the correct dimension to minimize gas exchange between the sample and the surrounding air. The stopper bottom was given a conical shape, similar to the design of typical Winkler bottles (Biochemical Oxygen Demand bottles), to allow air bubbles to readily escape. The stopper that we developed allowed simple mounting and dismounting of the Optode onto the sample bottle, without the trapping of air bubbles (Fig. 1). The Optode adaptor foot is inserted into the stopper tube and sealed by 2 O-rings toward the tube wall. The cable attachment screw will lock the Optode in the stopper from the outside.

A magnetic stirrer (Ikamag RET, www.ika.com) and 2 cm magnetic rods were used for each analysis bottle. The magnetic stirrer was set at 80 rpm to mix the water in the analysis bottle (Fig. 2). A stand was made to keep bottles in position during rough seas and maintain proper stirring and temperature control of the samples.

The thermostat heated water bath had inner dimensions of 32.5 cm (length), 30 cm (width), and 14.5 cm (depth) (Julabo 12 B, www.vwr.com), allowing the sample bottle to be covered by liquid up to the lower part of the neck. The bath was used in conjunction with an immersion cooler (Julabo FT 200, www.vwr.com) to control the incubator bath in a range of 0°-30°C with a precision of ± 0.03°C. To prevent freezing at temperatures approaching 0°C, 30% polypropylene glycol was used as the bath liquid. The bottles and immersion cooler probe were placed in a specially designed plastic holder to keep them in a fixed position and promote stable temperatures.

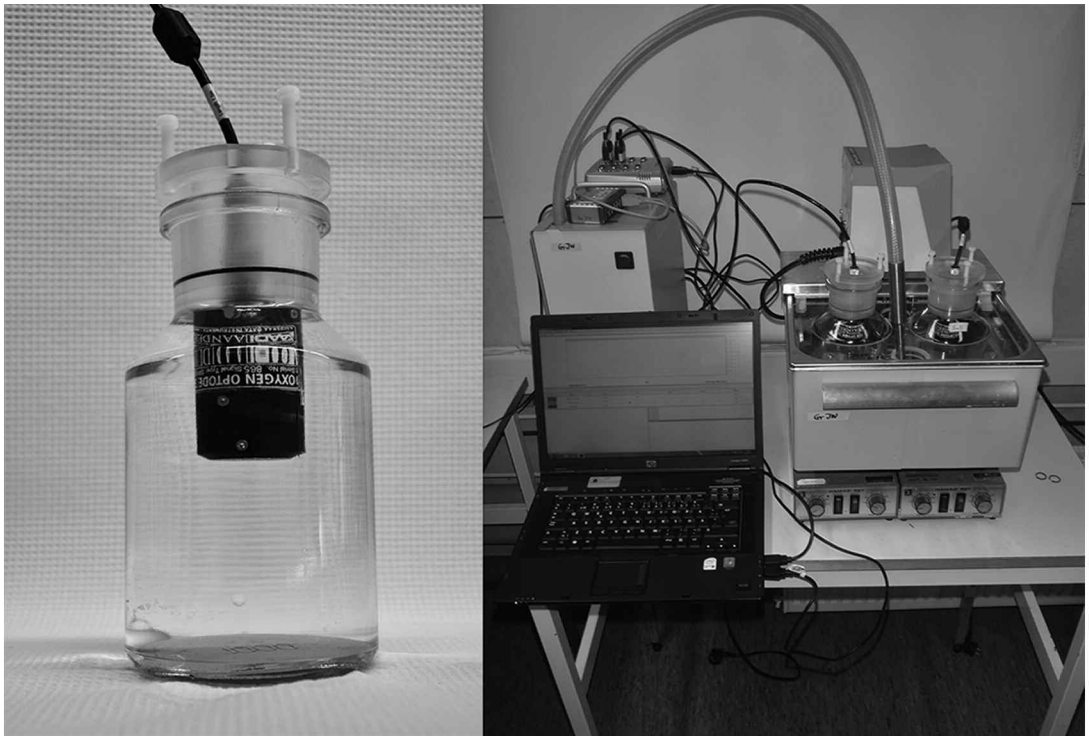

Fig. 1. The analysis unit used for respiration measurements. The left photograph shows an Optode mounted in 1 dm^3 glass bottles with the custom stopper made of acrylic plastic. This construction allowed rapid and simple mounting of the Optode without trapping air bubbles inside the bottles. A Nitrile O-ring mounted in the middle groove in the stopper provided a tight seal with the bottle walls. The screws can be used to help remove the stopper if it becomes stuck. The photograph to the right shows (from left) the immersion cooler (hubs on top), computer, and water bath with two glass bottles in a fitted plastic holder. Magnetic stirrers were placed underneath the water bath.

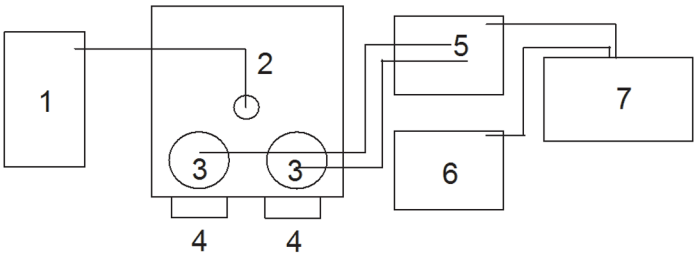

Fig. 2. Schematic diagram of the respiration measurement unit: 1, immersion cooler; 2, water bath with thermostat; 3, sample bottles; 4, magnetic stirrers; 5, serial hub; 6, USB hub for 5 V power to sensors; 7, portable computer.

Samples were protected from light with a cardboard box and black plastic bag. The level of light irradiation inside the cardboard box was below the detection limit (< 0.04 µmol m^{-2} s^{-1}) at an ambient room light of 17.6 µmol m^{-2} s^{-1}. Light inside the incubators were measured with a Li-Cor data logger (LI-1000) and a spherical quantum sensor for photosynthetic active radiation (Biosperical Instruments, QSPL 2101, www.biospherical.com) immersed in the water bath.

For Winkler titration measurements, 120 mL BOD glass bottles with glass stopper were used to sample the water using the procedures presented by Grasshoff et al. (1983). Briefly, samples were carefully transferred into 120 mL oxygen bottles by using tubing with the tip submerged, allowing 2 volumes of overflow. We then added 1 mL $Mn(II)SO_4$ (2.2 M) and 2 mL alkaline KI (1.8 M, 9 M NaOH) to the samples. After sedimentation of the precipitate to the lower 1/3 of the bottle, it was dissolved in 1.5 mL 50% H_2SO_4 and manually titrated (Eppendorf Topburet H) with 0.00125 M (± 0.2%) $Na_2S_2O_3$. The precision (SE) of this method is given as ± 0.22%.

To allow correction of field samples, system drift was determined by measurement of autoclaved samples at 121°C and 1.2 bar for 40 min. The samples were allowed to cool. No aeration was performed on these samples to minimize risk of contamination.

N_2 treatment

The potential that the observed drift was due to oxygen bound to plastic materials was tested by incubating the stopper with Optode mounted in N_2–bubbled Milli-Q water in an air-tight jar for 12 h before incubation. In addition, N_2 gas was applied above the stopper during incubation with a flow of 1.5 dm^3 min^{-1} and some overpressure using a rubber balloon. In a complementary test, the stopper and Optode were submerged 24 h in Na_2SO_3 solution (10 g dm^{-3}, O_2 saturation negative) using airproof glass jars prior to regular incubation as above in

air. All incubations were conducted in a land-based laboratory, temperature-controlled room, and temperature-controlled water bath as described above. During these experiments, the pressure inside the bottles was also measured in a parallel bottle. This was done with an Aanderaa pressure sensor (model. 4117 C, Aanderaa Data Instruments AS) mounted in the stopper as the oxygen Optode.

Statistical treatment

The rate of respiration was derived by a model I linear regression from the obtained time series. Respiration time series were analyzed for normal distribution with normal probability plots. The serial correlation was sometimes significant but considered to be of minor importance for the calculation of slope coefficients.

The total standard error (SE_{tot}) of samples and the system drift was calculated by

$$\mathrm{SE_{tot}} = \sqrt{\mathrm{SE^2_{sample}} + \mathrm{SE^2_{drift}}} \quad (1)$$

The total confidence interval was calculated in the corresponding manner.

To calculate the theoretical detection limit of the method (disregarding system drift), we computed the power to detect changes as a function of incubation time. The residual variance around the regression line was the main input variable in this calculation to avoid contribution from respiration or systematic drift. The standard deviation around the regression line (SD_r) was derived from the mean square error of the linear regression. The average SD_r from a set of representative incubations with autoclaved seawater samples was used.

The coefficient of variation for the slope coefficient (*CV*, %) was calculated by the SD_r divided by the mean oxygen concentration of the time series to be used in a power calculation. The incubation length required for a power (*1*-β) of 80% was calculated for a range of hypothetical respiration rates with the following function (Cohen 1988):

$$1-\beta = 1-\mathrm{NCDF.T}(\mathrm{IDF.T}(1\text{-}\alpha,\nu),\nu,\delta) \quad (2)$$

where NCDF.T is the non-central density function for the Student's t-distribution and IDF.T is the inverse density function for the Student's t-distribution as defined in the statistical software, SPSS. The level of a type I error (α; 0.05) and *v* the degrees of freedom (T-2) were also part of the calculation. The non-centrality parameter (δ) was equal to

$$\delta = \frac{\mathrm{q}}{\mathrm{CV}}\sqrt{\frac{\mathrm{T}\left(\mathrm{T}^2-1\right)}{12}} \quad (3)$$

where *q* is the trend in percent per time unit and *T* is the total incubation time in the same time unit. Data sets with a fixed *q*, associated series of *Ts*, and the determined *CV* were used to find the incubation length that provided a power of 80%. This was repeated for different values of *q* resulting in a data set with the lowest *q* detectable at a given *T*. The calculation was performed using the statistical software, SPSS (IBM Corp.).

Assessment

Temperature control

The Optode temperature sensor reading was required to be within ± 0.05°C of the overall temperature mean during respiration rate measurements. This was based on practical feasibility and observed temperature anomalies in an autoclaved sample due to activation of the compressor protection system of the immersion cooler (Fig. 3). A 0.14°C systematic temperature deviation resulted in a closely correlated change in the oxygen concentration of 1.0 µmol dm^{-3} (R^2 = 0.89, *n* = 39, 13.6-15.5 h). The slope coefficient of –5.9 µmol dm^{-3} $°C^{-1}$ matched the expected dependence of oxygen dissolution on temperature as defined in Grasshoff et al. (1999) and was therefore associated with the temperature-dependent mathematical algorithms of the sensor. The calculation of the O_2 solubility at standard air mixture and pressure, used in the calculation of relative O_2 saturation in the Optode, involves temperature factors raised to the power of 5 (Garcia and Gordon 1992). In addition, these algorithms also include temperature (*t*) dependent coefficients (C_x) for transforming the DLQ phase shift to oxygen units which are calculated according to:

$$C_x = C_{x0} + C_{x1}t + C_{x2}t^2 + C_{x3}t^3 \quad (4)$$

Thus, our temperature criterion of ± 0.05°C is related to the dependence of the Optode and limits variations in oxygen concentration to within ± 0.3 mmol dm^{-3} in accordance with the

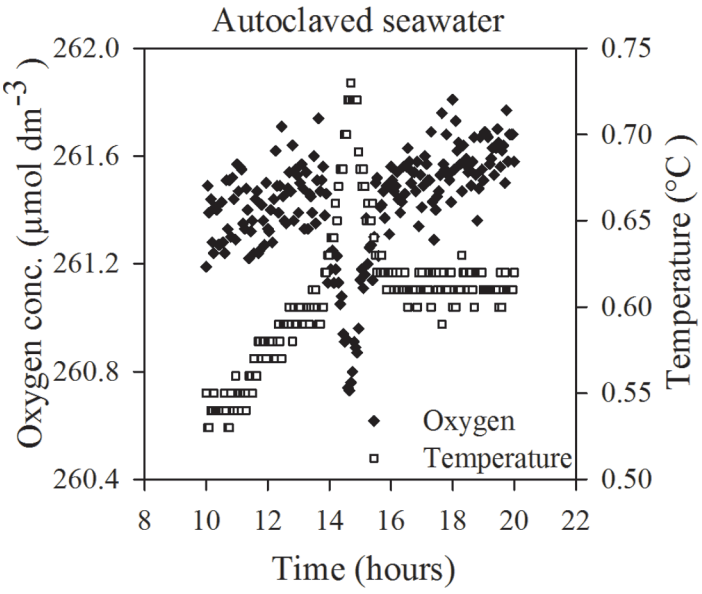

Fig. 3. Temperature and oxygen anomalies in autoclaved seawater induced by a temporarily engaged compressor protection system of the immersion cooler. This turns off the cooling for about 1 h after typically 15-20 h of operation.

precision of the method (see below). However, the temperature of samples typically initially differed tenths of a degree from the set incubator temperature. In the initial experiments, this required temperature equilibration for up to 60 min before stable temperature and oxygen measurements were achieved. Gentle stirring of the sample with a magnetic bead (2 cm long) at 80 rpm reduced time for temperature equilibration, decreased the initial variability in oxygen concentrations, and promoted a stable temperature throughout the incubation. Preconditioning of the analysis bottle, Optode and stopper in Seawater at close to in situ temperatures further reduced the initial period of variability to about 30 min. This routine also conditioned the dynamic luminescent membrane with oxygen in seawater, minimizing the time needed for equilibration with sample oxygen concentrations. The Optode membrane was kept wet at all times during sampling expeditions.

System drift

Over the 8-month period for which concomitant Winkler titrations were available, no long-term drift of the Optode could be demonstrated in 98 different incubations (–0.04 µmol d^{-1}, $P = 0.15$, $n = 96$). A short-term (< 24 h) drift of the experimental system was, however, discovered and assessed using autoclaved seawater. A gradual increase in the oxygen concentration was consistently observed during a 13-h incubation period in a research vessel laboratory (Fig. 4, field studies presented below). The increase amounted to 1.4 (± 0.048) and 2.4 (± 0.096) mmol O_2 m^{-3} d^{-1} (± 95% CI) for samples A and B, respectively. This level of drift was similar to the highest increases observed in untreated low productivity water samples taken from deep water and during the winter season (Table 3).

The level of system drift was confirmed using autoclaved samples from the field, covering sites with salinities between 2 and 35 g kg^{-1} (Fig. 5). The average drift was determined to be 2.1 mmol O_2 m^{-3} d^{-1} (SE ± 0.13, $n = 37$). The drift was independent of the oxygen concentration and temperature (Fig. 6). We assumed, thus, that the observed mean drift could also be applied at higher in situ oxygen concentrations.

Preconditioning of the stopper in N_2-bubbled water for 12 h, and incubation in N_2-atmosphere reduced the drift to an average of 0.41 (± 0.072) mmol m^3 d^{-1} (±SD) (i.e., drift reduced by 74%, $P = 0.043$, $n = 5$, Wilcoxon Signed Rank test, Table 4). The same effect was obtained with pretreatment in Na_2SO_3 during 24 h and regular incubation in air (0.44 ± 0.100 mmol m^3 d^{-1}). The cause of the relatively large drift during the incubation is, therefore, assigned to oxygen bound to the Optode and stopper plastic material.

We conclude from these observations that the measured oxygen increase in the autoclaved water samples was a short-term experimental system drift, independent of environmental conditions. The system drift should be subtracted from measured oxygen change. However, as discussed below, this drift correction will reduce the precision of the estimates and, thereby, will reduce the detection limit.

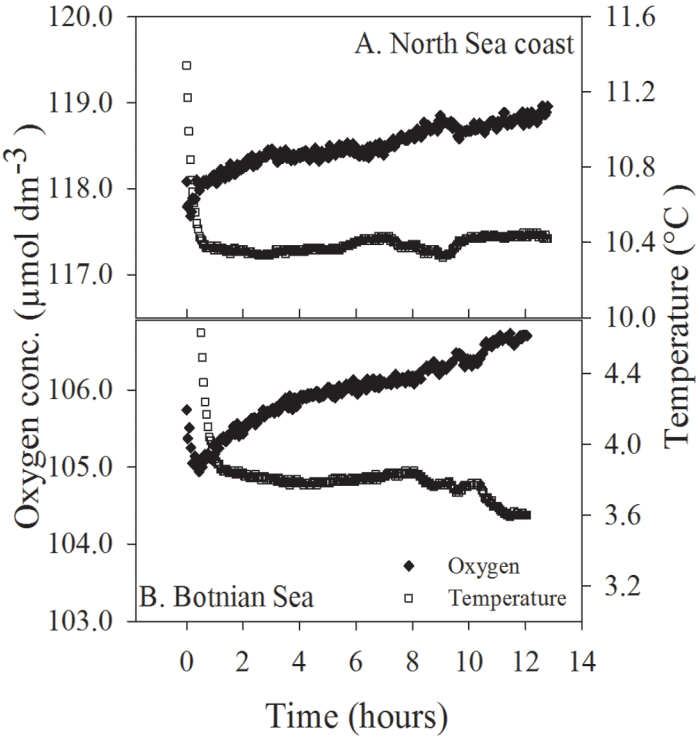

Fig. 4. Example of changes in oxygen concentration and temperature over time measured in sterile (autoclaved) seawater on board a research vessel. Shown are typical samples from oceanic (A, North Sea coast, Stn Å17, $n = 270$) and brackish (B, Bothnian Bay, Stn A5, $n = 242$) environments.

The median coefficient of variation (CV) was 0.066% for autoclaved samples and was used for the calculation of the statistical power. The CV was calculated as the SD around the regression line divided by the mean oxygen concentration, excluding the known variance due to the significant slope.

Drift-corrected Optode calibrated with Winkler measurements

The Optode measurements that were corrected for drift, outlined above, showed good agreement with respiration rates determined by the Winkler titration method (Fig. 7). The Optode-based rates were 105% and 101% that of the Winkler-based values at the in situ and +3° temperatures, respectively. This supported the conclusion that Optode measurements required drift correction to be comparable to measurements made by the Winkler method. It also shows that the estimated size of the mean short-term system drift was reasonable.

Expected increase with temperature

As a positive control, measurements of respiration rates with the Optode method were performed at different temperatures. This provided a controlled way to test the response of the method since an increase in the respiration rate is expected with elevated temperatures (Lefevre et al. 1994). Using samples from the same Niskin bottle, we found the expected exponential increase in oxygen consumption with increasing temperature (Fig. 8, Eq. 5, $R^2 = 0.992$):

Table 3. Statistical data for field measurements in the Baltic Sea (Stn B3, B7, and C14) and eastern North Sea (Stn Å17) at different seasons and depths (n = 24) are shown. The rate of change in oxygen was corrected for system drift. The 95% confidence interval of the oxygen slope coefficient (95% CI), oxygen concentration in the beginning of the incubation, number of reading per time series (n), standard deviation of temperature during the incubation, the incubation time used for rate measurement, and standard deviation around the regression line are shown. The coefficient of variation (CV) is the SD relative to the oxygen concentration. The temperature treatment is indicated in the station code for B3.

Station	Date	Depth (m)	Replicate	Oxygen change* (mmol m^{-3} d^{-1})	95% CI† (mmol m^{-3} d^{-1})	Oxygen conc. (mmol m^{-3})	n	SD temp. (± °C)	Incubation time (hours)	SD around regression line (mmol m^{-3})	CV (%)
C14	19 May 2008	5	1	–7.3	0.3	422.68	192	0.08	9.6	0.21	0.050
C14	19 May 2008	5	2	–9.0	0.3	424.71	193	0.08	9.6	0.24	0.057
C14	19 May 2008	60	1	5.3‡	0.4	398.53	186	0.06	9.2	0.46	0.115
C14	19 May 2008	60	2	–0.8	0.8	387.67	190	0.12	9.4	1.17	0.302
B3$^{+8°C}$	28 Jul 2008	5	1	–33.0	0.4	274.24	403	0.03§	20.1	1.15	0.418
B3$^{°-16°C}$	28 Jul 2008	5	1	–3.6	0.3	291.89	459	0.04§	22.9	0.36	0.125
B3$^{°-8°C}$	28 Jul 2008	5	1	–6.4	0.3	294.00	498	0.02§	24.8	0.18	0.061
B3$^{in\ situ}$	28 Jul 2008	5	1	–12.1	0.3	301.64	508	0.02§	25.3	0.16	0.054
B7$^{+3°C}$	16 Aug 2008	5	1	–11.4	0.3	281.21	425	0.02§	22.0	0.36	0.129
B7$^{+3°C}$	16 Aug 2008	5	2	–11.6	0.3	297.18	413	0.02§	22.0	0.22	0.075
B7$^{in\ situ}$	16 Aug 2008	5	1	–2.8	0.3	284.12	415	0.02§	21.8	0.09	0.033
B7$^{in\ situ}$	16 Aug 2008	5	2	–3.3	0.3	281.97	431	0.02§	22.7	0.09	0.031
Å17	09 Jun 2008	5	1	–2.4	0.3	349.71	251	0.11	12.5	0.19	0.053
Å17	09 Jun 2008	5	2	–3.8	0.4	344.47	248	0.10	12.4	0.43	0.126
Å17	09 Jun 2008	60	1	0.3	0.3	338.34	188	0.03	9.4	0.27	0.080
Å17	09 Jun 2008	60	2	–1.9	0.3	339.11	82	0.03	8.4	0.22	0.064
Å17	16 Feb 2009	5	1	–0.6	0.3	329.63	206	0.16	10.2	0.13	0.038
Å17	16 Feb 2009	5	2	–2.0	0.3	331.67	186	0.17	9.2	0.14	0.041
B7	03 Sep 2009	5	1	–9.5	0.3	287.93	261	0.02	13.0	0.10	0.035
B7	03 Sep 2009	5	2	–10.2	0.3	285.61	261	0.01	13.0	0.09	0.031
B7	03 Sep 2009	5	3	–10.4	0.3	282.63	253	0.01	12.6	0.06	0.021
B7	03 Sep 2009	5	4	–11.3	0.3	284.32	261	0.02	13.0	0.07	0.025
C14	10 Feb 2009	5	1	–0.4	0.4	391.11	124	0.04	6.2	0.19	0.048
C14	10 Feb 2009	5	2	0.0	0.4	396.25	105	0.04	5.2	0.16	0.040
Median				–3.7	0.3	315.63	252	0.03	12.6	0.19	0.054
Max.				5.3	0.8	424.71	508	0.17	25.3	1.2	0.42
Min.				–33.0	0.3	274.24	82	0.01	5.2	0.06	0.021

*Corrected for system drift
†Including error for system drift
‡Classified as outlier
§Incubated in temperature-controlled water baths inside a temperature-controlled room

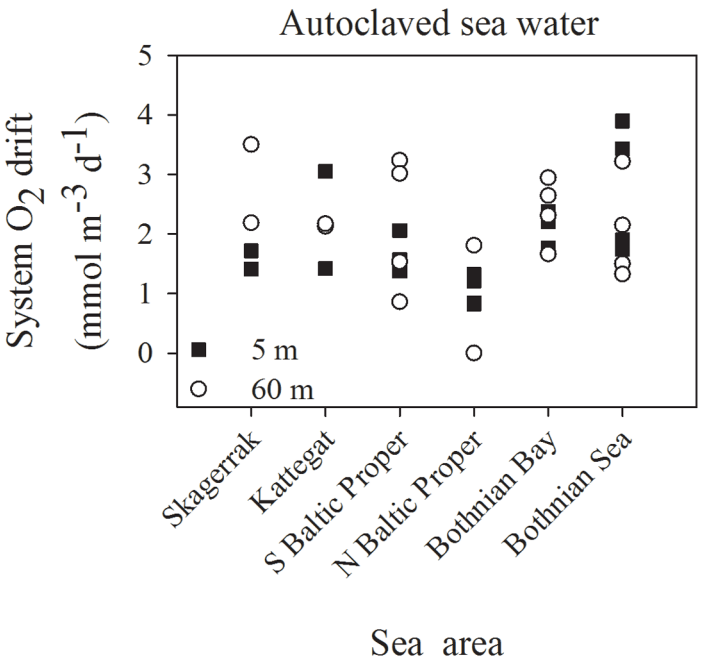

Fig. 5. Summary of the system oxygen drift in autoclaved brackish and oceanic environments at differerent depths.

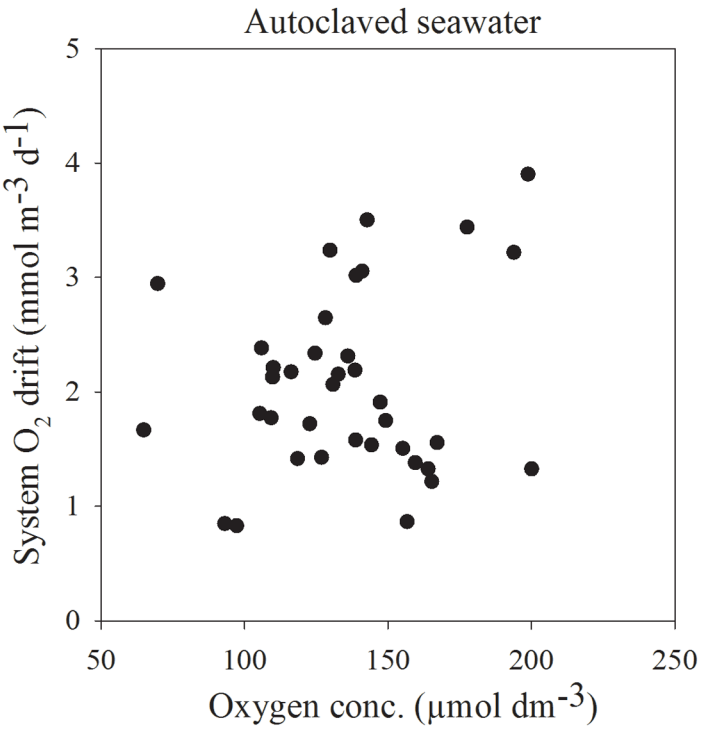

Fig. 6. Lack of dependence of oxygen change in autoclaved seawater samples (i.e., system drift) on oxygen concentration.

Table 4. Effect of preconditioning of stopper and Optode in low oxygen (7% O_2 saturation) water and incubation in N_2 atmosphere. The 95% CI is shown.

	N_2 treated		Control	
	Oxygen change	95% CI	Oxygen change	95% CI
Incubation	(mmol m^{-3} d^{-1})			
1	0.52	0.017	1.72	0.031
2	0.37	0.010	1.37	0.026
3	0.46	0.019	1.07	0.030
4	0.33	0.029	1.61	0.041
5	0.35	0.012	2.05	0.025
Average	0.41	0.097	1.57	0.46

$$\frac{dO_2}{dt} = 1.64 \times e^{(0,11 \times t)} \quad (5)$$

The temperature dependence of oxygen consumption corresponded to a Q_{10} –value of 3.0, comparable with the levels found in earlier studies (Lefevre et al. 1994; Vazquez-Dominguez et al. 2007). This result suggests that the Optode method accurately measures respiration rates with good precision when a system drift correction is applied.

Application to field studies

To test the Optode method during field conditions, measurements were conducted during environmental monitoring expeditions. The samples covered salinities between 2.5 and 35 g kg^{-1}and temperatures of 1.7-17.7°C (Table 5). At higher oxygen decline rates (over 7 mmol m^{-3} d^{-1}) and stable temperature conditions, overall linear decreases were readily identified (Fig. 9, 5 m; Table 3). These data could be used for slope estimation after 1 h and, in many cases, even earlier. Duplicate respiration estimates from the same Niskin bottle also showed good correspondence, with an average SD of 0.73 mmol m^{-3} d^{-1} (SD ± 0.50, n = 8).

When temperature control was reduced due to ship movements at rougher sea, and possibly, due to variations in the incubator power supply, temperature curves, and consequently, oxygen measurements showed more hour-to-hour variability (Fig. 10, 5 m). The temperature variability explained, on average, 37% (median R^2, n = 125, linear regres-

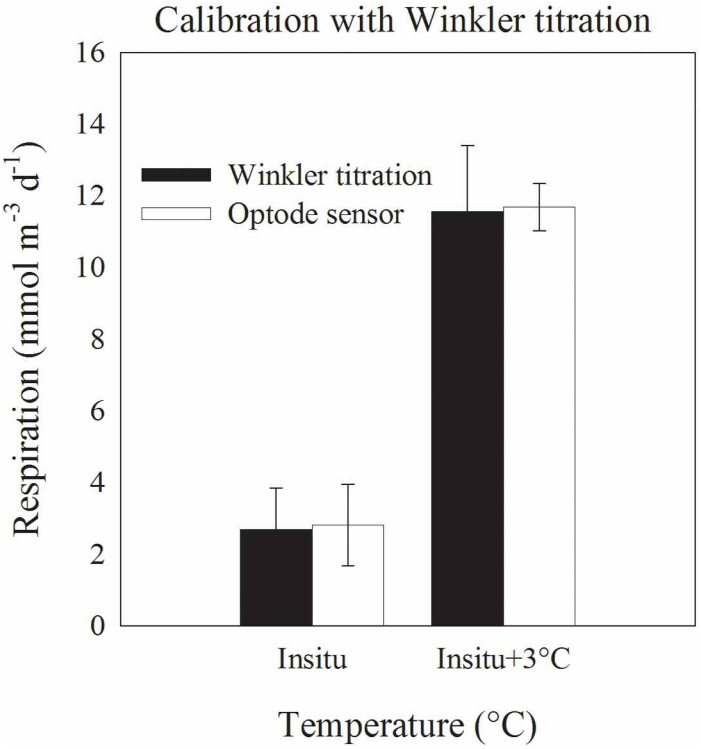

Fig. 7. Comparison between Winkler titration (n = 2×8) and the Optode sensor. The Optode values were corrected for system drift. The comparison was done at two temperatures to obtain different rates in parallel incubations. Error lines show the 95% confidence intervals. For the Optode, the sum of regression and drift confidence intervals were used for calculation of the 95% C.I. (n = 2).

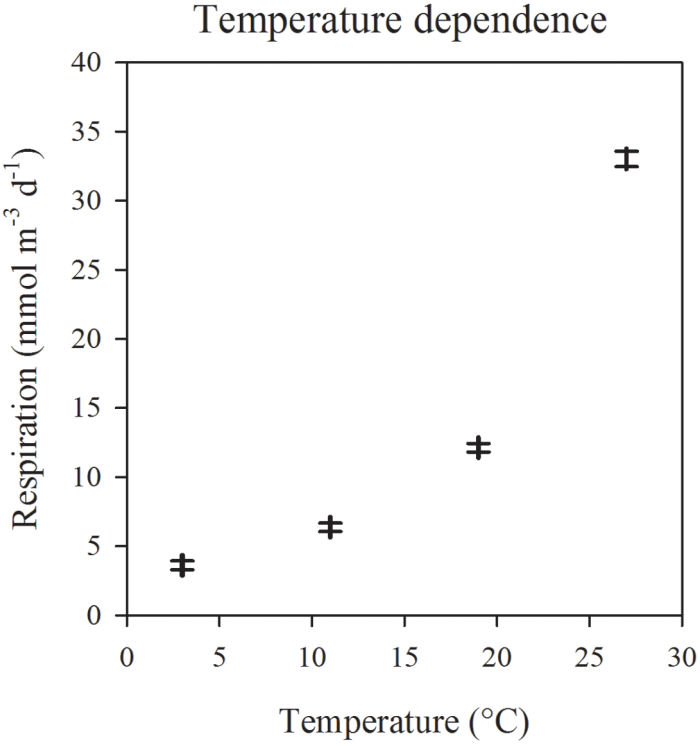

Fig. 8. The temperature dependence of oxygen consumption in seawater is shown. A system drift correction was applied. The sample was from the Bothnian Sea (Stn B3) taken at 1 m depth and incubated in parallel at 4 different temperatures in a temperature controlled room. The in situ temperature was 19°C. Error bars for respiration rates show the 95% confidence interval, including the system drift error. Error bars for the temperature were hidden by the vertical bars.

Table 5. Hydrographical and chemical data from the sampling stations. Oxygen shows in situ values based on Winkler titrations where available.

Station	Date	Depth (m)	Temp. (°C)	Salinity (g kg^{-1})	pH	Oxygen concentration (µmol dm^{-3})	Oxygen saturation (%)	Tot-P (µmol dm^{-3})	Tot-N (µmol dm^{-3})	Chl *a* (µg dm^{-3})
C14	19 May 2008	5	4.46	5.447	8.00	—	—	0.35	16.4	6.0
Å17	09 Jun 2008	5	8.86	33.640	8.32	367.85	126	0.34	10.0	0.6
Å17	09 Jun 2008	60	6.65	34.850	8.15	283.03	93	0.64	13.2	—
B3	29 Jul 2008	5	17.66	3.546	8.04	—	—	0.31	17.3	2.0
C14	10 Feb 2009	5	1.71	5.465	8.01	—	—	0.52	19.2	0.2
Å17	16 Feb 2009	5	3.92	32.226	8.15	322.32	97	0.84	15.6	1.4
B7	03 Sep 2009	5	13.67	4.263	7.90	—	—	0.39	17.1	2.1

sion) of the variation in the oxygen concentration at oxygen decline rates below 2 mmol m^{-3} d^{-1}. At rates higher than this value, the temperature variability explained 18% (n = 29) of the variation in the oxygen concentration. The increased noise at low rates introduced more subjectivity in the selection of the time period for regression analysis. The criterion to stay within ± 0.05°C of the mean temperature for the whole incubation was applied throughout the analyses to objectively select data for regression analysis. The opportunity to base respiration estimates on oxygen saturation rather than absolute oxygen values from the Optode was tested, but this method was not found to improve the precision or detection limit.

This result demonstrated that significant variability in the time series could disturb measurements at low respiration rates. This limitation was at least partly due to an inability to

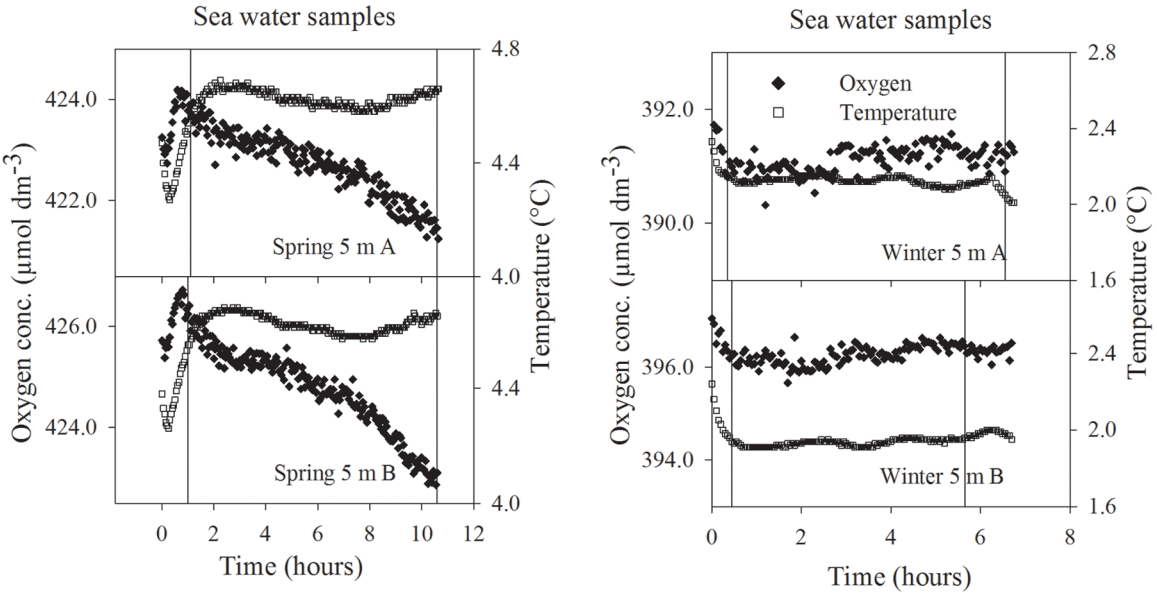

Fig. 9. Examples of primary data in duplicate time series from the same Niskin sample bottle during spring and winter from the same sampling station and depth (5 m). Both oxygen concentrations and temperature during the incubation are shown. No correction for drift was done in this case. Samples were collected from station. C14 in the northern Baltic Sea on 19 May 2008 and 10 Feb 2009. The full time series from the start of the incubations are shown. Vertical lines indicate the time period selected for determination of the rate of oxygen change where the sample temperature was within ± 0.05°C of the incubation temperature.

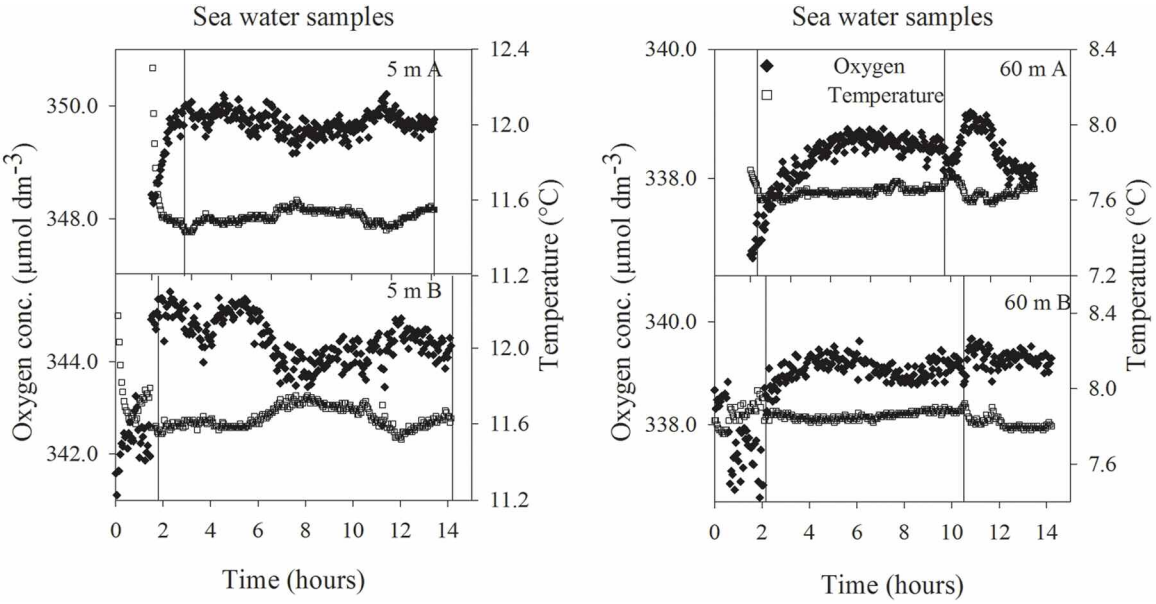

Fig. 10. Same as Fig. 8 but showing samples from station Å17 in the eastern North Sea on the 10 June, at 5 and 60 m depths.

maintain temperatures. In addition, as corroborated by the results of autoclaved samples, the occurrence of net increases in oxygen at low productivity conditions implied the presence of some short-term system drift.

The precision of respiration estimates (i.e., slope coefficient) was, on average, 0.3 mmol m^{-3} d^{-1} (95% CI) and varied little between different measurements (Table 3). The method, therefore, seemed to have good reproducibility, despite the variability observed in the time series. At this level of precision, one-third of the offshore measurements were at or below the detection limit. Pre-conditioning of the stopper and Optode in low oxygen water could, however, improve the detection limit to 0.15 mmol m^3 d^{-1}, close to the high precision Winkler titrations (Table 4).

The reason for the reproducibility of the precision was that the error of the applied system drift correction accounted for the majority of the uncertainty. The uncertainty of the oxygen change in each time series, excluding contribution from drift correction, was one third of the error (median 95% CI ± 0.15 mmol m^{-3} d^{-1}).

Oxygen consumption followed trophic gradients

The respiration derived by the Optode method generally followed expected variations with trophic gradients and temperatures (Tables 3 and 5). This was shown by a positive correlation with simultaneously measured Chl *a* concentrations ($r = 0.65$, $P = 0.040$, $n = 10$). A tendency for higher oxygen consumption in surface water when compared with the aphotic zone was observed based on 95% confidence intervals. Higher values were also found during late spring and summer when compared with winter at both locations. During summer in the eastern North Sea, values were about 2 times higher than in the winter. These observations were expected given that higher productivity and temperature, in general, should promote higher respiration rates on a volumetric basis (Robinson and Williams 2005).

The presented field studies in oceanic to low-brackish water salinities showed comparable rates of oxygen decrease to global marine and estuarine data sets (Table 3 and 7). Respiration rates in oceanic conditions (salinity 33-35) were in the range of 0-3.8 mmol m^{-3} d^{-1}. At the brackish water sites in the Baltic Sea (salinity 3.5-5.5), the range was 0-9.0 mmol m^{-3} d^{-1}. Our brackish water values were well within the range of those reported at different coastal locations (1.7-84 mmol m^{-3} d^{-1}) by Hopkinson and Smith (2005). They were also comparable with values for the Baltic Sea (NW Gulf of Finland; 0.8-9.3 mmol m^{-3} d^{-1}) that have been reported previously in spring to fall (Kuparinen 1987). Our measurements from the eastern coastal North Sea were also within the range found in marine coastal areas (0.1-75 mmol m^{-3} d^{-1}) (Robinson and Williams 2005).

Discussion

The investigated Optode with dynamic luminescence quenching (DLQ) technique showed a detection limit and precision similar to those obtained with the high-precision Winkler titration method (Table 6 and 7). Typical rates of respiration found in coastal waters and oceanic surface water during the productive seasons should, therefore, be above the detection limit.

However, this method does not currently have sufficient precision to routinely monitor respiration rates below the euphotic zone or in oligotrophic oceanic sites that often fall below 0.3 mmol m^{-3} d^{-1}. The same limitation applies to surface and coastal waters during the winter when productivity is lower. The detection limit of the Optode in the field was similar to most reported high-precision Winkler titrations, except from two field studies reporting a 3-fold lower detection limit (Smith and Prairie 2004; Williams et al. 2004, Table 7). The Optode sensor showed the best precision for sensor-based techniques that have been reported in the literature to our knowledge. Briand et al. (2004) also reported a high practical precision of oxygen concentration measurements for an oxygen microprobe (0.5 µM), but did not report the precision of the respiration measurement as such by accounting for the different time series variations they observed.

Table 6. Properties of the investigated Optode sensor. Accuracy shows the relative deviation from Winkler titrations when the drift correction is applied (Fig. 7). Precision shows the combined 95% confidence intervals for field incubations and the drift subtraction (Table 3). The detection limit is defined as one 95% confidence interval. The system drift is the mean of 37 measurements in autoclaved seawater samples (Fig. 5).

Accuracy (%)	Precision (mmol m^{-3} d^{-1})	Detection limit (mmol m^{-3} d^{-1})	System drift (mmol m^{-3} d^{-1})
103	± 0.3	0.3	2.1 (± 0.3)*

*95% CI

The field estimates for a Clark electrode reported in Langdon et al. 1995 was relatively high (6-8 µmol dm^{-3} d^{-1}) and lacked precision estimates or presentation of time series. In the original reference to the method by Langdon (1984), a precision of 2.4 µmol $dm^{-3}d^{-1}$ or higher is reported. This is about 10 times higher than the results we present for the Optode, and even higher compared with high-precision Winkler titration. The electrode drift reported is also a minimum estimate of the experimental system drift (i.e., the "productivity autosampler"), as other sources to changing oxygen concentrations in the whole experimental system are conceivable. The inherent oxygen consumption in older electrode types and lower precision of the Clark electrode is likely the reason that very few studies have applied electrodes in pelagic respiration measurements (del Giorgio and Williams 2005).

The main source of uncertainty was the estimated system drift that, in practice, set the detection limit. The system drift and initial temperature equilibration also limited the selection of data for regression analyses. The potential of the method to match the highest precision Winkler titrations was good provided that the system drift can be removed, markedly reduced, or routinely monitored. The preconditioning in low oxygen water (N_2-bubbling or Na_2SO_3 solution) improved the practical detection limit to 0.15 mmol m^{-3} d^{-1} at land-based laboratory conditions. This showed that the system drift mainly was due to oxygen bound in the Optode and stopper plastic material. Plastics have the characteristic to bind oxygen as previously observed in medical applications (Stevens 1992). Correction for the system drift based on a sufficiently precise estimate was the only measure we currently have for this problem. The correction, however, introduced a 3-times larger uncertainty in the estimates than that provided by the time series alone. Pretreatment of the stopper and Optode in low oxygen, however, showed a potential to approach the best detection limit reported (Table 7). Holtappels (2009) reported similar net

Table 7. Detection limits reported from the literature based on the 95% confidence interval or calculation of the extended measurement uncertainty (2×SE). Where coefficient of variation of the method was given as a percent, SE was calculated assuming 4 replicates at both end points and a mean oxygen concentration of 300 µM. The range of community respiration rates reported in these studies, and the methods used are also shown.

Method	Detection limit mmol O_2 m^{-3} d^{-1}	Respiration rates mmol O_2 m^{-3} d^{-1}	Reference
Photometric Winkler titration	0.1*	0-1.5	Williams et al. 2004
Automated Winkler titration	0.1	—	Smith and Prairie 2004
Potentiometric Winkler titration	0.2†	1.4-7	Biddanda et al. 1994[Shelf]
Optode sensor	0.3	0.3-33	This study
Potentiometric Winkler titration	0.5	0.3-0.8	Sherr and Sherr 2003
Potentiometric Winkler titration	0.6	0.5-14	Duarte et al. 2004
Potentiometric Winkler titration	0.9†	0-11	Biddanda et al. 1994[Slope]
Respirometer	1.0	4.5-54	Griffith 1988
Spectrophotometric Winkler technique	2.0‡	6-10	Maranger et al. 2005
Membrane inlet mass spectrometry	2.1	—	Kana et al. 1994
Respirometer	2.1	0-230	Taylor et al. 2004
Clark electrode	2.4	41-118	Langdon 1984
Automated Winkler	2.8	—	Coffin et al. 1993
Spectrophotometric Winkler	3.4	—	Roland et al. 1999

*Based on reported SE of 0.059 mmol O_2 m^{-3}.
†Assuming 15 samples used in the regression and df = n-2.
‡Using the reported SD of the mean as the SE. A factor of 2 was consistently used as t-value to calculate a confidence interval.

increases in oxygen at low oxygen consumption rates using a comparable Optode system in the Baltic Sea. However, no further investigation of this anomaly was reported. Also, release of oxygen from plastic containers has been observed in deep water sediment-water interface studies (Dominique Lefevre pers. comm.). In other systems with Optodes, such as bottom-landers, neither Drazen et al. (2005) nor Sommer et al. (2008) reported any significant Optode drift over more than 9 h. Signs of system drift of other sensors during the first 10 h can, however, be seen in control samples, although drift rates were not estimated or discussed by the authors (Griffith 1988; Briand et al. 2004).

According to the power analysis for the Optode time series a theoretical detection limit of 0.04 mmol m^{-3} d^{-1} at a 250 µM oxygen concentration in 24 h incubation is possible. This calculation was based on the median coefficient of variation around the regression line of autoclaved samples (CV = 0.066%). Robinson and Williams (2005) calculated a theoretical detection limit of 0.07 mmol m^{-3} d^{-1} for Winkler titrations. The dynamic luminescence quenching technique therefore has the potential to provide continuous measurements of respiration of highest known precision today. The possibility to investigate short-term variation is therefore an advantage of this method. However, according to the power analysis shorter incubation times elevate the detection limit, and rate differences as high as 1 mmol m^{-3} d^{-1} must, e.g., occur to be detected in 3-h incubations.

The primary advantage of the Optode method is that it provides a simple and operator-independent protocol to continuously measure respiration in natural water samples. The Optode can run for several years with annual calibration and the sensor foil alone can be renewed to extend the life-time of the Optode, promoting good economy. This may promote the incorporation of respiration measurements in field studies and make the use of respiration data in environmental monitoring programs feasible. Due to the ability to easily perform many measurements, the Optode technique could lead to new insights about factors that affect respiration rates. Additionally, with a higher rate of data collection in time and space, a better basis for establishing regional and global carbon budgets may be gained. Including respiration measurements in marine monitoring programs would also provide more reliable assessments of ecosystem productivity, and thereby, management of eutrophication.

The developed analytical unit and protocol was able to be used aboard research vessels, even during rough seas, with handling time less than 20 min (4 incubations). The avoidance of wet chemistry minimized operator influence on the results, resources, waste, and improved safety for technical staff. The Optode method, however, required stringent temperature control and evaluation of time series. The stable temperature (± 0.05°C) that was routinely achieved minimized the temperature influence on oxygen measurements. Temperature fluctuations were found to account for 33% of oxygen variability for the whole data set. Temperature equilibration, however, often required rejection of the first hour of the time series used for regression analysis of the rate of oxygen change. The temperature sensitivity of the Optode is mainly

associated with the applied temperature dependent algorithms that already have been improved in the most recent models (Fig. 3, Eq. 4).

One advantage of the presented Optode method is the 3-20 times larger sample volume (1 dm^3) obtained compared with Winkler titrations (0.05-0.3 dm^3). A larger sample includes more organisms. This is advantageous if a general estimate of plankton oxygen consumption is the study aim. A larger sample also reduces other containment effects and allows extended incubation time. A 1 dm^3 sample would include nanoplankton up to 50 µm in a statistically acceptable number of 10 dm^{-3} (Robinson and Williams 2005). Additionally, mesozooplankton may occur well above 10 individuals per dm^3 in coastal areas during the productive season, and therefore, can be included in the measurements, then comprising on average 99% of aquatic respiration. Typical volumes in Winkler titrations, however, would only contain a sufficient number of individuals less than 20 µm in size (i.e., mainly bacteria and flagellates). Theoretically, larger sampling volumes would result in higher respiration values than have been currently reported by Winkler titrations. However, given that the contribution from larger zooplankton, in general, is considered to be small, we currently do not see a need to reinterpret results based on Winkler titrations. The similarity in levels and responses to environmental factors between the Winkler and Optode techniques found in this study supports this conclusion.

The online continuous monitoring of the oxygen concentration by computer software precluded assumptions of linearity of oxygen change. This also enabled a correct mathematical model to be applied for rate determination. However, approximately linear development with time was typically observed at most sites investigated. This finding was partly disturbed by imperfect temperature control at low respiration levels and during temperature equilibration. Therefore, the assumption of linear decline often assumed in end point Winkler titrations seems valid, at least in low productivity ecosystems. The results from this study, therefore, do not indicate a need for revision of Winkler technique estimates.

Comments and recommendations

We recommend pre-incubating the stopper and Optode in low oxygen water 24 h before measurements to remove oxygen bound to the plastic material. This improved the detection limit to 0.15 mmol m^{-3} d^{-1}. Development of stopper without plastic material and using a titanium Optode is another option, that however may increase cost of the equipment. Use of a Fibox system with a PSt3-foil glued to the wall requires a specific evaluation as an alternative system, due to a risk of reduced signal strength by measuring through the glass wall, use of multiple fibers, and lack of internal temperature compensation.

Poor temperature control may introduce both elevated variance and drift in the time series. Access to high-precision incubators (i.e., ± 0.01°C) and temperature-controlled rooms may, therefore, limit the number of sample types or treatments that can be performed at different temperatures (e.g., depths). Alternatively, in sealed temperature-controlled samples manual calculation of oxygen concentration using a constant temperature may be applied.

The initial variability may derive from a temperature equilibration between the sample, indicator layer, and temperature sensor of the Optode. The temperature sensor of the 3835 Optode is located a few centimeters away from the oxygen sensor film, and this distance may initially influence the automatic temperature compensation. A temperature sensor located closer to the dynamic luminescence membrane and improved algorithms for calculating O_2 concentration at different temperatures have been included in the latest generation of Optodes (model no. 4330). In addition the latest generation of these sensor can be multipoint calibrated (e.g., at 5 temperatures and 8 oxygen concentrations), which should reduce the temperature dependence of the signal further.

The response time before accurate oxygen measurements are provided by the DLQ-membrane is 25 s, indicating that delays in the membrane chemistry are an unlikely explanation for the observed system drift or the initial oxygen anomalies (Tengberg et al. 2006). Pressure variation could neither contribute to the observed variations, as measured pressure in the experimental bottles varied within 2% (about 2 kPa) without correlation to oxygen concentration (data not shown).

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Submitted 27 June 2012
Revised 2 November 2012
Accepted 22 December 2012