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Intraspecific variability in the response of bloom-forming marine microalgae to changed climate conditions

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Abstract

Phytoplankton populations can display high levels of genetic diversity that, when reflected by phenotypic variability, may stabilize a species response to environmental changes. We studied the effects of increased temperature and CO_2 availability as predicted consequences of global change, on 16 genetically different isolates of the diatom *Skeletonema marinoi* from the Adriatic Sea and the Skagerrak (North Sea), and on eight strains of the PST (paralytic shellfish toxin)-producing dinoflagellate *Alexandrium ostenfeldii* from the Baltic Sea. Maximum growth rates were estimated in batch cultures of acclimated isolates grown for five to 10 generations in a factorial design at 20 and 24°C, and present day and next century applied atmospheric pCO_2, respectively. In both species, individual strains were affected in different ways by increased temperature and pCO_2. The strongest response variability, buffering overall effects, was detected among Adriatic *S. marinoi* strains. Skagerrak strains showed a more uniform response, particularly to increased temperature, with an overall positive effect on growth. Increased temperature also caused a general growth stimulation in *A. ostenfeldii*, despite notable variability in strain-specific response patterns. Our data revealed a significant relationship between strain-specific growth rates and the impact of pCO_2 on growth—slow growing cultures were generally positively affected, while fast growing cultures showed no or negative responses to increased pCO_2. Toxin composition of *A. ostenfeldii* was consistently altered by elevated temperature and increased CO_2 supply in the tested strains, resulting in overall promotion of saxitoxin production by both treatments. Our findings suggest that phenotypic variability within populations plays an important role in the adaptation of phytoplankton to changing environments, potentially attenuating short-term effects and forming the basis for selection. In particular, *A. ostenfeldii* blooms may expand and increase in toxicity under increased water temperature and atmospheric pCO_2 conditions, with potentially severe consequences for the coastal ecosystem.

Introduction

Human-induced climate change will significantly alter marine environmental conditions within the next century. Projected changes include a rise in sea surface temperature due to an atmospheric temperature increase of approximately 4°C, and elevated oceanic levels of free aqueous CO_2 as a consequence of the increase in atmospheric pCO_2 from the current 385 ppm, to 750 ppm at the end of this century (IPCC 2007). Warming of the upper ocean will enhance water column stratification with significant effects on light and nutrient conditions in the upper water column (Hoeg-Guldberg and Bruno 2010 and references therein). Increased CO_2 concentrations cause the pool of dissolved inorganic carbon to rise, shifting the carbonate equilibrium to higher CO_2 and HCO_3 levels, resulting in decreased CO_3^{2-} concentrations

and a drop in pH of 0.4 units by 2100 (Caldeira and Wicket 2003).

Such modified physical and chemical conditions will affect marine phytoplankton in different ways. Due to its influence on molecular kinetic energy, temperature acts directly on cell physiological processes and determines metabolic rates. Moderate increases in temperature, such as the 4°C rise projected by future climate scenarios, should enhance photosynthesis and phytoplankton growth (Beardall and Raven 2004). Studies on the effects of elevated temperature on algal growth have shown that particularly harmful warm water species thrive at elevated temperatures, whereas species naturally occurring at intermediate temperatures were negatively or not affected at all (Peperzak 2003; Fu et al. 2008). Cold water species with narrow temperature tolerances may be most severely affected as the projected temperature increase exceeds their tolerance limits considerably (Sundström et al. 2009). Oceanic warming will also influence phytoplankton by expanding the spatial and seasonal distribution of tropical and temperate warm water species (Hallegraeff 2010 and references therein).

The continuing increase in atmospheric pCO$_2$ affects the physiology of phototrophic organisms directly, as CO$_2$ is the primary substrate for photosynthesis. Increased concentrations of free CO$_2$ could potentially favor photosynthesis and growth (Riebesell 2004), since present CO$_2$ concentrations are not saturating for RUBISCO, the enzyme that catalyzes primary fixation of inorganic carbon (Badger et al. 1998). Most microalgae have developed strategies to counteract CO$_2$ limitation by employing CO$_2$-concentrating mechanisms (CCMs) (Giordano et al. 2005). CCMs in different species and phylogenetic groups vary considerably in efficiency and regulation (Badger et al. 1998; Ratti et al. 2007; Trimborn et al. 2008), and differences exist in CO$_2$ requirements between taxa, with respect to saturation levels and preferences of inorganic carbon source molecules (Paasche 2001; Rost et al. 2003). Such physiological diversity may explain the observed variability in phytoplankton sensitivity to elevated CO$_2$ levels (Riebesell et al. 2000; Leonardos and Geider 2005; Fu et al. 2010; Nielsen et al. 2010). The decrease in sea water pH associated with rising levels of free aqueous CO$_2$ particularly affects the calcification process of various phytoplankton species, as they depend on the availability of free carbonate for the production of calcite structures (Riebesell et al. 2000; Iglesias-Rodriguez et al. 2008; Langer et al. 2009). However, in terms of growth rates, marine phytoplankton generally appears unaffected by lowered pH (Berge et al. 2010).

Most of the laboratory studies investigating the effects of climate stressors on phytoplankton have been performed on single strains. The significant effects often found in such experiments are contrasted by the general lack of clear responses in natural populations (e.g., Engel et al. 2008). The higher tolerance of natural populations to environmental factors might be due to the ecophysiological variability of the diverse genotypes constituting the populations (Paasche 2001; Nielsen et al. 2010). Contradictory responses to changed climate conditions sometimes observed within the same species might be partly attributable to strain variability between or within populations (Langer et al. 2009). This emphasizes the need to consider variability in studies aiming to understand the effects of climate change on phytoplankton species.

Considerable variability has been shown in a number of ecologically important traits of phytoplankton, such as salinity tolerance (Brand 1984), toxicity (Bachvaroff et al. 2009), and growth requirements (Fredrickson et al. 2011). The genetic basis of such phenotypic variability has long been recognized (Brand 1982), and recently became the subject of focused investigation, revealing high levels of genetic differentiation among temporally and spatially separated populations (Rynearson and Armburst 2004; Alpermann et al. 2010; Godhe and Härnström 2010). Growing evidence suggests that considerable genetic and phenotypic diversity exist within the same population (Tillman et al. 2009; Alpermann et al. 2010). Such diversity is particularly important for a population to cope with changing environmental conditions. Genetically, diverse populations can resist environmental perturbations more effectively than genetically uniform populations (Hughes and Stachowicz 2004). Phenotypic variability can buffer the immediate effects of environmental fluctuations, while standing genetic variation should immediately influence the longer term selection (Barrett and Schluter 2007). Despite the relevance for adaptation, population level variability in phytoplankton has not been addressed in relation to climate change.

In this study, we examined response variability in growth and toxicity among multiple genetically different strains of two geographical populations (hereafter referred to as populations) of the marine diatom *Skeletonema marinoi* (Fig. 1A and B), and a Baltic population of the toxic dinoflagellate *Alexandrium ostenfeldii* (Fig. 1C and D), when exposed to increased supply of atmospheric CO$_2$ and increased temperature. Both species are widely distributed in temperate coastal waters where they form seasonal blooms. Despite their different life forms and life histories, both show high levels of genetic diversity (Godhe and Härnström 2010; P. Tahvanainen et al., unpubl. data) that, when reflected by phenotypic trait variability, may stabilize species response to environmental changes.

Materials

Culturing of clonal strains

Sixteen strains of *S. marinoi* were isolated from the NW Adriatic Sea and the Skagerrak (Table 1) as described in Godhe and Härnström (2010). Skagerrak cultures were maintained
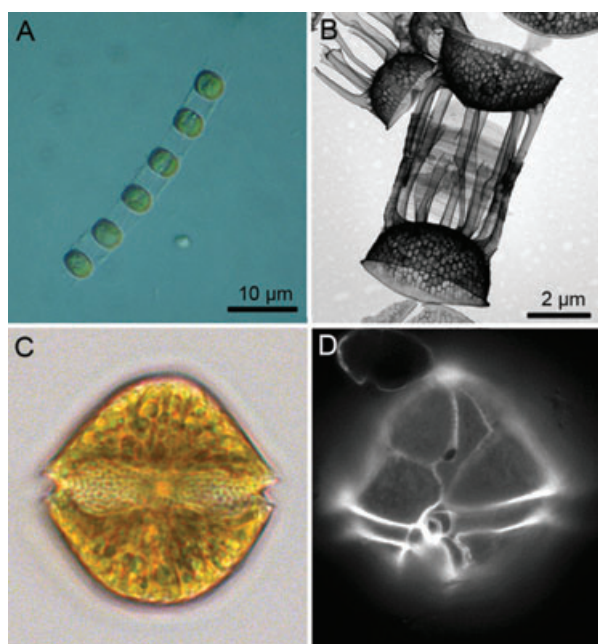
Figure 1. Micrographs of (A and B) the diatom *Skeletonema marinoi*: (A) a typical cell chain as observed in the light microscope, (B) silica frustules as shown by scanning electron microscopy (SEM), and (C and D) the dinoflagellate *Alexandrium ostenfeldii*: (A) cell as seen in a light microscope and (B) cell wall (theca) stained with calcofluor brightener and visualized by epifluorescence microscopy showing diagnostic thecal plate features.

Table 1. Diatom and dinoflagellate isolates used in this study.

Strain code	Geographic origin	Date of isolation
Skeletonema marinoi (Bacillariophyceae)	NW Adriatic Sea	
SM01–SM03	43°55.5′N, 12°53.5′E	19.11.2009
SM04–SM08	43°55.5′N, 12°53.5′E	15.4.2010
	Skagerrak (North Sea)	
SM09	57°33.0′N, 11°31.5′E	12.8.2009
SM10	58°15.2′N, 11°03.5′E	12.10.2009
SM11	58°20.3′N, 11°21.4′E	9.11.2009
SM12	58°15.6′N, 11°25.9′E	7.5.2009
SM13	58°15.2′N, 11°03.5′E	12.10.2009
SM14	58°15.2′N, 11°03.5′E	15.10.2009
SM15	58°15.6′N, 11°25.9′E	7.5.2009
SM16	58°15.6′N, 11°25.9′	15.5.2009
Alexandrium ostenfeldii (Dinophyceae)	Baltic Sea, Åland, Föglö	
AO01–AO08	60°05.9′N, 20°30.5′E	14.3.2009

To confirm clonal identities, the experimental strains of *S. marinoi* and *A. ostenfeldii* were genotyped by microsatellite (Almany et al. 2009; Godhe and Härnström 2010) and amplified fragment length polymorphism (AFLP) analyses (Vos et al. 1995), respectively. LSU rDNA was sequenced according to Godhe et al. (2006) to confirm the species identity of the northern Adriatic *S. marinoi* strains.

Experimental design and sampling

The effects of increased atmospheric $p\mathrm{CO_2}$ and temperature on growth and toxicity (the latter only for PST-producing *A. ostenfeldii*) were tested in batch culture experiments, where eight clonal strains of each species and population were grown in triplicates at four different temperature and $p\mathrm{CO_2}$ combinations (=treatments). A temperature of 20°C and ambient air $p\mathrm{CO_2}$ of approximately 385 ppm represented present spring-summer bloom phase climate conditions. This treatment served as the control, and below is referred to as such. In the second treatment (referred to as +$\mathrm{CO_2}$), an increased $p\mathrm{CO_2}$ of 750 ppm was applied to simulate future atmospheric $\mathrm{CO_2}$ concentrations, while temperature remained at 20°C. The third treatment (referred to as +T) was set up at 24°C with ambient $p\mathrm{CO_2}$ simulating the temperature increase anticipated by climate models (IPCC 2007). An additional treatment (referred to as +$\mathrm{CO_2}$, T) at 24°C and 750 ppm $p\mathrm{CO_2}$ examined the combined effects of these factors. *Skeletonema marinoi* from the Skagerrak was not exposed to this treatment, due to limited availability of the $\mathrm{CO_2}$-enriched gas mix. Experiments were performed at salinities reflecting the respective habitat conditions of each geographic population or species, that is, salinities of the isolation sites: 32 for *S. marinoi* from the northern Adriatic Sea and 26 for *S. marinoi* from the Skagerrak. *Alexandrium ostenfeldii*

at 10°C, 12:12 h light:dark cycle and 60 μmol photons $\mathrm{m^{-2}\,s^{-1}}$ in f/2 medium with a local salinity of 26; NW Adriatic cultures were maintained at 20°C, 100 μmol photons $\mathrm{m^{-2}\,s^{-1}}$, and a salinity of 32. The Adriatic Sea and the Skagerrak strains used in the experiment were randomly chosen from sets of 13 and 460 cultures, isolated from each of the respective area.

The cultures of *A. ostenfeldii* were established from a sediment sample collected in March 2009 from a bloom site in the Föglö archipelago, Åland, in the Northern Baltic Sea (Table 1). Single resting cysts were selected from sediment slurries and incubated wells of a tissue culture plates, each filled with 2 mL of f/8-Si enriched natural sea water (1/4 nutrient concentrations compared to standard f/2 medium) at a salinity of 6.5, and incubated at 16°C, 12:12 light:dark cycle and 100 μmol photons $\mathrm{m^{-2}\,s^{-1}}$. Once germinated, clonal strains were established by isolating single motile cells into a new culture well containing f/8-Si medium. Well-established clonal cultures were transferred to vented 50-mL polycarbonate tissue culture flasks and maintained in f/2-Si culture medium at 16°C, 12:12 light:dark cycle, and 100 μmol photons $\mathrm{m^{-2}\,s^{-1}}$. Eight strains were randomly selected from a set of 50 cultures established as described above from the Åland sediment sample.

strains had earlier been adjusted to the experimental salinity of 10.

Incubations were carried out in two climate controlled incubation chambers set to 20 and 24°C, respectively. Different pCO_2 conditions were achieved by gently bubbling air with ambient pCO_2, and a commercially purchased (AGA) gas mix with the pCO_2 adjusted to 750 ppm, respectively, into experimental batch cultures. Gas was distributed from central gas bottles (air, and air $+CO_2$) through silicon tubing and a microcapillary directly into the water phase of each batch culture following the setup of Torstensson et al. (2012). The design was chosen to simulate a bloom situation, that is, a situation with high biomass increase, and expected CO_2 drawdown under conditions of increased water temperature and supply of atmospheric CO_2 to the system.

Prior to the experiment, all strains were acclimated to the experimental conditions for two weeks. Acclimation was performed using the same conditions as in the experiment, that is, continuous bubbling of 385 and 750 ppm pCO_2 gas mixes into acclimation cultures at the two experimental temperatures. The fast growing *S. marinoi* (mean generation time of about one day) cultures were diluted several times to maintain the active growth stage. For the slower growing *A. ostenfeldii* with mean generation times of approximately three days, the acclimation period corresponded to approximately five generations.

From such cultures, an inoculum—resulting in initial cell concentrations of approximately 5000 cells mL^{-1} for *S. marinoi*, and 500 cells mL^{-1} for *A. ostenfeldii*—was transferred into three 250 mL tissue culture flasks per strain and treatment, containing 200 mL of f/2 culture medium (without silica for *A. ostenfeldii*). The flasks were placed in the respective climate chamber, and connected to the appropriate gas distributor. Three replicates without phytoplankton per culture medium and per treatment were used as controls, and kept for three days to check the dynamics of CO_2 equilibration. Experimental incubations lasted until cultures had reached stationary growth phase and biomass ceased to increase, five days for *S. marinoi* and 17 days for *A. ostenfeldii*.

Temperature, pH total scale (pH_{TS}), and total alkalinity (A_T) were measured every day from 24 randomly selected bottles covering all populations and treatments. The pH_{TS} was measured with a Metrohm (827 pH laboratory) pH electrode, calibrated with salinity adjusted seawater, TRIS, and AMP buffers following Dickson et al. (2007). The A_T measurements were conducted as described by Sarazin et al. (1999), with an accuracy of 10 μmol kg^{-1} seawater. Water-phase pCO_2 was calculated from pH and alkalinity using CO2SYS (Lewis and Wallace 1998), with dissociation constants from Mehrbach et al. (1973) and refitted by Dickson and Millero (1987). Throughout the duration of the experiments, light levels in the climate chambers were checked daily with an LI-COR LI-1400 Data Logger and Light Meter to ensure comparable and stable light conditions.

Samples for measurements of Chl *a* fluorescence were taken once a day from *S. marinoi* cultures, and every second day from *A. ostenfeldii* cultures. Flasks were gently shaken to distribute cells evenly before volumes of 200 μL were collected in 2-mL Eppendorf tubes filled with 1.8 mL of pure ethanol. Samples were allowed to extract for 1 h at room temperature in darkness, and were stored at –20°C until analysis.

Determination of growth rates

Growth was inferred from the development of Chl *a* fluorescence in each flask. Fluorescence was measured directly in a 1:9 culture:ethanol mix (Greenberg and Watras 1989; J. Seppälä et al. unpubl. data). Samples were measured in 96-well tissue culture plates on a PerkinElmer plate reader spectrophotometer at 450 nm excitation and 680 nm emission wavelengths. Relative Fluorescence Units were converted to cell numbers based on standard curves established for each separate plate run, using a linear series of culture:ethanol mixtures. For these standard curves, cell concentrations were obtained by manual cell counts. Growth rates, r, defined as instantaneous rate of increase, were calculated based on the longest possible period of exponential growth, using the equation $r = \ln(Nt/N0)/\Delta t$, where N = the number of cells per milliliter and t = time (Wood et al. 2005). The interval of exponential growth was determined from growth curves established for each experimental culture replicate.

Toxin measurements

At the end of the experiment, the remaining *A. ostenfeldii* cultures were filtered through Whatman GF/C filters (25 mm diameter) for PSP toxin analyses. Toxins were extracted from freeze-dried filters in 1 mL of 0.03 M acetic acid, using an ultrasonic bath (Bandelin Sonorex Digitec) at <10°C for 30 min. The filters were subsequently removed and the samples centrifuged at 12,000 $\times$ g for 5 min. The supernatant was then filtered through 0.45 μm GHP Acrodisc membrane filters (13 mm diameter, Pall Life Sciences, NY). PSP toxin analyses followed the protocol modified from Janiszewski and Boyer (1993) and Diener et al. (2006) as described in Hakanen et al. (2012). Analyses were performed using an Agilent HPLC system (Agilent, Santa Clara, CA) consisting of two series 1100 pumps, degasser, autosampler, photodiode array, and fluorescence detector. The optical detectors were preceded by a high sensitivity dual electrode analytical cell 5011A (ESA, Chelmsford, MA) controlled with an ESA Coulochem II multielectrode detector to achieve electrochemical postcolumn oxidation (Janiszewski and Boyer 1993).

Fluorescence emission signal was used in the PST quantification. Fluorescence detection was applied for the

Table 2. Seawater chemistry of culture media (mean ± SEM).

Treatment	Temperature (°C)	A_T (μmol kg^{-1})	pH$_{TS}$	pCO$_2$ (μatm)
Skeletonema marinoi—NW Adriatic				
Medium: salinity = 32 psu				
20°C/385 ppm	19.20 ± 0.02	2473.65 ± 13.02	8.10 ± 0.01	387.33 ± 7.22
20°C/750 ppm	19.07 ± 0.03	2451.88 ± 9.77	7.86 ± 0.01	738.67 ± 9.77
24°C/385 ppm	22.50 ± 0.06	2569.76 ± 14.82	8.13 ± 0.01	356.67 ± 14.68
24°C/750 ppm	23.57 ± 0.21	2414.86 ± 8.04	7.87 ± 0.01	730.67 ± 6.69
Skeletonema marinoi—Skagerrak				
Medium: salinity = 26 psu				
20°C/385 ppm	18.97 ± 0.07	1862.41 ± 11.46	8.05 ± 0.03	343.00 ± 24.85
20°C/750 ppm	19.10 ± 0.06	1854.62 ± 26.02	7.75 ± 0.01	754.33 ± 23.38
24°C/385 ppm	23.00 ± 0.15	1791.70 ± 8.83	8.03 ± 0.01	361.03 ± 79.4
24°C/750 ppm	23.70 ± 0.17	1860.30 ± 35.72	7.76 ± 0.01	744.12 ± 7.51
Alexandrium ostenfeldii				
Medium: salinity = 10 psu				
20°C/385 ppm	19.20 ± 0.06	820.59 ± 15.60	7.81 ± 0.02	348.67 ± 12.33
20°C/750 ppm	19.20 ± 0.01	808.28 ± 4.34	7.54 ± 0.03	761.06 ± 43.09
24°C/385 ppm	22.90 ± 0.17	795.39 ± 6.21	7.82 ± 0.04	344.67 ± 9.84
24°C/750 ppm	24.07 ± 0.03	783.14 ± 7.20	7.51 ± 0.02	745.67 ± 29.24

determination of PST oxidation products (Ex.: 335 nm, Em.: 396 nm, slits 1 nm). The samples were quantitatively analyzed by comparing with PSP standards of GTX (gonyautoxin) 1–4, NEO (neosaxitoxin), and STX (saxitoxin), purchased from the National Research Council Canada, Marine Analytical Chemistry Standards Program (NRC-CRMP), Halifax, Canada.

Statistical analysis

Analysis of variance (ANOVA II) was carried out to test treatment effects on seawater chemistry parameters calculated pCO$_2$, measured A_T, pH$_{TS}$, and temperature. One-way ANOVAs were performed using SPSS 15.0.1 for Windows to test for differences between control and treatment conditions in growth rates and cellular PST concentrations. Differences between treatments were examined using a Tukey's (HSD) post-hoc test with a significance level of $P < 0.05$.

Results

Seawater chemistry

Due to the different salinity requirements of each species and geographic population, the alkalinity conditions in the three growth media differed considerably, ranging from approximately 800 μmol kg^{-1} in the low saline Baltic *A. ostenfeldii*, to about 2500 μmol kg^{-1} in the units containing the Adriatic *Skeletonema* cultures (Table 2). No differences in alkalinity were detected among the treatments of either species or geographic population. The pH values were higher in the *Skeletonema* cultures compared to *A. ostenfeldii* (Table 2).

Generally, pH was lower in the treatments bubbled with the high pCO$_2$ gas mix. In the controls without phytoplankton, the target free aqueous CO$_2$ was reached in the medium within 24 h (Table 2 and Fig. S1). The two applied CO$_2$ concentrations generated different levels of free aqueous CO$_2$ in the experimental treatments. However, calculated levels of free aqueous CO$_2$ were generally lower than anticipated levels and decreased in all treatments over the time of the experiment (Fig. S1) due to the presence and continuous increase of CO$_2$-consuming algal biomass. In the Adriatic *Skeletonema* cultures, differences in free aqueous CO$_2$ between treatments were moderate but not significant. High pCO$_2$ resulted in significantly higher levels of free aqueous CO$_2$ in the respective treatments in the Skagerrak population of *Skeletonema* ($P < 0.0013$), and in *A. ostenfeldii* ($P < 0.0095$).

Growth of *S. marinoi* at different temperature and *p*CO$_2$ applications

The eight Adriatic Sea strains differed considerably in their growth rates. Under control conditions (20°C, pCO$_2$ of 385 ppm), maximum growth rates ranged from 0.43 days^{-1} in SM07, to 1.26 days^{-1} in SM05. The response of the tested *S. marinoi* strains to the different CO$_2$ and temperature treatments was also highly variable (Fig. 2), with nearly every strain showing a different pattern. Two of the tested strains (SM01 and SM02) were not significantly affected by any of the experimental manipulations. Elevated CO$_2$ alone had a moderately positive effect on SM04, and significantly increased growth in SM07 ($P = 0.005$). Growth of SM05 was somewhat reduced by this treatment. Higher temperature
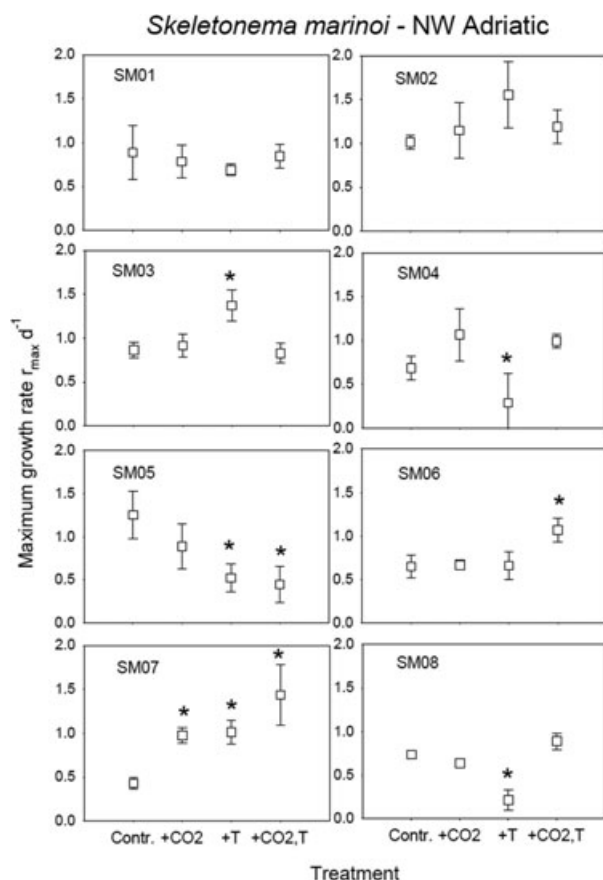
Figure 2. Maximum growth rates of eight strains of *Skeletonema marinoi* from the NW Adriatic under different treatments (means ± standard deviation, $n = 3$). Treatments that differ significantly from the control are marked with an asterisk (*). Labels on the *x*-axis represent experimental treatments: Contr. (Control, 20°C, 385 ppm), +CO₂ (high pCO_2, 20°C, 750 ppm), +T (high temperature, 24°C, 385 ppm), +CO₂, T (high temperature and pCO_2, 24°C, 750 ppm).

Figure 3. Whisker diagrams showing combined replicate growth rates of the eight Adriatic *Skeletonema marinoi* strains used in this study under different treatments. Whiskers above and below the boxes indicate the 90th and 10th percentiles, dots the respective 95/5 percentiles.

significantly favored growth of SM03 and SM07 ($P = 0.006$ and $P = 0.011$, respectively), but had a significantly negative effect on SM04 ($P = 0.017$), SM05 ($P = 0.02$), and SM08 ($P < 0.001$). The four remaining strains were not affected by this treatment. In SM06 and SM07, growth rates were significantly higher ($P = 0.016$ and 0.004) when both pCO_2 and temperature were increased compared to control conditions, whereas in SM05, growth was significantly negatively affected by these conditions ($P = 0.001$). The growth rates of five strains were unchanged in this treatment. When the combined growth rates of all strains and replicates were compared between treatments (Fig. 3), no significant treatment effects were detected.

Growth rates of Skagerrak *Skeletonema* strains at control conditions were in the same range as those measured for the Adriatic strains. However, this range was narrower in the Skagerrak population compared to the Adriatic population,
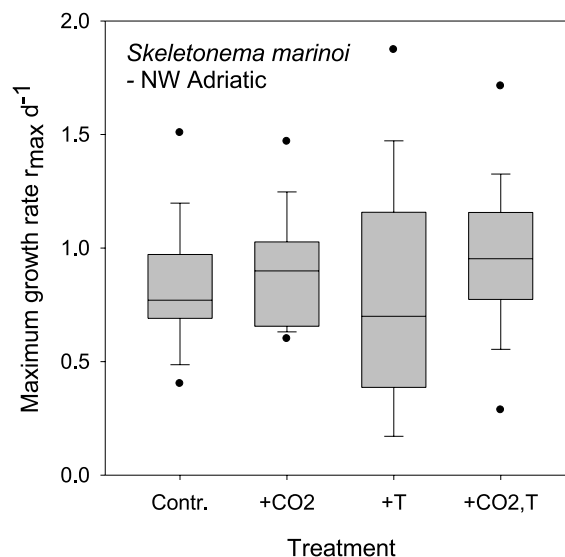
ranging from 0.63 to 1.04 days^{-1}. Similarly, the Skagerrak *Skeletonema* were affected more uniformly to the three experimental treatments (Fig. 4). Only in one of the strains was a significant difference in growth rates detected at higher applied pCO_2. This condition significantly enhanced the growth of *S. marinoi* strain SM16 ($P < 0.001$). Higher temperature at ambient pCO_2 resulted in significantly higher growth rates in four of the strains (SM09, $P = 0.002$; SM11, $P = 0.007$; SM12, $P = 0.004$; SM16, $P < 0.001$). Four strains were not affected by the applied changes in temperature or pCO_2 (SM10, SM13, SM14, and SM15). When comparing the combined growth rates of all Skagerrak strains and replicates in the three treatments, the favorable effect of temperature on half of the strains is reflected in the significant increase in growth rate ($P < 0.001$) in this treatment, compared to the control and the +CO₂ treatment (Fig. 5).

Growth of *A. ostenfeldii* in experimental treatments

Alexandrium ostenfeldii generally had much lower growth rates than *Skeletonema*, ranging from 0.1 under control conditions, to 0.33 days^{-1}. The experimental treatments also affected the tested strains of this species quite differently (Fig. 6). In three strains, including the slow growing AO01, AO02, and AO03, no significant differences in growth among treatments were detected. However, growth in strain AO02 was moderately enhanced by increased pCO_2, whereas AO04 experienced significant growth ($P = 0.013$). Temperature increase stimulated the growth of three other strains—AO06
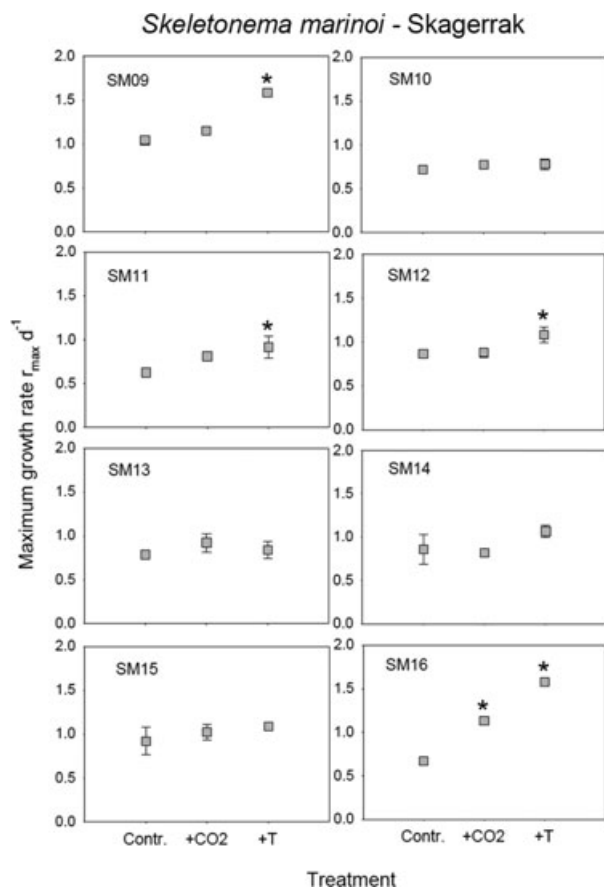
Figure 4. Maximum growth rates of eight strains of *Skeletonema marinoi* from the Skagerrak (North Sea) under different treatments (means ± standard deviation, $n = 3$). Treatments that differ significantly from the control are marked with an asterisk (*).
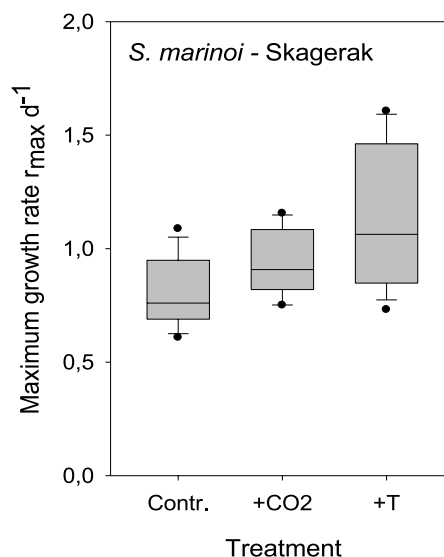

Figure 5. Whisker diagrams showing combined replicate growth rates of the *Skeletonema marinoi* strains from the Skagerrak used in this study under different treatments.

($P = 0.008$), AO07 ($P = 0.007$), and AO08 ($P < 0.001$). Temperature in combination with the higher pCO_2 had a positive effect on growth ($P = 0.001$) in AO06. These conditions also significantly enhanced growth of AO05 ($P = 0.004$). Despite the variability in strain-specific responses, increased temperature at both of the pCO_2 levels had an overall positive effect on growth of *A. ostenfeldii* ($P < 0.001$ at low applied pCO_2, and $P = 0.027$ at 750 ppm applied pCO_2; Fig. 7).

Toxin production of *A. ostenfeldii*

Although total cellular PST content and composition differed considerably between the eight tested strains, very few significant differences were observed in total PST content among the experimental treatments (Fig. 8). Responses were detected in only two strains. AO06 contained significantly lower toxin concentrations at higher pCO_2 at 20°C compared to ambient pCO_2 at the same temperature. The pCO_2 also affected AO07 at 24°C, where higher cellular toxicities were measured in the 750 ppm treatment compared to that with CO_2 at 385 ppm.

Treatments had more pronounced effects on the proportions of the major derivatives (GTX2, 3, and STX). In five of the strains, treatments triggered a significant relative increase in STX. High pCO_2 at 20°C increased the STX fraction in AO04 ($P = 0.006$) and AO08 ($P < 0.001$). These strains also had higher STX proportions at both 24°C treatments ($P \leq 0.001$). Furthermore, the higher temperature at both low and high pCO_2 levels led to higher relative STX amounts in AO03 ($P = 0.008$ and $P = 0.038$) and AO06 ($P = 0.002$ and $P = 0.011$). In AO02, the proportion of STX increased significantly at 24°C and high pCO_2 ($P = 0.023$). When comparing the STX proportions of all strains and replicates between treatments (Fig. 9), we found that all three treatments, that is, addition of CO_2 ($P = 0.028$), increased temperature ($P < 0.001$) and the combination of both ($P = 0.015$), promoted STX production in *A. ostenfeldii*.

Discussion

Here, the effects of changing climate conditions on multiple strains of two bloom-forming phytoplankton species were examined. In both species, individual strains were affected in different ways by increased temperature and pCO_2. The large response variability detected among the *S. marinoi* strains from the Adriatic buffered the overall effect of increased CO_2 supply and temperature. The more uniform response of Skagerrak *S. marinoi*, with many strains exhibiting increased growth rates with increased temperature, resulted in an overall positive effect of temperature on growth in this population. A general positive effect of increased temperature on growth was also detected for *A. ostenfeldii*, despite the variability
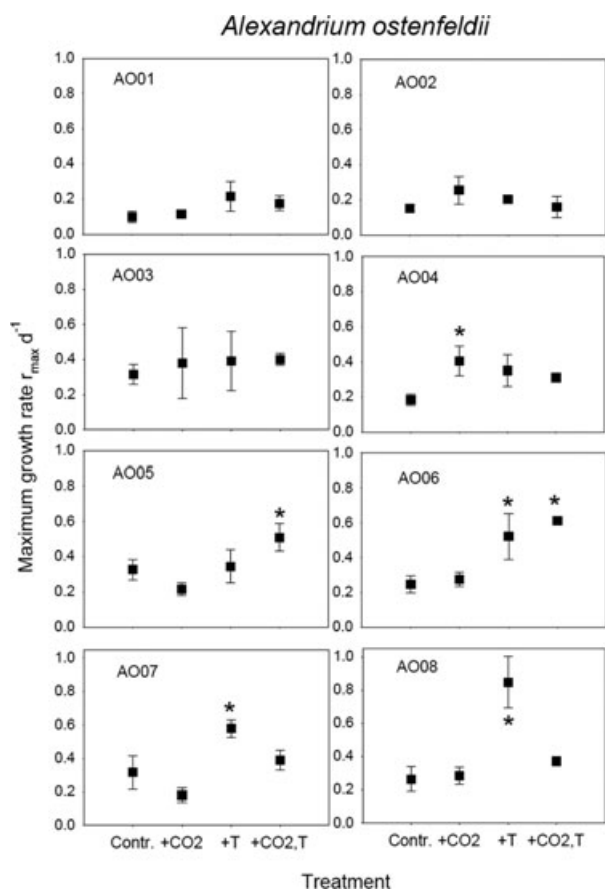
Figure 6. Maximum growth rates of eight *Alexandrium ostenfeldii* strains under different treatments (means ± standard deviation, *n* = 3). Treatments that differ significantly from the control are marked with an asterisk (*).
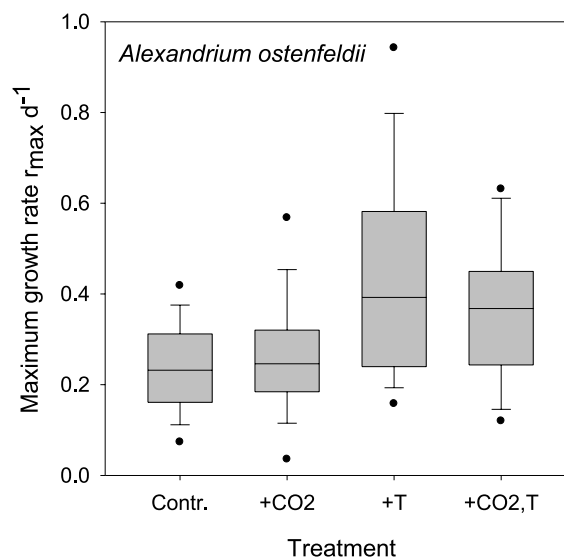

Figure 7. Whisker diagrams showing combined replicate growth rates of eight *Alexandrium ostenfeldii* strains used in this study under different treatments.

in strain-specific response patterns. While experimental manipulations only affected total cellular toxin concentrations in a few strains, toxin composition was consistently altered by increased CO_2 levels and temperature in the majority of strains, resulting in an overall promotion of saxitoxin production in these treatments. To our knowledge, this is the first study reporting considerable "within and between geographically separated populations" variability in the response of phytoplankton to climatic factors.

Growth stimulation resulting from increased pCO_2 has been reported for a number of phytoplankton species from different taxonomic groups (Leonardos and Geider 2005; Fu et al. 2010). Here, stimulating effects were only detected in a few individual strains of *S. marinoi* and *A. ostenfeldii*. To better understand the limited sensitivity toward elevated applied pCO_2, the effectiveness of CO_2 manipulations needs be evaluated. Since we worked with live and actively growing cultures of CO_2-consuming algae, the pCO_2 levels establishing in the water, eventually decreased well below anticipated

levels (Fig. S1). This was expected (Rost et al. 2008) and considered realistic, representing typical bloom situations in coastal nutrient-rich waters where high phytoplankton primary production may quickly lead to temporary exhaustion of free CO_2 and to high pH in a patch of water (Hällfors et al. 1983; Boyd et al. 2000; Hansen 2002; Fransson et al. 2009; Brutemark et al. 2011). Both of the species investigated here are typical bloom species: *S. marinoi* is one of the dominant members of the spring bloom phytoplankton community in temperate coastal waters. *Alexandrium ostenfeldii* forms dense late summer blooms (Kremp et al. 2009) that are likely to cause a considerable draw down of CO_2 in bloom patches. Differences in actual CO_2 concentrations of the same pCO_2 treatment at different temperature most likely reflect different biomass levels in the respective cultures. At higher temperature, where growth of many strains was enhanced, pCO_2 was generally lower than at control temperature despite equal levels of applied pCO_2.

Although *S. marinoi* and *A. ostenfeldii* growth quickly reduced initial levels of free pCO_2 by approximately two-thirds, pCO_2 in the next century treatments equilibrated at roughly twice the amount of ambient pCO_2 treatments. Hence, significantly more free CO_2 was available in treatments bubbled with the high pCO_2 gas mix and the treatments can be considered effective in terms of higher substrate availability that could potentially favor or prolong growth at sufficient inorganic nutrient concentrations. However, given the generally low, and potentially even limiting pCO_2 levels (Riebesell et al. 1993; Hansen et al. 2007) in our batch culture systems, the effects on the tested *S. marinoi* and *A. ostenfeldii* strains are
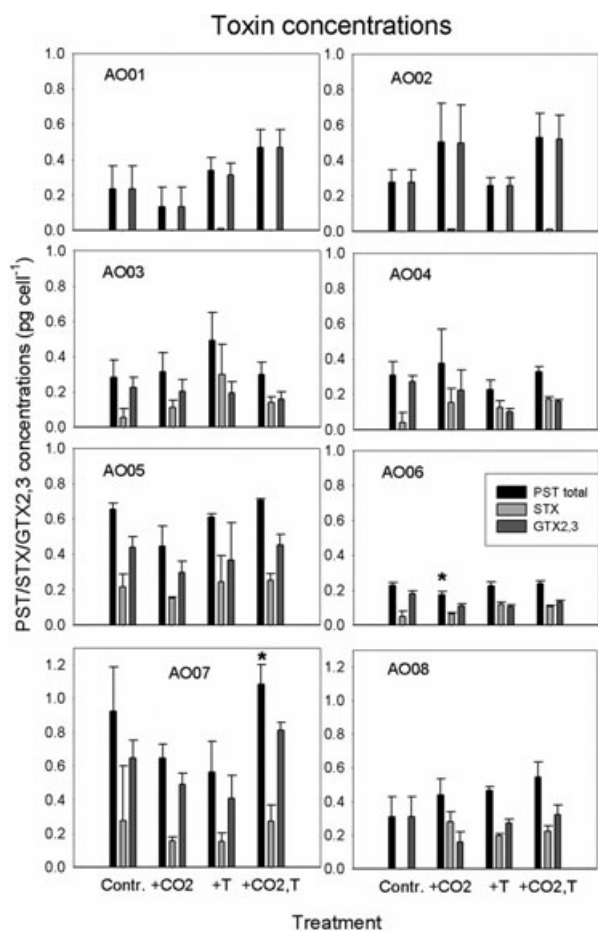
Figure 8. Cellular toxin concentrations (Total PST, STX, and GTX2,3) of *Alexandrium ostenfeldii* strains used in this study under different treatments (means ± standard deviation, $n = 3$).

Figure 9. Whisker diagrams showing statistical ranges of relative STX proportions of all measured *Alexandrium ostenfeldii* replicates and strains under different treatments.

most likely a result of C limitation and pH conditions instead of changed photosynthetic physiology and carbon acquisition mechanisms that may take effect at increased ambient pCO_2 in the water (Rost et al. 2003; Giordano et al. 2005; Ratti et al. 2007).

A significant correlation between CO_2 effect size (calculated as the ratio between growth rates at high and low pCO_2 for the same strain and temperature) and growth characteristics (Fig. 10) suggests that strain-specific growth rates determine, whether increased pCO_2 supply and availability would result in growth stimulation or not. Slow growing cultures of both species experienced the strongest growth enhancement at higher measured pCO_2 levels, whereas strains with intermediate and high growth rates in control conditions were not, or negatively, affected by increased pCO_2. This relationship obviously differs between *S. marinoi* and *A. ostenfeldii*, with the diatom experiencing much stronger growth stimulation at low growth rates compared to *A. ostenfeldii*, which is not surprising given the generally much lower growth rates of the
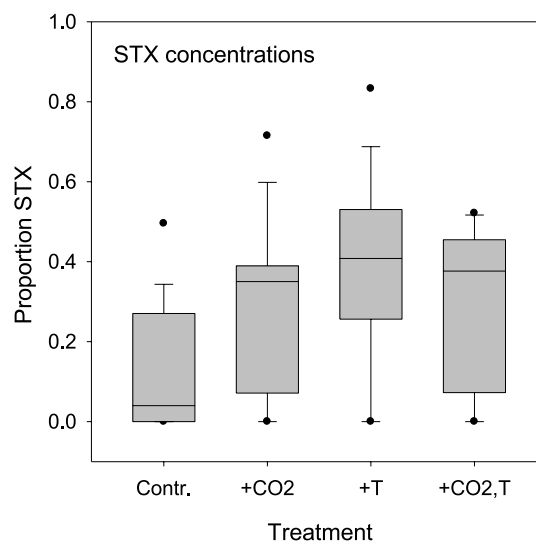
latter species. As suggested earlier, such relationships most likely reflect carbon limitation patterns. Fast growing strains will exhaust the C pool earlier than slow growing strains and may be negatively affected by the pH changes accompanying low concentrations of inorganic carbon (Søgaard et al. 2011).

Many studies report growth enhancement in phytoplankton from temperate environments when temperature increases moderately (e.g., Peperzak 2003). Most strains of *S. marinoi* from the Skagerrak and of *A. ostenfeldii* were favored by the 4°C increase applied in our experiments. Conversely, growth rates were reduced in several strains of *S. marinoi* from the Adriatic. Such differences between two geographic populations of the same species might be a result of different adaptation mechanisms. Organisms respond to the new environmental regimes either through inherited mechanisms of plasticity or by genetic changes. In the Skagerrak, *S. marinoi* grows in a large range of temperatures from late winter to early autumn, although their abundance is highest in early spring. Irrespective of their seasonal origin, strains from the Skagerrak generally respond positively to elevated temperature (Saravanan and Godhe 2010) indicating a high phenotypic plasticity in terms of temperature tolerance in this geographic population. Since the Skagerak population is exposed to high temperature variation, adjustment to changing temperature by plasticity is probably an advantage. In the temperate Adriatic Sea, where *S. marinoi* appears seasonally and is exposed to relatively more homogenous low temperatures, the strains may not possess this inherited mechanism of plasticity in the same extent. The presence of obviously different phenotypic responses among the Adriatic strains compared to the uniform Skagerrak population could be due
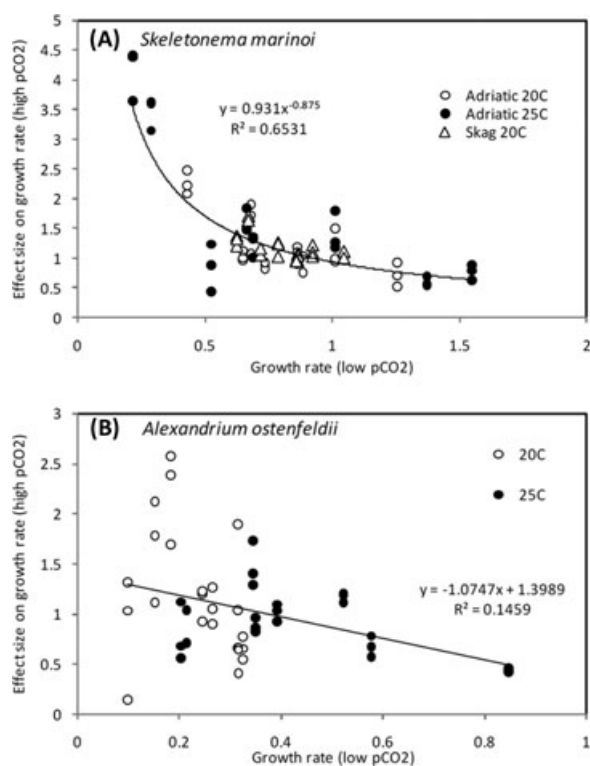
Figure 10. Relationship between growth rate at low pCO_2 condition and the elevated pCO_2 effect size (calculated as ratio of maximum growth rate at future atmospheric pCO_2 to maximum growth rate at present day atmospheric pCO_2) on growth rate. 1 = no effect, >1 = grow faster at high elevated pCO_2, <1 = grow slower at elevated pCO_2. (A) Two geographic populations of *Skeletonema marinoi*; (B) *Alexandrium ostenfeldii*.

to a higher general level of inherited phenotypic plasticity in the latter, as also reflected by larger growth differences of individual strains. Growth rates at control conditions differed by a factor of three in the Adriatic population, while the span was only half as wide among the Skagerrak strains. In *A. ostenfeldii*, the physiological variance indicated by different growth rates at control conditions was not reflected by a corresponding variability in the temperature response of strains. This might be attributable to the general preference of the Baltic *A. ostenfeldii* population for warm water. In the bloom region, significant growth of the species only occurs at water temperatures above 20°C (Hakanen et al. 2012).

The observed differences in cellular toxin concentrations among *A. ostenfeldii* strains involved both total PST content and the relative contribution of GTX2/3 and STX. This is in line with a previous report of strain level diversity in toxicity in *Alexandrium tamarense* (Alpermann et al. 2010). Intraspecific variability in toxin profiles and concentrations seems to be common among PST producers (Yoshida et al. 2001), but has also been shown for microalgae with other types of toxins (Bachvaroff et al. 2009). Despite the general variability in

strain-specific toxin characteristics, toxin responses to the applied temperature and pCO_2 manipulations within the tested *A. ostenfeldii* population were surprisingly uniform. In most strains, total cellular PST concentrations were unaffected by elevated temperature and pCO_2, whereas a significant increase in STX production was detected as a result of all three treatments. Changed PSP toxin profiles of *Alexandrium* spp. due to varying environmental conditions, such as nutrients, temperature, irradiance, and salinity, have been reported in several studies (e.g. Boczar et al. 1988; Etheridge and Roesler 2005).

The toxicity of a given strain or bloom is not only affected by the total PST concentration, but also by the relative proportion of individual toxins, since different PST variants vary considerably in their activity. Saxitoxin is the most toxic PST derivative and one of the most potent natural neurotoxins known (Wiese et al. 2010); hence, the observed promotion of saxitoxin production at elevated temperature and CO_2 availability may increase toxicity of *A. ostenfeldii* blooms despite unchanged total PST concentrations. Similar results were recently obtained by Fu et al. (2010), who found that increasing pCO_2, coupled with phosphorus limitation, stimulated production of more potent karlotoxin variants in *Karlodinium veneficum*, thus dramatically increasing the total cellular toxicity.

Both *S. marinoi* and *A. ostenfeldii* will be able to grow and even thrive under projected medium-term climate conditions. At the population level, the predicted temperature increase will be the primary factor influencing fitness of the two species, while CO_2 effects will be negligible. Although the NW Adriatic population of *S. marinoi* may not be directly affected by temperature and CO_2, the anticipated shifts in seasonal temperature development and changes in stratification patterns may indirectly confine the bloom peName, 0000riod due to a competitive advantage of warm-adapted species at higher water temperature, or promotion of motile life forms. Being directly favored by the temperature increase, the Skagerrak *S. marinoi* population may be expected to expand, provided that vertical mixing conditions are favorable for the immotile diatoms. However, in order to make precise predictions, the response of the co-occurring phytoplankton community will need to be taken into account. Increased summer temperatures should particularly promote Baltic *A. ostenfeldii* blooms in shallow stratified waters. In fact, in the past decade, summer blooms of *A. ostenfeldii* have been increasingly observed in shallow coastal embayments (Kremp et al. 2009). The increasing frequency of *A. ostenfeldii* mass developments coincides with a general trend of rising summer surface temperatures in the Baltic Sea (Suikkanen et al. 2007). As the blooms are toxic and may affect co-occurring biota in different ways, a climate-driven species expansion could have severe consequences on the coastal Baltic ecosystem. Changes in *A. ostenfeldii* toxin composition, mediated

by future pCO$_2$ and temperature conditions, might amplify the potential harmful effects of the toxins.

As mentioned above, the immediate response of organisms to environmental change can involve both acclimation based on phenotypic plasticity, and adaptation based on selection (Barrett and Schluter 2007). Although *S. marinoi* and *A. ostenfeldii* obviously possess enough phenotypic plasticity to prevail under future climatic conditions, it is not clear what the relative importance and predictive significance of such short-term acclimation potential is for a situation 100 years from now. The experiments presented here and similar instantaneous response studies do not assess an evolutionary response to climate change scenarios, since they do not allow gradual adaptation over many generations that may lead to new adaptive mutations (Collins and Bell 2004). It cannot be excluded that strains of *S. marinoi* and *A. ostenfeldii*, that here responded negatively or not at all, might over time evolve properties allowing them to better adapt to an environment of elevated temperature and pCO$_2$.

Our study shows that strains of one species, and even population, can be impacted in very different ways by climate stressors. A particularly wide response range was found in the population of *S. marinoi* from the NW Adriatic Sea, where temperature and pCO$_2$ caused positive, negative, or no effect at all. Depending on the strain of choice, experiments using single isolates from this population could have given opposite response patterns, which would have led to contrasting predictions. This emphasizes that responses observed in single strain experiments may not be representative, and that predictions for species behavior under future climatic conditions need to be treated with caution.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found online on Wiley Online Library.

Figure S1. Development of free aqueous CO_2 during the experiment in *Skeletonema marinoi* strains from 2 different geographic locations and *Alexandrium ostenfeldii* as measured from randomly chosen sets of experimental cultures.

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