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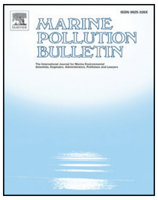

Effects of pH on the growth and NH_4-N uptake of *Skeletonema costatum* and *Nitzschia closterium*

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ABSTRACT

Ocean acidification (OA) and eutrophication intensifies in coastal sea under anthropogenic impact. OA coupled with the NH_4-N source effect in coastal water is likely to affect the planktonic ecosystem. In this work, *Skeletonema costatum* and *Nitzschia closterium* were chosen as typical species of diatom in Chinese coastal ecosystems to test the potential effect of OA and NH_4-N. Results showed that the growth and NH_4-N uptake of *S. costatum* and *N. closterium* were significantly inhibited by pH decline. The maximum uptake rate is higher than the maximum growth rate, implying that NH_4-N was assimilated faster for *S. costatum* and *N. closterium* with decreasing pH. Therefore, the inhibition rate of the growth of the two diatoms by the coupling effect of OA and eutrophication (pH 7.45) is higher that than in the coastal sea by the end of the 21st century (pH 7.71).

1. Introduction

Atmospheric CO_2 level continuously increases because of human activities, such as burning of fossil fuel and logging, and results in biological and environmental issues (Feely and Doney, 2011). In addition to global warming, which is a consequence of increasing carbon emission, Boytchev pointed out other worrisome issues that is ocean acidification (OA) (Boytchev, 2013). About one-third of anthropogenic atmospheric CO_2 is absorbed by the ocean, which is the most significant carbon sink in earth (Hönisch et al., 2009); this phenomenon, also known as OA, leads to pH drop and changes in the basic chemical balance in the ocean (Lefebvre et al., 2012). Orr reported that the pH decreased by 0.10 units worldwide since the 1880s and the H^+ concentration increased by 30% (Orr et al., 2005). The Intergovernmental Panel on Climate Change predicted that the atmospheric CO_2 concentration will increase to 800–1000 ppm at the end of the 21st century, whereas the average pH in the surface ocean will continue to decrease by 0.30–0.40 units (Caldeira and Wickett, 2005; Hönisch et al., 2012). Currently, OA occurs at the fastest speed compared with that in the past 300 million years mainly because human beings rapidly change the composition of the atmosphere and marine chemicals (Hönisch et al., 2012). The coastal ecosystem is a complex system influenced by natural and human factors (Aufdenkampe et al., 2011); marine nitrogen cycle is more significantly affected by OA than other nutrients (Hutchins et al., 2009). With social and economic development, human beings discharge large amounts of untreated domestic sewage and industrial wastewater into coastal sea, thereby increasing the NH_4-N concentration in coastal sea water (Caldeira and Wickett, 2005). Moreover, decrease in pH would change the concentration of NH_4-N. Cai proposed that eutrophication could increase the decline rate of OA, and the decline rate of pH in the coastal sea is faster than that in the open sea because of the coupling effect of OA, biological respiration, and hypoxia (Cai et al., 2011). According to the simulation results at the end of the 21st century, eutrophication can continuously decline the average pH by 0.47 units when the O_2 concentration approaches the value ($0\ \mu mol \cdot kg^{-1}$) (Cai et al., 2011). Under extreme conditions, the average pH decreases to 7.30–7.20 because of the coupling effect of OA and eutrophication, and the potential impact of OA could be larger. Basing on geological modification over the past 100 years, German biologist Ulf Riebesell indicated that the ocean is facing a great danger, and the marine chemical composition undergoes fundamental changes; algae, which are the basis of the food chain in the marine ecosystem, may be unable to adapt to these changes and face with survival crisis (Kroeker et al., 2013; Riebesell et al., 2013). OA may be the "culprit" that caused earth biological extinction 250 million years ago (Hand, 2015).

Marine phytoplankton, as the primary producers in the ocean, affect the global carbon cycle and absorb dissolved CO_2 in the ocean by photosynthesis, thereby slowing OA. Since the 1990s, the biological response of marine phytoplankton to OA has gained increased attention and is one of the important factors affecting the marine ecosystem (Gao et al., 1993; Gao and Zheng, 2010); OA exhibits toxic effects on marine organism and could alter the marine ecosystem (Kroeker et al., 2013). For instance, the calcified ecosystem of some reef systems could become negative because of the dissolution of $CaCO_3$ on account of OA (Andersson et al., 2005; Silverman et al., 2009); the composition of diatoms changes under different CO_2 concentrations and is biologically influenced by fed copepods (Rossoll et al., 2012). A decline in growth

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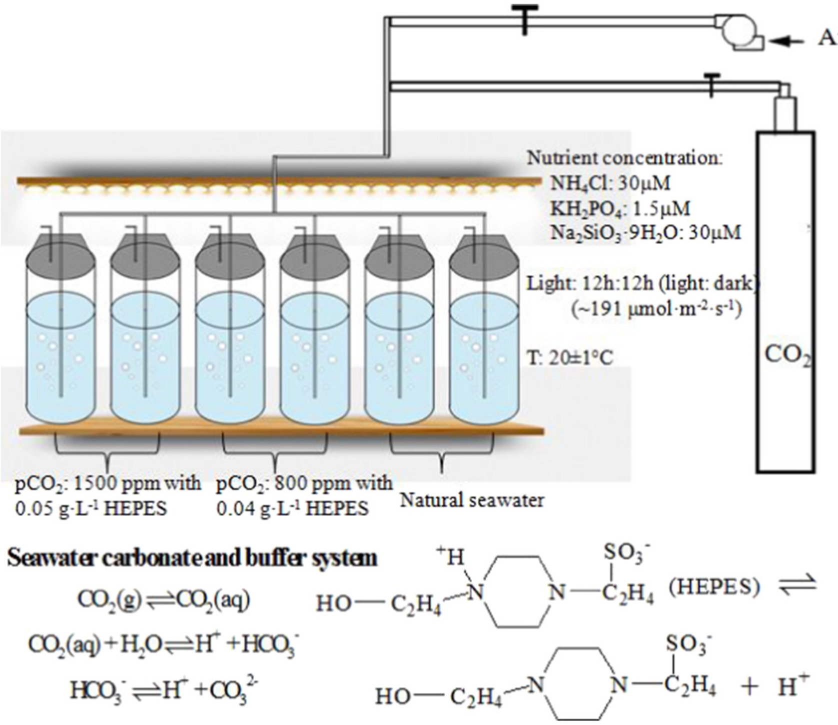

Fig. 1. Experimental facility. Here, seawater carbonate system is governed by a series of chemical equilibriums, high pCO_2 increasing CO_2(aq), HCO_3^-, and H^+ concentrations, and the pH lowers. Buffer agent HEPES is added to maintain pH. The 0.30–0.40 pH drop is equivalent to approximately 150% increase in H^+ concentrations at pCO_2 800 ppm with ~0.04 g L^{-1} HEPES, and the 0.70–0.80 pH drop is equivalent to approximately 400% increase in H^+ concentrations at pCO_2 1500 ppm with ~0.05 g L^{-1} HEPES.

rate of coastal marine phytoplankton, possibly due to adverse effects of decreasing pH, has observed at pCO_2 levels higher (Burkhardt et al., 1999), such as OA inhibits the growth of *Phaeochysglobosa* under high-intensity light and promotes the growth of these organisms under low-intensity light (Chen and Gao, 2011). Diatoms playa dominant role in marine primary productivity and are affected by OA; as photoautotrophs (Kroeker et al., 2013), diatoms reside near the water surface where OA occurs (Armbrust, 2009; Zhai et al., 2009; Caldeira and Wickett, 2003). Diatoms could also reflect the living condition of the water environment because they function as a biosensor for pH changes in seawater (Carpenter and Waite, 2000).

Generally, phytoplankton initially absorb NH_4-N because this compound can directly synthesize amino acids by transamination under the action of GS/GAGOT enzymes (Clayton et al., 1986); other nutrients such as NO_3-N and NO_2-N should be reduced to NH_4-N by the corresponding nitrate reductase and nitrite reductase (Berges and Mulholland, 2008). NH_4-N concentration is high in most coastal sea water undergoing eutrophication. Thus far, limited information is available regarding the effects of OA with NH_4-N on marine phytoplankton. In the present study, we selected *Skeletonema costatum* and *Nitzschia costatum* as representative diatoms for laboratory batch culture to assess the growth of phytoplankton and NH_4-N uptake kinetics under different pH conditions. We also evaluated the growth of *S. costatum* and *N. closterium* under different OA conditions. Results could provide experimental bases for further exploration on the potential impacts of OA on phytoplankton and marine ecosystems.

2. Materials and methods

2.1. Cultures

Marine diatoms *S. costatum* and *N. closterium* were obtained from the algal species room in the Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China and grown in *f*/2 medium. All experiments were maintained in an environmental room with a constant temperature (20 ± 1 °C) under 12 h: 12 h (light: dark) light cycle (~191 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Fig. 1). Qingdao coastal seawater (DOC = 3.94 $mg \cdot L^{-1}$, pH = 8.10, and Salinity = 27) was sequentially filtered through 2, 0.45, and 0.22 μm cellulose acetate fibers to remove adhering microorganisms. Subsequently, 70 mL of the algal suspension was added to 3.5 L of sterile seawater, where the equilibrium of the carbonate chemistry was reached before starting the experiment. The mixture was transferred into 5 L transparent glass culture vessels covered by qualitative filter paper (Φ11 cm). Generally, chlorophyll *a* (chl *a*) is used to estimate phytoplankton biomass, but its values in the natural habitat may not accurately indicate actual phytoplankton biomass. The phytoplankton biomass of *S. costatum* and *N. closterium* were ~3.53 and ~8.36 $\mu g \cdot L^{-1}$ (chl *a*) in the culture media, respectively. PO_4-P (1.5 μM, KH_2PO_4; Sinopharm Chemical Reagent Co., Ltd. (SCRC)), SiO_3-Si (30 μM, $Na_2SiO_3 \cdot 9H_2O$, SCRC) and NH_4-N (30 μM, NH_4Cl; SCRC) were enriched in the culture media. Considering the complex ecological environment in the coastal area, the pH values might decrease to ~7.7 with the effect of global OA and ~7.4 with the coupling effect of global OA and eutrophication at the end of the 21st century. Thus, three pH gradients (8.10, 7.71, and 7.45) were set, and each pH gradient had two parallel samples (Table 1). The experimental period lasted for 7–10 days, and sampling was performed 1–2 times$\cdot day^{-1}$. Then, 200 mL of the algal suspension was filtered through

Table 1
Experimental conditions of *S. costatum* and *N. closterium*.

pH		8.10		7.71		7.45	
Chl *a* concentration ($\mu g \cdot L^{-1}$) (*S. costatum*)		3.23	3.37	3.55	3.55	3.77	3.70
Chl *a* concentration ($\mu g \cdot L^{-1}$) (*N. closterium*)		8.69	8.73	8.66	8.66	6.22	6.67
Nutrient concentration ($\mu mol \cdot L^{-1}$)	NH_4Cl	30					
	KH_2PO_4	1.5					
	$Na_2SiO_3 \cdot 9H_2O$	30					
Photoperiod		12 h:12 h					
Temperature (°C)		20 ± 1					
Light ($\mu mol \cdot m^{-2} \cdot s^{-1}$)		191					

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