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The combined effect of ultraviolet B radiation and temperature increase on phytoplankton dynamics and cell cycle using pulse shape recording flow cytometry

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ABSTRACT

Temperature and ultraviolet radiation B (UVB) are expected to increase in the next few decades and will mostly affect mid and high latitudes. In order to study the combined effect of temperature and UVB increase, on the phytoplankton community in the Saint Lawrence Estuary, duplicates of four different treatments were applied to 2 m^3 mesocosms to simulate an overall 3 °C and a 77.8% UVB increase, and combined. Samples were collected every 6 h over 10 days and the phytoplankton community was then analysed using a conventional flow cytometer and a Cytosense flow cytometer. Flow cytometry distinguished 9 clusters (Pico, Nano I, C3, C4, CHAINS, C6, C7 and C8) of cells sharing similar optical properties with average sizes varying from 1.3 μm up to 101 μm for chain forming cells. Compared to untreated enclosures, the high UVB treatment induced lower cell abundances (up to −40%) for clusters Pico, Nano I, C4, CHAINS and C7, followed by an unexpected cell abundance increase in all the clusters during the last 3 days of the experiment (up to 46%). This increase was sustained by faster calculated periodicities of the cell optical characteristics and abundances, linked to a shorter cell cycle. In the samples from the high temperature treatment mesocosms, a positive delay was observed for the cell abundance increase in clusters CHAINS, C6 and C8, combined with higher average abundance values (up to 67% with respect to untreated mesocosms). During the last 3 days of the experiment, abundances decreased compared to the values observed in the high UVB mesocosms, with a slower trend in the periodicities, suggesting that high temperature inhibits the cell cycle. The combined temperature and UVB treatment emphasized the effects observed under high temperature treatments, maintaining temperature positive effects (*i.e.* higher abundances) on clusters C3, CHAINS, C6 and C8 suggesting a compensation from the positive temperature effects over the negative (*i.e.* lower abundances) UVB effects. Increasing temperature induced a negative effect on the abundance of clusters C4 and C7. In this case, trends in C6 and C8 cell periodicities were faster than under normal conditions while Nano I, C4 and C7 cycles were slower. Cells<3 μm were negatively affected by the combined exposure (up to −55% compared to untreated mesocosms) while most of the larger cells were positively affected (up to 75% compared to untreated mesocosms), suggesting a shift to an herbivorous food web (*sensu* Legendre and Rassoulzadegan, 1995). Results suggest that changes in cell cycles due to increases in temperature or UVB exposure may play an important role in controlling abundance.

1. Introduction

Aquatic microorganisms are affected by global climate change resulting from intense anthropogenic activity. Even if ozone concentration in the stratosphere is stabilized, the expected increase in temperature in the troposphere due to the greenhouse effect will continue to enhance ozone depletion by decreasing temperature transfer to the stratosphere (Christensen et al., 2007; Weatherhead and Andersen, 2006). Temperature and ultra violet radiation (UVR, 280–400 nm) increases will modify the structure of communities of living organisms, shifting species dominance and trophic interactions (Bothwell et al., 1994; Wängberg et al., 1996), and thus biogeochemical fluxes over a larger scale (Häder, 1993; Mostajir et al., 1999a). Positioned at the first stage of the marine food web, phytoplankton encompass thousands of species that develop by simple division at a rate of about once a day, thus potentially doubling their abundance every 24 h. Phytoplankton represent 2% of the Earths photosynthetic biomass, but they contribute up to ~50% of the global annual primary production (Falkowski and Raven, 2007; Field et al., 1998). Any significant perturbation at this level of the food web may modify the whole energy transfer and biogeochemical processes in the ocean.

UVR are harmful to most living organisms and may cause changes in population structures both on land and at sea (Bothwell et al., 1994). UVR will more likely affect the food web structure than bulk biomass because of different sensitivities among planktonic organisms (Belzile et al., 2006). Ultraviolet radiation B (UVB, 280–320 nm) has been

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shown to affect several ecologically important traits and functions in phytoplankton cells, both in mesocosm and laboratory studies. These effects include a decrease in photosynthetic rates, DNA damage and delays in cell division (Buma et al., 2003 and references there in). However, long term studies on the effects of UVB are sparse. When studying communities in mid-latitude natural ecosystems, (Davidson, 1998 and references therein), there was little evidence for UVB having a strong impact on phytoplankton production and biomass and most of the observed impacts were indirect and linked to an increase of dissolved organic matter degradation affecting the whole microbial community (Wangberg et al., 1999), or inhibiting algal consumers (Bothwell et al., 1994; Ferreyra et al., 2006; Mostajir et al., 1999a). In mid latitude coastal environments, both vertical mixing in the water column and high concentrations of dissolved organic matter, typical of such environments, limit the exposure of cells to UVR (Mohovic et al, 2006). Under such conditions, vertical mixing is the main feature that may explain the use of UVR (principally ultraviolet A radiations, UVA (320–380 nm)) as an energy resource for photosynthesis rather than a threat (Barbieri et al., 2002; Gao et al., 2007). Any of the acclimation processes found in phytoplankton cells are dependent on the exposure intensity and duration, as well as on the shape of the cells. Indeed, small cells were found to exhibit faster inhibition kinetics than large cells because of their higher surface to volume ratios (Helbling et al., 2001), but also to be more resistant to damage on the photosynthetic apparatus (Buma et al., 2003).

Temperature increases mostly show positive effects on phytoplankton growth, photosynthesis and abundance. Indeed, although higher temperatures are expected to decrease the mixed layer depth and thus, limit nutrient availability in the upper photic layer therefore enhancing exposure to UVR radiation (Doney, 2006; Häder et al., 2007), cells may recover through photo-repair processes which rely on temperature dependent enzymatic activity (Doyle et al., 2005). Growth rate and photosynthetic activity increase as a result of warming (Baulch et al., 2005), changing the timing of phytoplankton blooms (Lassen et al, 2010), inducing acceleration of physiological microorganism processes and biomass production.

Only a few studies have been carried out in order to estimate the combined effects of UVR and temperature increases on marine microorganisms (Doyle et al., 2005; Mohovic et al., 2006; Nouguier et al., 2007). Doyle et al. (2005) observed a decrease in phytoplankton growth rate under high UVR treatments in alpine lakes, which halted when temperature was increased to as much as 14 °C over usual values.

Single cell analysis using flow cytometry appears to be the best approach for achieving high resolution dynamics of aquatic microorganisms. Most of the bench top flow cytometers are suitable for picophytoplankton and nanophytoplankton analysis (mostly <10 μm, Grégori et al., 2001). New generations of automated flow cytometers are available and dedicated to larger phytoplanktonic microorganisms, being theoretically limited to up to ~800 μm in width and a few mm in length when considering chain forming cells (Cytosense, Dubelaar et al., 1999). Morphological information at the single cell level is provided by the recording of scatter and fluorescence signal shapes, and enables the description of several distinct groups of autofluorescing cells sharing similar optical properties (Thyssen et al., 2008). Regarding biogeochemical studies, shape, size, biomass and responses to environmental changes are the most important parameters to consider rather than taxonomical information in order to establish the microorganism relationships with organic matter fluxes and their role in global biogeochemical processes (LeQuéré et al., 2006; Weithoff, 2003).

This paper is dedicated studying the impact of UVB and temperature increase on phytoplankton dynamics. Experiments were run using 2 m^3 mesocosms, where changes in UVB and temperature were applied separately and combined, and monitored with respect to an unperturbed mesocosm using the natural community of the St-Lawrence estuary. Phytoplankton cells were analysed using a conventional flow cytometer and a Cytosense flow cytometer, to achieve the most possible accurate estimation of the picophytoplankton, nanophytoplankton and microphytoplankton community characteristics and abundances, using a frequency of one sample every 6 h to resolve the life cycle of those cells that divide, at most twice a day (Nyquist, 1928).

2. Materials and methods

2.1. Mesocosm experimental design and deployment

The experiment was performed on eight 2 m^3 (1.70 m deep, 1.2 m diameter) stainless steel mesocosms at the Aquaculture station of Pointe au Père/Institut des sciences de la mer de Rimouski (48°30 N, 68°28 W), Québec, Canada, from the 20th to the 29th of August 2008. Four experimental treatments were applied in duplicate, and will be referred to as follows: natural temperature and natural UVB (NTNUV), high temperature and natural UVB (HTNUV), natural temperature and high UVB (NTHUV), and high temperature and high UVB (HTHUV). All mesocosms were exposed to the natural incident solar light during the whole experiment, with the exception of the HUV treatment, where natural irradiance was supplemented with UVB lamps. Temperature in the mesocosms was independently controlled in each mesocosm with a N480D electronic controller and recorded every 60 s in a data logger. The temperatures in the mesocosms were adjusted and kept constant with an external heat exchanger with a stability characterised with an accuracy of 0.5 °C. This external heat exchanger was made of fibreglass outside with a series of stainless steel tubes inside, which allowed heat exchanges with an alternate warm-cool water external source. The temperature increase started on August 21 in the selected high temperature mesocosms (day 2).

The UVB treatments were applied on 4 mesocosms. A set of 4 UVB tubes (UVB fluorescent tubes, Philips TL40W-12RS, emission peak at 313 nm) per mesocosm were fixed 20 cm above the surface of the mesocosms (measured at the beginning of the experiment). In order to obtain similar solar radiation in the other mesocosms (NTNUV and HTNUV), a set of 4 dummy lamps were fixed over the untreated mesocosms. The UVB tubes were covered with acetate film (SABIC Polymershapes, cat. Nr. 70600605; Díaz et al., 2006) in order to block ultraviolet radiation C emission (UVC, 100–280 nm). The acetate films were changed daily in case of any potential acetate degradation. UVB lamps were switched on every day from 10:30 to 14:30 from August 23 (day 4) up to the end of the experiment. Ground and profiling radiometers (respectively models GUV-541 and PUV-542 T; Biospherical Instruments Inc., USA) were used to monitor irradiance during the experiment. The GUV monitored the natural incident photosynthetically available radiation, PAR (400–700 nm), UVA (at 340 and 380 nm) and UVB (at 305, 313 and 320 nm) irradiances and were continuously recording on the roof of the structure, allowing for cloud correction of the profile data. PUV vertical profiles in the water column of each mesocosm (same wavelengths as the GUV instrument) were obtained at local noon. The mesocosms were placed on the ground near the coast of the St-Lawrence and filled with 300 um Nitex filtered natural water from the maritime St-Lawrence estuary, from a depth of 15 m and ~1 km from the coast. The water distributor was a 150 L plastic container connected to 8 equally hoses in order to uniformly distribute water by gravity into the 8 mesocosms. The filling of the mesocosms took place on August 20 2008 at 9:00, and the openings of the mesocosms were protected during the night and rain events with plastic. The samples were collected through distinct outlet flows from a valve placed near the bottom of the mesocosms, and were manipulated by a control panel acting on a set of solenoid valves. The water column in the mesocosms was mixed with a pump at a turnover rate of 1000 $dm^3.h^{-1}$. In order to check for the homogeneity in the vertical distribution of water properties, profiles of pH, dissolved oxygen, temperature, salinity and Sigma-T (data not shown) were