UNIVERSITY OF TARTU

INSTITUTE OF ECOLOGY AND EARTH SCIENCE

Gerli Albert

THE RESPONSE OF THE DIATOM *ASTERIONELLOPSIS GLACIALIS* TO VARIATIONS IN CO_2 AND NITRATE AVAILABILITY

Master`s Thesis

Head supervisor: Joana Barcelos e Ramos, pHD

Supervisor: Kalle Olli, pHD

TARTU 2018

Information sheet

The anthropogenic CO_2 gas emissions lead to enhanced CO_2 concentration in the atmosphere and are expected further increase. As CO_2 concentrations rise in the atmosphere it equilibrates with the surface ocean, resulting in lower pH values. The changes in ocean chemistry (higher CO2/low pH values) are expected to affect primary producers. However, the responses to combined effects are still poorly understood. The aim of this study was to determine the potential effects of CO_2 (620, 840 and 1640 µatm) and nitrate availability (7 and 13 µmol l^{-1}), on the cosmopolitan colony forming diatom *Asterionellopsis glacialis*. The effect of CO_2 on growth rate didn't depend of the nitrate availability. Still, the cell buildup, growth rates and colony size increased significantly under elevated CO_2. Also, higher nitrate treatment showed enhanced cell buildup and growth rates. Chain length didn't differ between nitrate treatments. Thus, this study suggests that *A. glacialis* could benefit from ocean acidification, by enhanced carbon availability for growth. Moreover, it is likely that larger colonies sink faster and as a result, the carbon is removed more efficiently from the biological cycle and exported to the depth where it is stored for 100 years or more. Thus, *A.glacialis* is expected to a give a negative feedback to the ongoing increase in atmospheric CO_2.

Key words: climate change, CO_2, ocean acidification, phytoplankton, diatoms, growth rate, nitrate availability, colony size

CERS: B260 Hydrobiology

Infoleht

Inimtegevuse poolt õhku paisatud süsihappegaas (CO_2) on põhjustanud kiiresti tõusva CO_2 taseme atmosfääris. Heitkoguste praeguse kasvutempo jätkudes, võib aastaks 2100 CO_2 tase kahekordistuda. Süsihappegaasi kontsentratsioon atmosfääris on tasakaalus ookeani pindmise kihi CO_2 tasemega, põhjustades seeläbi pH alanemist. On leitud, et muutused vee keemilistes omadustes (suurenev CO_2/alanev pH tase) võivad avaldada mõju primaarprodutsentidele. Tänaseks on vähe teada millised on erinevate faktorite koosmõjud. Käesoleva töö eesmärgiks oli välja selgitada millised on CO_2 (620, 840, 1640 µatm) ja nitraadi (7 and 13 µmol l^{-1}) mõjud kolooniaid moodustavale kosmopoliitsele ränivetikale *Asterionellopsis glacialis.*

Vastupidiselt eeldusele, CO_2 mõju kasvule ei sõltunud nitraadi kontsentratsioonist. Leiti, et CO_2 suurenedes tõusis saagikus ning suurenes kasvukiirus. Samuti suurenesid kolooniad. Leiti ka, et kasv oli oluliselt suurem kõrgemal nitraadi kontsentratsioonil, aga see ei mõjutanud kolooniate suurust. Töö tulemustest järeldub, et suurenev süsiniku kättesaadavus mõjutab positiivselt *A. glacialise* kasvu ning kolooniate moodustamist. Eeldustekohaselt vajuvad suuremad kolooniad kiiremini ning seeläbi kiireneb orgaanilise aine settimine, mis eemaldab süsiniku aktiivsest bioloogiliselt ringest. Tänu sellele on võimalik anda negatiivset tagasisidet inimtekkelisele CO_2 hulga tõusule Maa atmosfääris.

Märksõnad: kliimamuutused, CO_2, ookeani hapestumine, fütoplankton, ränivetikad, kasvukiirus, nitraadi kontsentratsioon, koloonia suurus

CERS: B260 Hüdrobioloogia

Table of Contents

1. INTRODUCTION

1.1 Global change

1.1.1 The changes in the atmosphere and ocean

Atmospheric CO_2 levels have been rapidly increasing since the 280 ppm (ppm-parts per million) found previous to the industrial revolution (IPCC 2014). In 2010 the atmospheric CO_2 was ~380 ppm (IPCC 2014). In May 2018 the Mauna Loa Observatory in Hawaii reported 411.21 ppm of CO_2 at the surface ocean (Mooney 2018). These rapid changes in the atmosphere also affect ocean chemistry. Surface ocean pH has been rather stable before industrialization over the last 800 000 years, averaging 8.2 at the surface water. Since the industrial revolution, the pH has dropped ~0.1 units, so the present day value is ~8.1 (Gattuso, Hansson 2011; Riebesell *et al.* 2010). Based on business as-usual scenario, atmospheric CO_2 levels are expected to approach 800 ppm by the end of the century, which means that pH would drop further 0.3 to 0.5 units and reach 7.8 pH units (Feely *et al.* 2009, IPCC report 2014). Finally, the changes in ocean chemistry are not happening everywhere at the same pace. For example, in areas where the water temperature is lower, like the Arctic Ocean, CO_2 levels and concomitant acidification is increasing more rapidly (CO_2 dissolves better in colder water). This makes the Arctic one of the most efficient areas for the sink of anthropogenic CO_2 in the global ocean (Slagstad *et al.* 2011).

1.1.2 Ocean chemistry

Ocean acidification refers to the pH drop of the ocean, caused by the uptake of CO_2 from the atmosphere. When CO_2 dissolves in seawater it forms carbonic acid $CO_2 + H_2O \leftrightarrow H_2CO_3^-$. Carbonic acid rapidly dissociates (splits apart) to produce bicarbonate ions: $H_2CO_3 \leftrightarrow HCO_3^- + H+$. Bicarbonate ions can also dissociate into carbonate ions: $HCO_3^- \leftrightarrow CO_3^{2-} + H+$. Both of these reactions produce protons (hydrogen ions H^+) which decrease pH. As a result, the balance between the carbonate species of DIC (dissolved inorganic carbon) will change, with $[CO_2]$ and $[HCO_3^-]$ increasing and $[CO_3^{2-}]$, decreasing (Figure 1). In the present day HCO_3^- is the dominating species, representing about 90% of DIC, followed by CO_3^{2-} (~10% of DIC) and finally CO_2, which represents less than 1% of DIC (Feely *et al.* 2009; Barker, Ridgwell 2012).

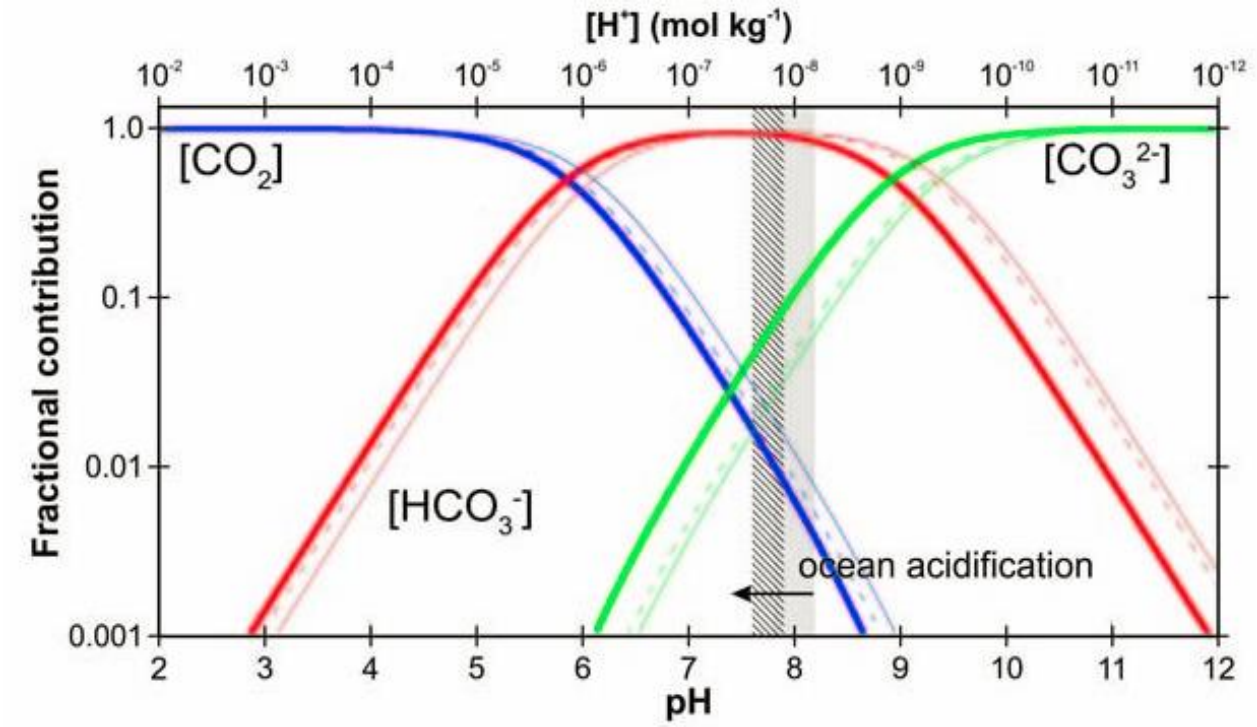

Figure 1. pH scale illustrating DIC species (Barker, Ridgwell 2012)

However, there is a natural capacity of seawater to buffer against changes in pH, called alkalinity. Seawater total alkalinity (TA) is commonly defined as "the excess base" in seawater, and its components ions are illustrated in the cloud of alkalinity (Figure 2). Seawater TA slows down, or buffers changes in ocean pH because it includes many different acid-base pairs. In other words, alkalinity reflects the ability of seawater to resist acidification. TA stays constant even when CO_2 is added to seawater because the charge balance of the solution stays unaltered, meaning that the number of positive ions generated equals the number of negative ions generated by this reaction (Holmes-Farely 2002).

$$A_T = [HCO_3^-]_T + 2[CO_3^{2-}]_T + [B(OH)_4^-]_T + [OH^-]_T + 2[PO_4^{3-}]_T + [HPO_4^{2-}]_T + [SiO(OH)_3^-]_T - [H^+]_{sws} - [HSO_4^-]$$

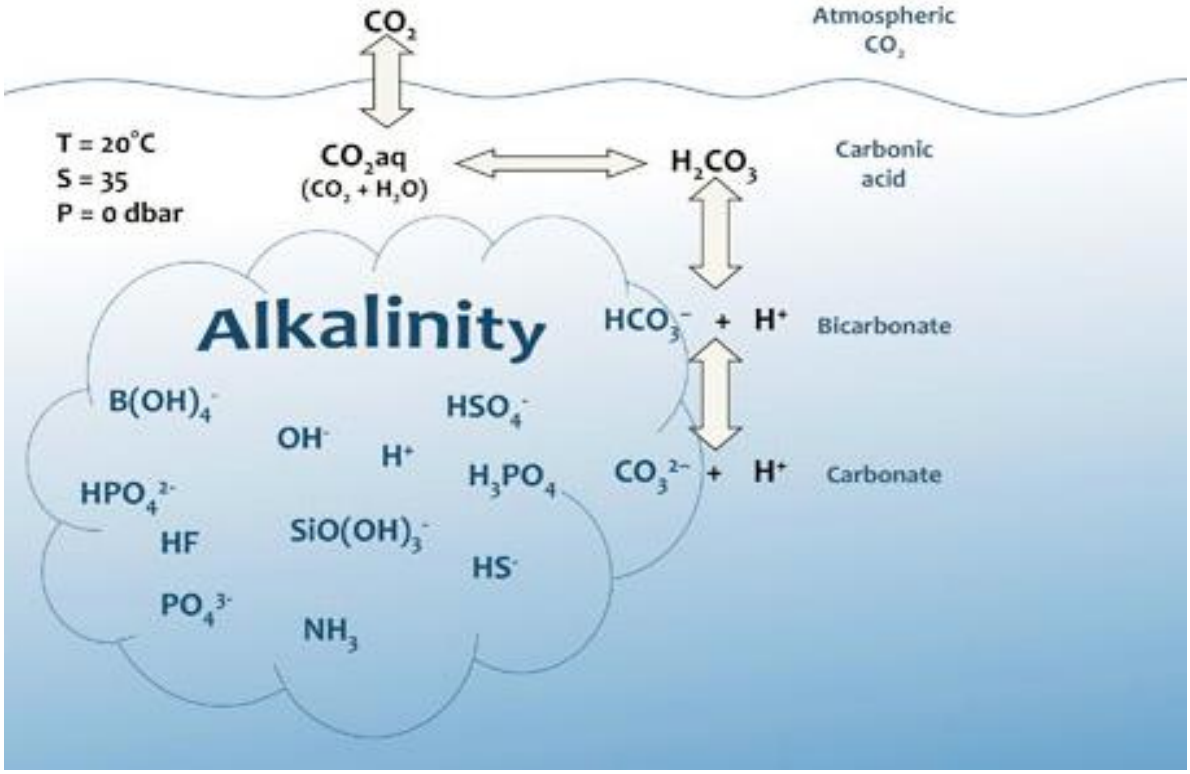

Figure 2. Total alkalinity as a buffer against changes in pH. *Source*:(http://www.whoi.edu)

1.2 Phytoplankton

1.2.1 An overview

Phytoplankton are microscopic single-celled protists, which live in oceans, seas, rivers or lakes. Their size can vary from < 1 μm^3 to over 109 μm^3 (Courties *et al.* 1994; Moore, Villareal 1996; Villareal *et al.* 1999). As primary producers they are at the base of the ocean food chain. Phytoplankton produce organic material from inorganic material, which they use for food and to make their cells. This process is called photosynthesis. For photosynthesis, they need water, light, carbon dioxide (CO_2) and nutrients. They live in the euphotic zone, which is the upper part of the water column where sunlight can penetrate. Below the euphotic zone light becomes a limiting factor. Allover, sunlight and nutrients are the hardest to obtain for phytoplankton (Reynolds 2006).

Phytoplankton can be organized according to different parameters, for example some have calcium carbonate shells (e.g., coccolithophores), some have silicate shells (e.g., diatoms), and some have no hard shells at all and have the ability to swim (e.g., dinoflagellates). The largest groups of phytoplankton are diatoms, cyanobacteria, and dinoflagellates (Pal, Choudhury 2014). Phytoplankton can be single-celled or colonial. Single cells attach to each other and thereby form colonies. Every cell in a colony has its own cell wall and no direct exchange of substances or information between the cells in a colony is taking place. Larger cells or colonies have smaller surface area to volume ratio and smaller cells/colonies have more surface area compared to cell volume. Smaller cells tend to have higher reproductive rate than larger cells, and larger cells or colonies tend to sink faster (Chisholm 1992; Gemmell 2016; Reynolds 2006). Also, larger size could be advantageous because there are fewer losses in the populations due to bigger zooplankton grazers (Strom *et al*. 2007; Gemmell 2016).

1.3 Parameters affecting phytoplankton growth

1.3.1 Light

The depth to which phytoplankton grows depends on how deeply the sunlight can enter into the water. The light environment for phytoplankton can vary enormously, due to absorbtion, scattering and shading by pigments and other material, variation in intensity and angle of insolation and many other factors. Natural water bodies are almost never still. Movement is often generated by warming or cooling, wind and Earth's rotation (Coriolis' force). This is causing phytoplankton to drift vertically and horizontally. Most of them don't have the ability to move so they drift with surface ocean currents.

There are large differences among species in what concerns their light utilization and photoprotective capacity. For example, diatoms are thought to perform relatively well under both limiting light and excessive light (Richardson *et al.* 1983) or fluctuating light (Litchman 1998). Smaller cells are thought to maintain higher photosynthetic rates under light limitation (Raven *et al.* 2000; Finkel 2001) while larger cells may be less susceptible to photoinhibition under excessive light. In general, growth increases with irradiance because of the light limitation. When photosynthesis is no longer light limiting growth saturates. Growth can also

decrease at higher irradiances due to photodamage from excess light and/or the costs of photoprotection (Key *et al.* 2010).

To get optimal light, phytoplankton has to avoid sinking out of the surface layer, due to their cells being slightly heavier than water. Many have different strategies to minimize the rate of sinking, such as 1) different body shapes 2) large surface area and small volume 3) gas filled vesicles and 4) oil droplets, retaining less dense ions etc. (Pal, Choudhury 2014). Generally, it is known that accumulated lipids are lighter than water and their presence in cells reduce excess density. Also, oil accumulation is responsible for the ability of colonies, like green alga *Botryococcus*, to float to the surface in small lakes (Reynolds 2006; Beardall *et al.* 2008). Although, there is a general agreement that sinking rates increase with a particle size (Sournia 1982; Kriest, Evans 1999) and by Stoke´s law the sinking rate of a spherical particle is related to the square of its radius (Reynolds 2006).

1.3.2 Nutrients

Along with light, phytoplankton also needs nutrients. In the ocean phytoplankton growth is mainly supported by either the recycling of nutrients or by reintroduction of nutrients from deeper waters by mixing or upwelling (Voss *et al.* 2013). Nitrogen and phosphorous are the nutrients needed in larger amounts and, both are essential to survival and reproduction. Nutrients are used to make proteins, nucleic acids and other cell parts that phytoplankton need to survive and reproduce. All phytoplankton have a requirement for the small amounts of silicon involved in protein synthesis, but diatoms need silicate the most, to build up their cell walls (Reynolds 2006). Phytoplankton has different systems to capture and assimilate nutrients. They have membrane transport systems for transporting nutrients from the boundary layer into the cell. Furthermore, different species have different physiological adaptions. Some are adapted to live in places with low nutrient concentrations, others with higher concentrations (Reynolds 2006).

Also, there are two acknowledged models describing the growth of phytoplankton in relation to limiting nutrient. Monod model (1942) states that the growth rate is related to the concentration of a single growth-limiting substrate. Phytoplankton cannot continue to grow when one or the other has been used up. Monod equation only takes account of the ambient

concentrations whereas the Droop model (1983) takes account of the level of nutrient already in the cells. The cells have the ability to store nutrients when they are available, so when nutrients become limiting in the environment, the cells are still able to divide by using those reserves. The actual growth rate of the phytoplankton depends on the nutrients in the cell (the cell quota), while the uptake rate depends on the ambient nutrient (Leadbeater 2006). Phytoplankton take up nutrients at a certain ratio, (also defined as the average of chemical elements in phytoplankton biomass or the stoichiometry, of photosynthesis and remineralization reactions) that varies between different species and environmental conditions, but on average occurs at 106 C: 16 N: 1 P (carbon: nitrogen: phosphorus) throughout the ocean, which is commonly referred to as the Redfield ratio (Redfield 2006). The stoichiometry of phytoplankton determines the nutritional quality for the secondary producers and the energy flow within next levels of the food chain (Basu, Mackey 2018).

The limiting nutrients differ within the ocean region. For example, Eastern Mediterranean Sea is phosphorous limited and growth stops when phytoplankton have used up all the phosphorous, even though there is still nitrogen in the water (Krom *et al.* 1991). Phosphorus is responsible for making new DNA and therefore the second most important growth limiting element. Phosphate is also important for synthesizing phospholipids. Bioavailable phosphorus forms occur in combination with oxygen in the ions of orthophosphoric acid. Many species can take up freely available phosphorus at very rapid rates (Reynolds 2006). For photoautotrophs, the available nitrogen forms are nitrate, nitrite and ammonium ions (NO_3^-, NO_2^- and NH^{+4}). Nitrogen fixers also use molecular N_2. Nitrate accounts approximately 88% of the dissolved inorganic nitrogen pool. Nitrogen is required for the biosynthesis of proteins and other macromolecules, including chlorophyll (Zhan *et al.* 2018).

Micronutrients (e.g., iron, zinc) occur naturally at low concentrations and are used in small quantities. In certain regions of the ocean, micronutrients are known to limit the growth of phytoplankton. An important trace component for algal cells is iron. One of the most energy-demanding processes in the cell is nitrogen reduction, which involves the participation of iron. Also, one of the most known symptoms of iron deficiency is the blockage of chlorophyll synthesis. The result of iron limitation is poor photosynthetic yields of fixed carbon and impaired growth potential (Pal, Choudhury 2014).

1.3.3 CO_2

Ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) is involved in the carbon fixation, a process by which atmospheric carbon dioxide is converted by photosynthetic organisms to energy-rich molecules (sugars) such as glucose (John, Wang *et al.* 2007). However, Rubisco is considered inefficient due to low affinity towards CO_2. Also, CO_2 diffuses easily through biological membranes and leaks out of the cell (John, Wang *et al.* 2007). For compensating Rubisco inefficiency, most phytoplankton species possess carbon concentrating mechanism (CCMs). CCMs elevate intracellular CO_2 levels to a concentration that nearly saturates Rubisco, but it´s energetically costly to operate, because first bicarbonate (HCO_3^-) has to be transformed to CO_2, so Rubisco could be able to fix carbon (Young, Morel 2015; Gattuso, Hansson 2011).

1.4 Diatoms

1.4.1 Biology

Diatoms are single celled or colonial algae with cell wall made of silica called frustule, and which has a characteristic species specific pattern. Their shapes and sizes vary widely. The frustule is made of two overlapping parts. The smaller inner part is called hypotheca and outer larger part epitheca. Diatoms have spread around the globe (oceans, lakes, rivers, streams, soils, wetlands) and diversified into hundreds of genera and around 100, 000 – 200, 000 species, which makes them the most species rich groups of phytoplankton. Most diatoms are non-motile and their relatively dense cell walls cause them to sink (Gemmell *et al.* 2016). It has been hypothesized that some diatoms resist sinking by exchanging ions with the seawater (Gemmell *et al*. 2016), but most species rely on turbulence to keep them suspended in the surface waters (Reynolds 2006). Often, marine diatoms form blooms during the spring and early summer and are dominant in upwelling areas. Diatoms are able to reproduce sexually and asexually, but they reproduce more frequently asexually by cell divison. In asexual reproduction each daughter cell receives one half of the parent cell's frustule, while the other missing part is regenerated. The generated half always becomes hypotheca. This leads to a decrease of the population average size after every cell division. By forming auxospore, diatoms are able to restore their original size (Round 2007).

1.4.2 Importance

Diatoms date back more than 200 million years when climate was much warmer and atmospheric CO_2 concentration was much higher than today. First, the planet was dominated by phytoplankton groups who´s cell were smaller. Approximately 150 million years ago diatoms and other groups with larger cells started to evolve. This lead to more rapid sedimentation of the cells (carbon burial) and caused carbon dioxide reduction in atmosphere and the increase of oxygen, which stayed rather stable in the next tens of millions years (Bhanu 2008). Diatoms are important for the export and burial of organic carbon for a number of reasons (Heureux, Rickaby 2015). They fix as much carbon dioxide as all the rainforests of the world combined, contributing for up to 25% of global primary production and about half of the marine primary production (Tréguer *et al.* 1995**;** Uitz *et al.* 2010). Their silica shells provide ballast material for sinking aggregates, and diatom blooms typically terminate in aggregation and mass settling of cells (Buesseler 1998; Baines *et al.* 2010; Heureux, Rickaby 2015).

1.4.2.1 Biological pump

CO_2 diffuses into the ocean from the atmosphere, and dissolved CO_2 taken up by phytoplankton becomes particulate organic carbon (POC) through photosynthesis. Organic matter is either remineralized by microbes, or consumed by zooplankton and their predators into fecal pellets, organic aggregates ("marine snow"), and other forms of POC are exported to the mesopelagic and bathypelagic zones by sinking and vertical migration. This process is called "biological pump", but it is relatively inefficient. It is estimated that the biological pump removes 11 Gt of carbon yr^{-1}, from the ocean's epipelagic waters (Buesseler, Boyd 2009; Turner 2015), but only about 10% of that flux reaches the bottom of the ocean (Martin *et al.* 1987) were carbon is stored for 100 years or more. Approximately 97% of net primary production that does not reach the deep sea, it is consumed or respired by bacteria (Ducklow 2000; Turner 2015), and zooplankton (Calbet, Landry 2004; Turner 2015) in the epipelagic zone (upper 100–200 m) or upper layers of the mesopelagic zone (100–200 to 1000 m) (De La Rocha, Passow 2007; Turner 2015). It has been hypothesized that when CO_2 increases it could potentially lead to higher primary production and as a result, increased carbon export (biological carbon pump) to the depth and thus give a negative feedback to the ongoing

increase in atmospheric CO_2. However, the export of carbon is determined by the C:N ratio of the sinking particles and the input of "new production" of nitrate to the euphotic zone (Haizheng *et al*. 2017).

1.5 CO_2 effect on diatoms and current study approach

Under present day carbonate chemistry the availability of CO_2 can limit the growth of diatoms (Riebesell *et al.* 1993; Barcelos e Ramos *et al.* 2014; Gallo *et al.* 2018, Haizheng *et al.* 2017). It has been found that the growth response of phytoplankton to increasing CO_2 levels range from positive (Haizheng *et al.* 2017; Barcelos e Ramos *et al.* 2014; Gallo *et al.* 2018; Low-Décarie *et al.* 2011; King, Jenkins *et al.* 2015) to negative (Gallo *et al.* 2018; Torstensson *et al*. 2012; von Dassow, Diaz-Rosas *et al*. 2018) or absent (Wei *et al.* 2012; Haizheng *et al.* 2017; King, Jenkins *et al.* 2015; Wynn-Edwards *et al.* 2014; Yang, Gao 2012), even under comparable experimental conditions. Most of the studies investigating the effect of ocean acidification on phytoplankton have been carried out in a narrow range ~400 ppm (present day) compared with ~800 ppm (estimated by year 2100) (Haizheng *et al*. 2017; Wei, Schippers *et al.* 2004; Wynn-Edwards 2004; Yang, Gao 2012; Low-Décarie *et al.* 2011; Torstensson *et al.* 2012; von Dassow, Diaz-Rosas *et al*. 2018; Hoppe et al. 2018).

Marine organisms are already exposed to higher CO_2 levels (lower pH) than present day atmospheric concentration – in upwelling areas where along with nutrients the water carries high load of CO_2 rich water (Norman *et al.* 2013; Lachkar 2014; Lauderdale *et al.* 2017; Feely *et al*. 2016; von Dassow, Diaz-Rosas *et al.* 2018). In these areas the pH is estimated to drop even below 7.8 by the year 2100 (von Dassow, Díaz-Rosas *et al*. 2018). CO_2 concentrations are also much higher near hydrothermal vents throughout the world. In the Azores shallow vents occur offshore the islands and in the seamounts. The characteristics of Azores shallow water (10-45 m) hydrothermal vents is their singularity, the major gas component of these vents is CO_2 (90%) (Cuoto *et al.* 2015; Cerqueira *et al.* 2017). The concentrations in these vents vary (shallow vent off Sao Miguel have ~577 µatm CO_2 J. Barcelos e Ramos personal communication), but it is much higher than surface ocean present average. Along with ocean acidification there are multiple effects which impact diatoms, like nutrient availability, light conditions, temperature, turbulence and community structure (Pardew, Blanco Pimentel *et al.*

2018; Hoppe *et al.* 2018, Sampaio *et al.* 2017; Tatters *et al.* 2018, Gallo *et al.* 2018; Heizheng *et al.* 2017). An important factor is enhanced nitrate concentrations in upwelling and coastal areas, as a result of the atmospheric deposition and agriculture runoff (Tae-Wook *et al.* 2011; Loughner *et al.* 2016). Therefore, it is important to address several factors and wider CO_2 range to better predict the response of the less studied colony-forming diatoms, since they influence global biogeochemical cycles and climate.

The goal of this work was to determine the potential effects of CO_2 and nitrate availability on the cosmopolitan chain-forming diatom species *Asterionellopsis glacialis* cell buildup, growth rate, cellular contents and colony formation. The following hypothesis were tested:

Hyp 1. Elevated CO_2 availability affects the cell buildup, growth rate and colony size of *A. galcialis.*

Hyp 2. Increased nitrate availability affects the cell buildup, growth rate and colony size of *A. galcialis.*

Hyp 3. The effect of CO_2 on *A. glacialis* growth performance is affected by the availability of nitrate.

2. MATERIAL AND METHODS

2.1 Preparation of the experiment

Experimental work was conducted in the year 2017, September to December at the University of the Azores (UAc), Terceira Island. We investigated the response of the cosmopolitan diatom *Asterionellopsis glacialis* to three CO_2 levels and 2 nitrate concentrations. All work was made under controlled laboratory conditions. First, stocks culture of *Asterionellopsis glacialis* were cultivated until stable exponential growth conditions, at which point growth rates were determined for each condition. This enabled the calculation of the starting number of cells and corresponding inoculum additions to achieve optimal conditions during the experiment.

North Atlantic water collected from south of Terceira was filtered through a 0.2 µm Polycap filter (Figure 3) in autoclaved bottles. After filtering, the water was enriched with approximately 4.5 µmol l^{-1} of phosphate, 130 µmol l^{-1} of silicate (the increase of total alkalinity upon addition of Na_2SiO_3 was compensated by HCl addition) and trace metals and vitamins as specified in the f medium (Guillard, Ryther 1962). Samples of pH, salinity and TA (total alkalinity) were taken and their analysis used to determine the carbonate system of the seawater and it´s manipulation which was achieved by combined additions of HCl and $NaHCO_3^-$ (Gattuso, Hansson 2011), to maintain total alkalinity constant while increasing dissolved inorganic carbon in a closed system following Schulz *et al*. (2009). Two nitrate treatments were prepared for the experiment with initial concentrations of ~7 µmol l^{-1} and 13 µmol l^{-1}. Also, three CO_2 concentrations were chosen for the experiment: 600, 800 and 1600 µatm, to investigate the response of the diatom under predicted CO_2 concentrations of the future (IPCC report 2014). Moreover, higher CO_2 values are already present today – upwelling areas and hydrothermal vents.

2.2 Experimental setup

The experimental design is depicted in Figure 4. First, three (5 L) bottles were filled with growth medium, enriched with 7 µmol l^{-1} of nitrate and CO_2 was adjusted in each bottle according to the treatments. After mixing, temperature and salinity was measured and aliquots for pH, TA, nutrients were taken to calculate the initial values of the carbonate system of the 7

µmol l^{-1} nitrate treatment. Then 500 ml bottles that would be used for the pre-culture I, II and the experiment of the first nitrate treatment (7 µmol l^{-1}) were carefully filled. After that, the same three (5 L) bottles were further enhanced with 6 µmol l^{-1} of nitrate and mixed. Again, samples of pH, TA and nutrients were taken to calculate the initial carbonate system chemistry of the second nitrate treatment (13 µmol l^{-1}). The corresponding 500 ml bottles of pre-cultures and experiment were carefully filled (Figure 4). All bottles were filled without headspace (to avoid equilibration with the gaseous phase) and stored in refrigerator at 4°C, until needed. At the beginning of each step (pre-culture I, II and the experiment) the bottles were placed inside the climate chamber for temperature equilibration. Cultures were grown at 20°C under 120 µmol m^{-2} s^{-1} light intensity and 14/10 h light/dark cycle. All cultures were gently rotated vertically (20 times) daily at the beginning of the light phase (10 times) and in the afternoon (10 times) to avoid aggregation, sedimentation and self-shading during the light phase.

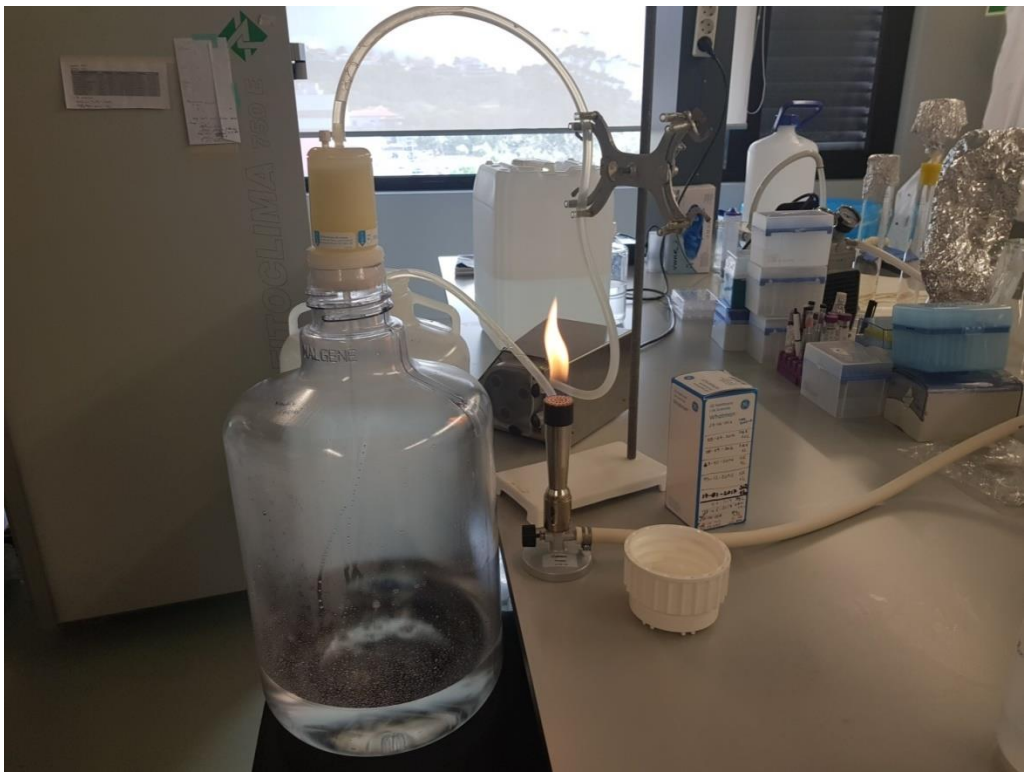

Figure 3. Seawater filtration with 0.2 µm Polycap filter.

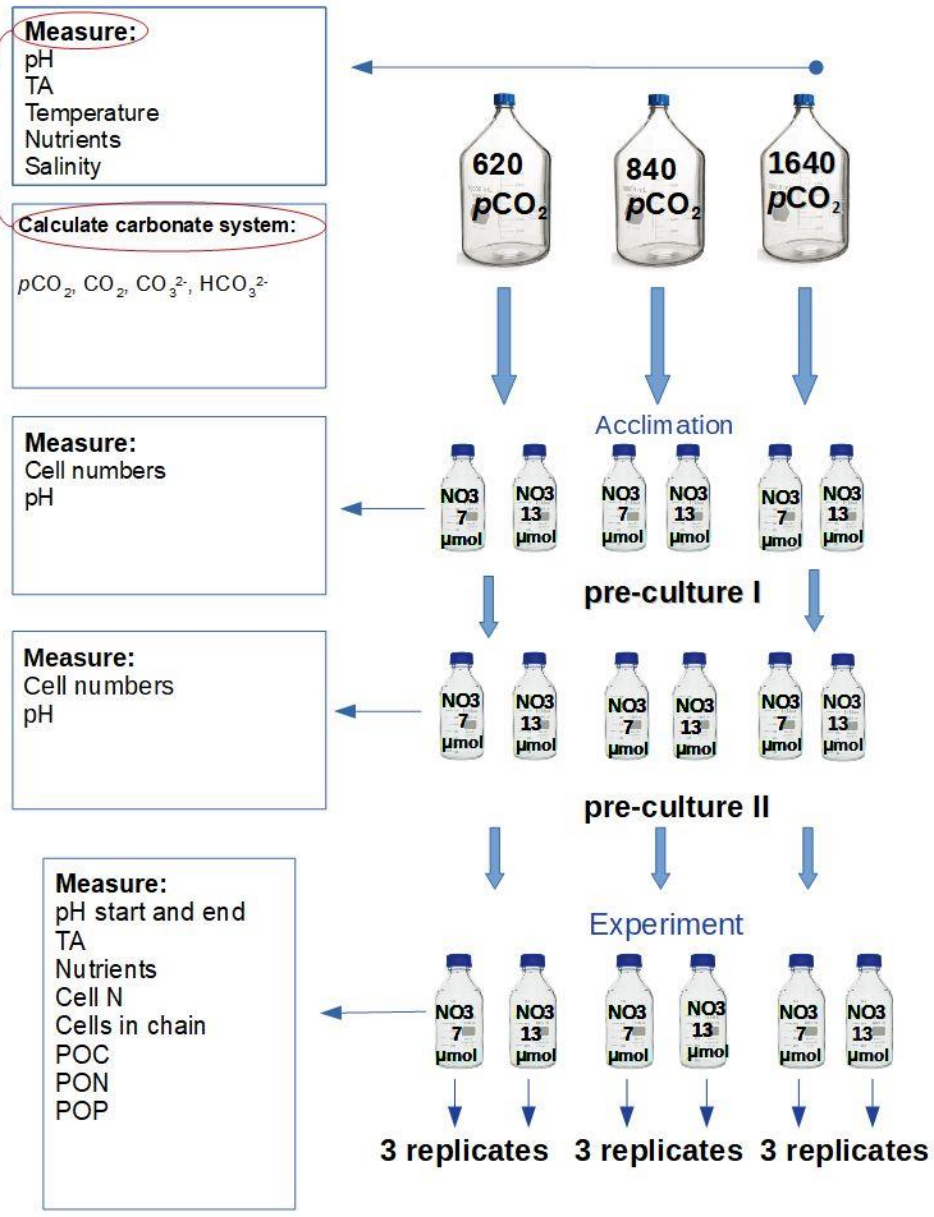

Figure 4. Design of the experiment. On top it is represented the setting of the carbonate system, followed by two acclimation phases (pre-culture I and II), and the final experiment.

2.3 Carbonate chemistry measurements and calculations

Total alkalinity was measured by potentiometric titration using a Metrohm 848 Titrino Plus equipped with Metrohm 869 Compact Sample changer. Total alkalinity measurements were corrected with certified reference material (Dickson 2010) at about 20 µmol kg^{-1} accuracy and 2 µmol kg^{-1} precision. Since the precision is important for calculating the carbonate system, two methods were used to measure pH 1) using a glass electrode (WTW 340i pH meter) and calibrated with a TRIS seawater buffer, supplied by A. Dickson 2) using the indicator dye m-cresol by (Gattuso, Hansson 2011). For the calculation of the carbonate chemistry (initial and final values), TA (total alkalinity), pH, temperature, salinity, phosphate and silicate were measured using the equilibrium constants determined by Mehrbach *et al.* (1973) as refitted by Dickson & Millero (1987), was calculated using CO_2SYS software. The pCO_2 levels referred in the graphs correspond to the average of the initial pCO_2 values shown in Table 1.

2.4 Cell numbers and growth rates

Cultures were grown 4 days (4-5 generations) at each step (pre-cultures and final experiment) before dilution or harvesting. To keep the carbonate system as constant as possible (with less than 5% change of the dissolved inorganic carbon (DIC)) the cultures were kept at low cell density (Gattuso, Hansson 2011).

A. glacialis cells were fixed with lugol (2% of final concentration) and cell abundance and the number of cells within a given colony was determined by counting, under the light microscope (Nikon Eclipse TS100, 200× magnification). On average, 800 cells were counted per sample. For determination of the colony size the weighted average was calculated.

Cell growth rate was calculated according to the equation:

$$\mu = \ln (Cf/Ci)/\Delta t \quad (1)$$

Where Cf and Ci represent the final and the initial cell concentrations, respectively, and Δt corresponds to the growth period in days.

2.5 Nutrients

Samples for the determination of nutrients were taken at the beginning and at the end of the experiment. All samples were filtered through 0.2 µm polyethersulfone syringe filters and stored at -20°C until analysis. Following Sharp (1974) reagents and standards were prepared for calibration and for determination of the concentrations of dissolved inorganic nitrate (NO_3), nitrite (NO_2), silicate (SiO_3) and phosphate (PO_4^{3-}). All nutrients were measured spectrophotometrically (Agilent Cary 60 UV-Vis) and concentrations were calculated according to the corresponding calibration curves.

2.6 Author role

All described work was accomplished by the author under supervisory guidance, except the measurements of pH m-cresol which were done by Joana Barcelos e Ramos and samples of POC/N will be analyzed in the Southern University by the collaborator Kai George Schulz.

2.7 Statistical analysis

Statistical analysis was done using R and STATISTICA 12 64-bit. Statistical significance of the data was tested by Two-way ANOVA and One-way ANOVA (significance determined as 95%, p-value < 0.05).

3. RESULTS

3.1 Carbonate system

During the incubation, drawdown of dissolved inorganic carbon (DIC) was less than 5%. Moreover, the manipulation of the carbonate system kept TA (total alkalinity) stable, with differences between treatments below 1% (Table 1). The comparison of pH electrode and spectrophotometric measurement (indicator dye m-cresol) at the beginning and end (Figure 5) of the experiment showed statistically significant correlation (r= 0.96, N=6, p= 0.006) and (r= 0.93, N=6, p= 0.006).

Table 1. Carbonate chemistry at the beginning and end of the experiment. Final values are expressed as average (± SE) of the replicates and pCO_2 is calculated based on the measurements of pH, TA (total alkalinity), nutrients (P, Si) and temperature. pH values correspond to method m-cresol.

	Nitrate treatment ($\mu mol L^{-1}$)	pCO_2 (μatm)	TA ($\mu mol\ kg^{-1}$)	pH	HCO3 ($\mu mol\ kg^{-1}$)	CO_3^{2-} ($\mu mol\ kg^{-1}$)	CO_2 ($\mu mol\ kg^{-1}$)	DIC ($\mu mol\ kg^{-1}$)
Initial	7	599	2421.66	7.914	2044.3	151	19	2215.0
	13	639	2421.36	7.890	2061.3	144	21	2226.2
	7	822	2408.70	7.791	2113.5	118	27	2258.0
	13	864	2409.11	7.772	2125.1	113	28	2266.4
	7	1642	2415.99	7.517	2247.6	67	53	2367.2
	13	1634	2416.09	7.520	2246.9	67	53	2366.6
Final (average)	7	619 ± 20	2430 ± 5.0	7.903 ± 0.01	2143 ± 10.6	148 ± 3.6	20 ± 0.7	2228 ± 8.1
	13	549 ± 10	2431 ± 0.7	7.947 ± 0.01	2027 ± 4.8	162 ± 2.2	17 ± 0.3	2207 ± 2.9
	7	786 ± 40	2422 ± 3.7	7.883 ± 0.03	2111 ± 12.8	123 ± 6.7	25 ± 1.6	2260 ±7.7
	13	719 ± 13	2423 ± 1.5	7.775 ± 0.01	2092 ± 5.7	132 ± 1.7	23 ± 0.4	2248 ± 4.4
	7	1818 ±100	2424 ± 0.6	7.478 ± 0.02	2268 ± 7.6	61 ± 3.0	58 ± 3.3	2389 ±7.9
	13	1581 ± 85	2429 ± 0.2	7.563 ± 0.02	2243 ± 8.9	74 ± 3.5	47 ± 2.8	2365 ± 8.1

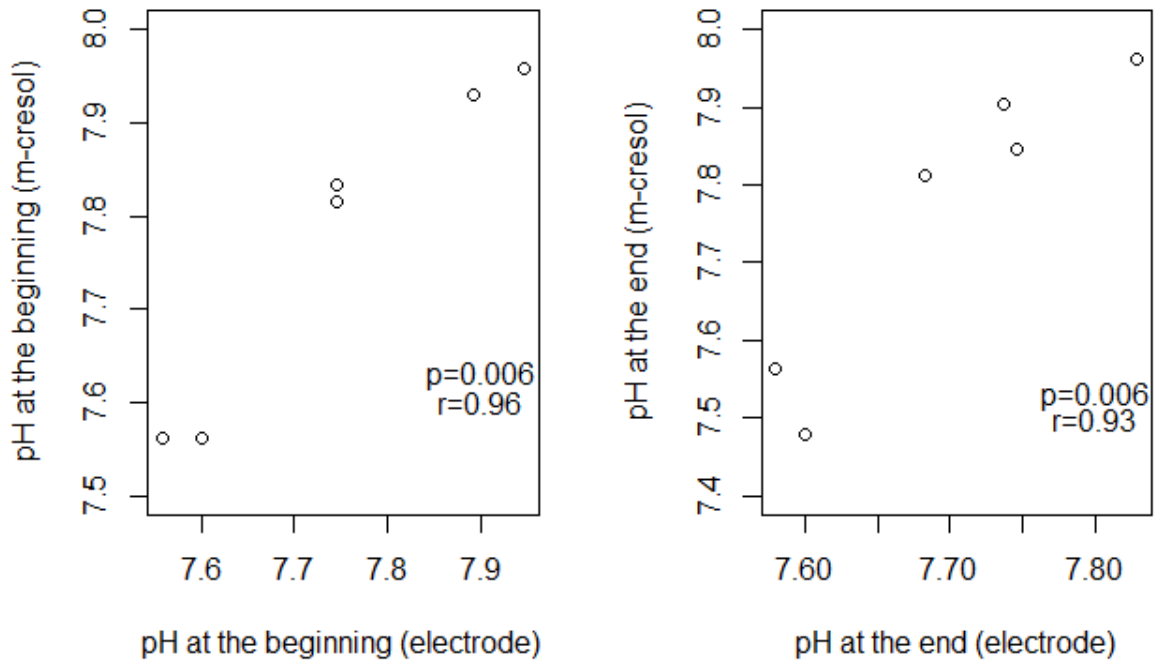

Figure 5. Comparison of two methods for measuring pH at the beginning and end of the experiment, with pH electrode and spectrophotometric measurement using indicator dye m-cresol.

3.2 Nutrients

Nitrate drawdown during the experiment was ~6.3 µmol l^{-1} in the lower nitrate treatment (7 µmol l^{-1}), decreasing on average 96% and 11 µmol l^{-1} in the higher nitrate treatment (13 µmol l^{-1}), decreasing 87%. At the same time the initial concentrations of phosphate decreased on average 22% in the lower nitrate treatment (7 µmol l^{-1}) and 23% in the higher nitrate treatment (13 µmol l^{-1}). Finally, the initial concentrations of silicate decreased on average 4% under lower nitrate treatment (7 µmol l^{-1}) and 8% under higher nitrate treatment (Table 2).

Table 2. Nutrient concentrations at the beginning and end of the experiment. Final values are expressed as the average (± SE) of the replicates.

	Treatment (CO_2 and $NaNO_3$)	Concentration (µmol l^{-1}) of phosphate	Concentration (µmol l^{-1}) of silicate	Concentration (µmol l^{-1}) of nitrate
Initial	620 and 7	4.3	138.4	6.9
	840 and 7	4.7	130.7	6.8
	1640 and 7	4.6	133.0	6.5
	620 and 13	4.6	138.7	12.7
	840 and 13	4.6	131.3	12.6
	1640 and 13	4.5	132.2	12.8
Final (average)	620 and 7	4.0 ± 0.1	134.6 ± 0.2	0.5 ± 0.06
	840 and 7	3.8 ± 0.2	125.2 ± 0.1	0.3 ± 0.1
	1640 and 7	3.7 ± 0.1	127.4 ± 0.2	0.2 ± 0.01
	620 and 13	3.6 ± 0.1	125.7 ± 0.1	2.2 ± 0.4
	840 and 13	3.4 ± 0.1	117.8 ± 0.3	1.3 ± 0.2
	1640 and 13	3.5 ± 0.1	125.7 ± 0.1	1.5 ± 0.03

3.3 Response of the biomass buildup

CO_2 (*two-way* ANOVA: F=9.9, p=0.002) and nitrate treatments (*two-way* ANOVA: F=23.6, p p<0.001) had a significant effect on cell buildup, which increased with elevated CO_2 and were always higher under the higher nitrate concentration (13 µmol l^{-1}). From 620 to 840 µatm of CO_2 the mean cell buildup increased 222% (nitrate 7 µmol l^{-1}) and 72% (nitrate 13 µmol l^{-1}). The difference between two nitrate concentrations was 94%. Between 840 and 1640 µatm of CO_2 no significant difference (p<0.001) was found. The *two-way* ANOVA showed no significant interaction between the two tested factors (CO_2 and nitrate concentration) on cell buildup (F=0.08, p>0.05) (Figure 6).

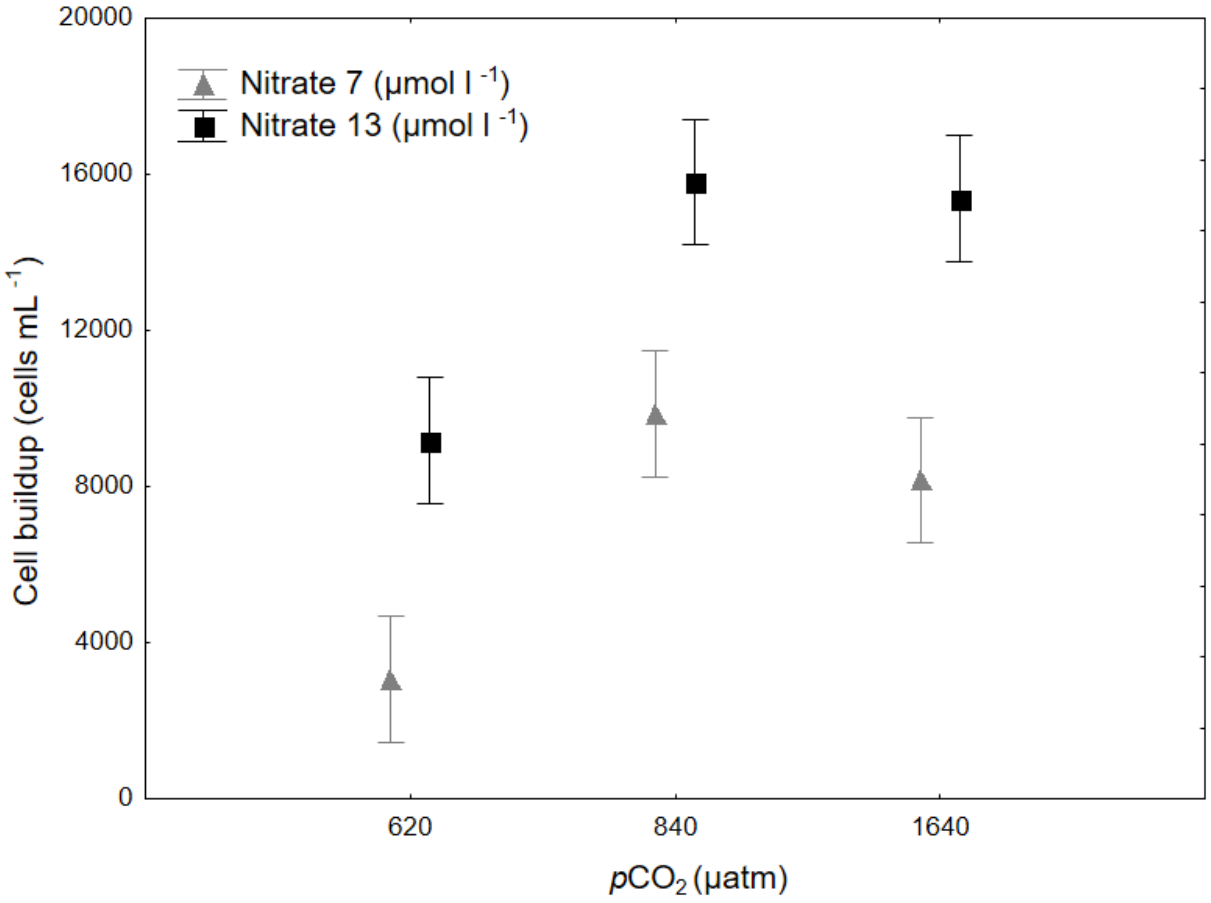

Figure 6. Cell buildup of *Asterionellopsis glacialis* at increasing pCO_2 levels and two nitrate treatments 7 µmol l^{-1} (grey triangles) and 13 µmol l^{-1} (black squares). The black squares and grey triangles express the mean values and with standard the errors (SE).

3.4 Growth rate

CO_2 (*two-way* ANOVA: F=5.7, p=0.018) and nitrate treatments (*two-way* ANOVA: F=25.6, p<0.001) had a significant effect on growth rate, which increased with elevated CO_2 and was always higher under the highest concentration (13 µmol l^{-1}). From 620 to 840 µatm of CO_2 the mean growth rate increased 20% (nitrate 7 µmol l^{-1}) and 14% (nitrate 13 µmol l^{-1}) and the difference between two nitrate concentrations was 34%. Between 820 and 1640 µatm of CO_2 no significant difference (p<0.001) was found in both nitrate treatments. The *two-way* ANOVA showed no significant interaction between the two tested factors (CO_2 and nitrate concentration) on growth rate (F=0.7, p>0.05) (Figure 7).

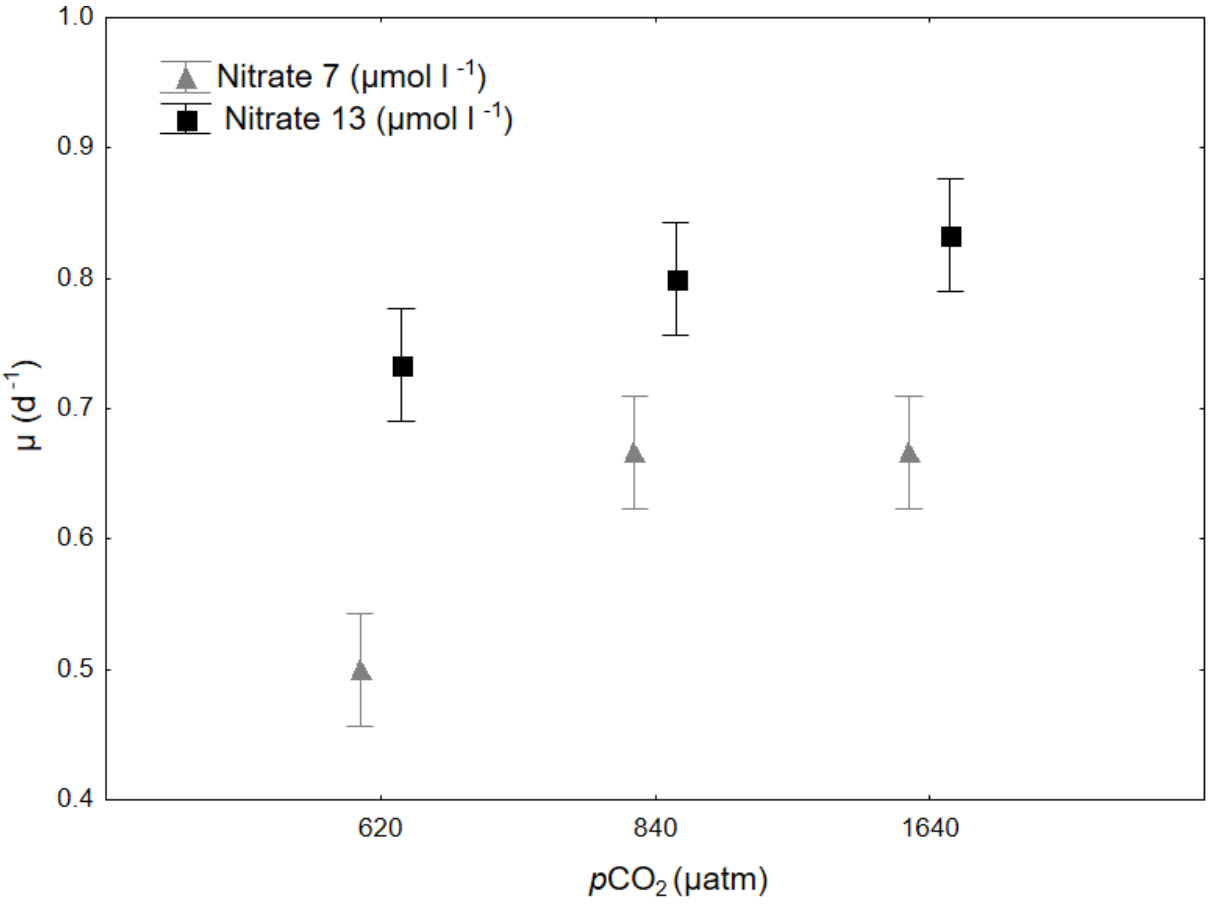

Figure 7. Growth rate of *Asterionellopsis glacialis* at increasing pCO_2 levels and two nitrate treatments 7 µmol l^{-1} (grey triangles) and 13 µmol l^{-1} (black squares). The black squares and grey triangles express the mean values with standard errors (SE).

3.5 Nitrate cell quota

Nitrate treatments didn't have a significant effect on nitrate cell quota (*Two-Way* ANOVA: F=1.49, p>0.05). However, CO_2 had a significant effect on nitrate cell quota (*Two-Way* ANOVA: F=16.02, p<0.001), which decreased 72% (nitrate 7 µmol l^{-1}) and 46% (nitrate 13 µmol l^{-1}) from 620 to 840 µatm of CO_2. Between 840 and 1640 µatm of CO_2 no significant difference (p<0.001) was found. The *two-way* ANOVA test showed no significant interaction between the two tested factors (CO_2 and nitrate concentration) on nitrate cell quota (F=3.43, p>0.05) (Figure 8).

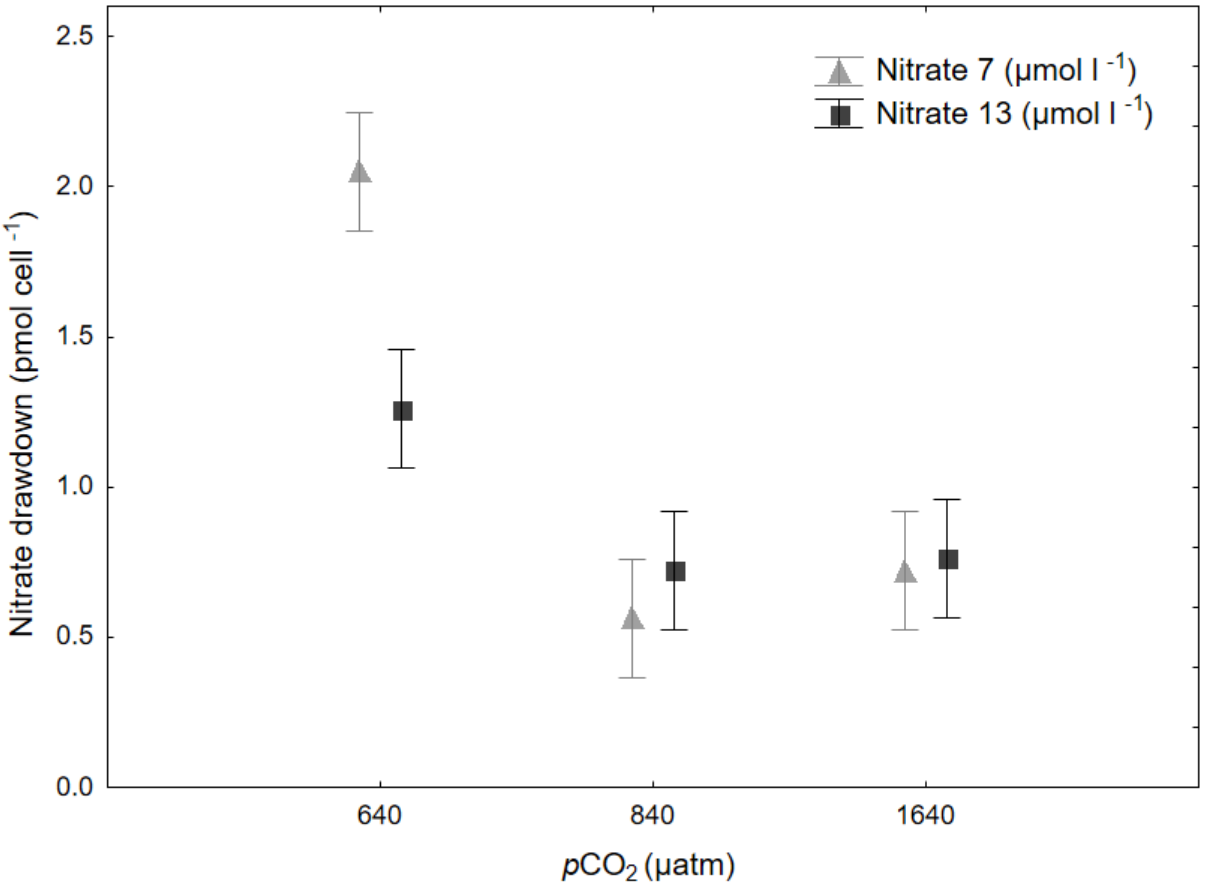

Figure 8. Nitrate drawdown of *Asterionellopsis glacialis* at increasing pCO_2 levels and two nitrate treatments 7 µmol l^{-1} (grey triangles) and 13 µmol l^{-1} (black squares). The black squares and grey triangles express the mean with standard errors (SE).

3.6 Nitrate concentrations at the end of the experiment

The *one-way* ANOVA showed a significant difference (82%) between the two nitrate treatments (7 µmol l^{-1} and 13 µmol l^{-1}) in relation to the nitrate concentrations at the end of the experiment ($F=14.2$, $p<0.001$). The average concentration left in the first treatment was 0.3 µmol l^{-1} and the second treatment had 1.7 µmol l^{-1} (Figure 9).

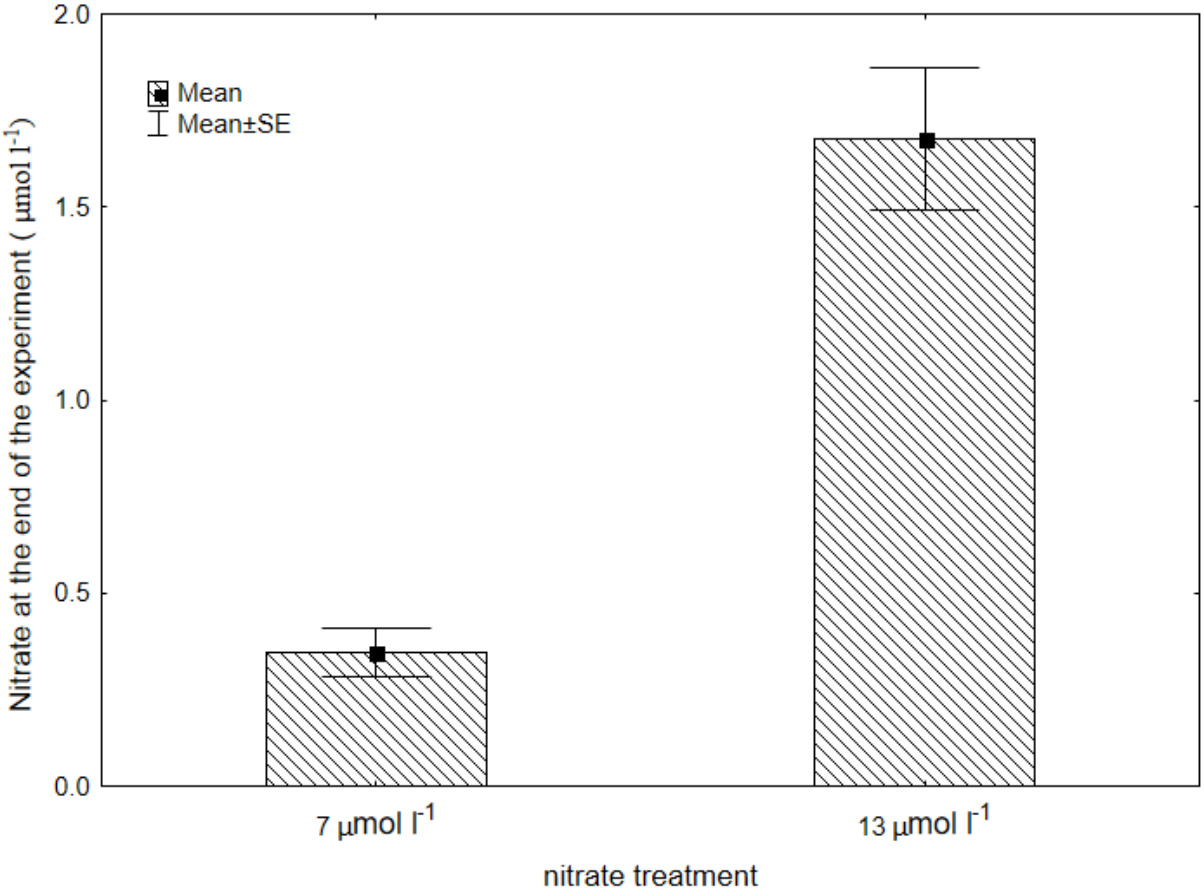

Figure 9. Nitrate concentration at the end of the experiment. The black squares express the mean values with standard errors (SE).

3.7 Average colony size

Nitrate treatments didn't have a significant effect on the average colony size (*Two-Way* ANOVA: F=0.004, p>0.05). However, CO_2 had a significant effect on colony size, which increased with elevated CO_2 (Two-Way ANOVA: F=13.3, p<0.001). From 620 to 840 µatm of CO_2 the average colony size increased 26% (nitrate 7 µmol l^{-1}) and 37% (nitrate 13 µmol l^{-1}). Between 820 and 1640 µatm of CO_2 the increase was 5% (nitrate 7 µmol l^{-1}) and 7% (nitrate 13 µmol l^{-1}). The *two-way* ANOVA showed no significant interaction between the two tested factors (CO_2 and nitrate concentration) on average colony size (F=0.1, p>0.05) (Figure 10).

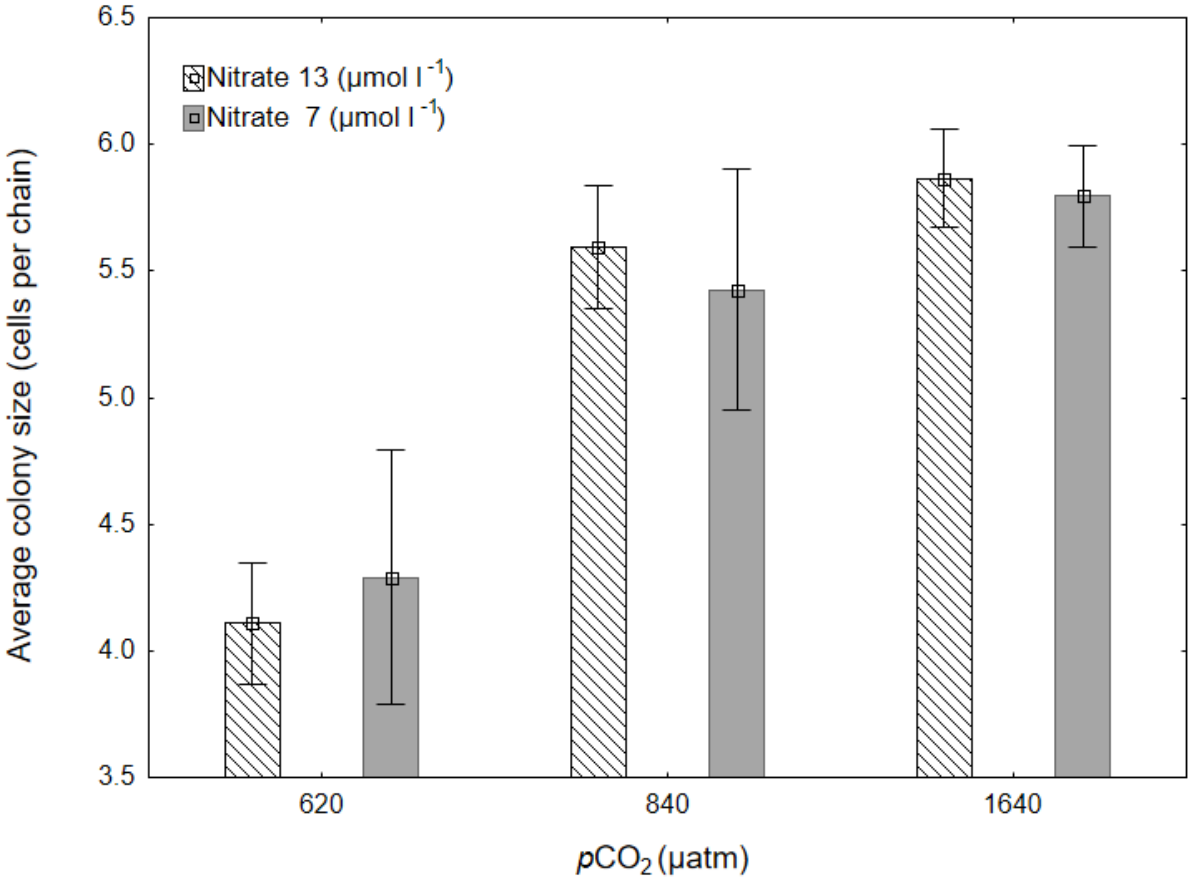

Figure 10. The (weighted) average colony size (cells per chain) of *Asterionellopsis glacialis* at increasing pCO_2 levels and two nitrate treatments. The black squares express the mean values with standard errors (SE).

4. DISCUSSION

4.1 Influence of elevated CO_2 on *Asterinellopsis glacialis*

The increasing CO_2 availability enhanced *Asterionellopsis glacialis* cell numbers and growth rate. Similarly, earlier studies with diatoms have shown increased growth rate under elevated CO_2 as expected for the year 2100 (Sampaio *et al*. (2017); Barcelos e Ramos *et al.* 2014; Gallo *et al.* 2018; Low-Décarie *et al.* 2011; King, Jenkins *et al.* 2015). It is taught that due to higher CO_2 availability carbon concentrating mechanisms (CCMs) might be down-regulated, and the saved energy used for other growth processes (Raven 2003; Rost *et al.* 2008; Reinfelder 2012; Hu *et al*. 2017; Gallo *et al*. 2018; Deppeler *et al.* 2018).

As CO_2 concentrations became higher (~1640 μatm) the growth rate was no longer enhanced. This is most likely related to the concomitant decrease in pH – which might be negatively affecting cell physiology (Barcelos e Ramos *et al.* 2014; Thoisen *et al.* 2015; von Dassow, Diaz-Rosas *et al.* 2018; Gallo *et al.* 2018) – and reaching carbon saturation point under the conditions tested. In other words, findings from this work indicate that CO_2 concentrations (e.g. 620 μatm) are limiting the growth of *Asterionellopsis glacialis* at concentrations higher than present. In previous work with *A. glacialis* and optimum was observed (Barcelos e Ramos *et al.* 2014; Gallo *et al.* 2018). However, in this work there was not decrease found in growth, due to the interval tested.

The increasing CO_2 availability enhanced the colony size of *Asterinellopsis glacialis.* As with the growth rate, the colony size increased from 620 to 840 μatm, and leveled thereafter. Larger colonies have been related to increased growth rate (Takabayashi *et al.* 2006; Thoisen *et al.* 2015). Like many diatoms, also *A. glacialis* cells are sticky, because of the exudation of polysaccharides (Barcelos e Ramos *et al.* 2014). When more carbon is available then the exudation is higher. Therefore, with increased growth rate and carbon availability there is also a higher possibility of adjacent cells to bind.

In previous work with *Asterionellopsis glacialis* under increasing CO_2 concentrations, Gallo *et al.* (2018) and Barcelos e Ramos *et al.* (2014) found also a positive correlation between increasing CO_2 and colony size. Furthermore, they found that even when growth rates

decreased at very high CO_2 values (~1300-3500 µatm) the number of cells per chain still increased. They related the increasing colony size with low pH values. When cells were exposed to more "extreme" pH values then larger colonies could be a strategy to increase pH near the cells (inner cells in chain are less exposed to bulk seawater) and thus protect the cells from acidification (Barcelos e Ramos *et al.* 2014; Gallo *et al.* 2018). In this work there was no decrease found in growth rate. Also, there was no significant increase in chain length at very at very high CO2/low pH, therefore it is likely that longer chains are in positive correlation with growth rate.

The effect on colony size might have implications to the biological pump of the future ocean. According to Stoke´s law, sinking rates increase with a particle size (Sournia 1982; Kriest, Evans 1999). This implies that larger colonies sink faster and as a result, the carbon is removed more efficiently from the biological cycle and exported to the bottom of the sea for long term storage. This would give a negative feedback to the ongoing increase in atmospheric CO_2 (Riebesell, Wolf-Gladrow 1992; Kriest, Evans 1999; Riebesell 2004).

Finally, CO_2 had a significant effect on nitrate cell quota. Under the lowest CO_2 concentration (620 µatm) the nitrate cell quota was significantly higher and the growth rate was significantly lower. When one of the nutrients is limiting (C, N, P) the growth of phytoplankton is suppressed (Leonardos, Geider 2004). Nitrate accumulated in the cell, since CO_2 was limiting the growth. Hence, as CO_2 increased the growth rate increased, and the nitrate cell quota decreased.

4.2 Influence of nitrate availability on *Asterionellopsis glacialis*

The increasing nitrate availability enhanced *Asterionellopsis glacialis* cell buildup and growth rate, which is in agreement with earlier studies (Justić *et al.* 1996; Haizheng *et al.* 2017). At the end of the experiment, there was significantly less nitrate in the lower nitrate treatment (7 µmol l^{-1}) than in the higher nitrate treatment (13 µmol l^{-1}). The growth rate of the nitrate treatment 7 µmol l^{-1} was lower due to the limitation of nitrate. Decreased nitrogen availability leads to decreased synthesis of proteins in algae, therefore the growth is suppressed (Li *et al.* 2013). When looking at nitrate cell quota, the difference between the two nitrate treatments was insignificant, suggesting that nutrient uptake was independent of nitrate concentrations.

It was also expected that increased nitrate availability affects positively the colony size, but the hypothesized wasn't confirmed. From previous work, it has been found that higher nitrate and phosphate availability increases the number of cell per chain (Takabayashi *et al.* 2006; Ma *et al.* 2014), but it was more related with phosphate concentration than nitrate. In this work the growth rate was significantly higher when there was more nitrate. But the colony size didn't differ between the nitrate treatments. Indicating that colony size is more dependent on carbon availability than growth rate. Different studies indicate that colony formation is a complex process and in nature probably affected by different factors and their combinations (e.g. light, grazing, buoyancy regulation, CO_2, pH). There is very little information about increasing CO_2 on colony formation. Therefore, it needs further investigation to better understand feedback effects of diatoms on climate change.

4.3 Combined CO_2 and nitrate availability on *Asterinellopsis glacialis*

Unexpectedly, we found no clear interaction between CO_2 and nitrate availability on *Asterionellopsis glacialis* growth. Fu *et al*. (2010) described the effect of increasing CO_2 and phosphate availability on *Karlodinium.* They found that *Karlodinium* may benefit from future increases in CO_2, but possibly only when nutrients are in excess. Growth is limited by the nutrient in least supply (Fu *et al.* 2010). Although, we found that even when nitrate was limiting (7 μmol l^{-1}), increasing CO_2 had a significant effect on growth. When CO_2 increases, the energy saved by a down-regulated CCM operation could be reallocated in nutrient transporters (Reinfelder 2012), which results higher nitrate drawdown from the environment. Therefore support higher growth under elevated CO_2. Even if the saved energy from CCM was reallocated into nutrient transporters, the effect applied to both nitrate treatments. Therefore, the differences in growth between the nitrate treatments (7 and 13 μmol l^{-1}) are similar under each CO_2 (620, 840 and 1640 μatm).

SUMMARY

Atmospheric CO_2 levels have been rapidly increasing since the industrial revolution. Based on business as-usual scenarios, atmospheric CO_2 levels are expected to further increase. Changes in the atmosphere also affect ocean chemistry (higher CO_2/lower pH values), and thereby different organisms. It has been found that the growth response of phytoplankton to increasing CO_2 levels range from positive to negative or absent. Along with ocean acidification, there are multiple effects which impact phytoplankton, such as nutrient availability, changes light conditions, temperature and turbulence. Since diatoms play a major role in carbon fixation, they influence global biogeochemical cycles and climate. Therefore, the goal of this work was to determine the potential effects of CO_2 and nitrate availability on of the cosmopolitan chain-forming diatom species *Asterionellopsis glacialis*. We investigated the response of three CO_2 (620, 840 and 1640 µatm CO_2) levels and 2 nitrate concentrations (7 and 13 µmol l^{-1}). Experimental work was conducted under controlled laboratory conditions at the University of Azores, Terceira Island. As was hypothesized, the increasing CO_2 and nitrate availability affected *Asterinellopsis glacialis* cell buildup, growth rate and colony size.

The cell buildup and growth rate of *A. glacialis* increased significantly from 620 to 840 µatm of CO_2, but leveled thereafter. This suggests that *A. glacialis* benefited from increasing CO_2, in terms of improved carbon supply. This is likely related to down-regulation of cellular carbon concentration mechanism (CCM) operation, which allowed to reallocate the saved energy for growth. As CO_2 concentrations became higher (~1640 µatm) the growth rate was no longer enhanced. This is most likely related to the concomitant decrease in pH, which might be negatively affecting cell physiology. Furthermore, findings from this work indicate that CO_2 concentrations are limiting the growth of *A. glacialis* at concentrations higher than present.

The colony size of *A.glacialis* increased significantly from 620 to 840 µtam of CO_2, and leveled thereafter in parallel with growth rate. Furthermore, the cell buildup and growth rate were significantly higher under the higher nitrate treatment. However, the colony size didn't differ between nitrate treatments, suggesting, that colony size is more dependent on carbon availability that growth rate. When more carbon is available the exudation of polysaccharides

is higher, thus the cells are more sticky and the possibility of adjacent cells to form larger colonies is higher.

In conclusion, the growth rate of *Asterinellopsis glacialis* increased significantly with elevated CO_2, until 840 µatm of CO_2. Therefore, if the CO_2 concentrations increase – until ~800 ppm, like it is expected by the year 2100 – *A. glacialis* could benefit from ocean acidification, by enhanced carbon availability for growth. It is likely that larger colonies sink faster and as a result, the carbon is removed more efficiently from the biological cycle and exported to the depth where it is stored for 100 years or more. Thus, *A.glacialis* is expected to give a negative feedback to the ongoing increase in atmospheric CO_2.

KOKKUVÕTE

Alates tööstusrevolutsioonist on CO_2 tase atmosfääris kiiresti tõusnud. Heitkoguste praeguse kasvutempo jätkudes suureneb CO_2 kontsentratsioon üha enam. Lisaks muutustele atmosfääris muutub ka ookean keemia (suurenev CO_2/madalam pH tase), mõjutades seeläbi mitmeid erinevaid organisme. On leitud, et suureneva CO_2 mõju fütoplanktonile võib olla nii positiivne, negatiivne kui puudulik. Lisaks ookeani hapestumisele mõjutavad fütoplanktonit mitmed teised faktorid (e.g. valgustingimused, temperatuur, turbulents ning toitainete kontsentratsioonid). Ränivetikatel on oluline roll süsiniku fikseerimisel, mõjutades seeläbi globaalseid biogeokeemilisi tsükleid ning Maa kliimat. Käesoleva töö eesmärk oli uurida millised on CO_2 (tasemetel 620, 840, 1640 µatm) ja nitraadi (7 ja 13 µmol l^{-1}) mõjud kolooniaid moodustavale kosmopoliitsele ränivetikale *Asterionellopsis glacialis*. Eksperimentaalne töö teostati Assooride Ülikoolis laboritingimustes.

Leiti, et *A.glacialise* saagikus ning kasvukiirus tõusid oluliselt, kui CO_2 tase tõusis tasemelt 620 µatm tasemele 840 µatm. Tänu suurenenud süsiniku kättesaadavusele, võis väheneda süsiniku kontsentreerimise mehhanismi (CCM) tähtsus *A.glacialise* metabolismis ning ülejääv energia oli võimalik suunata kasvu. Leiti veel, et süsihappegaasi suurenedes (kuni 1640 µatm) jäi kasv samale tasemele. Põhjuseks võib olla CO_2 tõusuga samaaegselt langenud pH tase, mis võis raku füsioloogiat negatiivselt mõjutada. Antud tulemustest järeldub, et tänasest paljud kõrgemad CO_2 kontsentratsioonid (e.g. 620 µatm CO_2) ei pruugi küllastada *A.glacialise* süsiniku vajadust.

Lisaks leiti, et *A.glacialise* kolooniad oli oluliselt suuremad, kui CO_2 tase tõusis tasemelt 620 µatm tasemele 840 µatm. Sarnaselt kasvukiirusega, kõrgemal süsihappegaasi kontsentratsioonil (1640 µatm) kolooniate suuruses olulisi muutuseid ei olnud. Lisaks süsihappegaasile oli oluline mõju nitraadil. Kõrgemal nitraadi kontsentratsioonil (13 µmol l^{-1}) oli *A. glacialise* saagikus ning kasvukiirus oluliselt suurem. Nitraadi manipulatsioon aga kolooniate suurust ei mõjutanud. Seega järeldub, et kolooniate suurused sõltuvad rohkem süsiniku kättesaadavusest kui kasvukiirusest. Süsiniku kättesaadavuse suurenedes on polüsahhariidide eksudatsioon suurem, seega on rakud rohkem kleepuvamad ning tõenäosus suuremate kolooniate moodustamiseks tõuseb.

Kokkuvõtvalt võib öelda, et *Asterinellopsis glacialise* kasv tõusis oluliselt, kui CO_2 tase tõusis. Seega, jätkuv CO_2 kontsentratsiooni tõus atmosfääris – eelduste kohastelt ~800 ppm aastaks 2100 – mõjutab positiivselt *A. glacialise* kasvu. Lisaks suurenevad kolooniad ning eelduste kohaselt vajuvad suuremad kolooniad kiiremini ning seeläbi kiireneb orgaanilise aine settimine, mis eemaldab süsiniku aktiivsest bioloogiliselt ringest. Tänu sellele on võimalik anda negatiivset tagasisidet inimtekkelisele CO_2 hulga tõusule Maa atmosfääris.

ACKNOWLEDGEMENTS

I would like to thank Joana Barcelos e Ramos who inspired and advised me through the process of this study. Also I would like to thank Kalle Olli who´s knowledge and time I highly value.

REFERNCES

A. Calbet, M.R. Landry (2004) Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems, Limnology and Oceanography, 49: pp. 51-57

Baines, S.B., Twining, B.S., M.A., Brzezinski, D.M Nelson and N.S, Fisher (2010) Causes and biogeochemical implications of regional differences in silicification of marine diatoms, Global Biogeochem Cycles, 24, GB4031

Barker, S., Ridgwell, A (2012) Ocean Acidification, *Nature Education Knowledge* 3:21

Barcelos e Ramos, J., Schulz, KG., Brownlee, C., Sett, S., Azevedo, E.B (2014) Effects of Increasing Seawater Carbon Dioxide Concentrations on Chain Formationof the Diatom Asterionellopsis glacialis. PLoS ONE 9(3): e90749

Basu, S.; Mackey, K.R.M. (2018) Phytoplankton as Key Mediators of the Biological Carbon Pump: Their Responses to a Changing Climate. *Sustainability* 2018, *10*, 869

Beardall, J., Allen, D., Bragg, J., Finkel, Z.V., Flynn, K., Quigg, A., Rees, A.V., Richardson, A., Raven, J.A. (2008) Allometry and stoichiometry of unicellular, colonial and multicellular phytoplankton, New Phytologist

Bhanu P. Jena (2008) Methods in Nano Cell Biology. Academic Press. Volume 90, Pages 1-473

K.O. Buesseler, P.W. BoydShedding (2009) Light on processes that control particle export and flux attenuation in the twilight zone of the open ocean Limnology and Oceanography, 54: pp. 1210-1232

Buesseler, K.O. (1998) The decoupling of production and particulate export in the surface ocean, Global Biogeochem. Cycles, 13, 297-310

Cerqueira, T., et al. (2017). "Sediment Microbial Diversity of Three Deep-Sea Hydrothermal Vents Southwest of the Azores." Microb Ecol 74(2): 332-349

Chisholm, S.W (1992) Phytoplankton size. In Primary productivity and biogeochemical cycles in the sea (eds P Falkowski, AD Woodhead), pp. 213–237. Berlin, Germany: Springer.

A. Calbet, M.R. Landry (2004) Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems, Limnology and Oceanography, 49: pp. 51-57

Cuoto, R.P., Rodrigues, A.S., Neto, A.I (2015) Shallow-water hydrothermal vents in the Azores (Portugal). Journal of Integrated Coastal Zone Management 15:4

Courties, C., Vaquer, A., Troussellier, M., Lautier, J., Chrétiennot-Dinet, M.J., Neveux, J., Machado, C., Claustre, H (1994) Smallest eukaryotic organism. Nature 370: 255

von Dassow, P., et al. (2018). "Over-calcified forms of the coccolithophore Emiliania huxleyi in high-CO2 waters are not preadapted to ocean acidification." Biogeosciences **15**(5): 1515-1534.

De La Rocha, U. Passow (2007)Factors influencing the sinking of POC and the efficiency of the biological carbon pump, Deep-Sea Research II, 54: pp. 639-658

Deppeler, S., Petrou, K., Schulz, K.G., Westwood, K., Pearce, I., McKinlay, J., Davidson, A (2018) Ocean acidification of a coastal Antarctic marine microbial community reveals a critical threshold for CO2 tolerance in phytoplankton productivity. Biogeosciences, 15 (1), pp. 209-231

Dickson, A.G., Millero (1987) Deep-Sea Research 34: 1733–1743

Dickson, A.G (2010) Standards for ocean measurement. Oceanography 23: 34−47

H.W. Ducklow (2000) Bacterial production and biomass in the oceans. Microbial Ecology of the Oceans, Wiley-Liss, New York pp. 85-120

Feely, R.A, Dooney, S.C, Cooley, S.R (2009) Present Conditions and Future Changes in a High-CO2 World , Oceanography, Volume 22, Number 4

Feely, R. A., et al. (2016). "Chemical and biological impacts of ocean acidification along the west coast of North America." Estuarine, Coastal and Shelf Science **183**: 260-270

Finkel, Z.V (2001) Light absorption and size scaling of lightlimited metabolism in marine diatoms. Limnol. Oceanogr. 46: 86–94

Fu FX, Place AR, Garcia NS, Hutchins DA (2010) CO2 and phosphate availability control the toxicity of the harmful bloom dinoflagellate Karlodinium veneficum. Aquat Microb Ecol 59:55-6

Gallo F, Schulz KG, Azevedo EB, Madruga J, Barcelos e Ramos J (2018) Responses of the diatom Asterionellopsis glacialis to increasing sea water CO2 concentrations and turbulence. Mar Ecol Prog Ser 589:33-44

Gattuso, J., Hansson, L (2011) Ocean acidification. Oxford University Press

Gattuso, J.P, Hansson, L., Fabry, V.J, Riebesell, U (2011) Guide to best practices for ocean acidification research and data reporting, Luxembourg: Publications Office of the European Union, 2011

Gemmell, J.B, Genesok Oh., Buskey, E.J., Villareal, T.A (2016) Dynamic sinking behaviour in marine phytoplankton: rapid changes in buoyancy may aid in nutrient uptake. Published 5 October 2016

Guillard, R.R.L., Ryther, J.H (1962) Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonulaconfervacea Cleve. Can. J Microbiol 8: 229–239

Haizheng, H., Li, D., Lin, W., Li, W., Shi, D (2017) Nitrogen nutritional condition affects the response of energy metabolism in diatoms to elevated carbon dioxide. MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser. Vol. 567: 41–56

Heureux, A.M.C., Rickaby, R.E.M (2015) "Refining our estimate of atmospheric CO2 across the Eocene–Oligocene climatic transition." Earth and Planetary Science Letters **409**: 329-338

Hoppe, C.J.M., Schuback, N., Semeniuk, D., Giesbrecht, K., Mol, J., Thomas, H., Maldonado, M.T., Rost, B., Varela, D.E., Tortell, P.D. (2018) Resistance of Arctic phytoplankton to ocean acidification and enhanced irradiance. Polar Biology, 41 (3), pp. 399-413

Hu S, Zhou B, Wang Y, Wang Y, Zhang X, Zhao Y, et al. (2017) Effect of CO2-induced seawater acidification on growth, photosynthesis and inorganic carbon acquisition of the harmful bloom-forming marine microalga, Karenia mikimotoi. PLoS ONE 12(8)

Hutchins, D.A. (2018) Interactive effects of temperature, CO 2 and nitrogen source on a coastal California diatom assemblage. Journal of Plankton Research, 40 (2), pp. 151-164

IPCC (2014): Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

John, D. E., et al. (2007). "Phytoplankton carbon fixation gene (RuBisCO) transcripts and air-sea CO2 flux in the Mississippi River plume." The Isme Journal **1**: 517

Key, T., A. McCarthy, D. A. Campbell, C. Six, S. Roy, and Z. V. Finkel (2010) Cell size trade-offs govern light exploitation strategies in marine phytoplankton. Environ. Microbiol. 12: 95–104

Krom M. D., Kress N., Brenner S., Gordon L. I (1991) Phosphorus limitation of primary productivity in the eastern Mediterranean Sea, Limnology and Oceanography, 36

Iris Kriest, Geoffrey T. Evans (1999) Representing phytoplankton aggregates in biogeochemical models, Deep Sea Research Part I: Oceanographic Research Papers, Volume 46, Issue 11

Lachkar, Z. (2014) Effects of upwelling increase on ocean acidification in the California and Canary Current systems, Geophys. Res. Lett., 41, 90–95

Lauderdale, J.M., Williams, R.G., Munday, D.R. et al. Clim Dyn (2017) The impact of Southern Ocean residual upwelling on atmospheric CO_2 on centennial and millennial timescales, Climate dynamics 48: 1611

Leadbeater, B. S. C. (2006). "The 'Droop Equation'–Michael Droop and the Legacy of the 'Cell-Quota Model' of Phytoplankton Growth." Protist **157**(3): 345-358

Leonardos, N., Geider, R. J (2004) Effects of nitrate:phosphate supply ratio and irradiance on the C:N:P stoichiometry of Chaetoceros muelleri , European Journal of Phycology, 39:2, 173-180

Li W, Gao K, Beardall J (2013) Correction: Interactive Effects of Ocean Acidification and Nitrogen-Limitation on the Diatom Phaeodactylum tricornutum. PLOS ONE 8(8)

Litchman, E (1998) Population and community responses of phytoplankton to fluctuating light. Oecologia 117: 247– 257

Loughner, C.P., Tzortziou, M., Shroder, S., Pickering, K.E. (2016) Enhanced dry deposition of nitrogen pollution near coastlines: A case study covering the chesapeake bay estuary and atlantic ocean coastline. Journal of Geophysical Research, 121 (23), pp. 14,221-14,238.

Low-Décarie E, Fussmann GF, Bell G (2011) The effect of elevated CO2 on growth and competition in experimental phytoplankton communities. Global Change Biology 17, 2525–2535.

Mehrbach (1973) LimnolOceanogr 18: 897–907

Moore, J.K, Villareal, T.A (1996). Size-ascent rate relationships in positively buoyant marine diatoms. Limnology and Oceanography 41: 1524–1520

Norman, M., Parampil, S.R., Rugersson, A., Sahlee, E. (2013) Influence of coastal upwelling on the air–sea gas exchange of CO_2 in a Baltic Sea Basin, Tellus B: Chemical and Physical Meteorology, 65:1

Pal, R., Choudhury, A.K (2014) An Introduction to Phytoplanktons: Diversity and Ecology, Springer

Pardew, J., et al. (2018). "Predictable ecological response to rising CO2 of a community of marine phytoplankton." Ecology and Evolution 8(8): 4292-4302.

Raven., J.A, Kubler, J.E, Beardall J (2000) Put out the light, and then put out the light. J Mar Biol Assoc UK 80:1–25

Raven JA (2003) Inorganic carbon concentrating mechanisms in relation to the biology of algae. Photosynthesis Research 77: 15–171.

Reynolds, C.,S (2006) The Ecology of Phytoplankton. Cambridge University Press

Reinfelder, J.R (2012) Carbon dioxide regulation of nitrogen and phosphorus in four species of marine phytoplankton. Mar Ecol Prog Ser, Vol. 466: 57–67

Richardson, K., Beardall, J., Raven, J.A (1983) Adaptation of unicellular algae to irradiance: an analysis of strategies. New Phytol. 93: 157–191

Richard J. Geider, C. Mark Moore & Oliver N. Ross (2009) The role of cost–benefit analysis in models of phytoplankton growth and acclimation, Plant Ecology & Diversity, 2:2, 165-178

U. Riebesell, D. Wolf-Gladrow (1992) The relationship between physical aggregation of phytoplankton and particle flux: a numerial model Deep-Sea Research, 39 (7/8) pp. 1085-1102

Riebesell U, Wolf-Gladrow DA, Smetacek V (1993) Carbon dioxide limitation of marine phytoplankton growth rates. Nature 361, 249–251

Riebesell, U. Journal of Oceanography (2004) Effects of CO_2 Enrichment on Marine Phytoplankton. Journal of Oceanography. Issue 4. 60: 719.

Riebesell, U., Fabry, V.J, Hansson, L., Gattuso, J.P, (2010) Guide to best practices for ocean acidification research and data reporting. Publications Office of the European Union, Luxemburg.

Round, F.E., Crawford, R.M., Mann, D.G (2007) Diatoms: Biology and Morphology of the Genera Cambridge University Press; 1 edition

Rost B, Zondervan I, Wolf-Gladrow D (2008) Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions. Mar Ecol Prog Ser 373: 227−237

Sampaio, E., Gallo, F., Schulz, K.G., Azevedo, E.B., Barcelos, J. (2007) Phytoplankton interactions can alter species response to present and future CO2 concentrations. Marine Ecology Progress Series, 575, pp. 31-42

Schippers P, Lürling M, Scheffer M (2004) Increase of atmospheric CO2 promotes phytoplankton productivity. Ecology Letters 7, 446–451

Schulz, K.G., Barcelos e Ramos, J., Zeebe, R.E, Riebesell, U (2009) CO2 perturbation experiments: similarities and differences between dissolved inorganic carbon and total alkalinity manipulations. Biogeosciences 6: 2145– 2153

Sharp, J.H (1974) Improved analysis for particulate organic carbon and nitrogen from seawater.Limnol Oceanogr 19: 984–98

Slagstad, D., Ellingsen, I.H., Wassmann, P (2011) Evaluating primary and secondary production in an Arctic Ocean void of summer sea ice: an experimental simulation approach. Prog Oceanogr 90: 117−131

Sournia, A. (1982) Form and function in marine phytoplankton. *Biol. Rev.*, 57, 347–394

Strom, S.L, Macri E.L, Olson, M.B (2007) Microzooplankton grazing in the coastal Gulf of Alaska: variations in top-down control of phytoplankton. *Limnol. Oceanogr.* **52**, 1480–1494

Zhan,Y., Fu, F., Hutchins, D.,Gao, K (2018) Interactive effects of seawater carbonate chemistry, light intensity and nutrient availability on physiology and calcification of the coccolithophore Emiliania huxleyi. Manuscript under review for journal Biogeosciences

Takabayashi M, Lew K, Johnson A, Marchi AL, Dugdale R, Wilkerson FP (2006) The effect of nutrient availability and temperature on chain length of the diatom, Skeletonema costatum. J Plankton Res 28: 831−840

Tatters, A.O., Schnetzer, A., Xu, K., Walworth, N.G., Fu, F., Spackeen, J.L., Sipler, R.E., Bertrand, E.M., McQuaid, J.B., Allen, A.E., Bronk, D.A., Gao, K., Sun, J., Caron, D.A., Wook, K., Kitack, L., Raymond, G., Najjar, H.D., Hae, J.J. (2011) Increasing N Abundance in the Northwestern Pacific Ocean Due to Atmospheric Nitrogen Deposition**.** *Science*, 22 September 2011

Thoisen C, Riisgaard K, Lundholm N, Nielsen TG, Hansen PJ (2015) Effect of acidification on an Arctic phytoplankton community from Disko Bay, West Greenland. Volume 520, pages 21-34

A. Torstensson, M. Chierici, A. Wulff (2012) The influence of increased temperature and carbon dioxide levels on the benthic/sea ice diatom Navicula directa. Polar Biology, 35: pp. 205-214

Tréguer, P., D. M. Nelson, A. J. Van Bennekom, D. J. DeMaster, A. Leynaert, and B. Quéguiner (1995), The silica balance in the world ocean: A reestimate, Science, 268, 375–379

Turner, J. T. (2015). "Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump." Progress in Oceanography **130**: 205-248

Uitz, J., H. Claustre, B. Gentili, and D. Stramski (2010), Phytoplankton class-specific primary production in the world's oceans: Seasonal and interannual variability from satellite observations, Global Biogeochem. Cycles, 24, GB3016

Villareal, T.A., Joseph, L., Brzezinski, M.A., Shipe, R.F., Lipshultz, F., Altabet, M.A, (1999) Biological and chemical characteristics of the giant diatom Ethmodiscus (Bacillariophyceae) from the Central North Pacific Gyre. Journal of Phycology 35: 896–902

Voss, Maren et al.(2013) "The Marine Nitrogen Cycle: Recent Discoveries, Uncertainties and the Potential Relevance of Climate Change." *Philosophical Transactions of the Royal Society B: Biological Sciences* 368.1621 (2013): 20130121. *PMC*. Web. 6 May 2018.

Wynn-Edwards C, King R, Davidson A, Wright S and others (2014) Species-specific variations in the nutritional quality of southern ocean phytoplankton in response to elevated pCO2. Water 6: 1840−1859

Yang G, Gao KS (2012) Physiological responses of the marine diatom Thalassiosira pseudonana to increased pCO2 and seawater acidity. Mar Environ Res 79: 142−151

Internet sources:

Holmes-Farely, R (2002) Chemistry and the Aquarium: What is Alkalinity? Advancedaquarist, February, volume I [WWW] https://www.advancedaquarist.com/2002/2/chemistry

Woods Hole Oceanographic Institution (2012) [WWW] http://www.whoi.edu/OCB-OA/page.do?pid=112136

Chris Mooney (2018) [WWW] https://www.washingtonpost.com/news/energy-environment/wp/2018/05/03/earths-atmosphere-just-crossed-another-troubling-climate-change-threshold/?utm_term=.5ddb54aa1447

Lihtlitsents lõputöö reprodutseerimiseks ja lõputöö üldsusele kättesaadavaks tegemiseks

Mina, Gerli Albert,

(*autori nimi*)

annan Tartu Ülikoolile tasuta loa (lihtlitsentsi) enda loodud teose

THE RESPONSE OF THE DIATOM *ASTERIONELLOPSIS GLACIALIS* TO VARIATIONS IN CO_2 AND NITRATE AVAILABILITY,

(*lõputöö pealkiri*)

mille juhendajad on Joana Barcelos e Ramos ja Kalle Olli,

(*juhendaja nimi*)

1.1. reprodutseerimiseks säilitamise ja üldsusele kättesaadavaks tegemise eesmärgil, sealhulgas digitaalarhiivi DSpace-is lisamise eesmärgil kuni autoriõiguse kehtivuse tähtaja lõppemiseni;
1.2. üldsusele kättesaadavaks tegemiseks Tartu Ülikooli veebikeskkonna kaudu, sealhulgas digitaalarhiivi DSpace´i kaudu kuni autoriõiguse kehtivuse tähtaja lõppemiseni.

2. olen teadlik, et punktis 1 nimetatud õigused jäävad alles ka autorile.

3. kinnitan, et lihtlitsentsi andmisega ei rikuta teiste isikute intellektuaalomandi ega isikuandmete kaitse seadusest tulenevaid õigusi.

Tartus, **22.05.2018**