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Patterns of dark respiration in aquatic systems

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Abstract. We used continuous measurements of dissolved oxygen (DO) in dark bottles to characterise patterns of the dark respiration rate (R_{dark}) for three marine phytoplankton monocultures and in natural-water samples from two marine coastal systems. Furthermore, patterns of ecosystem community respiration rate were determined from open-water changes in DO in a fjord and in a lake. We considered two models of R_{dark} to describe temporal changes in DO: constant R_{dark} and decreasing R_{dark}; increasing R_{dark}. In addition, the effect of incubation time on R_{dark} was investigated in bottle incubations. Constant R_{dark} was observed in short-term (12-h) bottle incubations in natural-water samples from two marine coastal systems. Declining R_{dark} was observed in marine phytoplankton cultures and open-water measurements in a lake. Increasing R_{dark} was observed in open-water measurements in a fjord, particularly during summer. Long-term (120-h) bottle incubations in natural-water samples showed an increase in R_{dark} after 48 and 72 h. We show that the conventional expectation of constant rates of respiration in darkness is far from typical, because non-linear changes are common under both controlled experimental conditions, as well as for open-water measurements of ecosystem respiration.

Additional keywords: bacteria, coastal ecosystems, lake, optode, phytoplankton.

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Introduction

Respiration is a key process in aquatic systems (del Giorgio and Williams 2005). In plants, the process occurs concomitantly with photosynthesis and provides energy for conversion of the immediate simple photosynthetic products into biomass and energy for uptake and, if necessary, assimilation of inorganic nutrients. Respiration by heterotrophs, primarily bacteria, is tightly coupled to remineralisation of organic carbon and carbon cycling. The balance between photosynthesis and respiration represents net ecosystem production, which contributes to the available carbon source for higher trophic levels in a food chain. Therefore, a better understanding of the dynamics of carbon cycles and the trophic conditions of aquatic systems requires knowledge of both photosynthesis and respiration. Nevertheless, far more attention has been given to photosynthesis than to respiration. Robinson and Williams (2005) showed that respiration measurements only accounted for 1% of the primary production measurements globally.

Despite the longstanding recognition that respiration in aquatic plants and aquatic systems can vary over hourly time scales (Jackson and McFadden 1954; Markager and Sand-Jensen 1989; Markager *et al.* 1992; Sampou and Kemp 1994), studies of respiration have often assumed that respiration is constant rather than variable at shorter time scales. However, short time patterns in the dark respiration rate (R_{dark}) may reveal information about the kinetics of the metabolic activity of autotrophic and heterotrophic organisms driving the respiration patterns (Sadro *et al.* 2011, 2014).

Aerobic respiration in aquatic systems is carried out by both autotrophic and heterotrophic organisms, but the substrates for their respiration are fundamentally different. According to the model suggested by Markager and Sand-Jensen (1989) and del Giorgio and Williams (2005), the substrate for respiration in algae is their internal pool of carbohydrates or lipids derived from photosynthesis, although uptake of simple dissolved organic compound can occur (Markager and Sand-Jensen 1990). Because these intracellular pools of organic compounds accumulate during daytime photosynthesis, algal respiration during daylight and shortly after sunset can be significantly higher than respiration rates after a long time (>12 h) in darkness, which will be dominated by physiological maintenance metabolism (Markager *et al.* 1992). The substrate for heterotrophic bacterial respiration is a combination of labile, semilabile and more recalcitrant organic matter supplied either directly from autochthonous processes, from allochthonous sources or based on old accumulated organic matter (del Giorgio and Williams 2005). Because auto- and heterotrophic activity and the source for respiration usually vary significantly over a day and a season or between systems depending on environmental conditions, patterns of night-time

respiration rates are expected to vary significantly (Sadro *et al.* 2011, 2014).

R_{dark} is traditionally determined by following the consumption of dissolved oxygen (DO). Approaches using electrodes, fibre-optic oxygen sensors (optodes; Markager and Sand-Jensen 1989; Markager *et al.* 1992; Briand *et al.* 2004; Sadro *et al.* 2011, 2014) or Winkler titration (Pomeroy *et al.* 1994; Hansen and Bendtsen 2014) to follow DO show that R_{dark} is not always constant over time in darkness. Algal respiration can be constant or decrease non-linearly over time depending on previous light exposure (Markager *et al.* 1992; Markager and Sand-Jensen 1994). However, evidence of increasing R_{dark} in community or bacterioplankton has also been found (Briand *et al.* 2004; Gattuso *et al.* 2002; Pomeroy *et al.* 1994) and is suggested to result from increasing bacterial biomass and production (Briand *et al.* 2004). Interestingly, this possibility of increasing respiration was excluded in a recent analysis of ecosystem respiration in lakes using open-water measurements (Sadro *et al.* 2014). The rationale was that any apparent increase in oxygen consumption overnight was most likely related to loss of oxygen due to mixing of surface water with deeper oxygen-depleted water rather than being caused by enhanced metabolic rates. Therefore, there is a need for studies that examine the time course of respiration during dark incubations and open-water measurements to identify patterns of R_{dark} over time and relate these patterns to ecosystem conditions under which these patterns dominate.

The aim of this study was to investigate the occurrence of different types of R_{dark} patterns in aquatic systems. We hypothesised four different scenarios, detailed below.

1. Because respiration is fuelled by a limited substrate pool, respiration would decrease exponentially with time as the substrate pool diminishes (Markager and Sand-Jensen 1989; Markager *et al.* 1992). This would be the case in a phytoplankton-dominated system after light exposure.
2. Respiration is dominated by a growing population with access to excess substrates. This could be a bacteria population (e.g. after a phytoplankton bloom). In this case, we expect an exponential increase in respiration rate.
3. There is a constant supply of substrate (e.g. in multicellular plants with internal reserves or a planktonic system with an external supply) or respiration is due to a grazer-controlled population of bacteria. Therefore, respiration rate is expected to be constant.
4. Finally, a combination of Scenarios 1 and 2 can result in overall constant respiration.

We compared time courses of respiration in phytoplankton cultures and natural samples of seawater using optodes in bottles and at the ecosystem level in both sea and lake water using open-water measurements. In addition, we investigated the patterns of R_{dark} over time in bottles incubated over short (12 h) and long (120 h) periods of time.

Materials and methods

Data sources

We determined R_{dark} from measurements of DO consumption over time using two different experimental techniques: (1) continuous measurement of oxygen concentration by fibre-optic oxygen sensors (optodes; Klimant *et al.* 1995) in bottles incubated in the dark; and (2) *in situ* measurement of oxygen by optodes freely deployed at the surface water (depth 1 m) during the night.

Bottle incubations

R_{dark} obtained from bottle incubations comprised two datasets: (1) incubations of natural surface (1 m) water samples ($n = 6$) collected at monthly intervals from January to December 2012 from two coastal marine waters, namely Roskilde Fjord (RF) and Great Belt (GB; these samples described a seasonal cycle and included the entire plankton community except zooplankton above 200 µm) and (2) data from three marine phytoplankton cultures (*Heterocapsa rotundata* (Dinophyceae), *Rhodomonas salina* (Cryptophyceae) and *Thalassiosira weissflogii* (Coscinodiscophyceae)) in the exponential growth phase incubated over 9 h. Cultures were maintained with L medium (Guillard and Hargraves 1993) under low irradiance (~10 µmol photons m^{-2} s^{-1}) under a 16-h light : 8-h dark cycle at 16°C. Respiration measurements were performed in replicates (in triplicate for *H. rotundata* and in duplicate for the others) after keeping the cultures in the dark for 16 h. Additional information about the respiration experiments is provided in Mantikci *et al.* (2017).

In addition, to examine the R_{dark} patterns occurring during bottle incubations, time courses of R_{dark} from RF and GB were analysed over two time scales leading to two datasets: (1) a dataset of up to 12-h incubations, so called 'short-term' incubations; and (2) a dataset that covered respiration up to 120 h, so called 'long-term' incubations. Experiments were performed in a temperature-controlled water bath in 60-mL biological oxygen demand (BOD) bottles. DO concentrations and temperature were measured continuously every 10 s using FireStingO2 optodes (PyroScience, Aachen, Germany) with a temperature extension module TeX4 (PyroScience). Optically isolated sensor spots were fixed to the inner wall of the BOD bottles. Oxygen concentrations were corrected against temperature using Firesting software (PyroScience; Mantikci *et al.* 2017).

Open-water measurements

DO concentrations were recorded in the fully mixed eutrophic RF at the same location sampled for bottle incubations between March and November in 2012 and in the epilimnion of the eutrophic Lake Vedsted (Obrador *et al.* 2014) during summer stratification between June and August in 2011. In both cases DO was measured using miniDOT oxygen optodes and temperature loggers (Precision Measurement Engineering, Vista, CA, USA) every 10 min near the surface (depth 1 m). In RF, 230 nocturnal time courses were analysed from one station, whereas in Lake Vedsted oxygen was measured at 19 stations, providing 394 nighttime measurements. DO concentrations were corrected for the flux of oxygen across the air–water interface through gas exchange (Cole *et al.* 1998). We limited our analysis to the period between sunset and sunrise, defined as surface irradiance equal to zero.

Calculation of time trends in respiration rates

Based on the hypotheses formulated above, one would expect to see either respiration rates that change exponentially with time in positive or negative directions (Model 1) or a constant

respiration (Model 2). Model 1 is derived as follows: if respiration is proportional to a limited substrate pool and the population is constant (phytoplankton during the night-time), the substrate pool will be (Markager *et al.* 1992):

$$S_t = S_0 - \int_0^t R_0\, e^{-at}\, dt \quad (1)$$

and

$$R_t = R_0\, e^{-at} \quad (2)$$

where S is the substrate pool, R is the respiration rate, t is time and a is a time constant. However, the same model is valid for a growing population where respiration is proportional to the population size (e.g. a bacteria population). Eqn 1 also applies to the external oxygen concentration and, by integration, the oxygen concentration is over time is:

$$c_t = c_0 + \frac{R_0}{a}(e^{-at} - 1) \quad (3)$$

where c is the oxygen concentration. The parameters in Eqn 3 are estimated directly from the oxygen concentrations. In this way, we avoid the calculation of $dc \div dt$, which will enhance noise in the recorded oxygen concentrations. Positive values of a indicate decreasing respiration rate, whereas negative values indicate increasing respiration over time. In the special case where $a = 0$, the respiration rate is constant (Eqn 4) and Eqn 3 is not valid. Then, both substrate and oxygen concentration will change linearly with time (Model 2):

$$S_t = S_0 - Rt \quad \text{and} \quad C_t = C_0 - Rt \quad (4)$$

Only the oxygen is measured here; however, see Mantikci *et al.* (2017) for the relationship between substrate and respiration.

From a statistical point of view it can be argued that the H_0 hypothesis is that $a = 0$ (i.e. a constant respiration) and it is undoubtedly often the case that uncertainty in a encompasses 0 (i.e. often we cannot prove a change in respiration over time). Thus, although we expect respiration to change over time, we also have fitted Model 2 to the data.

Data analysis

DO concentrations in the open-water measurements sometimes showed sudden decreases, increases or fluctuations. To avoid this error in the DO signal, datasets where the model had R^2 values smaller than 0.85 were removed. This filtration criterion was also applied to the data from bottle incubations for consistency. Consequently, 68% of RF open-water samples, 51% of Vedsted Lake open-water samples and 38% of bottle incubations were excluded. The DO consumption over the first 15 min was omitted because the signal was unstable in the beginning of bottle incubations.

Constant respiration over time ($a = 0$) in darkness was considered the null hypothesis and, to evaluate whether space and time have an effect on a, several statistical analyses were conducted. One-way analysis of variance (ANOVA) was used to test for differences between stations and a general linear mixed model (GLMM) was used to evaluate spatial variation between stations and seasonal (at a monthly resolution) variation as fixed factors, and variation among months and station–season interaction in short-term bottle incubations from RF and GB (Staehr *et al.* 2018). We used general linear models (GLM) for open-water measurements from RF and Lake Vedsted. In RF we tested for the effects of daily and seasonal variation and their interaction, whereas in Vedsted we tested for the effects of probe and weekly variation. All analyses were performed using SAS software (ver. 9.4, SAS Institute, Cary, NC, USA).

Results

All three patterns of R_{dark} over time were observed in our datasets (Fig. 1*a–c*). Constant R_{dark} over time ($a = 0$) was often observed in short-term bottle incubations where water samples were collected from coastal waters (Fig. 1*b*). Decreasing R_{dark} (time coefficient (a) > 0) was mostly observed in open-water measurements in the lake and in the phytoplankton cultures (Fig. 1*a*, 2, 3). Increasing R_{dark} ($a < 0$) was observed especially in open-water measurements from RF (Fig. 1*c*, 4). In addition, we observed a combination of the three R_{dark} patterns in long-term bottle incubations. This pattern was described with calculation of R_{dark} over different time intervals with Eqn 4 (Fig. 1*d*, 5). Values of a were positively correlated ($P < 0.001$) with the initial respiration rates (R_0) in bottle incubations and open-water measurements (Fig. 6).

Short-term bottle incubations

In short-term bottle incubations of water collected from GB and RF, replicate means of the coefficient for the change in R_{dark} over time (a) was mostly in the range -0.05 h^{-1} (increasing R_{dark}) to 0.05 h^{-1} (decreasing R_{dark}) with small variation over the 12 months (Fig. 4). There was a significant (ANOVA, $F = 5.61$, $P = 0.02$) difference in a between the two stations, but the effects of season or the station–season interaction were not significant (Table 1). Values of a were significantly ($P < 0.001$) different from 0 (estimated mean $a = 0.115$ h^{-1}) in RF, whereas they were not significantly ($P = 0.71$) different from 0 (estimate mean $a = -0.057$ h^{-1}) in GB. In RF, values of a close to 0.1 h^{-1} with a small variation were observed in March, where they coincided with spring bloom peak in phytoplankton biomass (chlorophyll (Chl)-$a > 7$ µg L^{-1}; see Bentzon-Tilia *et al.* 2015). Yearly average concentrations of Chl-a were 3.1 and 1.8 µg L^{-1} in RF and GB respectively (Table 2).

Experiments with the three algae cultures showed decreasing R with positive a values (Fig. 3). *H. rotundata* and *R. salina* showed similar a values at ~0.05 h^{-1}, whereas *T. weissflogii* showed an a value of 0.15 h^{-1}. Mean Chl-a concentrations were 208 µg L^{-1} for the phytoplankton cultures (Table 2), and oxygen concentrations varied between 235 and 100 µM during experiments.

Open-water measurements

R_{dark} patterns derived from open-water DO measurements in RF differed from patterns obtained using bottle incubations of natural water from RF. The monthly mean of the time coefficient a varied between -0.2 and 0.2 h^{-1} (Fig. 4). There was no significant temporal change in a with either day or season, and

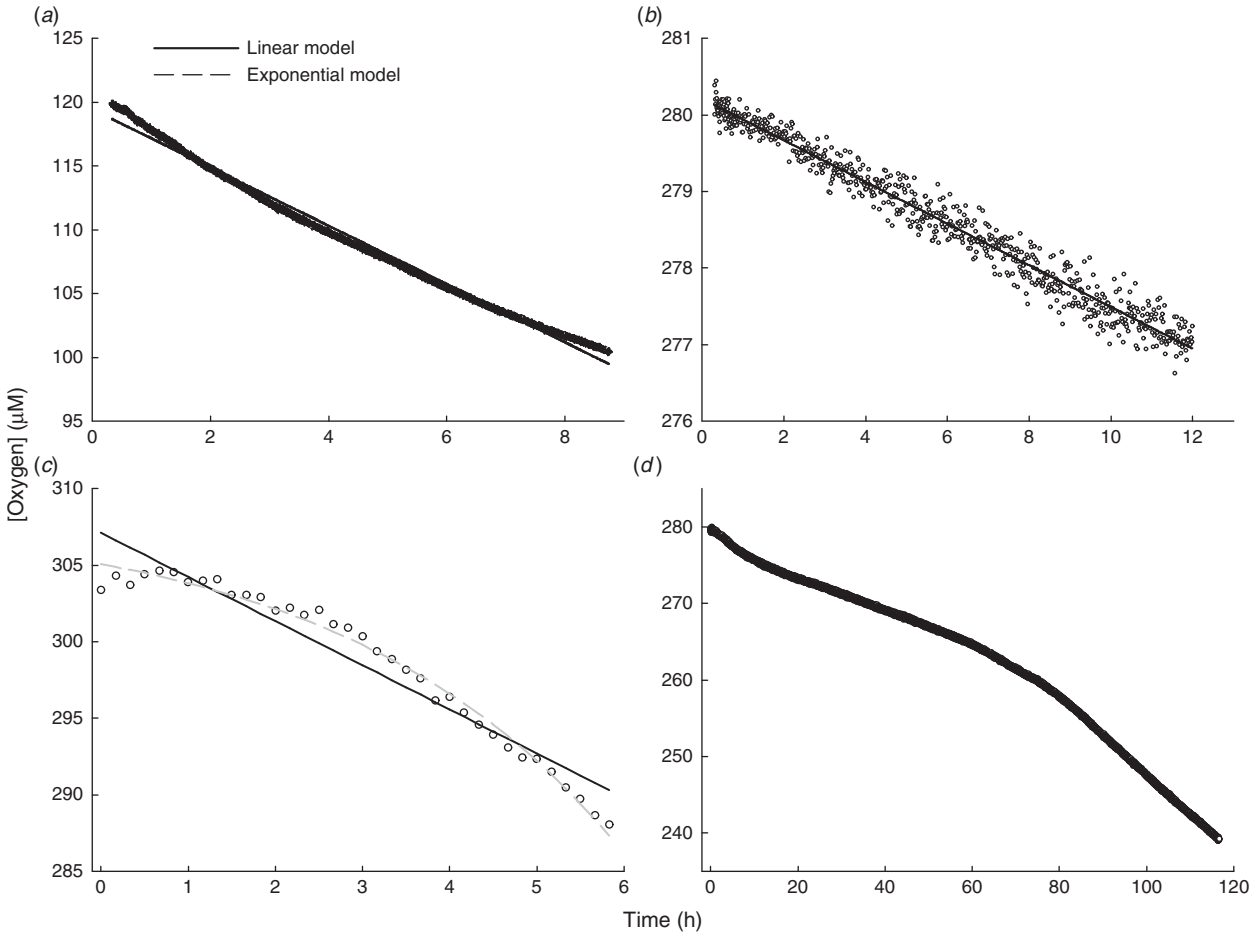

Fig. 1. Examples of dissolved oxygen time series from (*a*) phytoplankton culture, (*b*) short-term (12-h) bottle incubation, (*c*) an open-water measurement and (*d*) long-term (120-h) bottle incubation, and fitted linear (straight lines) and non-linear (dashed lines) models for some of the data.

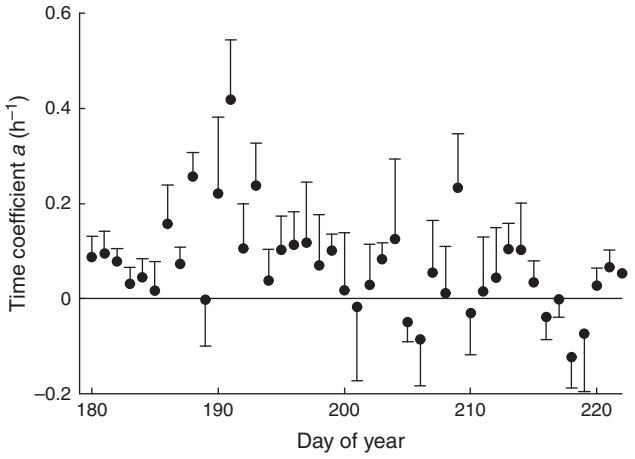

Fig. 2. Time coefficient a (h^{-1}) for a decrease ($a > 0$) or increase ($a < 0$) in respiration rate for open-water measurements from Vedsted Lake over 43 days. Data show the mean ± s.e.m. of replicate measurements.

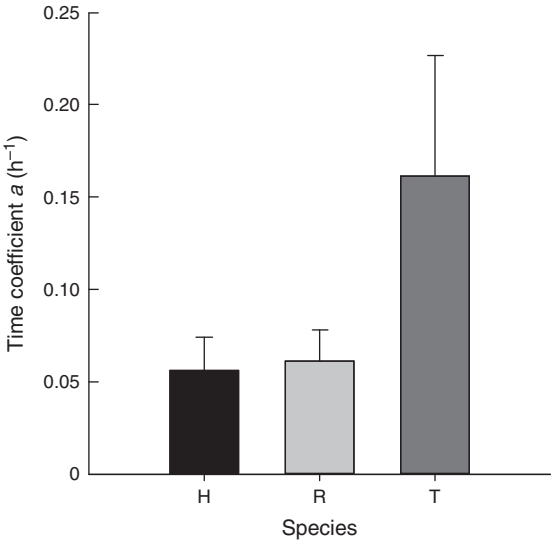

Fig. 3. Time coefficient a (h^{-1}) for a decrease ($a > 0$) in respiration rate for bottle incubations from the three phytoplankton cultures (H, *Heterocapsa rotundata*; R, *Rhodomonas salina*; T, *Thalassiosira weissflogii*) incubated for 9 h. Data show the mean ± s.e.m. of replicate measurements. Data are from Mantikci *et al.* (2017).

no overall effect of their interaction (Table 1). Although seasonally grouped values of a had different mean estimates, they were not significantly ($P > 0.14$) different from 0.

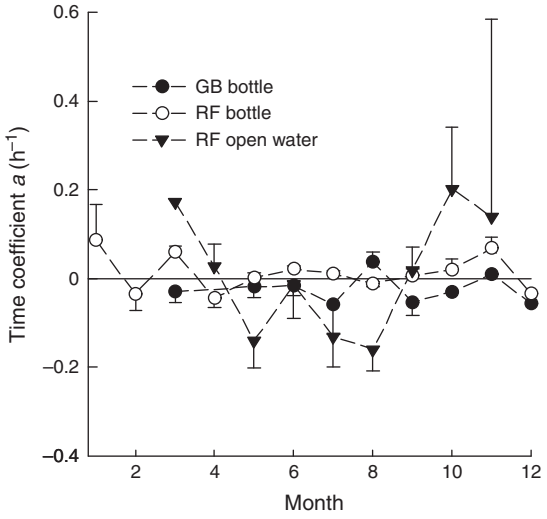

Fig. 4. Time coefficient a (h^{-1}) for a decrease ($a > 0$) or increase ($a < 0$) in respiration rate for short-term bottle incubations and open-water measurements in Roskilde Fjord (RF) and Great Belt (GB) over seasons. For the short-term bottle incubations in RF and GB, data are the mean ± s.e.m. of replicate measurements ($n = 6$); for the open-water measurements in RF, data are monthly mean ± s.e.m.

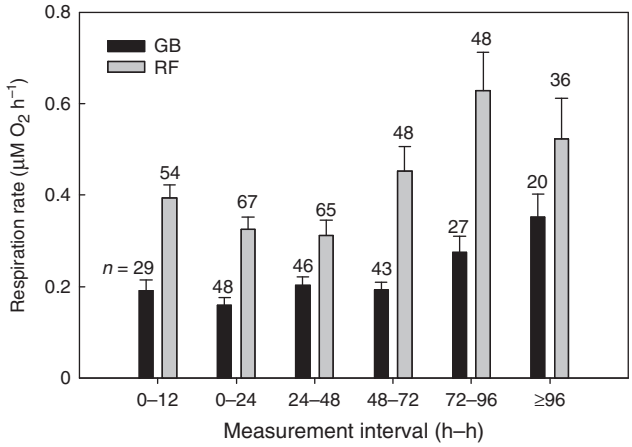

Fig. 5. Respiration rates over different time intervals during the bottle incubations from the Roskilde Fjord (RF) and Great Belt (GB) estuaries. The respiration rates were calculated by Model 1 (Eqn 1). Data are the mean ± s.e.m. of n measurements. Changes in sample size (n) are due to the selection criteria used in curve fitting (i.e. $R^2 < 0.85$ omitted).

In Lake Vedsted, the time coefficient a was significantly ($F = 6.76$, $P < 0.0001$) different from 0 (estimated mean $a = 0.07$ h^{-1}; decreasing R_{dark}) and ranged from −0.1 to 0.4 h^{-1} (Fig. 2). There was a significant weekly temporal development over a 43-day period (Table 1). In addition, there was no difference in a values for the 19 stations used to evaluate spatial variability (Table 1). The mean Chl-a concentration for Lake Vedsted was 41 µg L^{-1} (Table 2).

Long-term bottle incubations

Long-term bottle incubations of water from RF and GB showed three different R_{dark} patterns over time (Fig. 5). When rates were determined for time intervals up to 24 h, rates were higher at 0–12 h than at 0–24 h. After 24 h, rates were almost constant up to 48 h. Then, rates increased at time intervals starting from 48 to 72 h (GB) or from 72 to 96 h (RF). This pattern indicates that R_{dark} tended to increase after 48–72 h of incubation.

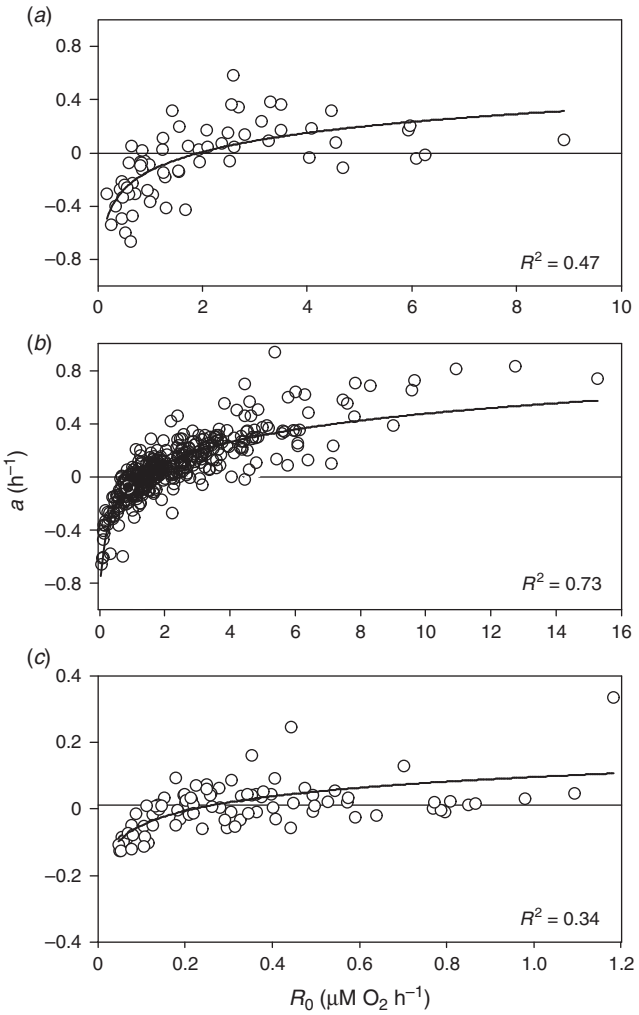

Fig. 6. Non-linear relationship between the time coefficient a (h^{-1}) and the initial respiration rate (R_0; µM O_2 h^{-1}) for open-water measurement from (*a*) Roskilde Fjord, (*b*) Lake Vedsted and (*c*) bottle incubations from Roskilde Fjord and Great Belt. The fitted lines are the ordinary least-squares regression; for (*a*), $a = 0.2055 \times \log(R_0) - 0.1347$ ($n = 66$, $P < 0.001$); for (*b*), $a = 0.234 \times \log(R_0) - 0.0641$ ($n = 394$, $P < 0.001$); and for (*c*), $a = 0.0634 \times \log(R_0) + 0.3398$ ($n = 91$, $P < 0.001$).

Discussion

The observed differences in temporal patterns corroborate the model of del Giorgio and Williams (2005) that characterised aquatic ecosystem respiration as the sum of multiple autotrophic, heterotrophic and baseline components. Accordingly, R_{dark} may vary over a dark period depending on the quantity or quality of organic substrates and distribution of respiratory activity among autotrophs and heterotrophs (Markager *et al.* 1992; Pomeroy *et al.* 1994; Briand *et al.* 2004; Sadro *et al.* 2011, 2014).

Table 1. General linear mixed model and general linear model results for effects of space and time on the time constant *a* (h^{-1})
Significant levels ($P < 0.05$) are highlighted in bold. Degrees of freedom (d.f.) for the numerator (Num) and denominator (Den) are shown along with *F*-values

	Num d.f.	Den d.f.	*F*	*P*-value
Short-term bottle incubations				
Station	1	76	1.93	0.169
Season	3	76	0.12	0.947
Station × season	3	76	0.16	0.921
Open-water Roskilde Fjord				
Day	1	60	2.74	0.103
Season	3	60	0.99	0.403
Day × season	2	60	2.34	0.105
Open-water Lake Vedsted				
Probe	1	386	1.12	0.289
Week	**7**	**386**	**2.68**	**0.01**

Table 2. Chlorophyll (Chl)-*a* concentrations from Lake Vedsted (mean of samples collected January–December 2008; Obrador *et al.* 2014), from three marine phytoplankton cultures (mean of seven culture experiments; Mantikci *et al.* 2017) and from Roskilde Fjord and Great Belt (yearly mean concentrations; Bentzon-Tilia *et al.* 2015)
Data are the mean ± s.d.

Data source	Chl-*a* ($\mu g\ L^{-1}$)
Lake Vedsted	41 ± 21
Phytoplankton cultures	208 ± 126
Roskilde Fjord	3.1 ± 1.5
Great Belt	1.8 ± 0.7

Therefore, differences in time patterns of R_{dark} provide valuable information on the dominating trophic components and the structural composition of the organic carbon pool (Hansen and Bendtsen 2014; Bendtsen *et al.* 2015) in a waterbody.

Our observations of variable temporal changes in R_{dark} give support to previous studies of respiration in phytoplankton cultures and natural planktonic communities (Markager and Sand-Jensen 1989; Markager *et al.* 1992; Pomeroy *et al.* 1994; Gattuso *et al.* 2002; Briand *et al.* 2004; Pringault *et al.* 2007; Sadro *et al.* 2011, 2014). The pattern of pelagic R_{dark} over time describes the sum of all respiratory processes of planktonic organisms contributing to the mineralisation of the different sources of organic matter in the incubated water or *in situ* in the water column. This includes intracellular stores of carbon being respired by the organism itself, such as recently fixed carbon in phytoplankton cells following light periods by photosynthesis (Falkowski *et al.* 1985*b*; Markager and Sand-Jensen 1989; Markager *et al.* 1992; Mantikci *et al.* 2017), as well as respiration of daily accumulated dissolved organic matter by heterotrophic bacteria (Sadro *et al.* 2011). Different kinetics may be expected for the different processes, and therefore the time course of oxygen consumption cannot be adequately described by one simple equation.

We argue that the temporal pattern of R_{dark} provides important information about which respiratory processes dominate in the waterbody: a decreasing respiration rate suggests a first-order process where one homogeneous pool of organic matter decays at a rate proportional to the lability of the pool and temperature, as described previously by Markager *et al.* (1992), Sampou and Kemp (1994) and Hansen and Bendtsen (2014). By contrast, an increasing respiration rate over time could be due to succession within the microbial community in response to concomitant changes in the quality and quantity of the substrate (Gattuso *et al.* 2002) or to changes in bacterial activity (Pomeroy *et al.* 1994; Gattuso *et al.* 2002; Briand *et al.* 2004). A constant R_{dark} could imply that the plankton community is in a steady state and the carbon source they degrade is fairly constant over the period of observation, or it could be due to a refractory substrate that degrades so slowly that it is impossible to distinguish between a first- and a zero-order process. Alternatively, a constant R_{dark} could also result from a combination of the two above patterns, whereby decreasing R_{dark} is balanced out by increasing R_{dark}.

Decreasing R_{dark} *over time*

The clearest patterns of decline in R_{dark} (positive time coefficient a (h^{-1}); $a > 0$) were observed from open-water measurements in the eutrophic Lake Vedsted, in the phytoplankton cultures and in some months from both the bottle incubations and the open-water measurements in RF. In particularly from both the bottle incubations and the open-water measurements in RF, positive values of a coincided with the spring bloom (Chl-a > 7 $\mu g\ L^{-1}$; see Bentzon-Tilia *et al.* 2015). In general, the pattern of R_{dark} was constant ($a = 0$) from open-water measurements in RF (Table 1).

A decreasing R_{dark} pattern has been observed previously in phytoplankton cultures (Falkowski *et al.* 1985*a*; Weger *et al.* 1989; Beardall *et al.* 1994; Xue *et al.* 1996; Ekelund 2000), in plankton incubations from both eutrophic and oligotrophic freshwater (Markager and Sand-Jensen 1989; Markager *et al.* 1992; Sadro *et al.* 2011) and marine (Pomeroy *et al.* 1995; Briand *et al.* 2004; Pringault *et al.* 2009) samples, in free water measurements of a lake ecosystem (Sadro *et al.* 2011, 2014) and in macroalgae (Markager and Sand-Jensen 1994). Despite observations of decreasing R_{dark} patterns in such diverse ecosystems and with different measurement methods, there is a common reason for the pattern: decreasing R_{dark} suggests depletion of labile substrate that could be due either to heterotrophic degradation of extracellular release of freshly produced photosynthate (Sadro *et al.* 2011) or autotrophic degradation of the organic matter pool accumulated during previous periods with photosynthesis (Markager *et al.* 1992; Mantikci *et al.* 2017). In a dense phytoplankton sample, Markager *et al.* (1992) found that the time coefficient a was 0.3 h^{-1} for the first 2 h in the dark and 0.04 h^{-1} for the following 10 h. By comparison, our measurements of R_{dark} in the three phytoplankton cultures conducted after 16 h of darkness resulted in a low time coefficient ranging between 0.05 and 0.15 h^{-1} (Fig. 3). This was likely caused by lower availability of labile photosynthetic products, which, by the time of the respiration measurements, had already been consumed, such that the respiration during the incubations represented maintenance respiration.

Spatial variances between 19 probe sites in Vedsted Lake were reflected as high variance over daily means of patterns in R_{dark}, which makes it difficult to interpret the results. Staehr *et al.* (2010) suggested that stronger relationships between

metabolic rates and driving variables may be found using weekly rather than daily rates. We also found that weekly data account for a significant portion of the variability in R_{dark} patterns in Vedsted Lake (Table 1). However, it was only in Week 28 that the decreasing pattern of R_{dark} became statistically significant ($t = 3.27, P < 0.001$). Furthermore, spatial heterogeneity in R_{dark} is common in lakes. For example, Van de Bogert *et al.* (2007) observed six- to sevenfold variations from site to site within the same lake. Therefore, expectation of small variations around the daily mean in the time coefficient a may not be reasonable. Although there was high variation in R_{dark} and patterns due to spatial heterogeneity during each day, there was a strong correlation between the initial respiration rate (R_0) and the time coefficient a (Fig. 6), suggesting that there is a mechanism affecting patterns in R_{dark}. According to the non-linear model (Fig. 6*b*), a decreasing pattern of R_{dark} was visible when R_0 was greater than 1.31 μM O_2 h^{-1}. Hence, fairly high respiration rates in Lake Vedsted may cause a decreasing pattern of R_{dark} although there were spatial variances. Therefore, the dominance of a decreasing pattern of R_{dark} (79%) in Vedsted Lake can be linked to a high phytoplankton biomass and primary production (Obrador *et al.* 2014), which could enhance both phytoplankton respiration (PR) and community respiration (CR) in the surface waters (1 m). Obrador *et al.* (2014) reported that the mean (±s.d.) R_{dark} was ~2.5 ± 1.5 μM O_2 h^{-1} at a depth of 1 in Lake Vedsted in 2008. Iriarte *et al.* (1991) also showed that CR can be dominated by autotrophs when Chl-*a* levels exceed 5 μg L^{-1}. This may be the case especially for the bottle incubations of water from RF, where a decreasing pattern of R_{dark} coincided with the spring bloom (Chl-*a* > 7 μg L^{-1}) and summer months (Chl-*a* > 3 μg L^{-1}; see Bentzon-Tilia *et al*. 2015). This agrees with our overall findings that decreasing R_{dark} is typically observed in phytoplankton-dominated aquatic systems, although we suggest that similar decreases in R_{dark} over time for plankton community and ecosystem (Pomeroy *et al.* 1994; Briand *et al.* 2004; Sadro *et al.* 2011) derive from source limitation for bacterioplankton caused by low quantity of dissolved organic carbon (Sadro *et al.* 2011).

Increasing R_{dark} *over time*

Observations of increasing R_{dark} over time in bottle incubations are rare in aquatic systems (Pomeroy *et al.* 1994; Gattuso *et al.* 2002; Briand *et al.* 2004) and, to our knowledge, such patterns have not been reported for open-water measurements, although Sadro *et al.* (2014) stated that increasing R_{dark} patterns were observed but excluded from their study. We observed increasing R_{dark} (negative values of a) in the open-water measurements from RF, in the bottle incubations and in samples on some days from Vedsted Lake. However, none of these observations was statistically significant (Table 1). Conversely, increasing R_{dark} after 48–72 h of bottle incubation was significant for RF and GB.

Although Pomeroy *et al.* (1994) and Gattuso *et al.* (2002) showed in their studies that changes occur in bacterial abundance, size and community, only Briand *et al.* (2004) correlated increasing bacterial respiration with increasing bacterial biomass and production during bottle incubations. Such changes could arise from bottle effects (Pomeroy *et al.* 1994), whereby the walls of the bottle provide a solid surface for planktonic organisms to attach themselves to and thereby promote succession towards an attached microbial community. Although we lack additional analyses for the bottle incubations, our observations of significant increasing R_{dark} after 48 h of incubation may be due to bottle effects. Therefore, increasing R_{dark} may be expected in the bottle incubations although such observations were not observed for night-time respiration in the open-water measurements. However, apparent increases in R_{dark} could also reflect mixing processes in the open-water measurements (Staehr *et al.* 2010). Mixing implies that the microbial community and the available substrate sources are not in steady state, and therefore succession is expected within the bacterial community.

Because increasing R_{dark} has previously been associated with bacterial activity (Pomeroy *et al.* 1994; Gattuso *et al.* 2002; Briand *et al.* 2004), we expected to observe this kind of pattern in heterotrophic-dominant systems. The ratio of respiration to light-saturated photosynthesis (R_{dark} : P_{max}) has been suggested as a useful indicator of the trophic balance of the plankton community, with values >0.2 occurring in heterotrophic-dominating plankton populations (Iriarte *et al.* 1991). Mantikci (2015) found values >0.2 during most months in GB and during the summer months in RF, which coincide with our observations of increasing R_{dark} (negative a values) from GB bottle incubations and RF open-water measurements. It has also been shown previously that active bacterial populations make potentially important contributions to community functions and biogeochemical cycles in GB and RF (Bentzon-Tilia *et al.* 2015; Traving *et al.* 2016), including respiration (Flindt and Nielsen 1992; Jensen *et al.* 1990), because both sites offer labile organic substrates in the form of bioavailable dissolved organic matter (Knudsen-Leerbeck *et al.* 2017). Under such conditions, it is likely that bacterial activity was limited by fresh production of photosynthate or allochthonous organic matter, rather than inorganic nutrients (Smith and Kemp 2003; Sadro *et al.* 2011). Pelagic plankton respiration, Chl-*a* and temperature have previously been shown to be strongly correlated in estuaries (Hopkinson and Smith 2005), including RF and GB (Mantikci 2015). A positive logarithmic relationship between the time coefficient a and R_0 (Fig. 6) suggests that increasing R_{dark} patterns occur with respiration rates below a certain level (where the fitted regression line crosses the x-axis, at $a = 0$). Although this level was similar for open-water measurements, discrepancy between open-water and bottle incubations may be due to differences in which component of the ecosystem respiration is measured (Staehr *et al.* 2012; Murrell *et al.* 2018). In general, open-water measurements give an estimate of sum of the pelagic and benthic respiration (total respiration), whereas bottle incubations estimate only pelagic respiration.

Interestingly, although R_{dark} measured in bottle incubations from RF showed constant or slightly decreasing R_{dark}, open-water measurements in RF mostly indicated increasing R_{dark}. There could be several explanations for this apparent inconsistency. First, open-water rates represented monthly averages of daily measurements, whereas bottle incubations only represented 1 day in a given month in RF. Daily variability in R_{dark} using open-water measurements of DO is known to be high and related to many factors, such as uncertainty of the respiration estimates, spatial heterogeneity in rates, biomass, activity and composition of the heterotrophic assemblage and the quantity and quality of the substrate for respiration (Van de Bogert *et al.* 2007; Staehr *et al.* 2010, 2018; Sadro *et al.* 2011; Solomon *et al.* 2013). Second,

open-water measurements were subject to natural changes in the water column, such as adjacent allochthonous inputs, close coupling with sediment, grazing and physical processes, which may affect observed R_{dark} (Hopkinson and Smith 2005). Particularly at RF, where the water column is shallow and well mixed (Pedersen *et al.* 2014; Staehr *et al.* 2018), a significant proportion of the open-water respiration has been shown to be derived from benthic habitats, such as eelgrass, micro benthic algae, macroalgae and the fauna associated with the seafloor (Murrell *et al.* 2018). In a recent study of RF, Staehr *et al.* (2018) reported that benthic habitat alone accounted for ~90% of R_{dark} at the eelgrass-dominated station that was 1 km away from the sampling station in the present study. Although total open-water estimates of R_{dark} were similar at both sites, Staehr *et al.* (2018) found less benthic contribution to total R_{dark} in the same station. In this case, respiration by the eelgrass meadows seemed to affect oxygen dynamics in adjacent sites (Staehr *et al.* 2018). Although we observed increasing R_{dark} in open-water measurements, it was not clear from the available data what caused this pattern. Therefore, there is a need for future studies to investigate the reasons for the differences in patterns of R_{dark} between bottle incubation and open-water techniques.

Physical factors effecting open-water measurements

Open-water estimates of metabolic rates are determined from changes in DO concentrations over time. However, not all variation in open-water DO measurements is due to biological processes. Significant variations in DO are also due to physical processes (Van de Bogert *et al.* 2007; Hanson *et al.* 2008; Staehr *et al.* 2010; Solomon *et al.* 2013). Vertical mixing, horizontal advection, temperature-dependent changes in the solubility of oxygen and precipitation are the main physical processes leading to inputs of high or low oxygenated water near the probe (Van de Bogert *et al.* 2007; Hanson *et al.* 2008; Staehr *et al.* 2010). Although RF and Vedsted Lake have different ecosystems and morphological properties (Obrador *et al.* 2014; Staehr *et al.* 2018), DO signals were likely influenced by advection of water masses with different concentrations of DO (Staehr *et al.* 2010). This could be caused by wind- or temperature-driven deepening of the active mixing layer in the case of the stratified Vedsted Lake (Obrador *et al.* 2014), and wind-driven horizontal water movements in the case of shallow RF (Staehr *et al.* 2018). In such cases, where biological processes have less effect than physical factors on DO signals, these data are generally excluded (Obrador *et al.* 2014; Sadro *et al.* 2014) because these factors are not considered in the model used. Because the models used in the present study do not consider physical effects other than the air–water gas exchange, we excluded data with poor model fit, likely to occur on days with a strong influence of physical exchanges of waterbodies with different DO content. We assumed that R_{dark} estimates on the remaining days with a good model fit were primarily affected by biological processes (Staehr *et al.* 2018).

Nevertheless, to evaluate the effect of physical process on DO in our analysis of the open-water R_{dark} estimates, we tested some of these against the available hydrographic and meteorological data. Advection and mixing of bottom water with low DO content to the surface during the night-time could be particularly important on windy nights due to a mixed water column (Van de Bogert *et al.* 2007; Staehr *et al.* 2010). However, the DO and temperature profiles of the water column and wind speed data did not correlate (linear regression analysis, $P > 0.05$) with R_{dark} (data not shown). Furthermore, we searched for a relationship between the temperature difference between day and night (ΔT), wind speed and the time coefficient a. However, no significant relationship was found between these variables ($P > 0.05$). If there had been an effect of advection by temperature, one could expect decreasing a, which suggests DO decrease, with increasing ΔT. We also examined the DO profiles for those days when we observed increasing oxygen consumption. However, the bottom water DO concentrations were the same as the surface DO. Therefore, upwelling bottom water could not have caused a decrease in the surface DO concentrations, which would result in an apparent increase in R_{dark} over time.

Conceptual models of R_{dark}

Based on our results, we have conceptualised the patterns of respiration occurring in aquatic systems with the assumption that CR is the sum of PR and bacterial respiration (BR; i.e. CR = PR + BR). Therefore, different simultaneous changes in PR and BR over time could give rise to the three observed temporal patterns of CR (Fig. 7).

Constant low respiration rate

Under limited levels of nutrients, substrate and light (especially for phytoplankton), both BR and PR are at levels near maintenance levels, causing CR to be low and constant over time (Fig. 7*a*). Such conditions can be observed mostly in the bottle incubations of natural oligotrophic waters where sampling time (e.g. sampling at dawn) probably resulted in observation of a maintenance respiration rate due to depleted intracellular carbon sources. Constant rates of respiration during bottle incubations may also derive from resource limitation of bacteria both to allochthonous and autochthonous organic carbon (Gattuso *et al.* 2002; Sadro *et al.* 2014). In addition, the absence of some heterotrophs other than bacteria due to small sampling volume may have affected the respiration pattern (Gattuso *et al.* 2002).

Mid to high constant respiration rate

Fig. 7*b* seems a good example of respiration rates occurring in aquatic systems, observed by open-water measurements. In this case both phytoplankton and bacteria respond to internal and external organic carbon sources respectively. As discussed above, decreasing R_{dark} is expected because of depletion of intracellular carbon sources in phytoplankton, whereas increasing R_{dark} is expected to arise from increasing bacterial activity, occurring with succession of microbial communities adapting to prevailing allochthonous carbon sources. As a result of these opposing processes, CR can remain constant, with the uncertainty of the measurements, if the magnitude of the increases and decreases is the same or depletion of carbon sources is balanced by bacterial growth.

Increasing CR

Increasing CR at the ecosystem level and in the bottles may arise when PR is low and may occur under nutrient-limited and bacterioplankton-dominated conditions (Fig. 7*c*). Given that

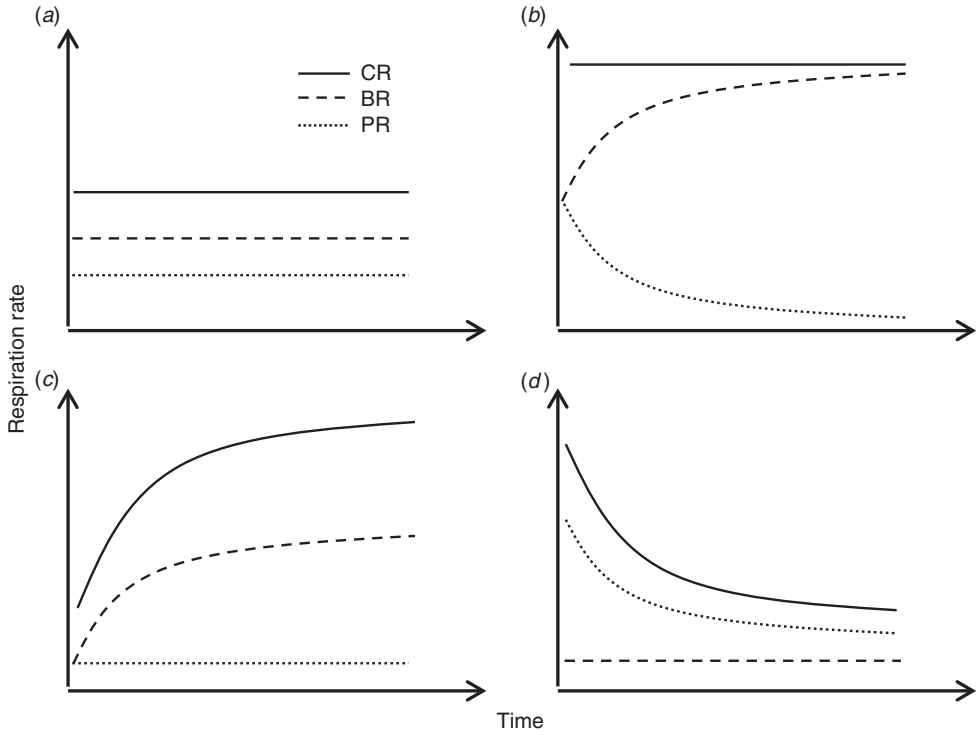

Fig. 7. Conceptual plots of the time course of community respiration (CR; straight line), bacterial respiration (BR; dashed line) and phytoplankton respiration (PR; dotted line) over time. CR was assumed to be the sum of BR and PR (i.e. CR = BR + PR). (*a*, *b*) CR is constant over time, (*c*) CR increases exponentially over time and (*d*) CR decreases exponentially over time.

bacterial activity is strongly dependent on availability of labile organic matter, BR may increase over time for the same reasons described above. Thus, CR tends to exhibit an increasing pattern over time.

Decreasing CR

When phytoplankton dominates (Fig. 7*d*), CR will exhibit a decreasing pattern over time. Unless phytoplankton are in a phase of maintenance respiration, they respire intracellular newly fixed carbon, which causes respiration rates to decrease exponentially over time concomitant with the exhaustion of intercellular pools. In this case, bacteria make a low contribution to CR, and most likely the system is eutrophic and phytoplankton respiration is dominant.

Conclusion

Our results show that R_{dark} can, indeed, be constant over time as generally assumed. However, assumptions of a constant R_{dark} often provide an incomplete description of respiration. This was most obvious in our experimental data, and more subtle and variable for the open-water measurements. This was likely because of physical disturbances, but also because of an interaction among the multiple sources of oxygen consumption (bacterial, plant, benthic). Furthermore, although different aquatic systems or plankton communities may have similar mean night-time R_{dark}, these may derive from different environmental factors affecting the quantity and quality of organic matter, and ultimately auto- and heterotrophic metabolic activities. In addition, we showed that the duration of measurement has implications on the derived daily rates. Accordingly, representative estimates of nocturnal respiration require incubations with the same duration as the night period. From our understanding of the conditions that shape a constant CR, this should, in fact, be an exception rather than the general case, because this is only expected when both phytoplankton and bacteria are in maintenance phase or low in numbers (Fig. 7*a*) or exactly balance each other out (Fig. 7*b*). Thus, small changes in the quality or quantity of the organic carbon pool or the microbial community will likely affect the time course of CR in aquatic systems.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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