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Phytoplankton-driven dark plankton respiration in the hypoxic zone off the Changjiang Estuary, revealed by in vitro incubations

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ABSTRACT

Hypoxia in near-bottom waters has been increasing globally. Dark plankton respiration is a key aspect of hypoxia studies. In situations where the general background eutrophication level is high, more blooms are found in estuaries and adjacent coastal zones, suggesting an increase in respiration from phytoplankton and heterotrophs. An assessment of the phytoplankton biomass-specific rate of dark plankton respiration is therefore of considerable value in terms of environmental assessments and modeling. During the summer of 2011 a series of concentrated in vitro incubation experiments were conducted on board a ship off the Changjiang Estuary and in the adjacent coastal zone, to simulate phytoplankton-driven dark plankton respiration under elevated phytoplankton biomass (i.e. high Chlorophyll *a* concentration) conditions and to further quantify the relationship between dark plankton respiration and phytoplankton biomass (measured as Chlorophyll *a*). A power function was used to elucidate the relationship for the concentrated incubation system. Based on our results we determined that the value for this constant was 0.67, which is similar to a previous value derived from other estuaries. Given the strong allochthonous (i.e. terrestrial) material input and the specific incubation condition, an empirical formula is suggested, which applies to conditions in which a high chlorophyll *a* concentration prevails and in situations where diatoms are the dominant phytoplankton.

1. Introduction

Against a background of worldwide eutrophication, a large number of studies have focused on increased algal growth accompanied with hypoxia in near-bottom environments (Diaz, 2001; Rabalais et al., 2014). Dark plankton community respiration (hereafter refer to as DPR) is one of the two processes that consume dissolved oxygen (DO) in near-bottom waters (with the other being sedimentary respiration), so quantification of DPR is important for understanding the oxygen depletion process in hypoxia studies. Since hypoxia is an environmental problem of public concern, parameterization of the DPR in hypoxia research will be an appropriate analytical technique for carrying out environmental assessments relating to coastal management and predictive modeling.

In estuaries and shallow coastal waters where diatoms are widespread, phytoplankton have a tendency to sink beneath the pycnocline (Sarthou et al., 2005). During summer-stratification conditions, phytoplankton that have sunk down to deeper waters (beneath the pycnocline) are unlikely to re-enter the upper mixed layer. It should also be noted that phytoplankton located at these deeper levels, together with their breakdown products, become an important source of fresh organic matter for heterotrophs in near-bottom waters, promoting DPR beneath the pycnocline (via direct phytoplankton and heterotrophic respiration). Stimulated by terrestrial input, the Chlorophyll *a* (Chl *a*) concentration in estuaries and coasts often exceeds 4 μg L^{-1}, or can even reach levels of over 30 μg L^{-1} during summer blooms, with diatoms being the predominant group (e.g., Anderson and Taylor, 2001; Tian et al., 2009). Although the main contributors to DPR are heterotrophs, the system is reliant on autotrophs. The DPR is thus powered by, or could be influenced by, autotrophs. In estuaries and coastal zones, such scenarios can occur during elevated phytoplankton biomass (i.e., high Chl *a*, or bloom conditions) when autochthonous organic matter becomes a more dominant source of carbon than allochthonous matter. Given the increasingly high occurrence of blooms in estuaries and adjacent coastal zones, there is a corresponding increase in the potential for phytoplankton-driven DPR. In the Louisiana continental shelf, DPR was found to be clearly related to Chl *a* concentrations (Murrell et al., 2013). A review of over 22 estuaries of various trophic status and from different locations further confirms a power relationship between plankton respiration and phytoplankton biomass (i.e., Chl *a*; Hopkinson and Smith, 2005), suggesting the potential of phytoplankton-driven water column DPR. The phytoplankton biomass, or the concentration of Chl *a*, at least under some circumstances, can therefore be used as one of the potential parameters on which to base a rapid assessment of plankton respiration in estuarine and coastal

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hypoxia studies. Given the usually notable allochthonous organic matter input in estuaries, the assessment of plankton respiration may be a more reliable approach to use, under high Chl *a* conditions. Although autotrophs may also contain notable autotrophic bacteria, the phytoplankton biomass (or Chl *a*) can be more easily monitored via various detection approaches (for example, continuous measurement with in situ fluorescence probes, discrete samples for later laboratory instrumental measurement, or the use of remote sensing for large-area monitoring). Until further development of fast and convenient methods for assessing total autotroph populations, it is more feasible and reasonable to use Chl *a* as the primary variable for such an assessment.

Hypoxia in the Changjiang (Yangtze River) Estuary and adjacent coastal zone was found to be severe, covering an area of up to 15,400 km^2 in summer (Zhu et al., 2011), comparable to areas covered in other well-known hypoxic zones, such as the Gulf of Mexico. In recent decades the summer algal blooms, stimulated by strong terrestrial input, have been frequently observed, with Chl *a* concentrations often reaching concentrations in excess of 24 μg L^{-1} (Zhou et al., 2008). It can also be expected that the frequency of blooms will increase, due to increasing eutrophication trends in the Changjiang River (Wang, 2006). Although a number of parameters have been suggested for the assessment of plankton respiration and Chl *a* relationships (Hopkinson and Smith, 2005), reports on the biomass-specific rate of DPR in the Changjiang Estuary and adjacent coastal zone are limited (Chen et al., 2003, 2006, 2009). It has been shown that the DPR is not clearly coupled with phytoplankton biomass alone, but phytoplankton does have an important impact on DPR, particularly in productive regions (Chen et al., 2006). The highest plankton respiration generally occurs in early summer, when plankton biomass is high (Chen et al., 2006). It is thus important that the contribution of phytoplankton should also be taken into account when determining the extent of 'background' eutrophication (Zhu et al., 2014). There is, however, a lack in understanding of the effects of phytoplankton-driven DPR in this region, but this can be assessed via concentrated in vitro incubation.

During the summer of 2011 we encountered several blooms in the region off the Changjiang Estuary and its adjacent coastal zone, in the hypoxic zone (Zhu et al., 2011). We took this opportunity to collect phytoplankton and carry out a series of in vitro dark incubation experiments on board a ship, in order to measure the phytoplankton-driven DPR rate, using the classic Winkler titration method (Bryan et al., 1976). The concentrated in vitro incubation was carried out with gradient Chl *a* concentration, the purpose being to obtain a clear phytoplankton-driven trend. The elevated Chl *a* concentration in the incubation system also enabled us to obtain an obvious DO concentration difference over a relatively short time period (7–9 h), so as to avoid problems relating to respiration detection limits (Murrell et al., 2013; Robinson and Williams, 2005). Our initial purpose was to examine the factors that influenced the system and to use this knowledge to derive an empirical formula. This would be compared to similar formulae derived from other regions. Such analyses are important for the determination of recommended application limits.

2. Materials and methods

2.1. Sampling stations and in vitro incubations

The study area covers the offshore region near the Changjiang Estuary and the adjacent coastal zone where oxygen depletion has been reported in near-bottom waters (Zhu et al., 2011) (Fig. 1). All in vitro incubation experiments, including both concentrated in vitro incubation and original-concentration in vitro incubation, were carried out on board R/V *Beidou* during August, 2011. Incubations were carried out at nine stations (Fig. 1), as summarized in Table 1.

To simulate phytoplankton-driven DPR, we undertook the concentrated in vitro incubation experiment. Phytoplankton in the water column at the station was first collected via a plankton net (pore size: 76 μm) vertically trawled from 10 m above the seabed to the surface. The liquid in the net was carefully transferred to a pre-cleaned bottle. In most cases, vertical trawling was repeated several times, to obtain an elevated biomass of phytoplankton. After several collections the volume of the phytoplankton-abundant liquid was usually brown in color, with a volume of about 1 L. This liquid was immediately filtered through a 200 μM mesh filter to remove zooplankton, and then diluted with 200-μM-filtered sea-surface water, to prepare three concentrated phytoplankton water samples that differed in terms of phytoplankton abundance (measured as a gradient Chl *a* concentration). The three concentrated phytoplankton water samples were then siphoned into ten parallel biochemical oxygen demand (BOD) bottles (65 mL in volume), wrapped in black bags and aluminum foil, and placed into a water tank that had been equilibrated with sea surface water (i.e., incubated in dark conditions at the sea-surface temperature). DO was measured by titration at 0, 1, 3, 5, and 7 h. To check variation of phytoplankton (in terms of both community structure and biomass) during the incubation period, phytoplankton pigment samples were collected from some of the incubation experiments (at Stations C0, O11, and B2) at the beginning and end of the experiments. For the purpose of pigment

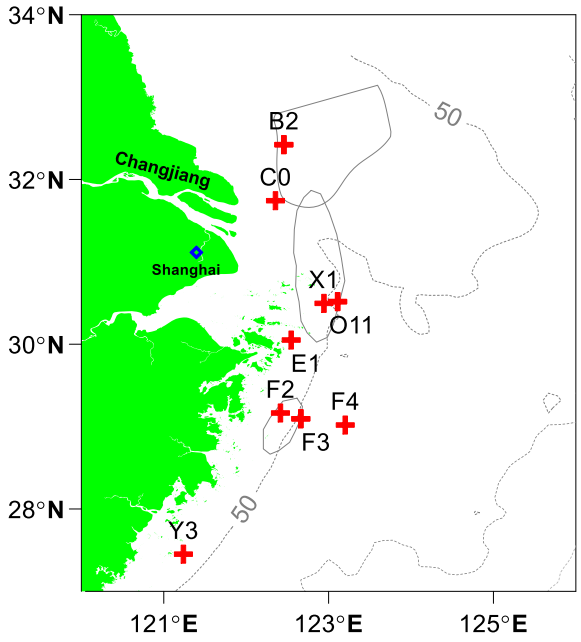

Fig. 1. The study area and incubation stations in August, 2011, with grey line indicating the locations where bottom hypoxia was reported previously by Li et al. (2002) and Zhu et al. (2011).

Table 1
Incubation stations in this study (O: original-concentration in vitro incubation, C: concentrated in vitro incubation).

Station	Longitude (°N)	Latitude (°E)	Date	Depth (m)	Temperature (°C)	Incubation
Y3	121.238	27.455	14th	30	27.3	O
F3	122.663	29.098	19th	54	25.0	O
F2	122.416	29.168	19th	33	25.0	O
F4	123.202	29.022	19th	72	28.3	C
E1	122.546	30.054	20th	22	25.0	O
X1	122.944	30.499	21st	57	26.4	C + O
C0	122.355	31.745	24th	24	25.2	C + O
B2	122.457	32.426	26th	27	23.7	C + O
O11	123.110	30.520	27th	59	26.8	C