Global warming is expected to affect the metabolism and oxygen concentrations in natural streams. Higher temperature may lead to higher respiration of biota and thus a higher risk of oxygen depletion. It is, therefore, crucial to obtain reliable data on the oxygen dynamics in the different stream compartments. Determination of sediment oxygen demand (SOD) is typically based on lab or field measurement using cores or benthic chamber in which the actual physical conditions in the streams are not possible to mimic perfectly. We compared SOD based on lab core incubations with SOD measured in situ in stream sections where the oxygen exchange between water and air was eliminated artificially. The in situ SOD increased with increasing oxygen concentrations and both the temperature and the oxygen dependency of SOD increased with increasing organic content in the surface sediment. The laboratory rates reached 17 - 83% of the rates obtained in situ. The percentages were especially low at low stream velocity, likely reflecting a pure imitation of the physical conditions near the sediment in the lab when the sediment organic content was high (at low velocity). Therefore, alternative methods, simulating the natural horizontal water flow, are needed to provide reliable information on SOD in streams.

**Keywords:** Metabolism, running waters, sediment, water, oxygen
aeration was eliminated by covering the stream surface with a plastic film during the experiment. It is well established that SOD is dependent on the temperature, oxygen concentration and organic matter in the sediment [10]. Although, the relationships established are all based on measurements in incubation chambers. To what extent such relationships can be transferred to the field is an open question given the different physical conditions mentioned above. Therefore, our second aim was to re-examine the dependence of the field estimated SOD on the oxygen concentration, temperature and sediment organic matter content.

EXPERIMENTAL

Study area and in situ experimental set-up: This study was performed in the upper part of the River Suså, which is situated on Southern Zealand, Denmark [11]. The river runs through agricultural areas and receives a moderate amount of sewage, mainly from diffuse sources. Three 75 m long stream sections were chosen, varying in surface slope from 0.39 to 1.18 km⁻¹ (Table 1).

Table 1. Hydraulic and hydrometric data for the three sections studied in the Suså watershed in July-October

| Substratum type   | Section 1 | Section 2 | Section 3 |
|------------------|-----------|-----------|-----------|
| Surface slope (m km⁻¹) | 0.69      | 0.39      | 1.18      |
| Mean velocity (m sec⁻¹)  | 0.14 - 0.34 | 0.06 - 0.12 | 0.09 - 0.21 |
| Mean depth (m)         | 0.15 - 0.25 | 0.20 - 0.26 | 0.14 - 0.34 |
| Mean width (m)         | 2.5       | 4.0       | 2.0       |
| Discharge (L sec⁻¹)    | 49 - 240  | 65 - 136  | 23 - 107  |
| Substratum type        | mud, sand | mud, silt | on silt   |

Fig. 1. Diagram of the experimental set-up in situ.

a. To prevent plant re-establishment and benthic algal or macrophyte growth, the sections were kept dark by covering the experimental sections of the streams with wood frames (3.6 × 5.4 m) with tarpaulins, positioned on the stream banks; b. To avoid exchange of oxygen between water and air (re-aeration) a plastic film was rolled out on the water surface after removing the wood frames and measurements were conducted in upstream and downstream with the same water mass (see further details in the text).

Sections 1 and 2 were situated in the River Suså itself, whereas section 3 was situated in a small tributary, River Sneslev Lilleå. Variations in stream velocity, depth and discharge during the study are given in Table 1. All sections were characterised by dense vegetation of macrophytes and filamentous algae in summer. The study was conducted from June to October. In June, the vegetation of three sections was cut and removed. To prevent plant re-establishment and benthic algal or macrophyte growth, the sections were kept dark by covering the experimental sections of the streams with a number of wood frames (3.6 × 5.4 m) with tarpaulins, positioned on the stream banks (Fig. 1a). The sections were adapted to the new conditions for three weeks before initiating the measurements.

In situ measurements: The in situ measurements were conducted over 1-3 diurnal periods (Table 2). In each section, the tarpaulin frames were removed, and a dark polyethylene plastic film with 0.1 mm thickness was rolled out on the water surface from the downstream to the upstream end of the sections by two persons, one on each bank, to avoid disturbing the sediment (Fig. 1b). The film was fastened at the banks with canes, and all air pockets were removed by pouring water over the film. The oxygen permeability of the plastic film is negligible, and the film can therefore be considered to eliminate re-aeration as well as photosynthesis (under dark condition). Then, the residence time of the water in each section was estimated by injecting an acetic-methanol solution of Rhodamine B in a transversal line 15 m upstream the beginning of the sections and following the traverse of the substance through the sections. At intervals (8 to 19 times during the 1-3 diurnal periods), a water volume was followed from upstream to downstream of the experimental reach, and temperature and oxygen were measured when the water entered and left the plastic covered sections, the latter based on the residence time obtained with the Rhodamine B injections. The oxygen concentration was measured by a chemical method, the Winkler technique [12]. Since re-aeration was eliminated using dark plastic film, the total oxygen consumption could be estimated as the difference in the oxygen transport between the upstream and downstream of the section. The oxygen consumption of the water was estimated from incubations of stream water in 125 ml Jena glass bottles covered with tinfoil based on Winkler titration before and after incubation. Sets of three samples were incubated at different temperatures (±0.5 °C), reflecting the variation in stream temperatures. Turbulence was obtained by magnetic stirring, and the incubation period (5-15 h) was adjusted to reduce the oxygen concentration by 10-20%. The discharge in the different section was estimated either from measurements of stream velocity with an Ott-propeller or from measurements of residence time and mean depth.

Lab measurements: In the lab, SOD was measured on undisturbed sediment cores taken with Perspex tubes.
(length 40.5 cm, diameter 5.2 cm and thickness 0.3 cm) (Fig. 2). Six to twelve cores were taken randomly in each section on each sampling occasion. The depth of the sediment was 10-15 cm. The cores were placed in the dark at the actual stream temperature (±1.5°C) not later than 1.5 h after sampling. About 80% of the water was replaced by GF/C-filtered (0.45 µm) stream water without disturbing the sediment. Homogeneity in the water was maintained by a magnetic stirrer, placed outside the core (with a magnet inside) (Fig. 2) as close as possible to the sediment but without disturbing it [13].

Fig. 2. Diagram of the core experimental system used in the lab.
1. Magnetic stirrer;
2. Cored sediment.

The cores were stirred for about half an hour before they were sealed with rubber corks. Stirring continued during the incubation as well. The incubations lasted for 2-10 h and a 0.5-1.5 mg O₂ L⁻¹ reduction of the oxygen concentration was obtained. SOD was measured before and after the experiments by Winkler method. The mean concentrations of the two measurements were used to describe the experimental oxygen concentration.

Following the SOD estimates, the upper 3 cm of the sediment was collected and analysed for ash free dry weight (AFDW) by first drying at 105 °C to constant weight and then burning at 550 °C for 6 h. Baity (1938) [14] found only increasing oxygen consumption in the interval 0-3 cm, and others [1] found no changes in the oxygen consumption between 2.5 and 10 cm in sediments from a river similar to River Suså. The upper 3 cm, therefore, appear to be a reasonable thickness to consider.

RESULTS AND DISCUSSION

In situ SOD was estimated by subtracting the water oxygen consumption from the total oxygen consumption as found by the in situ mass balances. The water oxygen consumption varied between 1 and 20 mg O₂ m⁻² h⁻¹ and was always 1-2 orders of magnitude lower than SOD (Table 2). The relationship between temperature (T, °C) and water oxygen consumption (Y, µg O₂ L⁻¹ h⁻¹) was Y = 0.0095 T²⁻¹.³³ (r² = 0.81, n=34) based on data from all sections and periods. SOD varied between 0.05 and 1.53 g O₂ m⁻² h⁻¹ (Table 2) with maximum values in July-August. In all sections, the diurnal variations in SOD were considerable. The maximum values were 2.1-4.8 times higher than the minimum values. As expected, the oxygen consumption was higher when the oxygen concentration and temperature were high (e.g., afternoon) and low when the oxygen concentration and temperature were low (before sunrise) (data not shown).

| Date       | Oxygen consumption (mg O₂ m⁻² h⁻¹) | Oxygen concentration (mg O₂ L⁻¹) | Temp. (°C) | Mean Temp. (°C) |
|------------|----------------------------------|---------------------------------|------------|-----------------|
|            | Water                            | Sediment                        |            |                 |
| Section 1  |                                  |                                 |            |                 |
| 25-26/7    | 8-20                             | 3.9-17.8                        | 16.3-21.1  | 18.3            |
| 10-13/8    | 3-11                             | 3.7-10.1                        | 12.2-18.4  | 15.5            |
| 29/8-1/9   | 2-7                              | 6.5-10.9                        | 10.4-14.8  | 11.9            |
| 26-30/9    | 2-6                              | 9.0-11.5                        | 9.0-12.6   | 10.9            |
| Section 2  |                                  |                                 |            |                 |
| 10-13/8    | 6-12                             | 3.3-20.1                        | 13.0-16.9  | 15.0            |
| 29-31/8    | 3-9                              | 5.3-10.8                        | 9.9-14.8   | 12.4            |
| Section 3  |                                  |                                 |            |                 |
| 10-13/8    | 3-7                              | 2.6-9.1                         | 11.5-15.3  | 12.5            |
| 29/8-1/9   | 4-6                              | 3.7-6.9                         | 10.4-12.8  | 11.6            |
| 27-30/9    | 1-3                              | 5.2-7.6                         | 8.2-11.0   | 9.6             |
| 13-15/10   | 1-3                              | 6.4-9.1                         | 8.8-11.0   | 9.8             |

Table 2. Minimum and maximum values of the in situ oxygen consumption, oxygen concentration and temperature

Temperature and especially oxygen concentration showed great diurnal variations (Table 2). The in situ SOD dependency of these two variables in each period and section were, therefore, estimated (least squares fit) from the frequently used equation [15]:

\[
SOD = a (\text{ox})^b \theta^{(\text{Temp}-20)}
\]

where: SOD is mg O₂ m⁻² h⁻¹, oxygen concentration (ox) is mg O₂ L⁻¹, temperature (temp) is °C, and a, b and θ are constants.

The regression coefficients varied between 0.53 and 0.98 (Table 3), being high in periods with pronounced variations in temperature and oxygen concentration and low with small variations. The estimated θ-values varied between 0.97 and 1.20 (Table 3).

Table 3. Linear regression on log-transformed data using Eq. 1; r² is the determination coefficient, and a, b, θ are constants. Also shown is the mean water temperature during the periods (for variation see Table 2)
In section 3, which had highly silty sediment, the $\theta$-values were generally high compared with the other sections. Except for one measurement, there was a tendency to obtain increasing values of $\theta$ with decreasing mean temperature (Table 3). The estimated $b$-values varied between 0.62 and 2.30 (Table 3). Both $\theta$- and $b$-values varied widely between sections at the same time and in each section with time.

The $b$-values were significantly correlated with the sediment AFDW (Fig. 3), with about 61% of the variance being explained. SOD was standardised to 10 °C and 8 mg O$_2$ L$^{-1}$ (to be able to compare the results) based on Eq. 1 and the parameters are given in Table 3 for all sections and sampling periods. There was no significant correlation between SOD and sediment AFDW ($p > 0.05$) (Fig. 4). However, there was a significant decrease in SOD with decreasing temperature (Fig. 5).

For each date and section, the SOD found by the laboratory method was compared to that obtained by the in situ method. The laboratory data were corrected to actual field temperatures and oxygen concentrations using established relationships between laboratory SOD, temperature (temp) and oxygen concentration (ox):

$$SOD = a (ox)^{0.75} (temp-20)$$

($n=37$, $r^2=0.85$, $p < 0.001$)  \(2\)

In all cases, the laboratory SOD estimates were lower than the in situ estimates, ranging from 17% to 83% of the in situ estimates, and the percentage increased significantly with increasing mean stream velocity (Fig. 6).

In our simplified stream ecosystems without re-aeration and photosynthesis, the oxygen consumption of the water was 1-2 orders of magnitude lower than of SOD (Table 2). The low contribution of the oxygen consumption in the water agrees with other studies from natural streams [16]. SOD in small streams during the summer is reported to vary between 0.7 and 82 g O$_2$ m$^{-2}$ d$^{-1}$ and mostly ranges within 10-35 g O$_2$ m$^{-2}$ d$^{-1}$ [17]. Our findings from streams without macrophytes varied between 0.05 and 1.53 g O$_2$ m$^{-2}$ h$^{-1}$ (Table 2), corresponding to 1.3 and 37 g O$_2$ m$^{-2}$ d$^{-1}$, and is thus within the range previously reported. In 34 Australian waters [5], however, using an in situ respirometer found overall lower values of 0.1-1.3 (mean 0.6) g O$_2$ m$^{-2}$ d$^{-1}$ in systems dominated by benthic algae; and the values between 1 and 7 g O$_2$ m$^{-2}$ d$^{-1}$ were recorded in various sections of the Jordan River in Utah using different
types of in situ respirometers [4]. Thus, their values were overall lower than our field SOD estimates in summer (Table 2). We found no significant correlation between the level of in situ SOD, at fixed temperature and oxygen concentration, and the sediment AFDW (Fig. 4). This concurs with [18] and [19], while [3] found a significant linear relationship with total organic carbon. Different results may reflect variation in the degradability of the organic matter [20, 21], water exchange in the sediment [22] as well as the numbers and activity of the macroinvertebrates present [1].

We found large variations in the oxygen dependency of in situ SOD. The b-values calculated for sections 1 and 2 varied between 0.62 and 1.56 (Table 3), although the latter was not significantly greater than 1.0 (p>0.05). Using a mud core laboratory method, a mean b-value of 0.45 for the sediments from three unpollluted rivers and b-values close to 1.0 in a river polluted with wastewater were found [1], indicating a shift from an oxygen consumption limited by organic matter to a consumption limited by the oxygen concentration, i.e., an approximately 0 order and 1 order reaction, respectively [23]. In another study b-values between 0.30 and 0.66 were found [19].

We found major differences between in situ SOD and the mud core lab method, with decreasing deviance with increasing stream velocity (Fig. 3). This might be explained by the placing of the stirrer in the lab set-up. At lower stream velocities, the upper sediment consisted of fine particles, and the stirrer was placed high above the sediment surface to avoid sediment resuspension. This leads to a locally high mixing just below the stirrer and a strong velocity gradient from this point. At higher stream velocities, the upper sediment consisted of coarse particles, and the stirrer was placed just above the sediment surface. Consequently, the velocity gradient across the sediment surface was smaller and probably gave a better simulation of natural stream conditions. Supporting this view, Mackenthun and Stefan (1995), using a lab channel system, found a flow effect on SOD with a major increase in SOD with increasing velocity up to a certain threshold that depended on the type of sediment and biota [24], and with the highest effect of velocity on organic sediment, as in our study. Furthermore, they found that SOD in cores were approximately the same as those obtained with a lab flow-through system when the sediment was sandy [2], but lower when it was organic rich. Others have used closed benthic respirometers of various kinds [2-5], but such chamber systems are also sensitive to resuspension if the sediment is organic rich, and the stirring has to be adjusted accordingly. Thus, in a comparison of SOD in cores, an in situ respirometer and an in situ tunnel respirometer in English streams [25], showed that both the core and the in situ respirometer method underestimated SOD compared to the rates obtained with the tunnel respirometer and from mass balance calculations, the results from the laboratory core method showing the greatest percent deviation. In their study, the mass balances were more uncertain than in our study, however, as correction for re-aeration had to be done to be able to make the comparison with the other methods. Nevertheless, their results clearly indicate that the tunnel method, allowing horizontal flow over the sediment and in the surface sediment, was superior to the others. There are, however, three serious problems with this method: 1) the tunnel cannot be placed in the stream without disturbing the sediment, 2) the water volume in the tunnel is difficult to measure, and 3) the tunnel must be long enough e.g., 20-30 m, depending on sediment SOD to trace significant changes in the oxygen concentration from upstream to downstream. An alternative is to use flow-through chambers [26], perhaps positioned at the stream banks to minimise the effect of transport and to create more realistic light conditions [7].

CONCLUSIONS
Our results demonstrate 1) that lab measurements of SOD substantially underestimate the actual SOD in natural streams, not least when the sediment is organic rich and easy to resuspend; and 2) that the horizontal flow-through systems, perhaps positioned at the bank of the stream, may provide more realistic SOD uptake values.

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