Importance of bacterial maintenance respiration and baseline respiration for development of coastal hypoxia

Kevin Vikström
“Valid criticism does you a favor.”
- Carl Sagan
# Table of Contents

Abstract.................................................................................................................. 2
   English .................................................................................................................. 2
   Svenska............................................................................................................... 3

List of papers and author contributions................................................................. 4
   Paper I: ............................................................................................................... 4
   Paper II: ............................................................................................................. 4
   Paper III: .......................................................................................................... 4
   Paper IV: ........................................................................................................... 4

List of abbreviations and glossary........................................................................... 5

Background............................................................................................................. 6
   Oxygen and the Baltic Sea ...................................................................................... 6
   Current knowledge of hypoxia .............................................................................. 7
   Respiration.......................................................................................................... 8
   Energetics of bacteria .......................................................................................... 10
   The Pirt-model and the ecological concept of baseline respiration.................... 11
   Measuring oxygen and respiration ...................................................................... 13

Aims and Research Questions.................................................................................. 16

Materials and Methods ......................................................................................... 17
   Respiration rate .................................................................................................. 17
   Bacterial Production, Abundance and Volume .................................................... 17
   Nutrient and Carbon concentration analysis ..................................................... 18
   Phytoplankton production .................................................................................. 18

Results ..................................................................................................................... 20
   Paper I: Improved accuracy of optode-based oxygen consumption measurements by removal of system drift and non-linear derivation ......................................................... 20
   Paper II Importance of Bacterial Maintenance respiration in a Subarctic Estuary: A Proof of Concept from the Field ........................................................................................................ 22
   Paper III: Coastal filter effect by microbial mineralization of riverine DOC in a sub-arctic river-estuary gradient ......................................................................................... 24
   Paper IV: High influence of baseline respiration in a sub-Arctic coastal system ...... 25

Discussion............................................................................................................... 27

Acknowledgements................................................................................................. 34

References .............................................................................................................. 35
Abstract

English
Reduced oxygen concentrations and increasing hypoxic zones have become more common in the sea due to climate change and eutrophication. The main cause of oxygen loss in oxygenated environments is respiration. Respiration rates can be estimated using optode methodologies which utilize dynamic luminescence quenching to estimate the oxygen concentration declines in dark incubations. A published optode methodology was improved by using optodes with titanium housing instead of plastic housing plausibly trapping oxygen. Drift was highly reduced by the titanium casings leading to a higher precision and lower detection limit of 0.97 mmol O$_2$ m$^{-3}$ d$^{-1}$. 28% of measurements were shown to have non-linear oxygen concentration declines. The rate of oxygen change was derived with a 2nd degree polynomial at 1 hour from the incubation start. The majority of non-linear declines were concave and due to carbon substrate limitation. Analyzing non-linear trends linearly, a common practice, leads to an underestimation of respiration by up to 64%.

Bacterial maintenance respiration ($R_m$) was studied using an ecophysiological model unverfied in natural environments. The model was applicable at high productivities but a quadratic model was demonstrated to give a better fit. $R_m$ was found to represent a significant part in the sub-arctic estuary contributing to 58% of the annual specific bacterial respiration. Therefore, $R_m$ may be more important in nature than previously recognized. The ecophysiological model is driven solely by the bacterial specific growth rate ($\mu$) where the relative influence of $R_m$ is elevated as $\mu$ decreases. As a consequence, I hypothesize that a reduction in nutrients may not decrease the oxygen consumption but rather shift bacterial growth based respiration to $R_m$ as $\mu$ approaches zero.

Baseline respiration ($R_{bl}$), defined as ecosystem respiration disconnected from contemporary primary produced carbon, was also studied. $R_{bl}$ was shown to be largely supplied by allochthonous carbon in a coastal ecosystem and had a contribution of 50% to the annual plankton community respiration in the sub-arctic estuary studied. I claim that $R_{bl}$ and $R_m$ are crucial to include for understanding and managing development of aquatic hypoxia in an effective and economic manner.
Svenska

Minskande syrehalten samt större områden med syrefria bottnar har blivit alltmer vanligt i våra hav. Respiration hos levande organismer orsakar den största konsumtionen av syre och är därmed viktig att studera. Respirationshastigheter kan uppskattas med optoder. Metodiken använder sig av dynamisk fasförskjutning av luminisens för att mäta syrehalten i flaskor som inkuberas i mörker. Den publicerade metoden förbättrades med nya optoder med titanholjen istället för optoder med plasthölje, som kan binda syre. Detta resulterade i minskad drift vilket i sin tur gav en bättre detektionsgräns på 0.97 mmol O₂ m⁻³ d⁻¹. Dessutom visade 28% av mätningar en icke-linjär minskning av syrehalt. De flesta icke-linjära nedgångar var konkava i sin karaktär och analyserades med andragradspolynom. Syrekonsumtionshastigheten deriverades vid 1h från den kvalitetssäkrade inkubationens start, för att få en så sann skattnings sannolikhet. Om icke-linjära nedgångar analyseras linjärt, vilket är vanligt för andra metoder, skulle det medföra en 64% underskattning av respirationshastigheter. Tillsatsförsök med kolsubstrat till inkubationer, indikerade att brist på kolsubstrat kan orsaka de observerade konkava nedgångarna av syre.

Bakteriell underhållsrespiration (Rₘ) studerades med hjälp av en ekofysiologisk modell, som ej än verifierats i naturen. Modellen fungerade vid hög produktion sommartid, men en kvadratisk funktion passade data bättre. Med den kvadratiska funktionen kunde vi beräkna att den bakteriella underhållsrespirationen bidrog med 58% till den årliga bakteriella respirationen. Rₘ är därmed en viktigare process i naturen än vad man hittills har känt till. Den ekofysiologiska modellen drivs endast av specifika bakteriella tillväxthastigheten (μ), där betydelsen av Rₘ ökar då μ närmar sig noll. Detta innebär att en reducering av närsalter, och därmed μ inte nödvändigtvis resulterar i högre syre koncentrationer utan snarare en förskjutning från tillväxtbaserad respiration till Rₘ, där konsumtion av syre hålls på samma eller liknande nivå.

Baslinjerespiration (Rᵇˡ), definierad som respiration frikopplad från primärproduktion, studerades också. Rᵇˡ utgjorde 50% av den årliga plankton-respirationen. Rᵇˡ drejs i huvudsak av organiskt kol från tillrinnande älvvatten. Jag hävdar att baslinje- och underhållsrespiration är avgörande att beakta för att förstå och förvalta utvecklingen av hypoxia på ett ändamålsenligt och ekonomiskt sätt.
List of papers and author contributions

**Paper I:**
**Respondent part:** Analyzes of non-linearity, contributed to design of testing of optode pre-treatment, measurements of linearity comparison, drift measurements and pre-treatments, statistical analyses of non-linearity and pretreatment experiments, contributed to statistical analysis of drift data and writing of the manuscript.

**Paper II:**
Respondent part: Contributed to formulation of research questions and design of the field study, conducted the field study for all gradient variables except bacterial growth rates. Performed most of the data analysis and was the main author of the manuscript.

**Paper III:**
Johan Wikner, Lumi Haraguchi, Kevin Vikström, Veljo Kisand, Colin Stedmon and Jacob Carstensen. *Manuscript planned for Estuarine Coastal and Shelf Science.*
**Respondent part:** Contributed to formulation of research questions and design of the field study, conducted the field study for all gradient variables, except bacterial growth rates. Contributed to data analysis and writing of the manuscript.

**Paper IV:**
Kevin Vikström, Ines Bartl, Jan Karlsson and Johan Wikner. *Manuscript Submitted for Marine Ecology Progress Series.*
**Respondent part:** Contributed to formulation of research questions and design of the field study, was responsible for the field measurement of plankton respiration. Contributed to develop the strategy for specific measurement of baseline respiration. Performed all of the data analysis and was the main author of the manuscript.
List of abbreviations and glossary

**R_b**: Bacterial respiration

**R_m**: Bacterial maintenance respiration

**R sb**: Specific bacterial respiration

**R bg**: Bacterial growth based respiration

**R_p**: Pelagic respiration of organisms smaller than zooplankton

**R_bl**: Baseline respiration, respiration not driven by contemporary primary production

**BGE**: Bacterial growth efficiency, the efficiency with which bacteria convert carbon to biomass

**P_b**: Bacterial production

**µ**: Cell-specific bacterial growth rate

**PP**: Phytoplankton production as measured by the \(^{14}\text{C}\) incorporation methods

**GPP**: Gross primary production, the total productivity of primary production in the ecosystem

**P_{max}**: Estimated maximum uptake rate of CO\(_2\) the estimated by the \(^{14}\text{C}\) incorporation method

**K_{p/i}**: Light intensity response factor of primary production calculated from a tangential formula in the \(^{14}\text{C}\) incorporation method

**PMMA**: Polymethylmethacrylate plastic

**POM**: Polyoxyethylene plastic

**Allochthonous**: Originating from another place than where it is found.

**Hypoxia**: Reduced oxygen concentration (\(<1.4 \text{ cm}^3 \text{ dm}^{-3}\)) in a body of water detrimental to aerobic organisms

**Eutrophication**: Elevated organic production as a result of increased nutrient input to the system
Background

**Oxygen and the Baltic Sea**

Large scale declines in oxygen concentrations have been recorded throughout our oceans (e.g. Keeling et al. 2010, Katsumata et al. 2015). These declines are largely attributed to changes in oceanic ventilation and temperature increases reducing oxygen solubility while increasing stratification. Biological factors such as increased productivity also contribute to the oxygen declines observed. Increased productivity elevates the oxygen demand and is influenced by nutrients such as nitrogen and phosphorus but also carbon quality and availability (Kirchman 2008, Robinson 2008). The current consensus of modelling studies is that physical changes dominate the recorded reduction in oxygen (Johnson & Gruber 2007, Deutsch et al. 2015). However, eutrophication has been seen as a major contributor to oxygen loss in the Baltic Sea alongside physical characteristics resulting in long water renewal times (30-40 yrs, Snoeijs-Lejonmalm et al. 2017). The Baltic Sea is an enclosed sea with a drainage area of 1.78 km million km² and a human population of 80-90 million living along its coasts (Leppäranta & Myrberg 2009, Snoeijs-Lejonmalm et al. 2017). The large drainage area has brought excess nutrients that have increased the importance of biological factors to the Baltic Sea basins. This increase in nutrients has been active since industrial fertilization became widespread during the 1950s. Since then, a doubling of nitrogen and tripling of phosphorous during the last century have been recorded, assumed to increased productivity in the Baltic Sea and is known as eutrophication (Schneider & Kuss 2004, Savchuk et al. 2008). Further complicating the ecology and oxygen dynamics of the Baltic Sea is the regular occurrence and increase of nitrogen fixing cyanobacterial blooms (Finni et al. 2001), after the limiting nutrient for the Baltic proper, nitrogen, has been consumed by eukaryotic phytoplankton. Nitrogen fixing cyanobacteria can produce the nitrogen required from gaseous nitrogen (N₂) and are thereby, primarily limited by the phosphorous concentration and light availability for survival or growth. The increased biomass due to eutrophication is eventually remineralized in the water column of the Baltic Sea, consuming large amounts of oxygen.
Oxygen enters the water column primarily via dissolution by wind driven mixing and photosynthesis by phytoplankton in the surface layers of the oceans (Valiela 1995). Phytoplankton production (PP) also produces attractive carbon substrates to support respiration by heterotrophic organisms. Respiration in the aquatic environment occur mainly in microorganisms (< 1 mm in size fraction) which includes bacteria, virus, protozoa and some phytoplankton organisms (del Giorgio & le B Williams 2005, Kirchman 2008). Out of these, bacteria are the main consumers of oxygen and therefore, bacterial respiration ($R_b$) plays a key role in the oxygen dynamics of the oceans. Consequently, $R_b$ and microbial community respiration are linked to the formation of low oxygen zones, known as hypoxic zones. However, PP is often not enough to provide the whole water column with carbon equivalent to measured oxygen consumption rates in coastal systems (Duarte et al. 2001, González et al. 2001, Serret et al. 2006). In the Baltic Sea, freshwater input from rivers are especially large in the northern and eastern areas, with an average riverine inflow of 500 km$^3$ yr$^{-1}$ bringing both nutrients and carbon to the nearby ecosystem.

**Current knowledge of hypoxia**

The occurrence of hypoxia, a reduction of oxygen concentrations below a threshold (i.e. 1.4 cm$^3$ dm$^{-3}$ / 2 mg O$_2$ dm$^{-3}$ / 60 µmol O$_2$ dm$^{-3}$) where higher organisms cannot survive, is a worldwide phenomenon with grave implications for ecosystems. Hypoxia has occurred throughout history e.g. in deeper basins, fjords and highly productive lakes (Kamykowski & Zentara 1990, Zillén et al. 2008), but the last few decades have seen an increase in hypoxic zones. Coastal hypoxic zones have increased with 6% (Vaquer-Sunyer & Duarte 2008) and hypoxic zones in the Baltic Sea has had a four times increase in area between the 1960s and early 1990s (Jonsson et al. 1990).

Reported thresholds also vary depending on original species composition present (Vaquer-Sunyer & Duarte 2008, Brennan et al. 2016). The ecological effects of hypoxia are often seen as ecological deserts in the benthos as a result of a loss in biomass and habitats for higher order organisms (Karlson et al. 2002). Increased stress is also recorded and a change in behavior as oxygen levels get lower is a well-known response by organisms (e.g. Eriksson & Baden 1997, Domenici et al. 2007).
Baltic Sea large deposits of phosphorus bound to iron are also released as oxygen levels decrease. This phosphorus fuel the eutrophication present in the Baltic Sea, hindering remediation efforts for this inland sea.

Hypoxia in the Baltic Sea is believed to be caused by a complex hypsography of the Baltic Sea basins and the complexity of the archipelago (Savchuk 2010, Conley et al. 2011), combined with ongoing eutrophication of the Baltic Sea (Carstensen et al. 2014). The mixing processes of the Baltic Sea are highly dependent on the weather conditions and its influence on in- and outflow of the Baltic Sea which can only occur through the Danish straits (Wulff et al. 2001, Storch & Omstedt 2008). A longer period of strong eastern winds has the potential to push out water from the Baltic Sea through the Danish straits, lowering the sea level in the Baltic. The difference in sea level between the Baltic Sea and the Kattegat may then result in an inflow of dense saline waters replenishing bottom waters oxygen concentrations in the Baltic Sea basins. These inflow events vary in magnitude with the latest major Baltic inflow occurring in 2014 (Fig. 16 in Mohrholz et al. 2015).

Coastal hypoxic zones have also been recorded with an uncertain increase in the Baltic Sea. The areas where a significant oxygen decline has been recorded are dominated by a precursor to hypoxia, referred to as episodic hypoxia (Conley et al. 2011). Coastal hypoxic zones surrounding the Danish straits are reported to be a result of strong stratification, due to salinity, and nutrient loadings sustaining algal biomass (Vahtera et al. 2007). On the other hand, coastal hypoxia surrounding the Finnish coast are reported to be influenced largely by restricted water circulation rather than stratification, and freshwater input of nutrients sustaining high biomass and respiration (Norkko & Bonsdorff 1996). Overall, this illustrates the complex coupling between biological and physical factors affecting the oxygen dynamics of the aquatic ecosystem, of which respiration is central in explaining oxygen loss.

**Respiration**

Respiration is a key metabolic process producing energy for organisms in the aquatic ecosystem. Physiologically respiration is an electron flow through membrane-associated transport molecules, shifting electrons from donors to acceptors resulting in a proton gradient which ultimately
leads to production of energy in the form of ATP (Adenosine triphosphate). Several different acceptors can be utilized such as Iron (Fe(III)), Cobalt (Co(III)) and manganese (Mn(IV)). Here we focus on a far more common acceptor, oxygen (O$_2$), providing the highest energy yield. Aerobic respiration is a multipart metabolic pathway where carbohydrates undergo the anaerobic process of glycolysis resulting in ATP molecules and an end product of pyruvic acid. Two pyruvic acids forms the acetyl coenzyme A complex, which enters the Krebs cycle consisting of several enzymatic conversions eventually transferring electrons to nicotinamide adenine dinucleotide (NAD) and Flavin adenine dinucleotide (FAD), subsequently resulting in NADH and FADH molecules. The latter two molecules are used in the electron transport system forming H$_2$O as the final electron acceptor. Oxygen accepts electrons, netting the cell 18 ATP (del Giorgio & le B Williams 2005, White et al. 2011). This energy may be utilized for a myriad of cellular processes such as motility, enzymatic activity, repairing cell damage and growth (Russell & Cook 1995).

For bacteria, the relationship between respiration and growth is often assumed to be a constant (cf. Stock et al. 2014) and thus is often calculated using a constant from bacterial growth efficiency (BGE, Eq. 1).

$$BGE = \frac{P_b}{P_b + R_b} \quad \text{Eq. 1}$$

Bacterial BGE is a function of both bacterial production ($P_b$) and $R_b$. However, BGE has been shown to vary widely between ecosystems and similar $P_b$ (del Giorgio & Cole 1998, Roland & Cole 1999, Manzoni et al. 2012). Thus, the variable BGE within similar spans of $P_b$, cited above, must be driven by a change in respiration. Disentangling the complex control of respiration becomes vital to develop accurate models and environmental management strategies.

Current knowledge states that temperature, nutrient stoichiometry as well as the quality and concentration of carbon governs $R_b$ rates (Robinson 2000, Duarte et al. 2004, Hopkinson & Smith 2005, Vazquez-Dominguez et al. 2007). The $R_b$ in the Hudson Bay was elevated when glucose was added in tandem with elevated temperatures (Roland & Cole 1999) while $R_b$ increased as a function of carbon to phosphorus ratios in Canadian lakes (Smith & Kemp 2003, Smith & Prairie 2004). Temperature coupled
with nutrients has also been shown to increase $R_b$ (Berggren et al. 2010, Kritzberg et al. 2010). Furthermore, temperature effects on respiration may not be a linear function as shown by Pomeroy and Wiebe (2001), who found temperature manipulations to have a stronger effect on $R_b$ at lower natural temperature ranges (e.g. the Arctic). Similar effects were also discussed by Panigrahi et al. (2013) who found elevated temperature sensitivity ($Q_{10}$) in respiration during winter (November-March) in an sub-arctic estuary located in the north-westerns Bothnian Sea (a basin of the Baltic Sea). These studies illustrate the complexity of $R_b$ control in the environment.

On an ecosystem scale the rate of respiration is often assumed coupled with the level of PP (e.g. Robinson 2008, Bendtsen & Hansen 2013, Caffrey et al. 2014, Du & Shen 2015). However, in coastal systems riverine inflow of carbon can be consumed by heterotrophs weakening the coupling between PP and respiration (del Giorgio & le B Williams 2005). Carbon introduced from the terrestrial system (TDOC) has a long history of being utilized before entering the coastal system. Therefore, it may be more recalcitrant and may incur an extra cost, in energy, to utilize than autochthonously primary produced carbon. Simultaneously UV-light and other environmental conditions can transform the latent TDOC into a more bioavailable form for bacterial degradation (e.g. Moran & Zepp 1997, Bertilsson et al. 1999), further promoting respiration of TDOC, and disconnecting respiration from PP within the ecosystem.

**Energetics of bacteria**

Energy produced by cells have a myriad of pathways contributing to the oxygen demand of the bacterial cell. It is commonly assumed that the majority of energy produced goes to bacterial growth or storage of energy for periods of starvation (Russell & Cook 1995). However, processes such as motility, enzymatic activity and repairing cell damage also require a certain amount of energy. The latter mentioned processes can be viewed in the context of maintenance energy, or in other words energy not directly related to bacterial growth.

As early as the 1920s scientists observed a lack of efficiency in bacterial cell cultures despite frequent transfer intervals and it was suggested that bacteria have an energetic need to maintain their cellular functions
McGrew and Mallette (1962) tried to estimate the minimum amount of glucose needed to sustain optical densities in cultures, being some of the first to discuss the concept of maintenance energy for organisms. This concept of maintenance energy requirements in bacteria was further investigated by Neijssel and Tempest (1976) in continuous cultures which resulted in an ecophysiological model explaining, in part, the maintenance cost of bacteria.

The experiments by Neijssel and Tempest (1976) showed maintenance cost to vary between limiting nutrients and carbon substrates resulting in a conclusion that the maintenance cost depend on at least two factors: i) maintenance of cell integrity and (ii) maintenance of growth potential. The idea that maintenance energy could vary caused Pirt (1982) to separate the original maintenance cost variable into growth rate-independent (m₁) and growth rate-dependent component (m’), allowing for a variable maintenance cost. The literature so far is solely focused on laboratory experiments with single model organisms and has yet to be fully tested in the environment.

The Pirt-model and the ecological concept of baseline respiration

Here, I will describe the ecophysiological model by Pirt (1982) more in depth as it pertains to paper II. I will also introduce the concept of baseline respiration (del Giorgio & le B Williams 2005) which was central in paper IV.

The original Pirt-model (Eq. 2) describes a linear relationship between specific bacterial growth rates (µ) and utilization of a substrate (in our case oxygen, q(O₂)).

\[
q(O₂) = \left(\frac{1}{Y_g} - \frac{m'}{r_m}\right) \times \mu + m_1 + m' \quad \text{Eq. 2}
\]

According to the model the biomass synthesis of a specific bacteria is the ratio between the dynamic growth rate dependent maintenance cost (m’) and the observed maximum growth rate (µ’m) subtracted from the inverse of the maximum growth yield (Yₔ) and multiplied by the specific growth rate (µ) of a bacteria. As such, the growth related maintenance cost (m’) increases as µ approaches zero. The maintenance cost is represented by
the sum of m’ and m. In order for us to apply this model to the natural environment we must expect the factors within the brackets to be represented by the slope of a range of µ and bacterial specific respiration (Rs) measurements (Eq. 3). It is also difficult, if not impossible, to discern between m’ and m, in the natural environment and therefore these were summed up to a constant referred to as Rm in paper II. The equation used for paper II was thus.

\[
R_{sb} = \left( \frac{1}{\frac{m'}{\mu_m}} - \frac{m'}{\mu_m} \right) \times \mu + m_1 + m' \rightarrow R_{sb} = R_m + b\mu \quad \text{Eq. 3}
\]

It is noteworthy that the model by Pirt-model is solely driven by µ and the assumption that the environmental conditions are reflected by µ. Furthermore, accepting that the Pirt-model holds true under natural conditions, we may have an additional tool with which to untangle the relationship between bacterial growth and Rb. It may also prove to be a valuable tool in understanding the cost of living for bacteria in different environments, increasing our understanding of oxygen dynamics and the energetics of ecosystems.

Oxygen consumption based on the secondary carbon supply allochthonous sources and older algal produced carbon was discussed by del Giorgio and Williams (del Giorgio & le B Williams 2005). They referred to this respiration as baseline respiration and was defined as “a steady consumption of organic matter that is relatively independent of the contemporary primary production”. This aspect of ecosystem respiration was explored in paper IV as the level of baseline respiration (Rbl) is currently unknown for the marine environment and coastal systems. The concept of Rbl is largely based on the gross primary production (GPP) of several lake studies, and is seen as a third component of ecosystem respiration. The two other components are the respiration of autotrophs (primary producers) and the respiration of contemporary PP carbon by heterotrophs. The level of Rbl may vary substantially between ecosystems, and the carbon fueling Rbl may vary substantially in age and quality (del Giorgio & le B Williams 2005). The relationship between GPP
and $R_{bl}$ is assumed to be non-linear in nature as the previously discussed maintenance cost of bacteria, and most likely of other organisms as well, becomes dominant in ecosystems with lower GPP. Investigating the level of $R_{bl}$ may answer questions regarding an ecosystems connectivity to PP, the ecosystems lower energetic need and the presence of a continuous oxygen loss in different systems.

To study $R_{bl}$ can be a challenge, as it is difficult to discern between contemporary PP and non-contemporary PP. One may utilize the respiration present during winter conditions to form a baseline level of respiration, but only if the ecosystem has clear seasons and a contemporary PP close to naught during winter. This does, however, not answer the question of $R_{bl}$ during other seasons as this may vary. Environments without net photosynthetic production, like deep water, could be applicable in forming an estimate of $R_{bl}$ during other seasons than winter or in areas where seasons aren’t clearly defined. The concept of $R_{bl}$ and how to define baseline levels is also explored in paper IV.

**Measuring oxygen and respiration**

A classical way of measuring oxygen in water is the Winkler titration method still in use today. This Winkler method was first proposed in the late 19th century and has been further improved upon by e.g. Carpenter (1966). The method is based on oxidation of Mn$^{2+}$ to Mn$^{3+}$ in an alkaline solution and subsequently oxidizing iodide into iodine by way of Mn$^{3+}$ when the sample is acidified with sulphuric acid. Traditionally, this was performed by hand using titrations, but automated titration was developed in the 1990s and a potentiometric Winkler titration method eventually published improving the accuracy of the Winkler method (Furuya & Harada 1995). Incubating water samples over a period of time and measuring the difference in oxygen before and after incubation using Winkler titration allows for estimation of respiration rate. However, measuring respiration rates using the Winkler method can quickly become laborious as several replicate sub-samples are required to increase detection limits. This method is further complicated if additional sampling occasions are needed during incubation and a small sample size excluding larger organisms. The reported detection limit of this methodology can be as low as 0.07 mmol O$_2$ m$^{-3}$ d$^{-1}$ but in practice this
Another way of estimating respiration rates is the electron transport system (ETS) activity method. This method is based on the reduction of tetrazolium salt 2-para (iodophenyl)-3(nitrophenyl)-5(phenyl) tetrazolium chloride, which is a membrane-permeable salt and thereby, passively enters through the cell membrane (Dufour & Colon 1992). The dehydrogenase enzymes of the electron transport system has the capacity to turn this salt into a stable formazan crystal which in turn is detectable by spectrophotometry or bright-field microscopy (Zimmermann et al. 1978, Posch et al. 1997). The increase in formazan crystals has been reported to follow respiration with a significant correlation (Arístegui & Montero 1995, Martínez-García et al. 2009). The ETS method has the advantage of in situ measurements which do not require pre-filtration, known to distort respiration rates, as well as a much shorter incubation time of 2-5h compared to other methods (Martínez-García et al. 2009). However, the ETS method requires an uncertain conversion factor of a potentially toxic substance (tetrazolium) and as of the time of writing, this is an estimate of $R_b$ rather than on a pelagic level.

The two methods described above, while viable, both lack temporal resolution throughout the incubation process without frequent and laborious sampling. Therefore, these may not reveal the full dynamic of the oxygen decrease. An alternative to measure oxygen concentration in water is the use of optode technology which utilizes dynamic luminescence quenching (DLQ) for measuring oxygen concentrations in water. The optode uses a specially designed ruthenium foil which freely reacts to oxygen in the water (Demas et al. 1999). As the foil is excited by blue light the backscatter timing of the foils fluorescence changes depending on the amount of oxygen bound. Measuring the shift in fluorescence timing using a red light sensor, and applying the internal algorithm by Uchida et al. (2008), allows the optode to automatically measure the concentration of oxygen with high resolution ($1 \text{ min}^{-1}$). The methodology by Wikner et al. (2013) utilizes this high resolution optode technology together with specially designed stoppers in polymethylmethacrylate (PMMA) mounted with the model 3835 optode (Aanderaa Data instruments AS, Norway), and stringent temperature control for incubations ($\pm 0.1 ^\circ \text{C}$). However, this methodology suffers
from an elevated detection limit (0.3 mmol O$_2$ m$^{-3}$ d$^{-1}$) due to significant drift (2.1 mmol O$_2$ m$^{-3}$ d$^{-1}$ ± 0.3 95% C.I), most likely due to the plastic housing of the 3835 optode trapping oxygen (Stevens 1992, Wikner et al. 2013).
Aims and Research Questions

The overall aim was to assess the role of maintenance respiration ($R_m$) and baseline respiration ($R_{bl}$) in development of hypoxia. High-resolution oxygen measurements was used to estimate the respiration rates in field samples. The results should support environmental management of hypoxic zones, and advance our understanding of the role $R_m$ and $R_{bl}$ play in the oxygen dynamics of the sea.

- **Paper I**: Can the precision and detection limit of optode-based respiration measurements be improved? Can the existing background drift in the optode method be reduced by changing plastic housed optodes to titanium housed optode? Can pre-treatment and stopper materials effect the drift of the optode method? How often and when do non-linear oxygen declines occur in respiration incubations? What forms non-linear oxygen declines?

- **Paper II**: Is the Pirt-model applicable in the field under high and low productivity? What is the estimated $R_m$ of a sub-arctic estuary? What is the annual contribution of $R_m$ to the annual $R_b$ (free-living)? And what effect can a reduction in nutrients have on the $R_b$ if the Pirt-model is applicable?

- **Paper III**: How and where do hydrographical, biogeochemical and biodiversity shifts occur in the river to estuary transect? Do these shifts vary between high and low productivity? Are the changes due to simple mixing or biological processes? Does the estuary work as a filter for carbon and nutrients?

- **Paper IV**: How can $R_{bl}$ be defined? What is the importance of $R_{bl}$ in a sub-arctic estuary? What supplies the $R_{bl}$ with carbon substrates? What are the implications of a high $R_{bl}$ for environmental management?
Materials and Methods

Respiration rate
Plankton community respiration ($R_p$) was measured using the optode methodology briefly described above. However, the methodology was improved in paper I using titanium cased optodes that reduced drift and improved the detection limit of the methodology. Water samples were collected from the field in hydrochloric acid washed polycarbonate bottles and transferred to the laboratory. Here, 1 dm$^3$ glass bottles were carefully filled with sample water and sealed with a specially designed stopper in either PMMA or polyoxymethylene (POM) with the model 4330 optode attached to the stopper. Samples were subsequently incubated in temperature controlled baths (Julabo ED, Julabo 13) connected to an immersion cooler (Julabo FT 200). This resulted in a stringent temperature control (± 0.1 °C) at in situ temperatures. The optode sampled oxygen concentration once min$^{-1}$ over 12-24 hours and the slope of the decline in oxygen was then determined to get a respiration rate. Revisiting the method also allowed for analysis of non-linear oxygen declines. In cases where non-linearity was observed we derived the polynomial function fitted to 1 hour from the start of the quality assured dataset, often corresponding to the first hour of the incubation. To obtain specific bacterial respiration ($R_{sb}$) we used the same methodology, dividing $R_p$ with the bacterial abundance of the sample.

The commercially available model 4330 optodes used in the studies presented here have an accuracy in determining oxygen concentration of ± 1.5% according to the manufacturers specifications (Tengberg & Hovdenes 2014). This as compared to both Winkler titrations and other commercially available sensors (Bittig & Körtzinger 2015, Johnson et al. 2015, Bittig & Körtzinger 2017). Paper I, where we studied respiration rate incubations showed a detection rate of 0.97 mmol m$^{-3}$ d$^{-1}$ for individual optodes and zero drift. Allowing for precise estimates of respiration rates in pelagic and bacterial samples

Bacterial Production, Abundance and Volume
Bacterial biomass production ($P_b$) was calculated by uptake of $^3$H-thymidine in triplicate 1 cm$^3$ samples. Controls were pre-treated with
trichloroacetic acid (TCA) at 5% final concentration, and samples then incubated with $^3$H-thymidine for 1 hour at in situ temperature. $^3$H-thymidine was chosen as specific conversion factors were available and were close to that of reported conversion factors (Wikner & Hagstrom 1999). Thymidin has also been shown to not differ significantly from leucine estimates (Bell 1993). Furthermore, the Pirt-model is based on bacterial specific growth rate, and thereby, thymidine was more appropriate as it measures production of cells (Kirchman 2008). The uptake rates were then converted to cell production by multiplying the carbon density per cell from abundance and cell volume according to (Simon & Azam 1989, Norland 1993). In order to obtain specific bacterial growth rates ($\mu$), BP was divided by the bacterial abundance of each sample. Abundances and volumes were determined using epifluorescence microscopy of cells stained with acridine orange staining of the whole cell (Hobbie et al. 1977, Blackburn et al. 1998). Automatic image analysis of cell abundances and volumes were calculated according to (Blackburn et al. 1998).

**Nutrient and Carbon concentration analysis**

Nutrient and carbon concentration analysis of the sample water was preformed on the dissolved fraction. Nutrient samples were sterilized by filtering the sample water through 0.2 µm filters and then autoclaved with potassium peroxodisulphate additions that simultaneously oxidize the sample. The nutrient concentrations were then determined according to Grasshoff et al. (1983) using a four-channel autoanalyser (QuAAtro Marine, Bran & Luebbe®, Sweden). Dissolved organic carbon (DOC) was analysed in 0.2 µm filtered sample water acidified by hydrochloric acid. The DOC was determined using a high-temperature catalytic oxidation instrument with non-dispersive infrared (NDIR) detection and a TOC-L instrument (Schimadzu Corporation, Kyoto, Japan) (Sugimura & Suzuki 1988, Norrman 1993).

**Phytoplankton production**

Phytoplankton production (PP) was determined by the environmental monitoring group at Umeå Marine Sciences Centre using integrated hose samples from 1-10 m. The methodology uses incorporation of $^{14}$C, incubated in 500 µE m$^{-2}$ s$^{-1}$ light intensity in 11 glass vials with varying light absorption (0-100%). Incubations were stopped with additions of
HCl and excess $^{14}$CO$_2$ expunged before measuring dpm using Liquid Scintillation Analyzer TriCarb 2910TR (Perkin-Elmer, Singapore). PP at each depth throughout the water column was calculated by applying Beer’s law and a hyperbolic tangent function (Jassby & Platt 1976). For a more in depth look at the methodology please refer to paper IV.

We have not found a published detection limit for the $^{14}$C incorporation method. Therefore, detection limit for the $^{14}$C method was estimated assuming the average standard error of the estimated maximum uptake rate of CO$_2$ ($P_{\text{max}}$) and the light intensity response factor ($K_{p/I}$) to account for the major uncertainty (Eq. 3 in Paper IV). The determination of detection limit was close to where an increase in respiration occurred in paper IV supporting the estimate. However, the two main sources of error ($P_{\text{max}}$ and $K_{p/I}$) are not the only sources of error in the methodology. Additional errors may be added and can elevate the detection limit.
Results

Paper I: Improved accuracy of optode-based oxygen consumption measurements by removal of system drift and non-linear derivation

The introduction of titanium casings for the model-4330 optodes significantly reduced drift and thereby improved the detection limit for the respiration estimates when using the modified methodology by Wikner et al. (2013). Additionally, drift corrections were unnecessary when using the model-4330 optodes, resulting in an improved detection limit of 0.97 mmol O₂ m⁻³ d⁻¹ for a single optode. This can be reduced by replication down to 0.1 mmol O₂ m⁻³ d⁻¹. This estimate was conservative as the uncertainty if autoclaved control samples was included, despite lack of drift correction. There was no significant difference between stopper materials polymethylmethacrylate (PMMA) and polyoxymethylene (POM) nor was there a significant effect in pre-treatment solutions for storage between Milli-Q water, Hydrochloric acid (HCl) and Sodium sulphite (Na₂SO₃). However, we recommend the use of 0.3 mol dm⁻³ HCl as it can simultaneously clean the optodes between measurements.

Figure 1 Example of a non-linear trend in oxygen concentration decreases and the effect of analyzing the non-linear decline linearly
Investigating the dynamics of oxygen decrease within respiration incubations showed non-linear declines in 28% of the measurements having. In cases where respiration showed a non-linear decline the estimated slope (rate of oxygen loss = respiration rate) was derived at 1 hour. This resulted in a significantly higher respiration rate (64%) compared to a more traditional linear approach (e.g. Winkler titration). The majority of trends during incubations were concave and most likely due to limitation of carbon substrate.

The improved method for optode based respiration measurements, has the advantage of avoiding drift correction, improving precision and more accurate analysis of non-linear dynamics. Furthermore, samples of 1 dm$^3$ allows for including respiration from higher organisms up to zooplankton, while being a low effort, easily applied methodology.
**Paper II Importance of Bacterial Maintenance Respiration in a Subarctic Estuary: A Proof of Concept from the Field**

The concept of bacterial maintenance respiration ($R_m$) as described by Pirt (1982) was shown to be applicable under high productivity conditions. However, a 2nd polynomial function fit the dataset better. Using the 2nd polynomial function resulted in a first estimate of $R_m$ of 0.58 fmol O$_2$ d$^{-1}$ cell$^{-1}$ during the productive season. The annual contribution of $R_m$ to the $R_{sb}$ was calculated to 58% in the sub-arctic estuary. The annual calculation was based on a weighted sum of the 2nd polynomial function driven by $\mu$ values from environmental monitoring data in the estuary. The Pirt-model was not applicable at low productivity, and showed elevated $R_{sb}$ in comparison to similar $\mu$ values summer. This would indicate stress or a shift in the growth related maintenance cost ($m'$) suggesting elevated energy expenditure during starvation.

![Graph](https://via.placeholder.com/150)

**Figure 2** The Pirt and quadratic model fitted to data sets with specific growth rates ($\mu$) and specific bacterial respiration ($R_{sb}$). Models applicable at high productivity. Elevated respiration was observed at lower productivity.

The elevated $R_m$ by individual limiting nutrients, as postulated by Pirt (1982) and Neijssel an Tempest (1976), could not be fully confirmed in this study. The availability of the limiting nutrient, phosphorus, occasionally explained some of the variation in $R_{sb}$. Significant relationships to total dissolved phosphorus and carbon to phosphorus
ratios suggested a higher energetic need for bacteria as the limiting nutrient decreased.

Results of this study show that the annual contribution of $R_m$ can be a large part of $R_{sb}$, and suggests the Pirt-model to be applicable under high productivity conditions. The consequence of a high influence by $R_m$ on $R_{sb}$ is that a small decrease in ecosystem productivity may not necessarily have the expected result on respiration, and thereby the oxygen concentration. Instead, a shift from growth based respiration ($R_{bg}$) to $R_m$ may occur leaving the total oxygen concentration unchanged while reducing BGE. The governing factors and temporal variations for $R_m$ in nature are still unclear, warranting further studies focused on $R_m$ and improvement of ecophysiological models under natural conditions.
Paper III: Coastal filter effect by microbial mineralization of riverine DOC in a sub-arctic river-estuary gradient

The transect representing high productivity (August) showed changes within the first 10 km (salinity 3) in many variables. 48% of the changes observed were due to active transformation of carbon and nutrients. The low productivity transect of (April) showed most changes to occur closer to the rivermouth. Here, the majority of the changes (34%) could instead be attributed to simple mixing between freshwater and brackishwater.

Respiration during both seasons was significant and resulted in an average respiration rate of 2.6 mmol O₂ m⁻³ d⁻¹. This was interpreted as extensive respiration of DOC, mainly introduced to the estuary through river discharge. Nutrients showed limited effects on nitrogen and no significant decline in phosphorus was observed. However, a reciprocal relationship bacterial community growth and the limiting phosphorus for the whole data set indicates stringent bacterial control of the limiting nutrient concentration.

The bacterial community growth (BCG) rates were positively influenced by temperature also at low productivity indicating energy limitation. BGE was generally low (1-16%), mainly influenced by BCG at high productivity, but by bacterial community respiration (BCR) at low productivity. Within the transect phytoplankton showed pronounced variation in biomass and chlorophyll-a in April, in accordance with the spring bloom community. April was dominated by centric diatoms while this dominance was shifted to flagellates during August.

In conclusion, we found that the estuary acts mainly as a remineralization zone for riverine DOC transforming it to CO₂. This was supported by a relatively high $R_b$ during both productivity levels (and low BGE), likely influenced by increased $R_m$ during limited substrate supply (i.e. stress), especially in April.
Paper IV: High influence of baseline respiration in a sub-Arctic coastal system

Four methods to estimate $R_{bl}$ resulted in an average rate of 4.2 mmol O$_2$ m$^{-3}$ d$^{-1}$ corresponding to a significant contribution of 50% by $R_{bl}$ to the annual plankton respiration ($R_p$) in the estuary (Table 1). Wintertime respiration represented 25% of the annual $R_p$ in the estuary, showing unexpectedly active metabolism throughout the year. Dynamic estimates for the estuary using a hypsographic map together with the detection limit for the H$_{14}$CO$_3$ - incorporation method or the compensation depth as definitions of $R_{bl}$, captured both the depth variation in $R_p$ as well as the shift in baseline between seasons.

<table>
<thead>
<tr>
<th>Method</th>
<th>$R_{bl}$</th>
<th>SE</th>
<th>$R_{bl}/R_p$</th>
<th>$R_{bl}$ definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter respiration</td>
<td>4.2</td>
<td>0.48</td>
<td>76$^a$/38$^b$</td>
<td>November to March</td>
</tr>
<tr>
<td>$^{14}$C detection limit</td>
<td>4.2</td>
<td>0.34</td>
<td>51</td>
<td>PP &lt; $^{14}$C detection limit</td>
</tr>
<tr>
<td>Compensation depth</td>
<td>4.0</td>
<td>0.30</td>
<td>47</td>
<td>Sample depth &gt; compensation depth</td>
</tr>
<tr>
<td>Regression PP vs. $R_p$</td>
<td>4.6</td>
<td>0.21</td>
<td>83</td>
<td>y-intercept of Figure 2</td>
</tr>
</tbody>
</table>

$^a$Using a constant $R_{bl}$ . $^b$Assuming halved $R_{bl}$ during spring and summer.

A simple carbon budget showed PP to contribute with 16% to the $R_p$. Consequently, the estuary utilizes allochthonous carbon sources for energy and carbon production. For $R_p$ to be a robust tool for management of eutrophication related hypoxia, it must be free from interference of PP by definition. We therefore proposed a modification of the original concept (Eq. 4).

$$C_{bl} = C_{Lat} + C_A \rightarrow C_{bl} = C_A$$

Eq. 4

The only carbon source supplying baseline respiration ($C_{bl}$) is that of allochthonous carbon ($C_A$) while the primary produced carbon is excluded. As a consequence, the level of $R_{bl}$ will be set solely by $C_A$, a carbon source difficult to manage with nutrient reduction. In contrary, $C_{lat}$
is now included in the manageable part of the carbon sources for respiration, despite influence on respiration at longer time scales.

The results of this study suggest that $R_{bl}$ is an important aspect of an ecosystems oxygen dynamics. It also means that $R_{bl}$ is crucial in understanding the response of a coastal ecosystems to eutrophication, and thereby actions to reduce the oxygen consumption by nutrient load. This is in particular true if $R_{bl}$ is defined to only utilize allochthonous carbon sources, decoupled from the PP of the system. An ecosystem with a high $R_{bl}$ is already exposed to significant oxygen consumption, and thereby less resilient to additional nutrient load. Secondly, an already eutrophied waterbody with significant $R_{bl}$ may not benefit from a reduction of nutrients, depending on the level of productivity and nature of the association with ecosystem respiration.
Discussion

The original definition of \( R_{bl} \) by del Giorgio and le B Williams (2005) proved difficult to apply in a management context. While the original definition can help explain a reduced connection between contemporary PP and respiration in different ecosystem (le B. Williams et al. 2004, del Giorgio & le B Williams 2005), the change to the concept as presented in paper IV was made with environmental management in mind. According to the proposed improvement to the concept, \( R_{bl} \) is governed mainly by non-PP carbon. As such, one may expect \( R_{bl} \) levels to be the lowest in offshore ecosystems e.g. the oceanic environment, as these systems are less influenced by allochthonous carbon. In contrast, areas with excessive allochthonous carbon would have the highest \( R_{bl} \), potentially increasing its sensitivity to physical and biological changes that are known to cause formation of hypoxia (e.g. Keeling et al. 2010, Savchuk 2010). We found a significant contribution by \( R_{bl} \) to the annual \( R_p \) in the sub-arctic estuary studied in paper IV, highly influenced by allochthonous carbon, supporting the concept of \( R_{bl} \). Furthermore, we also found a similar non-linear response of \( R_p \) to gross primary production (GPP) to that found by del Giorgio & Le B Williams (Figure 14.52005). This indicates a lower limit of \( R_p \) in an ecosystem that would remain unchanged as long as the abundance of organisms remains stable.

To clarify the proposed changes in relation to environmental management a scenario can be considered as presented in figure 3. The physical characteristics sets an allowed maximum respiration rate after which hypoxia occurs (grey zone, Norkko & Bonsdorff 1996, Johnson & Gruber 2007, Deutsch et al. 2015). In an ecosystem with limited influence from allochthonous carbon we can expect a proportional respiration response to PP that ideally intercepts the y-axis close to, but not reaching zero (i.e., \( R_{bl} \approx 0 \), Solid line, Fig. 3). The line would not reach zero due to the quadratic response of respiration to growth demonstrated in paper II (for bacteria but plausibly for other plankton as well), and the non-linear trend found in paper IV between \( R_p \) and PP. A reduction in nutrients (dotted lines, Fig. 3), eventually reducing PP, would shift \( R_p \) towards the y-axis bringing the rate of respiration below the hypoxic threshold in an environment with limited allochthonous carbon (\( R_{bl} \approx 0 \)).
Now consider another ecosystem largely influenced by allochthonous carbon where we expect a quadratic response to PP as in paper IV, with a clearly higher baseline respiration (dashed line, $R_{bl2}$). In contrast to the $R_{bl1}$ environment, the response in respiration of a system highly influenced by allochthonous carbon ($R_{bl2}$) would continue to reside in a hypoxic state. In the case of $R_{bl2}$, management strategies focusing on nutrient reduction would not have the desired effect as a large part of $R_p$ is not driven by PP carbon from within the system. $R_{bl}$ may also make the ecosystem more sensitive to further increases in PP from within the system, stimulating oxygen respiration. Thus, due to the quadratic increase even a small increase in PP may result in a decrease in oxygen concentration or even hypoxia. In order to reduce the oxygen demand of the system a reduction in allochthonous carbon would instead be needed. As the allochthonous carbon is introduced by riverine input and groundwater input this may be difficult. Future climate change is predicted to elevate temperatures and change the frequency and amount of precipitation which can increase allochthonous carbon inputs to many northern coastal ecosystems (e.g. Cubasch et al. 2001, Meier 2006, Solomon 2007, Räisänen 2017) Thus, $R_{bl}$ may become more important in these ecosystems.
The relationship between \( R_p \) and PP is critical in understanding the level of \( R_{bl} \). Thus, a lack in parallel measurements of \( R_p \) and PP from the field complicates studies addressing the energetics on an ecosystem scale. The possibility to connect measured PP (provided by national environmental monitoring program) and \( R_p \) in paper IV allowed us to study \( R_{bl} \). Although not specifically discussed, there are some indications of the level of \( R_{bl} \) in the environment in published literature where PP is measured, or at least calculated, in parallel to \( R_p \). Du and Shen (2015) estimated a respiration rate corresponding to 4.7 mmol O\(_2\) m\(^{-3}\) d\(^{-1}\) during the winter in the Chesapeake Bay, comparable to our baseline respiration estimate of 4.2 mmol O\(_2\) m\(^{-3}\) d\(^{-1}\). However, the chlorophyll-\( a \) values presented in Du and Shen (2015) suggest some contemporary PP to still being active during the winter season. An incubation study on batch cultures mended by waste water treatment plant effluent water from the southern Baltic proper, allowed for a rough estimate of \( R_{bl} \) to be calculated (extracted values from Fig. 2 and Fig. 4 in Vaquer-Sunyer et al. 2016). This would correspond to a high productive ecosystem with a moderate influence of allochthonous carbon and resulted in an approximate \( R_{bl} \) contribution of 27\% to \( R_p \). Furthermore, three estuaries studied on the north American coast also provided data for a rough calculation of \( R_{bl} \) contribution to \( R_p \) amounting to 15\% However, respiration and PP values were not directly measured, but calculated from changes in dissolved oxygen and ecosystem productivity, and did not include production values close to zero (Fig. 9 in Caffrey et al. 2014). These two issues obscure the response of \( R_p \) to PP when approaching the y-axis. Thus, the 15\% \( R_{bl} \) estimated from this data may not represent the true \( R_{bl} \) present in their system.

The river-estuary transect study conducted in paper III observed a small difference in \( R_b \) between a low and high productivity state in the annual cycle. However, \( R_b \) switched from a random variation at low productivity, partly influenced by carbon:nutrient ratios, to one related to \( P_b \) at the high productivity state. This was in line with the results from Paper II analyzing process rates per cell. The median BGE values were also comparable with earlier estimates in the same estuary by independent methods, and within the range reported in literature (del Giorgio & Cole 1998, Roland & Cole 1999, Wikner et al. 1999).

The overall low BGE reported, and the shift from BCR controlled BGE at low productivities to BCG controlled BGE at higher productivities in paper
III, can be explained by the ecophysiological model by Pirt (1982) studied in paper II. A high level of $R_m$ would directly influence the BGE as bacteria respire more for each cell or biomass produced. In the ecophysiological model $R_m$ becomes increasingly important (Fig. 4) as $\mu$ approaches zero. If we assume that $\mu$ is a reflection of nutrients available, then in meso-eutrophic and eutrophic systems a reduction of nutrients would have a direct effect on the level of $R_{sb}$, as the contribution from $R_m$ is small in replete environments. In contrast, oligotrophic systems and mesotrophic systems are influenced by a higher share of $R_m$. Here, a reduction in nutrients would result in a shift from $R_{bg}$ to $R_m$ with a minor net change in $R_b$.

Neijssel and Tempest (1976) postulated that the level of $R_m$ would differ depending on the limiting nutrient. An ecosystem with phosphorus limitation would have a higher $R_m$ cost than one limited by nitrogen. Our study could not corroborate a nutrient effect on $R_m$, but phosphorus could occasionally explain the variations in $R_{sb}$. Furthermore, measuring the concentration present in the water mass may not be sufficient to investigate the effects of limiting nutrient. Instead, the uptake rate of the specific nutrient in nature may better reflect the role of nutrient supply. Varying associations between $R_b$, nutrients and carbon have been reported by prior studies. The Chesapeake Bay and Canadian lakes show

![Figure 4 Conceptual presentation of the importance of $R_m$ to $R_{sb}$ over a range of trophic levels, from ultraoligotrophic to mesotrophic. As ecosystem productivity decreases the role of $R_m$ increases and $R_{bg}$ becomes less dominant.](image)
elevated carbon-to-phosphorus (C:P) ratios elevating $R_b$ (Smith & Kemp 2003, Smith & Prairie 2004). A similar effect was indicated in table 2 of paper II where C:P ratios and phosphorus inconsistently elevated $R_{sb}$. The C:P ratio connection to $R_{sb}$ also indicates carbon to play a role in the level of $R_{sb}$. This is somewhat in line with respiration measured by Pomeroy and Wiebe (2001) who presents a relationships between $R_b$, carbon substrate as well as temperature changes. Our studies could not relate temperature to $R_{sb}$ but we found a relationship between temperature and $R_b$ during high productivity in paper III. The temperature range during the low productivity may have been too low for a significant relationship to be revealed, and as such, we can disregard a large influence of temperature in April. It is also worth noting that, the reduced connectivity between $R_b$ and many measured variables in paper II and III. This was also shown by del Giorgio et al. (2011) where $R_b$ rates varied less than $R_p$ rates over an inshore-offshore transect in the northern Pacific Ocean.

Few, if any studies, have focused on $R_m$ in the marine environment. Maintenance cost was discussed as a cause for higher respiration rates compared to $P_b$ in a transect study, indicating an elevated energetic need at sampling stations with lower available energy further offshore (del Giorgio et al. 2011). Another paper discusses maintenance respiration in terms of an energetic need maintaining functions during energy limitation (Lever et al. 2015). The latter connects to the low productivity season (April) in paper II where we showed elevated $R_{sb}$ in April plausibly due to stress and thereby, a change in the m’ factor of the Pirt-model (Pirt 1982).

Cajal-Medrano and Helmut (1999) estimated an $R_m$ rate 3 times higher than the one presented in paper II. However, this estimate may be questioned as the authors have not measured $R_{sb}$, but rather calculated respiration from $\mu$ and growth yield, resulting in autocorrelation in the data they base their estimate on.

The contribution of $R_b$ to $R_p$ was roughly calculated from paper III, using $R_b$ values of the two closest stations to the ones used in paper IV. These were then integrated over depth and the average of these were subsequently compared to the average values of $R_p$ integrated over depth for April and August in paper IV. This resulted in an $R_b$ contribution of 50% (April) and 80% (August) to $R_p$. This is in line with literature stating that heterotrophic bacterioplankton are main consumers of oxygen in the marine environment (del Giorgio & le B Williams 2005, Robinson 2008).
The contribution was also in line with an average contribution of 79% by $R_b$ on $R_p$ in an Pacific Ocean inshore-offshore transect (del Giorgio et al. 2011). Similarly, the contribution of $R_b$ to $R_p$ also fit the reported contributions of 40-76% summarized by Robinson (2008), omitting one reported value of 23% from the Saragasso sea. The $R_p$ measured in paper IV were all within the range of published scientific literature (e.g. Sherr & Sherr 1996, Smith & Kemp 2003, Preen & Kirchman 2004, Apple et al. 2006, Caffrey et al. 2014, Carvalho et al. 2017). The rates that were in the lower ranges of the literature cited was plausible due to the oligotrophic status of the studied sub-arctic estuary, showing low PP (Paper IV) and $P_b$ (paper II, Wikner & Hagstrom 1999). It was however also due to a coverage of both cold seasons and deep waters, representing low productivity environments.

The detection limit for the model 4330 optodes presented in paper II was seen as a conservative estimate as we included the drift error ($SD_{\text{sample}}$, Eq. 1, paper I), despite the negligible drift for all optodes combined precluding drift corrections. If the SD for drift was removed from the calculations we would have a detection limit of 0.1 mmol $O_2$ m$^{-3}$ d$^{-1}$. However, we still had a significant decline in oxygen concentrations over 24h for individual optodes and therefore, presented the conservative estimate of 0.97 mmol $O_2$ m$^{-3}$ d$^{-1}$. Also, the lowest quality assured measurement in our study had a significant respiration rate of 0.46 mmol $O_2$ m$^{-3}$ d$^{-1}$, indicating that the conservative detection limit may infact be lower than the one presented. To study the drift of model-4330 optodes we had to assume that the drift measurements and background error ($SD_{\text{sample}}$ & $SE_{\text{background}}$, Eq 1, paper I) performed in autoclaved water are representative in natural water samples. If we assumed that the oxygen trends in autoclaved water are irrelevant to natural water samples, then using $2 \times SE$ form wintertime mesurements (with the lowest respiration rates) we would get a detection limit of 0.1 mmol $O_2$ m$^{-3}$ d$^{-1}$. However, it is noteworthy that we could not demonstrate a difference in variation between winter and autoclaved seawater in paper I.

Purchasing several optodes and the incubator set-up with coolers can still result in a substantial initial cost for the method. The handling of optodes and sample water prior to incubation also needs to be considered in order to not introduce more oxygen to the flasks elevating the airsaturation above 100%. We have observed that 18 out of 249 measurements (7%) had
above 100% in initial air saturation. However, we have not been able to confirm if this is due to a calibration error, due to poor handling of sample water or simply high photosynthethic O₂ production in relevant cases. To clearly avoid influence of oversaturation we discarded these estimates prior to analysis. Temperature shifts during the incubation can also produce non-linear trends. Thus, there may be a need to precondition the sample water prior to sealing the sample with the stoppers when performing e.g temperature increase experiments. Nevertheless, the use of the oxygen optode methodology for respiration measurements presented by Wikner et al. (2013), improved in paper I, allowed for precise (in terms of low drift and a detection limit) measurements, invaluable for the studies conducted. Continuous sampling (frequency 1 min⁻¹) allowed adaptive non-linear derivation, decreasing potential overestimation (i.e., convex curve shape) or underestimation (i.e., concave curve shape) of respiration rates. The improved method is an easy and fast tool for robust estimation of planktonic respiration rates in natural waters, including trophic levels up to zooplankton.

In conclusion, by using high frequency sampling of oxygen concentration declines our studies revealed that $R_{bi}$ and $R_m$ may play a key role in setting the level of oxygen demand in an ecosystem. Nutrient reduction strategies seem to have a limited effect on oxygen consumption when an ecosystem has a high contribution of $R_{bi}$ to $R_p$ (Fig. 3). Similarly, heterotrophic bacteria showed a small net change in oxygen consumption due to a $R_b$ shift from $R_{bg}$ based respiration towards $R_m$ based respiration. This was relevant at relatively low $\mu$ range and coastal environment rich in carbon. A nutrient reduction would thereby have a small impact also for the main consumers of oxygen (i.e. heterotrophic procaryotes). Thus, understanding the energetics of the ecosystem can aid environmental management of the oxygen concentrations in our oceans. However, further studies focusing on $R_{bi}$ is required in order to confirm or discard the concept presented. Similarly, the Pirt-model was not applicable at low productivities and needs further development before it can be fully applicable as a tool to predict oxygen consumption also in low productivity environments.
Acknowledgements

The research presented was funded by the Kempe foundation, EcoChange and funding from Umeå University.

No venture is successful without great support from the people around you. My family has always supported me throughout hectic times, project work and a long term pursuit of an academic career. It has to be said, that the lack of pressure from my parents to go into a “more lucrative” field has allowed me to find and work in a field I find fascinating. I cannot thank my parents and family enough for their support.
I believe my fascination in marine ecology has its roots in a series of unsuccessful experiments named “Aphanizomenon gone bad” which resulted in me moving to Sweden in order to study marine ecology at Gothenburg University. I must admit I’ve seldom been the best of students but somehow I’ve managed to get this far. I owe a thank you to Angela Wulff who kept me studying marine ecology through my masters while acting as a guiding hand to work on the microorganism scale.
I was also fortunate to find what I consider my second family during my years in Gothenburg. I had to leave you behind when moving to Umeå for this opportunity to start my PhD studies, I want to thank you for being very supportive and having no relatives close by was hard at times so thank you for supporting me by being always being there.

Naturally, I would like to thank Johan Wikner and Jan Karlsson for choosing me to do this PhD position. To this day I am not sure why I was chosen from the other applicants as I am quite sure I wasn’t the most apt at bacterial ecology. I don’t think it is an overstatement to claim that Johan has spent a lot of time and energy to get me up to speed with bacterial ecology for this dissertation. We have very different personalities and your patience and scientific practice has taught me a lot! The phrase “It’ll be fine” has many times been uttered by me and I think this describes my general attitude to many things in life. Johan has many times pointed out “things are only solved when someone solves them” Therefore, a final thanks to all at Umeå Marine Sciences Centre for your support, comradery and interesting discussions, both at and outside of work. In particular I would like to thank Henrik, Martina, Siv and Jocke for always answering my strange questions and helping with my studies.
References


Buchanan RE, Fulmer EI (1928) Physiology and biochemistry of bacteria. The Williams and Wilkins company, Baltimore, USA


del Giorgio PA, le B Williams PJ (2005) Respiration in aquatic ecosystems: history and background. Oxford Univ Press, 198 Madison Avenue, New York, Ny 10016 USA


Eriksson SP, Baden SP (1997) Behaviour and tolerance to hypoxia in juvenile Norway lobster (Nephrops norvegicus) of different ages. Marine Biology 128:49-54
Kamykowski D, Zentara S-J (1990) Hypoxia in the World Ocean as Recorded in the Historical Data Set, Vol 37


