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Respiration, growth and grazing rates of three ciliate species in hypoxic conditions

Emma Rocke, Hongbin Liu *

Hong Kong University of Science and Technology, Division of Life Science, Clear Water Bay, Kowloon, Hong Kong

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ABSTRACT

Marine hypoxic episodes are affecting both marine and freshwater bodies all over the world. Yet, limited data exists with regard to the effects of decreasing oxygen on protist metabolism. Three ciliate species were therefore isolated from Hong Kong coastal waters. Controlled hypoxic conditions were simulated in the lab environment, during which time growth, respiration and grazing rates were measured. *Euplotes* sp. and a *Oxytrichidae*-like ciliate showed decreased growth and respiration below 2.5 mg O_2 L^{-1}, however *Uronema marinum* kept steady growth and respiration until below 1.5 mg O_2 L^{-1}. *Euplotes* sp. and the *Oxytrichidae*-like ciliate had the highest ingestion rate, which dropped significantly below 3.0 mg O_2 L^{-1}. *U. marinum* grazing rates were affected at and below 1.5 mg O_2 L^{-1}, correlating with their drop in growth and respiration at this lower concentration. This study illustrates the slowing metabolism of key grazing protists, as well as species-specific tolerance in response to hypoxia.

1. Introduction

Hypoxia is becoming a growing coastal threat to marine communities. Protists are unicellular eukaryotes which occupy a key place in the microbial food web (Pomeroy, 1974). Protist species composition is shifting, and in some cases increasing in species diversity in low oxygen marine waters (Edgcomb et al., 2011; López-García et al., 2001; Moon-van der Staay et al., 2001). Bacterivorous protists in particular, through grazing, recover dissolved and particulate organic matter lost through excretion and other processes into the 'bigger' food chain (Azam et al., 1983; Sherr and Sherr, 2002). Protists also contribute significantly to nutrient remineralisation (Caron et al., 1990; Strom, 2000). Many ciliates are known to be active during hypoxic disturbances (Stauffer et al., 2013), and as active micrograzers are therefore contributing to top down control of protist and prokaryote communities in these areas during hypoxic disturbances (Anderson et al., 2013). In order to incorporate the response of the marine microbial loop to hypoxia into current models and understand how biogeochemical cycling is affected here, understanding how the physiological state of these micrograzers is affected during hypoxic episodes is vital.

Ciliates in particular are easily cultured, are relatively large in size, have short life cycles and lack complex developmental stages and therefore are the best studied protist in simulated hypoxic and anoxic environments so far (Fenchel et al., 1990; Forster et al., 2012; Stoecker and Michaels, 1991; Xu et al., 2009). Many field studies to date focussing on anoxic to hypoxic habitats have found ciliates to persist significantly here (Esteban et al., 2009; Forster et al., 2012; Stauffer et al., 2013). In addition, it has been observed previously in molecular studies that the Oligohymenophorea ciliates increased during hypoxia in field conditions (Stauffer et al., 2013; Rocke et al., 2014).

Very few studies have explored respiration rates of individual protist species. Fenchel and Finlay (1983) compiled the most comprehensive survey to date of protistan grazing rates. During balanced growth, energy metabolism of protists was found to be linearly proportional to the growth rate constant. No published study has yet focussed on how protist respiration rates react to decreasing oxygen concentrations however.

Grazing rates of ciliates is well documented, and both Oligohymenophorea and Spirotrichea are well known bacterivores (Fenchel, 1980a). Spirotrichea (represented in this study by *Euplotes* sp. and an *oxytrichidae*-like ciliate) are characterised as 'upstream' filter feeders, whose filter feeding apparatus selects food particles based on the structure of their feeding apparatus (Fenchel, 1980b,c; Jonsson, 1986; Wilks and Sleigh, 1998). As a result of this they are omnivores, requiring a high concentration of bacteria to survive, frequently taking advantage of patches of bacteria (Fenchel and Jonsson, 1988).

* Corresponding author. Tel.: +852 23587341; fax: +852 23581559.
E-mail address: liuhb@ust.hk (H. Liu).

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Scuticociliates (represented here by *Uronema marinum*) are microphagous bacterivores primarily found below the oxycline, in eutrophic habitats (Beaver and Crisman, 1982). They will take advantage of sinking detritus, and some species are able to consume up to 500 prokaryote cells per ciliate per hour in freshwater habitats (Simek et al., 1996).

These studies demonstrate how the presence of micrograzers in suboxic habitats has the potential to dominate trophic interactions here. However until now no published study has addressed this.

Oligohymenophorea, Spirotrichea and the third ciliate isolated for this study all occupy the range of smaller size fractions occupied by ciliates. Their physiology is less studied in comparison to other ciliate size classes studied so far (Fenchel and Finlay, 1983), and no known study has focussed on their physiological response to decreased oxygen concentrations. For this study we were able to isolate a representative species of Oligohymenophorea, and two from class Spirotrichea from local Hong Kong waters in order to measure their physiological responses to hypoxic conditions, in an attempt to understand what species specific physiological changes or adaptations are occurring in low dissolved oxygen waters.

The goal of this study was therefore to expose local isolated ciliate species of different sizes and microhabitats to various DO concentrations, and monitor their growth, respiration rates and grazing rates with the goal of learning more about their physiological response to hypoxic conditions.

2. Methodology

2.1. Isolates

Ciliate species *Euplotes* sp., *Uronema marinum,* and a 9–12 μm long *oxytrichidae*-like ciliate were isolated from Hong Kong waters (Fig. 1). *Euplotes* sp. was isolated from the Port shelter site (PS in Fig. 1), and the remaining two species were taken from Tolo Harbour surface waters (TH in Fig. 1), a tidal inlet prone to stratification and seasonal hypoxia, which can last from several weeks to several months during late summer. Tolo Harbour isolates were sampled in May 2011, when surface water DO was 7.1 mg O_2 L^{-1}, and water temperature was 27 °C. Pure cultures were obtained through picking single cells and inoculating fresh autoclaved seawater with 0.005% yeast extract. Cultures were kept at 24 °C, and different batches were used for different experiments.

2.2. DNA extraction, amplification and sequencing

Pure cultures were filtered through 3 μm pore sized polycarbonate filters (GE Water & Process Technologies) using a vacuum pump. DNA was immediately extracted from the filters using a modified phenol: chloroform extraction and alcohol precipitation procedure (Boström et al., 2004). The 18S rDNA fragments were amplified by polymerase chain reaction (PCR) using the two universal eukaryote primers Euk82f (5′ GAA ACT GCG AAT GGT TCA TTA AAT CAG 3′) and EUK516r (5′-ACC AGA CTT GCC CTC C-3′) (Casamayor et al., 2002; Lepere et al., 2006). Briefly, the PCR reaction was carried out with a 20 μl master mix containing 2.5 μl of 10 × Buffer, 1 μl of $MgCl_2$ (25 mM) and dNTPs (5 mM), 0.5 μl of BSA and forward and reverse primer (10 μM), 0.1 μl of Taq polymerase (Invitrogen) and 2 μl of DNA template. The PCR program consisted of an initial incubation step at 94 °C for 3 min, followed by 29 cycles with a denaturation step at 94 °C for 20 s, an annealing step at 55 °C for 30 s, then an extension step at 72 °C for 40 s. These cycles were followed by a final extension step at 72 °C for 8 min. Amplifications were verified on a 0.8% agar gel stained with ethidium bromide.

PCR-amplified extracts were purified using the Purelink® PCR gel Purification Kit (Invitrogen). Purified PCR products were then sent to Techdragon for sequencing using the Euk82f primer. Sequences were assembled, aligned and a maximum likelihood phylogenetic tree was constructed using MEGA 5.1, using 400 bp sequences. Bootstrap values were calculated using 1000 replicates (Tamura et al., 2011) (Fig. 2.).

18s rDNA sequences generated by this study have been filed in Genbank under the accession numbers KJ754150-52.

2.3. Dissolved oxygen level control used for all experiments

Low DO was achieved by bubbling N_2 and air into the seawater at specific rates, using a negative feedback mechanism. Oxygen concentrations were monitored using a Cole Parmer Polarographic DO probe, and seawater DO concentrations were controlled by regulating gas flow rates through a digital dissolved oxygen controller (B&C electronics). Normoxic controls (6.0–6.5 mg O_2 L^{-1}) were acquired by bubbling air into the culture flask.

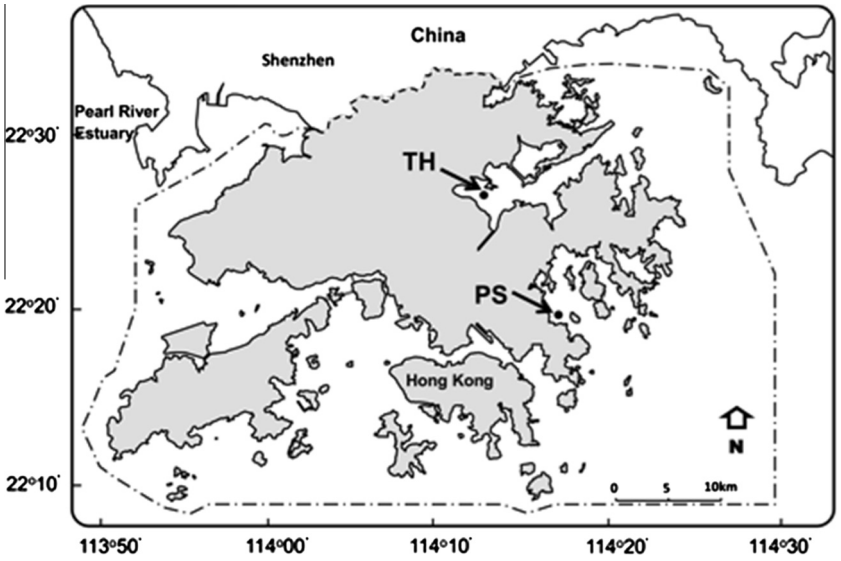

Fig. 1. Map of Port Shelter (PS) and Tolo Harbour (TH), Hong Kong.

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