Coastal microbial respiration in a climate change perspective

Anna Nydahl
To my family
List of papers

This thesis is based on the following papers, which will be referred to in the text by their roman numerals


II. Nydahl, A., Panigrahi, S., Wikner, J. Increased microbial activity in a warmer and wetter climate enhance the risk of coastal hypoxia. In revision FEMS Microbiology Ecology


IV. Panigrahi, S., Nydahl A., Wikner, J. Strong seasonal effect on plankton respiration by moderate experimental warming in a temperate estuarine plankton community. Manuscript
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Abstract

In a climate change perspective increased precipitation and temperature are expected which should influence the coastal microbial food web. Precipitation will have a strong impact on river flow and thereby increase the carbon input to the coastal zone as well as lowering the marine salinity by dilution with freshwater. Simultaneously temperature may increase by 2-5 °C, potentially influencing e.g. metabolic processes. Consequences of this have been evaluated in this thesis with focus on microbial respiration in paper II and IV. A temperature increase of 3 °C will have a marked effect on microbial respiration rates in the coastal zone. The effect of temperature on microbial respiration showed a median $Q_{10}$ value of 25 with markedly higher values during winter conditions (around 0°C). These $Q_{10}$ values are several-fold higher than found in oceanic environments. The conclusion was in accordance with a consistent temperature limitation of microbial respiration during an annual field study, however, shifting to DOC limitation at the elevated temperature. Neither bacterial production nor phytoplankton production showed a consistent temperature effect, suggesting that the biomass production at the base of the food web is less sensitive to a temperature increase. Results from both a field study and a fully factorial microcosm experiment supported the conclusion. Our results suggested that areas dealing with hypoxia today will most likely expand in the future, due to increased respiration caused by higher temperatures and larger riverine output of dissolved organic carbon.

Pelagic respiration measurements in the sea are relatively scarce in the literature, mainly due to the lack of sufficiently good and user friendly techniques. New methods such as the dynamic luminescence quenching technique for oxygen concentration have been developed. This makes it possible to obtain continuous measurements of oxygen in an enclosed vial. Two different commercially available systems based on the dynamic luminescence quenching technique were evaluated from the aspect of precision, accuracy and detection limit when applied to respiration measurements in natural pelagic samples. The Optode setup in paper III showed a practical detection limit of 0.30 mmol m$^{-3}$ d$^{-1}$, which can be applied to measure respiration in productive coastal waters (used in paper IV). This included development of a stopper where the sensor was attached, stringent temperature control, proper stirring and compensation for an observed system drift. For controlled laboratory experiments with organisms smaller than 1 µm the Sensor Dish Reader (paper I) has sufficient detection limit of (4.8 mmol m$^{-3}$ d$^{-1}$). This required a stringent temperature control and manual temperature correction. The Sensor Dish Reader gives the opportunity to perform multiple treatments at low cost (used in paper II), but the precision is too low for field studies due to the between ampule variation.
Introduction

Expanding hypoxia in the Sea

Dissolved oxygen levels in coastal waters have changed drastically over the last decades (Diaz and Rosenberg 2008). Literature shows that the number of coastal sites where hypoxia has been reported have increased with a rate of 5.4 % year\(^{-1}\) (Vaquer-Sunyer and Duarte 2008). Hypoxia in the coastal zones of the Baltic Sea has increased during the past 50 years and 20% of all known hypoxic sites around the world is located in the Baltic Sea (Conley et al. 2011). Local, regional and global changes in dissolved oxygen levels will have fundamental effects on ecosystems and have been more intensively studied during recent years (Körtzinger et al. 2004, Diaz and Rosenberg 2008, Riser and Johnson 2008, Keeling et al. 2010). Hypoxia i.e. oxygen concentrations under 2 mg ml\(^{-1}\) will have impact and kill bottom living organisms (Vaquer-Sunyer and Duarte 2008) and the consequences of hypoxia on nutrient biogeochemical cycles are substantial (Conley et al. 2002). The large-scale hypoxia is an inherent property of the Baltic Sea caused by geographically and climatically determined insufficiency of oxygen supply to the deep water layers (Savchuk 2010). To understand occurrence of hypoxic waters respiration plays a fundamental part. Respiration is one of the most important processes in the biosphere, from the level of individual organisms to regulation of CO\(_2\) an O\(_2\) levels in the atmosphere. Further, the balance between auto- and heterotrophic processes may be measured by light and dark changes in oxygen. It is of importance to study the downward transport of biomass through sedimentation, and thereby the role of the sea as a global sink or source for CO\(_2\) (g). (Karl et al. 2003, Stramma et al. 2008, Reid et al. 2009). The development of hypoxia as well as emissions of CO\(_2\) is thought to be promoted by future climate driven increase in temperature and discharge of freshwater (Conley et al. 2002, Zillén et al. 2008).

Future climate projections

Climate change projections suggest some changes in ocean physics and chemistry such as higher sea surface temperature and CO\(_2\) concentration, lower pH and enhanced upper ocean stratification (Solomon et al. 2007). Furthermore, precipitation may increase by approximately 20 % by the end of this century, leading to a larger riverine discharge to the sea, potentially resulting in higher inputs of riverine dissolved organic matter (rDOM) and lower salinity in the coastal zone (Meier 2006, Raymond and Saiers 2010). In addition, a temperature increase of approximately 2-5 °C is projected for northern Scandinavia. Microbial activity will likely be influenced by these climate related land-sea drivers individually or in concert. This change may in turn have a profound impact on food-web efficiency, carbon flow and nutrient recycling in marine surface waters (Wikner and Andersson 2012). These potential changes motivate the study of the effects of the major environmental drivers for microbial growth and respiration.
The marine microbial food web

Life in the oceans is dominated by microbes (e.g. < 0.1 mm in size), comprising viruses, bacteria, protozoa and some phytoplankton. In fact microorganisms are thought to account for 90 % of the respiration in the water column (Robinson and Williams 2005). These small, single-celled organisms constitute the base of the marine food web and catalyze the transformation of energy and matter in the sea. The role that the microbial community plays in the global marine carbon cycle was recognized in the end of the 1970-ies, partly as a result of a large share of marine respiration occurring in the microbial size fraction (Pomeroy 1974, Williams 1981, Azam et al. 1983). In the microbial food web heterotrophic bacteria use dissolved organic carbon and nutrients, primary Nitrogen (N) and Phosphorous (P) as their energy source. The carbon can either be derived autochthonously from phytoplankton (or other planktonic organism groups) (Azam et al. 1983) or from allochthonous sources such as riverine input (Sandberg et al. 2004, Findlay et al. 1991). The bacteria are grazed on by heterotrophic flagellates and ciliates and they are in turn eaten by zooplankton, creating a link between bacteria and zooplankton (Fig. 1). The largest fraction of the carbon taken up by bacteria is respired to CO₂ during consumption of oxygen, but the production of bacterial biomass is also an important process to make dissolved carbon available to the food web. (Kirchman 2000).

![Fig 1. Illustration of the aquatic food web and carbon transfer. Illustration by Kristina Viklund.](image-url)
Marine respiration

As mentioned above microorganisms are responsible for the major part (90%) of respiration in the water column and respiration is a fundamental part of biospheric metabolism. In a physiological perspective, respiration encompasses electron flow through membrane-associated transport systems from donors to acceptors, creating a proton gradient. The energy in proton gradients can be coupled to ATP-synthesis or can for example transport substrate or drive flagellar motors (White 2000). In this thesis focus is on aerobic respiration (i.e. oxygen consumption) where oxygen is the electron acceptor. There are also different types of anaerobic respiration where other components are used as electron acceptors, which occur mainly under anoxic conditions and in aquatic systems, mainly in sediments. Examples of anaerobic respiration are N-oxide respiration such as denitrification, metal reduction, sulphate respiration and carbon dioxide reduction. (Del Giorgio and Williams 2005).

Measurements of pelagic respiration rates are scarce and dominated by measurements in the northern hemisphere (Del Giorgio and Williams 2005). Robinson and Williams (2005) reviewed a variety of observations of respiration in marine surface (<10 m) waters and reported a mean value of 4.9 (±0.23) mmol m⁻³ day⁻¹. Observed rates of respiration in the coastal zone fall within the range 1.7-84 mmol m⁻³ day⁻¹ (Hopkinson and Smith 2005). Respiration rates found in the Baltic Sea varies from 1.7 to 14.6 mmol m⁻³ day⁻¹ (Kuparinen 1987, Jensen et al. 1990, Olesen et al. 1999).

Several studies indicate that temperature has a strong influence on respiration rates across coastal and estuarine systems. Marked temperature dependence of bacterial respiration has been observed in cold water and temperate coastal systems (Pomeroy and Deibel 1986, Pomeroy et al. 1991, Pomeroy et al. 1995). Mesocosm experiments further support a positive effect of temperature on respiration to occur over longer time scales and including a larger part of the food web (Hoppe et al. 2008, Wohlers et al. 2009). Since bacterioplankton account for half of the plankton respiration, they are an important target for both temperature and substrate changes. Temperature has consequently been found to be the dominant factor regulating the seasonality of bacterial respiration and bacterial carbon consumption, whereas both temperature and substrate supply influence the bacterial production and growth efficiency in a salt marsh estuary (Apple et al. 2006). Kritzberg et al. (2010) showed that in situ bacterial growth and bacterial respiration are both positively related to temperature. However, temperature and substrate concentration may influence growth and respiration in bacteria in an interactive way, motivating the simultaneous study of these factors (Pomeroy and Wiebe 2001, Kirchman et al. 2005).

To be able to propose potential consequences of climate change in the coastal zone more studies is needed on how climate related drivers such as temperature, salinity and rDOM effects the microbial activities.
Respiration measurements

Measurements of planktonic respiration can be obtained in a number of ways: the rate of production or consumption of a product; the assay of an appropriate respiratory enzyme; predictions from biomass and from invers models of the community composition and activity. The major challenge to measure microbial respiration is that marine pelagic ecosystems requires accurate respiration rate measurements, because the ambient oxygen pool may change as little as 0.01 \% day\(^{-1}\) in oceanic deep water or at winter conditions in surface waters (Robinson and Williams 2005). In absolute units, this demands that rates between 0.02 to 75 mmol O\(_2\) m\(^{-3}\) day\(^{-1}\) have to be measured.

The classic way to determine respiration rates in aquatic environments is by end-point Winkler titration. The Winkler method was first described in 1888 (Winkler 1888) and has ever since been the standard method for oxygen measurements. The winker method is a chemical method for estimating the dissolved oxygen in seawater. The well-known method is founded on the quantitative oxidation of Mn\(^{2+}\) into Mn\(^{3+}\) in alkaline solution followed by the oxidation of iodide to iodine by Mn\(^{3+}\) in acificified solution. The released iodine is titrated with a standard solution of Sodium thiosulfate. Classically the titration is performed by hand and the endpoint is found visually. However, more accurate measurements have been achieved by high precision titration with potentiometric (Furuya and Harada 1995) detection. A major part of respiration data in aquatic environments available in literature is measured by an automated system with photometric detection developed by Williams and Jenkinson (1982). The precision and detection limit of the Winkler technique are also adequate for oceanic deep water measurements in the ocean. A theoretical detection limit of 0.07 mmol O\(_2\) m\(^{-3}\) day\(^{-1}\) has been reported for automated Winkler titration, while practical precision reported from the field ranges between 0.1 and 2 mmol O\(_2\) m\(^{-3}\) day\(^{-1}\) (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 (i.e. 8 to 16 pcs.). 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.

A new technique for oxygen concentration is the dynamic luminescence quenching (DLQ) technique, which makes it possible to obtain continuous measurements of oxygen in an enclosed vial (Klimant et al. 1995, Holst et al. 1997, Wolfbeis 2006). The principle of DLQ, refers to the ability of certain molecules to influence the fluorescence of other molecules. For O\(_2\), a ruthenium complex is used in the sensing foil. Due to its ability to fluoresce the sensing foil (i.e. the ruthenium complex) will return a red light when it is excited with a blue-green light (505 nm). If there is O\(_2\) present this fluorescent effect will be quenched and the returning light will therefore be dependent on the concentration of oxygen. Since the returned light is delayed with respect of the
excitation light, the presence of $O_2$ will also influence this delay. This property is called luminescence decay time and is a function of the phase of the returned signal. (Klimant et al. 1995, Holst et al. 1997, Demas et al. 1999). The DLQ technique has been successfully used to measure the respiration of bacterial isolates and aquatic samples with satisfying results (Warkentin et al. 2007, Koster et al. 2008, MdAmin et al. 2012).

The DLQ method has never, to our knowledge, been thoroughly evaluated when it comes to precision, accuracy and detection limit on rates of respiration in natural pelagic samples. This is needed to get a robust method to be used on routine basis for respiration estimates.

**Objectives of this thesis**

The main objective of this thesis was to find a continuous and accurate method for respiration measurements, easily conducted in the field and to study the influence of climate related factors on microbial respiration.

In paper I we investigate the potential of the commercially available Sensor Dish Reader® to measure low oxygen consumption rates of aquatic samples.

In paper II we investigate the short-term interactive effects of salinity, temperature and riverine dissolved organic matter on microbial respiration, growth and abundance in an estuarine community.

In paper III an analytical setup for respiration rate measurements was developed and evaluated in pelagic water samples using a commercially available optical oxygen sensor (Optode™).

In paper IV the aim was to study how temperature in interaction with riverine dissolved organic matter influenced respiration, bacterial growth and primary production in the field throughout a full seasonal cycle in the northern Baltic Sea.

**Major findings and Discussions**

**Climate effects on respiration (paper II and IV)**

In a climate change perspective we expect a potential temperature rise of 2-5 °C and increased precipitation, which will impact river flow and thereby increase the carbon input to the coastal zone as well as lowering the marine salinity by dilution with freshwater. All these factors will likely affect the microbial foodweb in general and the microbial respiration in particular.
In paper II a lab experiment was created where the interactive influence of changes in salinity, temperature and rDOM on microbial (organisms < 200 µm) respiration, bacterial growth and abundance was investigated. The experimental microcosm design was fully factorial to allow statistical analysis of interaction effects. The different treatments were chosen from a climate change perspective and correspond to the predicted changes for this area with a moderate reduction of anthropogenic CO₂ emissions (Meier 2006). Although temperature and autochthonous DOM have been investigated in many previous studies, our design is unique in also studying rDOM, salinity and the relative importance of these factors when varied simultaneously.

Paper IV investigated in the field how temperature simultaneously influenced plankton (unfiltered sample) respiration, phytoplankton primary production and bacterial growth. The study was performed in a temperate estuary with high dissolved organic carbon (DOC) concentration and high C:P ratio over a full annual cycle. Seasonal and annual Q₁₀ values of respiration, bacterial growth and primary production are presented and discussed in relation to published values from other aquatic environments including DOC concentration.

Elevated temperature showed a marked short-term effect on respiration in the microcosm study as well as the coastal zone (paper II and IV). A positive effect of temperature on respiration is well established and has been reported previously both in in situ and mesocosm experiments (Pomeroy and Wiebe 2001, Hopkinson and Smith 2005, Robinson and Williams 2005, Apple et al. 2006, Hoppe et al. 2008, Wohlers et al. 2009, Kritzberg et al. 2010). Corresponding Q₁₀ values on the respiration were found to be high and similar in both studies (median values of 25 in paper II and 26 in paper IV) and very high, 332 (median winter values paper IV), under winter conditions. High values of Q₁₀ (142) has been reported previously both for respiration (Christia and Wiebe 1974) and bacterial growth. Corresponding Q₁₀ values in paper III (2453 in paper IV and 2370 in Paper II) representing both field data and laboratory experiment. Vaque et al. (2009) reported a Q₁₀ of 5747 for production in the cold (-0.5-1 °C) environment of Gerlache Strait, Antarctica supporting that our extremes are not unrealistic. DOC levels in our studies is high (around 300 µM) if compared to what is found in oceanic waters (Lemee et al. 2002), and may fuel respiration and promote a larger temperature response. In paper IV we found a shift in respiration control from temperature to DOC due to as little as 3°C increase in temperature, resulting in 220% median increase in plankton respiration. We therefore argue that there might be two types of Q₁₀ values to consider. Q₁₀ values over a small temperature interval from the in situ temperature in combination with high DOC concentrations actually measures the temperature sensitivity of respiratory enzymes, where respiration is carbon saturated. When Q₁₀ values instead are estimated over a larger temperature interval the temperature sensitivity for supply of the
carbon substrate to the respiratory system is measured. Current data in the literature may therefore often (i.e. at high DOC levels) underestimate the potential effect of temperature on respiration. The temperature effect on respiration (i.e. $Q_{10}$) would consequently be expected to be greater in coastal environments than in oceanic environments. Further experiments are needed to confirm this hypothesis.

Additionally, in paper II with defined medium and controlled treatments, also rDOM had positive effect on microbial respiration, as well as bacterial growth and abundance. Bacterial growth on rDOM may not be expected as the rDOM in the area consists of approximately 80 % humic substances (Pettersson et al. 1997). Some of the carbon in the rDOM, however, seemed capable of promoting microbial activity without experimental pre-treatment with light, suggesting a shift to substrate limitation at higher temperatures with longer incubation time. This therefore corroborated the results from the field study in paper IV. Bacterial growth was in paper II stimulated by the interaction of rDOM and salinity, with increased bacterial growth with increasing salinity especially at the higher rDOM level. This was in accordance with Wikner et al. (1999) showing greater utilization of allochthonous DOC in estuarine compared to limnic conditions in the study area. Stepanauskas et al. (1999) also demonstrated enhanced utilization of organically bound riverine nutrients stimulating bacterial growth at higher salinity. Bacterial growth rates are also reported to increase when bacteria and substrate from the riverine environment are exposed to higher salinity (Langenheder et al. 2003). Taken together, increased ionic strength appears to enhance the bioavailability of rDOM with a mechanism not fully understood.

In a well cited review, Pomeroy and Wiebe (2001) proposed an interactive effect of temperature and substrate primarily on bacterial growth when approaching the freezing point. We could not find support for this model in our microcosm experiment in paper II. Important factors differing between our experiments were the type of substrate used, the C:N:P ratio and temperature level in the experiment. In experiments similar to our paper, where natural rDOM was used, Wiebe et al. (1992) used proteose peptone-yeast extract as substrate whereas Autio (1998) used sucrose. Different compounds vary in bioavailability for bacteria and in addition C:N:P ratio, potentially explaining the different results on bacterial activity. Also we used a small temperature change of 3 °C, while many studies estimate $Q_{10}$ values from experiments of 0-20 °C. Our results indicate that respiration may shift from temperature limited to resource limited already after a 3°C increase. The temperature effect on respiration observed in many studies could in fact be a temperature effect on the supply of limiting nutrient rather than direct on the process of intracellular metabolism. Therefore we advocate that the C:N:P ratio in added substrate is important to have under experimental control in future experiments. Further, it is of major importance to keep bacterial growth and respiration separated as the respiration process per se does not need N and P, but only a carbon source
whereas bacterial growth requires many nutrients (Lopez-Urrutia and Moran 2007).

Putting our overall findings in a climate-change perspective, areas dealing with hypoxia today will according to our results most likely expand, especially in coastal areas where hypoxia has been seen to expand already (Diaz and Rosenberg 2008, Conley et al. 2011). If global climate change has the potential to affect respiration in all aquatic systems, it is unlikely to affect respiration in the same way and at similar time scale everywhere. As the time scale of climate change is over long period (i.e. 100 years) and temperature will increase slowly one could anticipate that the temperature effect would have a smaller impact because the organisms have time to adapt and evolve in their warmer environment. Assuming that our findings in paper II are applicable in a future climate change perspective both increased microbial respiration and bacterial growth are expected, although driven by different environmental factors. How bacterial abundance will be affected is not completely clear, because the rDOM stimulated abundance while temperature reduced it (Paper II). We suggest that the latter may be due to increased grazing pressure from bacterivores. Furthermore, the response of temperature and substrate in a given environment may not be possible to translate to other environments, and a better understanding of the adaptive and evolutionary ecology of microorganisms is needed before any general theories of climate change response can be drawn.

**Methods for respiration measurements (paper I and III)**

The DLQ technique was used for respiration measurements in two different systems, with varying results. In paper I the commercially available system, Sensor Dish Reader® (SDR®, PreSens GmbH, Germany) was used and in paper III we developed an analytical setup using an optical oxygen sensor, Optode™. For the Optode™ setup this required the development of a gas tight stopper to connect the sensors to a 1 dm³ glass bottle. The theoretical detection limit of 0.04 mmol m⁻³ d⁻¹ for the Optode™ (paper III) was found lower to what has been reported for high precision Winkler titration (Robinson and Williams 2005). The practical detection limit was 0.3 mmol m⁻³ d⁻¹, slightly higher than reported for the Winkler method (0.1 mmol m⁻³ d⁻¹). This is the best detection limit and precision reported for sensor based respiration measurement. For the SDR® (paper I) the detection limit was higher, 4.8 mmol m⁻³ d⁻¹.

There are several advantages with the two systems. They provide continuous measurements that avoid assumptions of linearity of the oxygen consumption rates. The on-line measurement gives the opportunity to take decisions during the incubation about the time needed to get statistically reliable respiration rates. As the methods are free of chemicals and wet chemistry as well as needs little handling time they are a clear advantage for field measurements at sea conditions. For the SDR® (paper I) another advantage is the replication, where one SDR® plate gives 24 replicates and with the ability to serially connect up to
10 plates. Different temperatures can be used for each plate providing a large experimental setup with the option of 240 replicates at 10 different temperatures. For the Optode\textsuperscript{TM} setup (paper III) the sample volume provides the advantage of a larger sample including 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\textsuperscript{3} sample would include nanoplankton up to 50 µm in a statistically acceptable number of 10 per dm\textsuperscript{3} (Robinson and le B Williams 2005). Additionally, mesozooplankton may occur well above 10 individuals per dm\textsuperscript{3} in coastal areas during the productive season and, can therefore 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).

As the sample volume is an advantage in paper III it can be a draw back for the SDR\textsuperscript®} in paper I, where only a 5 ml sample is used which means that you will not get has high number of individuals in your sample. In a 5 ml sample you will mainly catch organisms smaller than one µm (i.e. bacteria), however about 50 % of the respiration in aquatic environments is dedicated to organisms smaller than 0.8 µm (Robinson and Williams 2005). There will also be a potentially higher containment effect urging the need for as short incubation time as possible. Temperature is affecting both oxygen levels, in form of solubility, \textit{per se} (Weiss 1970) as well as the sensor foil in the Optode\textsuperscript{TM} (paper III) and the SDR\textsuperscript®} (paper I). Poor temperature control can introduce both elevated variance and drift in the time series from both methods. This requires a temperature control of at least ±0.2 °C which we could obtain with incubators (paper I) and water baths (paper III, ±0.05 °C), and preferable also temperature controlled rooms to avoid temperature fluctuations from the surroundings. However this requires rather large equipment during field work which may limit the number of samples types or treatments to be performed at different temperatures. The higher detection limit found in paper I was mainly due to variation between sample vials, which can potentially be improved by individually calibrate each vial to a specific position on the SDR\textsuperscript®}.

In paper III a drift was reported for the Optode method, which in practice set the detection limit and provided a 3-times larger uncertainty in the method as compared to automated Winkler titrations. Similar drift has been reported earlier but not taken further into consideration (Holtappels 2009). The system drift was found mainly be due to oxygen bound in the optode and stopper plastic material. By pre-conditioning of the optode and the stopper in low oxygen (N\textsubscript{2} (g) or Na\textsubscript{2}SO\textsubscript{3} the best detection limit reported may be approached. Further ways to try to eliminate the drift can be by creating a stopper in glass and use Optodes with titanium housing.
Conclusions

The overall findings suggest that areas dealing with hypoxia today will most likely expand in the future based on climate-change projections and new oxygen minimum zones may arise. Especially in coastal areas respiration may increase due to both higher temperatures and larger riverine output, resulting in a synergistic effect. The effect of temperature on respiration ($Q_{10}$-values) may also be markedly higher in areas with high discharge and high C:P ratio in the DOM. Respiration was further shown to be temperature limited at a coastal field site, but shifting to resource limitation (i.e. DOC) by a 3 °C temperature increase. Our findings in paper II suggests that both increased microbial respiration and bacterial growth are expected, although driven by different environmental factors. Bacterioplankton growth was not temperature limited in neither the natural environment investigated nor the microcosm experiment, but responded positively to rDOM increase in combination with elevated salinity. Taken together, our results supported a model were bacterial respiration and growth are regulated by temperature and resource concentration independently of each other.

Further the observations suggest that two types of $Q_{10}$ values occur. $Q_{10}$ values measured over a small temperature interval from the *in situ* temperature, in combination with high DOC concentrations (i.e. resource saturated), measures the temperature sensitivity of respiratory enzymes. When $Q_{10}$ values are estimated over a larger temperature interval, imposing resource limitation, this instead measures the temperature sensitivity of the process(es) providing carbon substrate to the intracellular metabolism. The experimental evidences for such a functional division in temperature responses are still quite meagre, and further controlled experiments in different environmental conditions are needed.

The Optode™ method (paper III) can be used to measure respiration in productive coastal surface waters, covering plankton respiration up to mesozooplankton. However, samples from oceanic, cold or deep waters often fall under the detection limit. Even though the SDR® (paper I) has the ability to measure respiration in high productive waters the recommendation is to use the SDR® in controlled laboratory experiments, where you can keep a high temperature control. Further the SDR® will give you the opportunity to perform multiple treatments studies in an easy way.

Acknowledgements

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References


Östersjön är ett brackvatten hav som sträcker sig från Bottenviken i norr till de danska sunden i söder och omsluts av en landmassa som representerar av nio länder. Denna miljö är på många sett unik genom stor sötvattenpåverkan och litet utbyte med världshaven (30 års omsättningstid). Östersjön utsätts framförallt för tillförsel av ämnen från såväl naturliga som antropogena aktiviteter. Något som ofta uppmärksammas är problem med syrefria områden och döda havsbottnar. Detta anses påverkas av både klimatförändringar och övergödning. En av de biologiska prosesser som påverkar syresituationen i haven är respiration, syreförbrukning, som utförs av de flesta levande organismerna i Östersjön. Den här avhandlingen presenterar resultat på hur bakteriers syreförbrukning påverkas av de förändringar vi förväntar oss i vårt klimat i framtiden. Det är framförallt ökad temperatur och ökat vattenflöde i våra floder som i sin tur leder till snabbare omsättning och tillförsel av näring åt bakteriesamhället. Resultaten från artiklarna II och IV visar att den potentiella temperaturökningen som väntas skulle öka syreförbrukningen i kustnära områden. Den blir extra stor i kustområden, troligen på grund av stor tillgång på organiskt material från älvarna. Även den högre tillförseln av näringsämnen kan öka syreförbrukningen enligt artikel II. De områden som idag är syrefattiga kommer på grund av detta att expandera, framförallt längs kusterna där nya områden kan uppstå. Eventuellt kan det vara en förklaring till den ökande ytan av syrefria bottnar i i Östersjön och världshaven.

För att kunna utföra mätningar av syreförbrukning krävs väldigt precisa och gärna användarvänliga metoder som lätt kan tillämpas i fält. I avhandlingen presenteras hur två olika mätmetoder optimeras för att göra tillförlitliga förbrukningsmätningar av syre. Ny teknik gör att syrehalten kan mätas med en ljusbaserad metod som skiljer sig från dagens kemiska bl.a. genom att resultaten kan följas löpande på en dator. De båda metoderna kräver en väldigt precis temperaturkontroll. Optod uppsättningen presenterad i artikel III innefattar en volym på 1 liter och organismer upp till en storlek på 50 µm omfattas i den uppmätta syreförbrukningen. Denna metod rekommenderas för fältmätningar, och användes förfältmätningar i Artikel IV. I utvecklingen ingick utformning av en kork för att montera optod-sensorn i. I artikel I presenteras en utrustning som baseras på en mindre volym (5 ml) vilket innebär att endast mätningar på bakterier och organismer mindre än 1 µm kan anses tillförlitliga. Detta i kombination med viss variation mellan mätflaskor gör att den framförallt rekommenderas för användning i laboratoriemiljö. Det systemet användes för mätningarna av syreförbrukning i laboratorieexperimentet som presenteras i artikel II.
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