

BIOLOGICAL REGULATION IN THE FORMATION OF HYPOXIA

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Abstract. As part of a larger study investigating hypoxic potential in waters of Long Bay, South Carolina, the magnitude and variability of oxygen demand due to heterotrophic respiration in nearshore waters of this region were quantified during summers (Jul-Aug) of 2006-2008. Respiration rates were measured as short-term (4-6 h) oxygen consumption rates in the dark at ambient water temperatures. Rates were spatially patchy, although tended to be greater in nearshore waters (0.3 km) than in offshore (3 km) waters. Variability in respiration was strongly correlated with variations in organic matter (especially particulate organic matter) concentrations. The ultimate sources of this organic matter remain uncertain, however. Water column chlorophyll generally accounted for a small portion (mean = 20.2%) of total particulate organic matter concentration and oxygen time-series rarely showed any indication of significant net *in situ* production. To experimentally test the ability of inorganic nutrients to stimulate heterotrophic metabolism, a series of short-term (1h) enrichment bioassays were conducted using bacterial production (^3H -leucine incorporation) as a response variable. Significant enrichment effects were observed in 15 of 25 experiments, with enrichment effects ranging from 22-275% over control treatments. Nutrient addition, especially PO_4^{+} addition, more often resulted in greater increases over controls than did carbon addition (added as glucose). Results of both field observations and laboratory experiments are consistent with a conceptual model in which conditions that act to elevate concentrations of terrestrially-derived organic and inorganic substrates in the immediate nearshore waters of Long Bay result in enhanced oxygen consumption rates, which can promote the formation of low dissolved oxygen conditions. This agrees with observations made during the August 2009 pronounced hypoxia/anoxia event, when concentrations of dissolved and particulate matter were, on average, 40 – 200 % greater than concentrations observed throughout the summers of 2006 – 2008. In addition, results of this study suggest that conventional models of coastal hypoxia formation may need to be revised to account for the direct effects of nutrient availability on microbial heterotrophy in carbon-rich coastal waters.

INTRODUCTION

Hypoxia (the depletion of dissolved oxygen; generally defined as $< 2 \text{ mL O}_2 \text{ L}^{-1}$) in aquatic ecosystems occurs when oxygen consumption associated with microbial respiration exceeds the rate at which it can be replenished, either by oxygen production associated with photosynthesis or by physical re-aeration processes. This depends on a net input of organic matter to the ecosystem, or specific water mass, as well as the ability of the microbial community to utilize this supply of organic matter for their respiratory needs. In most cases of coastal hypoxia, the input of organic matter is generated from algal blooms stimulated by nutrient enrichment (e.g., Mallin et al. 2006), but it has also been known to occur through enhanced loading of terrestrial organic matter in the absence of significant algal production (e.g., Verity et al. 2006).

The microbial community, primarily the heterotrophic bacteria and archaea community, is responsible for the bulk of the respiratory oxygen demand in aquatic ecosystems (Williams 1984). In contrast to the wealth of information regarding the regulation of photosynthesis in marine microbial communities much less is known about the regulation of rates of microbial respiration in aquatic ecosystems (Williams and del Giorgio 2005). Since respiration is dependent on a number of enzymatic reactions at the cellular level, microbial respiration at the community level has been shown to be strongly dependent on ambient temperatures, and temperature alone can explain the majority of variability in microbial respiration rates across a range of aquatic ecosystems (e.g., Hopkinson and Smith 2005). Microbial metabolic rates will also depend on the source, composition, and chemical characteristics of the substrates available to fuel respiratory demand (e.g., Amon and Benner 1996). In addition, there is growing experimental evidence that the supply of inorganic nutrients can affect microbial respiration rates by facilitating the use of semi-labile, carbon-rich organic matter substrates (e.g. Smith and Kemp 2003). Therefore, understanding the regulation of heterotrophic microbial activity is key to elucidating the conditions and mechanisms that regulate organic matter

cycling, and by extension oxygen dynamics, in a given aquatic ecosystem.

The present study was motivated by the discovery in 2004 of hypoxic conditions in the immediate nearshore of Long Bay, a coastal embayment that borders the greater Myrtle Beach area in northeastern South Carolina (Sanger et al. *In Press*, Libes and Kindelberger 2010). Available data indicated that this event occurred during a period of upwelling-favorable meteorological conditions, suggesting the importance of physical processes in promoting the hypoxia event (Voulgaris and Sanay 2010). The role of biogeochemical conditions and processes could not be ascertained, however, due to a lack of data. The purpose of the present study was therefore to: 1) quantify rates of oxygen consumption associated with microplankton community respiration in nearshore waters of Long Bay during peak periods of likely hypoxia development (July – August); 2) assess the role of organic and inorganic substrate availability in regulating the magnitude and variability in microplankton respiration rates; and 3) specifically test the hypothesis that nutrient availability in this naturally organic rich environment may directly influence organic substrate availability to microbial communities. In attempting to quantify biogeochemical dynamics in these waters, we chose to specifically focus on rates of respiration since 1) respiration is the proximate biological process responsible for hypoxia formation; and 2) because measures of respiration integrates all organic carbon sources to an ecosystem it is likely the best index of carbon flow and trophic state in ecosystems receiving significant external organic inputs (Jahnke and Craven 1995, Williams and del Giorgio 2005).

METHODS

Sample collection

Water samples were collected as part of a high-resolution spatial sampling of waters off the Grand Strand in summer (July – August) of 2006, 2007, and 2008 (Koepfler et al. 2010). Sampling locations were paired to compare nearshore (0.3 km) and offshore (3 km) waters at various locations along the Grand Strand. In 2006, water samples were collected from surface (~1m below sea surface) and bottom (~1 m above sediment surface) at each location. In 2007 and 2008, water samples were collected from bottom waters only, to maximize spatial coverage of sampling.

Microplankton respiration rate measurements

Respiration rates were determined from standard dark-bottle oxygen consumption rates during short-term (4-6 h) incubations in 300-mL borosilicate glass BOD bottles. Respiration rate was calculated as the difference in oxygen concentrations between initial (n=4) and final (n=4) replicate bottles, over the course of the incubation.

Oxygen concentrations were determined by automated Winkler titrations of whole bottle samples using a Metrohm 798 Titrino with potentiometric end-point detection. Temperatures during incubation were maintained at ± 1 °C of the average *in situ* temperature for all sample collections, which ranged from 26 – 28 °C over the course of the study.

Nutrient and organic matter analyses

Subsamples of water collected for respiration rate measurements were analyzed for total nitrogen (TN), total phosphorus (TP), orthophosphate, nitrate + nitrite, ammonium, total suspended sediment (TSS), dissolved organic carbon, and chlorophyll *a* (Chl) concentrations. TN, TP, orthophosphate, nitrate + nitrite, and ammonium were measured on filtered (GF/F) samples using a Bran Luebbe Technicon AAIII autoanalyzer. TN and TP were also measured on unfiltered samples to distinguish between total (TNW and TPW) and dissolved (TNF and TPF) nutrient fractions. TSS concentrations were determined by standard gravimetric methods, with the organic fraction of TSS (taken as a measure of total particulate organic matter, POM) determined by loss on ignition at 450 °C. DOC concentrations were determined on filtered (GF/F) water samples by high-temperature combustion on a Shimadzu TOC-VCPN. Concentrations of chlorophyll *a* (Chl) and pheophytin (Pheo) were determined fluorometrically on acetone-extracted samples.

Enrichment bioassays

On four occasions (7/24/07, 7/10/08, 7/25/08, 8/29/08), the potential for direct stimulation of bacterial metabolism by inorganic nutrients was examined through a series of short-term nutrient enrichment bioassays. Treatments administered were: +N treatment, 50 μM additions of NH_4 as $(\text{NH}_4)_2\text{SO}_4$; +P treatment, 5 μM additions of PO_4 as KH_2PO_4 ; and +C treatment, 500 μM additions of C as glucose. Enrichment effects were determined as significant changes in rates of bacterial production, relative to control treatments (no enrichment). Bacterial production rates were determined via the ^3H -leucine method (Smith and Azam 1992). Rates were measured in 1 h incubations at *in situ* temperatures.

RESULTS AND DISCUSSION

Variability in respiration rates

Rates of respiration measured during the summers of 2006 – 2008 were highly variable, both within and across sampling events. For the entire data set (n=77) respiration rates ranged from 5.6 – 73.6 $\mu\text{g O}_2 \text{ L}^{-1} \text{ h}^{-1}$ and showed a log-normal distribution, with a geometric mean of 20.2 $\mu\text{g O}_2 \text{ L}^{-1} \text{ h}^{-1}$. Respiration rates measured in inshore waters were significantly greater than those measured in offshore waters (paired t-test, $p < 0.01$). In contrast to this

pronounced onshore-offshore gradient in respiration rate, differences between surface and bottom water respiration rates measured in 2006 tended to be much smaller and, on average, not significantly different.

In contrast to the wealth of data on pelagic production rates in nearshore coastal waters, data on respiration rates in these environments is generally lacking. The only other pelagic respiration rate data that exist for the South Atlantic Bight come from the waters off the coast of Georgia. Rates reported in this study encompass the range in rates reported by Jiang et al. (2010) for surface waters of the inner continental shelf off Georgia in summers of 2003 – 2006 ($20.5 - 28.3 \mu\text{g O}_2 \text{ L}^{-1} \text{ h}^{-1}$). Jiang et al. (2010) also observed respiration rates to decrease significantly with distance offshore and found a significant inverse relationship between respiration rates and salinity for inner shelf waters, which they attributed to the role of terrestrial inputs in fueling respiration.

Substrate regulation of respiration rates

In the present study, ambient water temperatures across all of the sampling cruises only varied between 26 and 28 °C. This allowed us to use relationships between respiration rate and *in situ* nutrient and organic matter concentrations to explore the role of substrate regulation in controlling the magnitude and variability in respiration. Across all sampling events, rates of respiration were significantly correlated ($p < 0.001$) to concentrations of POM, Chl, Pheo, DOC, TN, and TP. Concentrations of these substrates tended to show significant co-variation, however, with the exception of DOC which, interestingly, was not significantly related to either Chl or Pheo. This suggests that conditions that enhance the concentration of one substrate type tended to enhance the concentrations of all substrate types, and made the use of simple correlation analysis problematic in determining which resource most strongly regulate the magnitude of *in situ* respiration.

To further explore the role of these substrates in regulating respiration rate, the log-transformed dataset was subject to Principle Component Analysis. This identified two composite variables (PC1 and PC2) that explained 85% of the variability in the dataset, with 52% and 26% attributed to PC1 and PC2, respectively. PC1 had high positive loadings for Respiration, POM, Chl, and Pheo, whereas PC2 had high positive loadings for TPW, TNW, TNF, TPF and DOC. The tight grouping of mean factor loadings for Respiration, POM, Chl and Pheo suggest the importance of living and detrital particulates in determining variability in respiration rates, which is consistent with the fact that POM and Chl had the highest individual correlation coefficients with respiration rate (0.66 and 0.70, respectively) of the entire dataset.

Concentrations of POM and Chl were themselves highly correlated. This is perhaps not surprising, given that the later is a component of the former. Assuming that

Chl represents 1.15 % of the ash-free dry weight of phytoplankton biomass (Reynolds 2006), the fraction of POM represented by phytoplankton averaged just 20.2 ± 11.1 % across the entire dataset. That living phytoplankton represented a relatively minor fraction of the total POM pool is consistent with the idea that oxygen demand associated with microbial consumption of detrital material exceeds oxygen production due to phytoplankton in this generally net heterotrophic system. Concentrations of Pheo were relatively low in all samples, suggesting this large detrital POM pool was not of recent phytoplankton origin. The source of this particulate material remains unclear at present; limited evidence suggests that export from swashes or resuspension of marine sediments might both be possible sources.

Effects of nutrients on bacterial metabolism

A total of twenty-five enrichment experiments were performed among the four sampling dates (equaling 6 – 7 locations per sampling date). In fifteen of these experiments (60%) there were significant ($p < 0.01$) treatment effects relative to controls. Most of these showed significant enrichment effects for more than one treatment, as has been observed in other locations with similar types of experiments (e.g., Pomeroy et al. 1995). Of these fifteen experiments, ten showed the greatest stimulation of bacterial production upon phosphorus enrichment, with a mean stimulation over control of 84%; two showed the greatest stimulation to nitrogen enrichment, with a mean stimulation over control of 70%; and two showed the greatest stimulation to glucose enrichment, with a mean stimulation over control of 69%.

Phosphorus limitation of bacterial growth and metabolism is not an uncommon finding in coastal waters rich in organic material (Pomeroy et al. 1995, Smith and Kemp 2003). Heterotrophic bacteria tend to have high P requirements relative to phytoplankton and they will readily take up inorganic nutrients in order to metabolize low-nutrient organic matter resources. Long Bay, and the South Atlantic Bight in general, receives large quantities of organic matter from blackwater rivers and coastal salt marshes (Mallin et al. 2000). As these substrates tend to be carbon-rich, their utilization by heterotrophic bacteria likely requires the availability of inorganic nutrients. Increased inputs of inorganic nutrients, particularly phosphorus, are therefore likely to enhance the utilization of this organic matter by heterotrophic bacteria.

CONCLUSIONS AND IMPLICATIONS

Results of the present study support a conceptual model, developed by the Long Bay Working Group (Sanger et al. *In Press*), which describes hypoxia formation in Long Bay as the result of both regional-scale physical process and local scale biogeochemical

processes. Wind-driven upwelling enhances stratification and acts to constrain cross-shelf dispersion of terrestrial inputs delivered to nearshore waters by swashes, stormwater discharge pipes, groundwater, etc. This then elevates organic and inorganic substrates and directly stimulates heterotrophic oxygen demand, which results in the formation of hypoxic conditions in nearshore waters. Although primary production data are currently lacking, this appears to occur without the formation of a substantial phytoplankton bloom, as is the case in conventional models of coastal eutrophication and hypoxia formation.

Further support for this model comes from the August 2009 hypoxia event (see Libes and Kindelberger 2010). Although respiration rates were not measured during this event, limited sampling was conducted for organic matter and nutrient analyses. Ambient concentrations of POM, Chl, TNW, TNF, TPW, TPF and DOC during this event were, on average, significantly elevated relative to mean concentrations measured during the 2006-2008 sampling. These increases were not uniform, however. The mean increase in POM concentration (123%) was much greater than that of DOC (42%) but, interestingly, the relative contribution of Chl to POM for the 2009 data was almost identical that for 2006-2008. Mean increases in TPF (200%) were substantially greater than mean increases in TNF (34%). If respiration vs. POM relationships determined from the 2006-2008 data held for the 2009 event, respiration rates during the 2009 event would greatly exceed any previously observed rates.

From our results and conceptual model, it is clear that increasing organic and inorganic inputs to the waters off the Grand Strand, as is likely to occur with projected increases in population and development for this region, will act to decrease the physical threshold necessary for hypoxia formation. This should be considered as local municipalities consider options for better stormwater management practices.

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