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

Running head: phenotypic variability and climate conditions

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Abstract

Phytoplankton populations can display high levels of genetic diversity which, 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 8 strains of the PST producing dinoflagellate *Alexandrium ostenfeldii* from the Baltic Sea. Maximum growth rates were estimated in batch cultures of acclimated isolates grown for 5 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 *p*CO2. 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.

51 Introduction

52 Human induced climate change will significantly alter marine environmental conditions within the
53 next century. Projected changes include a rise in sea surface temperature due to an atmospheric
54 temperature increase of approximately 4 °C, and elevated oceanic levels of free aqueous CO_2 as a
55 consequence of the increase in atmospheric pCO_2 from the current 385 ppm, to 750 ppm at the end
56 of this century (IPCC 2007). Warming of the upper ocean will enhance water column stratification
57 with significant effects on light and nutrient conditions in the upper water column (Hoeg-Guldberg
58 & Bruno 2010 and references therein). Increased CO_2 concentrations cause the pool of dissolved
59 inorganic carbon (DIC) to rise, shifting the carbonate equilibrium to higher CO_2 and HCO_3 levels,
60 resulting in decreased CO_3^{2-} concentrations and a drop in pH of 0.4 units by 2100 (Caldeira &
61 Wicket 2003).

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

74 The continuing increase in atmospheric pCO_2 affects the physiology of phototrophic
75 organisms directly, as CO_2 is the primary substrate for photosynthesis. Increased concentrations of

76 free CO_2 could potentially favor photosynthesis and growth (Riebesell 2004), since present CO_2
77 concentrations are not saturating for RUBISCO, the enzyme that catalyzes primary fixation of
78 inorganic carbon (Badger *et al.* 1998). Most microalgae have developed strategies to counteract
79 CO_2 limitation by employing CO_2 concentrating mechanisms (CCMs) (Giordano *et al*. 2005).
80 CCMs in different species and phylogenetic groups vary considerably in efficiency and regulation
81 (Badger *et al.* 1998, Ratti *et al*. 2007, Trimborn *et al.* 2008), and differences exist in CO_2
82 requirements between taxa, with respect to saturation levels and preferences of inorganic carbon
83 source molecules (Paasche 2001, Rost *et al.* 2003). Such physiological diversity may explain the
84 observed variability in phytoplankton sensitivity to elevated CO_2 levels (Leonardos & Geider 2005,
85 Fu *et al.* 2010, Riebesell *et al.* 2000, Nielsen *et al*. 2010). The decrease in sea water pH associated
86 with rising levels of free aqueous CO_2 particularly affects the calcification process of various
87 phytoplankton species, as they depend on the availability of free carbonate for the production of
88 calcite structures (Riebesell *et al.* 2000, Iglesias-Rodriguez *et al*. 2008, Langer *et al*. 2009).
89 However, in terms of growth rates, marine phytoplankton generally appears unaffected by lowered
90 pH (Berge *et al.* 2010).
91 Most of the laboratory studies investigating the effects of climate stressors on phytoplankton
92 have been performed on single strains. The significant effects often found in such experiments are
93 contrasted by the general lack of clear responses in natural populations (e.g. Engel *et al*. 2008). The
94 higher tolerance of natural populations to environmental factors might be due to the
95 ecophysiological variability of the diverse genotypes constituting the populations (Paasche 2001,
96 Nielsen *et al.* 2010). Contradictory responses to changed climate conditions sometimes observed
97 within the same species might be partly attributable to strain variability between or within
98 populations (Langer *et al*. 2009). This emphasizes the need to consider variability in studies aiming
99 to understand the effects of climate change on phytoplankton species.

100 Considerable variability has been shown in a number of ecologically important traits of
101 phytoplankton, such as salinity tolerance (Brand 1984), toxicity (Bachvaroff *et al.* 2009) and growth
102 requirements (Fredrickson *et al.*2011). The genetic basis of such phenotypic variability has long
103 been recognized (Brand 1982), and recently became the subject of focused investigation, revealing
104 high levels of genetic differentiation among temporally and spatially separated populations
105 (Rynearson & Armburst 2004, Alpermann *et al.* 2010, Godhe & Härnström 2010). Growing
106 evidence suggests that considerable genetic and phenotypic diversity exist within the same
107 population (Tillman *et al.* 2009, Alpermann *et al*. 2010). Such diversity is particularly important for
108 a population to cope with changing environmental conditions. Genetically diverse populations can
109 resist environmental perturbations more effectively than genetically uniform populations (Hughes &
110 Stachowicz 2004). Phenotypic variability can buffer the immediate effects of environmental
111 fluctuations, while standing genetic variation should immediately influence the longer-term
112 selection (Barrett & Schluter 2007). Despite the relevance for adaptation, population level
113 variability in phytoplankton has not been addressed in relation to climate change.
114 In this study we examined response variability in growth and toxicity among multiple
115 genetically different strains of two geographical populations (hereafter referred to as populations) of
116 the marine diatom *Skeletonema marinoi* (Fig. 1 a-b), and a Baltic population of the toxic
117 dinoflagellate *Alexandrium ostenfeldii* (Fig 1 c-d) , when exposed to increased supply of
118 atmospheric CO_2 and increased temperature. Both species are widely distributed in temperate
119 coastal waters where they form seasonal blooms. Despite their different life forms and life histories,
120 both show high levels of genetic diversity (Godhe & Härnström 2010, Tahvanainen *et al,*
121 unpublished data) that, when reflected by phenotypic trait variability, may stabilize species response
122 to environmental changes.
123
124 Materials

125 ***Culturing of clonal strains*** - Sixteen strains of *Skeletonema marinoi* were isolated from the NW
126 Adriatic Sea and the Skagerrak (Table 1) as described in Godhe & Härnström (2010). Skagerrak
127 cultures were maintained at 10°C, 12:12 h light:dark cycle and 60 µmol photons m^{-2} s^{-1} in f/2
128 medium with a local salinity of 26; NW Adriatic cultures were maintained at 20°C, 100 µmol
129 photons m^{-2} s^{-1} and a salinity of 32. The Adriatic Sea and the Skagerrak strains used in the
130 experiment were randomly chosen from sets of 13 and 460 cultures, isolated from each of the
131 respective area.
132 The cultures of *Alexandrium ostenfeldii* were established from a sediment sample collected
133 in March 2009 from a bloom site in the Föglö archipelago, Åland, in the Northern Baltic Sea (Table
134 1). Single resting cysts were selected from sediment slurries and incubated wells of a tissue culture
135 plates, each filled with 2 mL of f/8 –Si enriched natural sea water (1/4 nutrient concentrations
136 compared to standard f/2 medium) at a salinity of 6.5, and incubated at 16°C, 12:12 light:dark cycle
137 and 100 µmol photons m^{-2} s^{-1}. Once germinated, clonal strains were established by isolating single
138 motile cells into a new culture well containing f/8-Si medium. Well established clonal cultures were
139 transferred to vented 50 mL polycarbonate tissue culture flasks and maintained in f/2 –Si culture
140 medium at 16°C, 12:12 light:dark cycle, and 100 µmol photons m^{-2} s^{-1}. Eight strains were randomly
141 selected from a set of 50 cultures established as described above from the Åland sediment sample.
142 To confirm clonal identities, the experimental strains of *S. marinoi* and *A. ostenfeldii* were
143 genotyped by microsatellite (Almany *et al.* 2009, Godhe and Härnström 2010) and AFLP
144 (Amplified Fragment Length Polymorphism) analyses (Vos *et al.* 1995, Tahvavnainen *et al*. in rev.)
145 respectively. LSU rDNA was sequenced according to Godhe *et al.* (2006) to confirm the species
146 identity of the northern Adriatic *S. marinoi* strains.
147 ***Experimental design and sampling***: – The effects of increased atmospheric pCO_2 and
148 temperature on growth and toxicity (the latter only for PST producing *A. ostenfeldii*), were tested in
149 batch culture experiments, where eight clonal strains of each species and population were grown in

150 triplicates at four different temperature and pCO_2 combinations (= treatments). A temperature of 20
151 °C and ambient air pCO_2 of approximately 385 ppm represented present spring-summer bloom
152 phase climate conditions. This treatment served as the control, and below is referred to as such. In
153 the second treatment (referred to as + CO_2), an increased pCO_2 of 750 ppm was applied to simulate
154 future atmospheric CO_2 concentrations, while temperature remained at 20 °C. The third treatment
155 (referred to as +T) was set up at 24°C with ambient pCO_2 simulating the temperature increase
156 anticipated by climate models (IPCC 2007). An additional treatment (referred to as + CO_2, T) at
157 24°C and 750 ppm pCO_2 examined the combined effects of these factors. *S. marinoi* from the
158 Skagerrak was not exposed to this treatment, due to limited availability of the CO_2 enriched gas
159 mix. Experiments were performed at salinities reflecting the respective habitat conditions of each
160 geographic population or species, i.e. salinities of the isolation sites: 32 for *S. marinoi* from the
161 northern Adriatic Sea and 26 for *S. marinoi* from the Skagerrak. *A. ostenfeldii* strains had earlier
162 been adjusted to the experimental salinity of 10.
163 Incubations were carried out in two climate controlled incubation chambers set to 20 and
164 24°C respectively. Different pCO_2 conditions were achieved by gently bubbling air with ambient
165 pCO_2, and a commercially purchased (AGA) gas mix with the pCO_2 adjusted to 750 ppm
166 respectively, into experimental batch cultures. Gas was distributed from central gas bottles (air, and
167 air +CO_2) through silicon tubing and a micro-capillary directly into the water phase of each batch
168 culture following the set-up of Torstensson *et al.* (2011). The design was chosen to simulate a
169 bloom situation, i.e. a situation with high biomass increase, and expected CO_2 draw-down under
170 conditions of increased water temperature and supply of atmospheric CO_2 to the system.
171 Prior to the experiment, all strains were acclimated to the experimental conditions for 2
172 weeks. Acclimation was performed using the same conditions as in the experiment, i.e. continuous
173 bubbling of 385 ppm and 750 ppm pCO_2 gas mixes into acclimation cultures at the two
174 experimental temperatures. The fast growing *S. marinoi* (mean generation time of ca. 1 day)

175 cultures were diluted several times to maintain the active growth stage. For the slower growing A.
176 ostenfeldii with mean generation times of approximately 3 days, the acclimation period
177 corresponded to approximately 5 generations.

178 From such cultures, an inoculum - resulting in initial cell concentrations of approximately
179 5000 cells mL^{-1} for *S. marinoi*, and 500 cells mL^{-1} for *A. ostenfeldii* - was transferred into three 250
180 mL tissue culture flasks per strain and treatment, containing 200 mL of f/2 culture medium (without
181 silica for *A. ostenfeldii*). The flasks were placed in the respective climate chamber, and connected to
182 the appropriate gas distributor. Three replicates without phytoplankton per culture medium and per
183 treatment were used as controls, and kept for three days to check the dynamics of CO_2 equilibration.
184 Experimental incubations lasted until cultures had reached stationary growth phase and biomass
185 ceased to increase, 5 days for *S. marinoi* and 17 days for *A. ostenfeldii*.

186 Temperature, pH total scale (pH_{TS}) and total alkalinity (A_T) were measured every day from
187 24 randomly selected bottles covering all populations and treatments. The pH_{TS} was measured with
188 a Metrohm (827 pH lab) pH electrode, calibrated with salinity adjusted seawater, TRIS and AMP
189 buffers following Dickson *et al.* (2007). The A_T measurements were conducted as described by
190 Sarazin *et al.* (1999), with an accuracy of 10 µmol kg^{-1} seawater. Water-phase pCO_2 was calculated
191 from pH and alkalinity using CO2SYS (Lewis & Wallace 1998), with dissociation constants from
192 Mehrbach *et al.* (1973) and refitted by Dickson & Millero (1987). Throughout the duration of the
193 experiments, light levels in the climate chambers were checked daily with an LI-COR LI-1400 Data
194 Logger & Light Meter to ensure comparable and stable light conditions.

195 Samples for measurements of Chl *a* fluorescence were taken once a day from *S. marinoi*
196 cultures, and every second day from *A. ostenfeldii* cultures. Flasks were gently shaken to distribute
197 cells evenly before volumes of 200 µL were collected in 2 mL Eppendorf tubes filled with 1.8 mL
198 of pure ethanol. Samples were allowed to extract for 1 h at room temperature in darkness, and were
199 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 & Watras 1989, Seppälä et al. unpublished). 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 mL^{-1} 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 ºC for 30 min. The filters were subsequently removed and the samples centrifuged at 12,000 x g for 5 min. The supernatant was then filtered through 0.45 µm GHP Acrodisc membrane filters (13 mm diameter, Pall Life Sciences, USA). PSP toxin analyses followed the protocol modified from Janiszewski & Boyer (1993) and Diener *et al.* (2006) as described in Hakanen *et al*. 2012. Analyses were performed using an Agilent HPLC system 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, USA) controlled with an ESA Coulochem II multi-electrode detector to achieve electrochemical post-column oxidation (ECOS; Janiszewski & Boyer 1993).

Fluorescence emission signal was used in the PST quantification. Fluorescence detection was applied for the 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 pCO2, measured AT, pHTS 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 ca. 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 24h (Table 2, 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

249 resulted in significantly higher levels of free aqueous CO_2 in the respective treatments in the
250 Skagerrak population of *Skeletonema* ($p < 0.0013$), and in *A. ostenfeldii* ($p < 0.0095$).
251 ***Growth of* Skeletonema marinoi *at different temperature and* pCO_2 *applications***: The
252 eight Adriatic Sea strains differed considerably in their growth rates. Under control conditions
253 (20°C, pCO_2 of 385 ppm), maximum growth rates ranged from 0.43 d^{-1} in SM07, to 1.26 d^{-1} in
254 SM05. The response of the tested *S. marinoi* strains to the different CO_2 and temperature
255 treatments was also highly variable (Fig. 2), with nearly every strain showing a different pattern.
256 Two of the tested strains (SM01 and SM02) were not significantly affected by any of the
257 experimental manipulations. Elevated CO_2 alone had a moderately positive effect on SM04, and
258 significantly increased growth in SM07 ($p = 0.005$). Growth of SM05 was somewhat reduced by
259 this treatment. Higher temperature significantly favored growth of SM03 and SM07 ($p = 0.006$ and
260 $p = 0.011$ respectively), but had a significantly negative effect on SM04 ($p = 0.017$), SM05 ($p =$
261 0.02) and SM08 ($p < 0.001$). The four remaining strains were not affected by this treatment. In
262 SM06 and SM07, growth rates were significantly higher ($p = 0.016$ and 0.004) when both pCO_2 and
263 temperature were increased compared to control conditions, whereas in SM05, growth was
264 significantly negatively affected by these conditions ($p = 0.001$). The growth rates of five strains
265 were unchanged in this treatment. When the combined growth rates of all strains and replicates
266 were compared between treatments (Fig. 3), no significant treatment effects were detected.
267 Growth rates of Skagerrak *Skeletonema* strains at control conditions were in the same range
268 as those measured for the Adriatic strains. However, this range was narrower in the Skagerrak
269 population compared to the Adriatic population, ranging from 0.63 – 1.04 d^{-1}. Similarly, the
270 Skagerrak *Skeletonema* were affected more uniformly to the three experimental treatments (Fig. 4).
271 Only in one of the strains was a significant difference in growth rates detected at higher applied
272 pCO_2. This condition significantly enhanced the growth of *S. marinoi* strain SM16 ($p < 0.001$).
273 Higher temperature at ambient pCO_2 resulted in significantly higher growth rates in four of the

274 strains (SM09, $p = 0.002$; SM11, $p = 0.007$, SM12, $p = 0.004$, SM16, $p < 0.001$). Four strains were
275 not affected by the applied changes in temperature or pCO_2 (SM10, SM13, SM14 and SM15).
276 When comparing the combined growth rates of all Skagerrak strains and replicates in the three
277 treatments, the favorable effect of temperature on half of the strains is reflected in the significant
278 increase in growth rate ($p < 0.001$) in this treatment, compared to the control and the + CO_2
279 treatment (Fig. 5).

280 ***Growth of* Alexandrium ostenfeldii *in experimental treatments***: *A. ostenfeldii* generally
281 had much lower growth rates than *Skeletonema*, ranging from 0.1 under control conditions, to 0.33
282 d^{-1}. The experimental treatments also affected the tested strains of this species quite differently (Fig.
283 6). In three strains, including the slow growing AO01, AO02 and AO03, no significant differences
284 in growth among treatments were detected. However, growth in strain AO02 was moderately
285 enhanced by increased pCO_2, whereas AO04 experienced significant growth ($p = 0.013$).
286 Temperature increase stimulated the growth of three other strains - AO06 ($p = 0.008$), AO07 ($p =$
287 0.007) and AO08 ($p < 0.001$). Temperature in combination with the higher pCO_2 had a positive
288 effect on growth ($p = 0.001$) in AO06. These conditions also significantly enhanced growth of
289 AO05 ($p = 0.004$). Despite the variability in strain-specific responses, increased temperature at both
290 of the pCO_2 levels had an overall positive effect on growth of *A. ostenfeldii* ($p < 0.001$ at low
291 applied pCO_2, and $p = 0.027$ at 750 ppm applied pCO_2; Fig. 7).

292 ***Toxin production of* A. ostenfeldii** – Although total cellular PST content and composition
293 differed considerably between the eight tested strains, very few significant differences were
294 observed in total PST content among the experimental treatments (Fig. 8). Responses were detected
295 in only two strains. AO06 contained significantly lower toxin concentrations at higher pCO_2 at
296 20°C compared to ambient pCO_2 at the same temperature. The pCO_2 also affected AO07 at 24°C,
297 where higher cellular toxicities were measured in the 750 ppm treatment compared to that with CO_2
298 at 385ppm.

299 Treatments had more pronounced effects on the proportions of the major derivatives (GTX2,
300 3 and STX). In five of the strains, treatments triggered a significant relative increase in STX. High
301 pCO_2 at 20°C increased the STX fraction in AO04 ($p = 0.006$) and AO08 ($p < 0.001$). These strains
302 also had higher STX proportions at both 24°C treatments ($p \leq 0.001$). Furthermore, the higher
303 temperature at both low and high pCO_2 levels led to higher relative STX amounts in AO03 ($p =$
304 0.008 and $p = 0.038$) and AO06 ($p = 0.002$ and $p= 0.011$). In AO02, the proportion of STX
305 increased significantly at 24°C and high pCO_2 ($p = 0.023$). When comparing the STX proportions
306 of all strains and replicates between treatments (Fig. 9), we found that all three treatments, i.e.
307 addition of CO_2 ($p = 0.028$), increased temperature ($p < 0.001$) and the combination of both ($p =$
308 0.015), promoted STX production in *A. ostenfeldii.*
309
310 **Discussion**
311 Here, the effects of changing climate conditions on multiple strains of two bloom-forming
312 phytoplankton species were examined. In both species, individual strains were affected in different
313 ways by increased temperature and *p*CO2. The large response variability detected among the *S.*
314 *marinoi* strains from the Adriatic buffered the overall effect of increased CO_2 supply and
315 temperature. The more uniform response of Skagerrak *S. marinoi*, with many strains exhibiting
316 increased growth rates with increased temperature, resulted in an overall positive effect of
317 temperature on growth in this population. A general positive effect of increased temperature on
318 growth was also detected for *A. ostenfeldii*, despite the variability in strain specific response
319 patterns. While experimental manipulations only affected total cellular toxin concentrations in a few
320 strains, toxin composition was consistently altered by increased CO_2 levels and temperature in the
321 majority of strains, resulting in an overall promotion of saxitoxin production in these treatments. To
322 our knowledge, this is the first study reporting considerable "within and between geographically
323 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 & 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 towards elevated applied pCO2, 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. *A. ostenfeldii* forms dense late summer blooms (Kremp *et al.* 2009) which 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

349 the tested *S. marinoi* and *A. ostenfeldii* strains are most likely a result of C limitation- and pH
350 conditions instead of changed photosynthetic physiology and carbon acquisition mechanisms, that
351 may take effect at increased ambient pCO_2 in the water (Rost *et al*. 2003, Giordano *et al.* 2005,
352 Ratti *et al.* 2007).

353 A significant correlation between CO_2 effect size (calculated as the ratio between growth
354 rates at high and low pCO_2 for the same strain and temperature), and growth characteristics (Fig.
355 10) suggests that strain specific growth rates determine, whether increased pCO_2 supply and
356 availability would result in growth stimulation or not. Slow growing cultures of both species
357 experienced the strongest growth enhancement at higher measured pCO_2 levels, whereas strains
358 with intermediate and high growth rates in control conditions were not, or negatively, affected by
359 increased pCO_2. This relationship obviously differs between *S. marinoi* and *A. ostenfeldii*, with the
360 diatom experiencing much stronger growth stimulation at low growth rates compared to *A.*
361 *ostenfeldii,* which is not surprising given the generally much lower growth rates of the latter
362 species. As suggested earlier, such relationships most likely reflect carbon limitation patterns. Fast
363 growing strains will exhaust the C pool earlier than slow growing strains and may be negatively
364 affected by the pH changes accompanying low concentrations of inorganic carbon (Søgaard *et al.*
365 2011).

366 Many studies report growth enhancement in phytoplankton from temperate environments
367 when temperature increases moderately (e.g. Peperzak 2003). Most strains of *S. marinoi* from the
368 Skagerrak and of *A. ostenfeldii* were favored by the 4°C increase applied in our experiments.
369 Conversely, growth rates were reduced in several strains of *S. marinoi* from the Adriatic. Such
370 differences between two geographic populations of the same species might be a result of different
371 adaptation mechanisms. Organisms respond to the new environmental regimes either through
372 inherited mechanisms of plasticity or by genetic changes. In the Skagerrak, *S. marinoi* grows in a
373 large range of temperatures from late winter to early autumn although their abundance is highest in

374 early spring. Irrespective of their seasonal origin, strains from the Skagerrak generally respond
375 positively to elevated temperature (Saravanan & Godhe 2010) indicating a high phenotypic
376 plasticity in terms of temperature tolerance in this geographic population. Since the Skagerak
377 population is exposed to high temperature variation, adjustment to changing temperature by
378 plasticity is probably an advantage. In the temperate Adriatic Sea, where *S. marinoi* appears
379 seasonally and is exposed to relatively more homogenous low temperatures, the strains may not
380 possess this inherited mechanism of plasticity in the same extent. The presence of obviously
381 different phenotypic responses among the Adriatic strains compared to the uniform Skagerrak
382 population could be due to a higher general level of inherited phenotypic plasticity in the latter, as
383 also reflected by larger growth differences of individual strains. Growth rates at control conditions
384 differed by a factor of three in the Adriatic population, while the span was only half as wide among
385 the Skagerrak strains. In *A. ostenfeldii* the physiological variance indicated by different growth
386 rates at control conditions, was not reflected by a corresponding variability in the temperature
387 response of strains. This might be attributable to the general preference of the Baltic *A. ostenfeldii*
388 population for warm water. In the bloom region, significant growth of the species only occurs at
389 water temperatures above 20°C (Hakanen et al. 2012).
390 The observed differences in cellular toxin concentrations among *A. ostenfeldii* strains
391 involved both total PST content and the relative contribution of GTX2/3 and STX. This is in line
392 with a previous report of strain level diversity in toxicity in *Alexandrium tamarense* (Alpermann *et*
393 *al.* 2010). Intraspecific variability in toxin profiles and concentrations seems to be common among
394 PST producers (Yoshida *et al.* 2001), but has also been shown for microalgae with other types of
395 toxins (Bachvaroff *et al.* 2009). Despite the general variability in strain specific toxin
396 characteristics, toxin responses to the applied temperature and pCO_2 manipulations within the tested
397 *A. ostenfeldii* population were surprisingly uniform. In most strains, total cellular PST
398 concentrations were unaffected by elevated temperature and pCO_2, whereas a significant increase in

399 STX production was detected as a result of all three treatments. Changed PSP toxin profiles of
400 *Alexandrium* spp. due to varying environmental conditions such as nutrients, temperature,
401 irradiance and salinity, have been reported in several studies (e.g. Boczar *et al*. 1988, Etheridge &
402 Roesler 2005).

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

412 Both *S. marinoi* and *A. ostenfeldii* will be able to grow and even thrive under projected
413 medium-term climate conditions. At the population level, the predicted temperature increase will
414 be the primary factor influencing fitness of the two species, while CO_2 effects will be negligible.
415 Although the NW Adriatic population of *S. marinoi* may not be directly affected by temperature and
416 CO_2, the anticipated shifts in seasonal temperature development and changes in stratification
417 patterns may indirectly confine the bloom period due to a competitive advantage of warm adapted
418 species at higher water temperature, or promotion of motile life forms. Being directly favored by
419 the temperature increase, the Skagerrak *S. marinoi* population may be expected to expand, provided
420 that vertical mixing conditions are favorable for the immotile diatoms. However, in order to make
421 precise predictions the response of the co-occurring phytoplankton community will need to be taken
422 into account. Increased summer temperatures should particularly promote Baltic *A. ostenfeldii*
423 blooms in shallow stratified waters. In fact, in the past decade, summer blooms of *A. ostenfeldii*

424 have been increasingly observed in shallow coastal embayments (Kremp *et al.* 2009). The
425 increasing frequency of *A. ostenfeldii* mass developments coincides with a general trend of rising
426 summer surface temperatures in the Baltic Sea (Suikkanen *et al.* 2007). As the blooms are toxic and
427 may affect co-occurring biota in different ways, a climate driven species expansion could have
428 severe consequences on the coastal Baltic ecosystem. Changes in *A. ostenfeldii* toxin composition,
429 mediated by future pCO_2 and temperature conditions, might amplify the potential harmful effects of
430 the toxins.

431 As mentioned above, the immediate response of organisms to environmental change can
432 involve both acclimation based on phenotypic plasticity, and adaptation based on selection (Barrett
433 & Schluter 2007). Although *S. marinoi* and *A. ostenfeldii* obviously possess enough phenotypic
434 plasticity to prevail under future climatic conditions, it is not clear what the relative importance and
435 predictive significance of such short-term acclimation potential is for a situation 100 years from
436 now. The experiments presented here and similar instantaneous response studies do not assess an
437 evolutionary response to climate change scenarios, since they do not allow gradual adaptation over
438 many generations that may lead to new adaptive mutations (Collins and Bell 2004). It cannot be
439 excluded that strains of *S. marinoi* and *A. ostenfeldii*, that here responded negatively or not at all,
440 might over time evolve properties allowing them to better adapt to an environment of elevated
441 temperature and pCO_2.

442 Our study shows that strains of one species, and even population, can be impacted in very
443 different ways by climate stressors. A particularly wide response range was found in the population
444 of *S. marinoi* from the NW Adriatic Sea, where temperature and pCO_2 caused positive, negative or
445 no effect at all. Depending on the strain of choice, experiments using single isolates from this
446 population could have given opposite response patterns, which would have led to contrasting
447 predictions. This emphasizes that responses observed in single strain experiments may not be

448 representative, and that predictions for species behavior under future climatic conditions need to be
449 treated with caution.
450
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Tables

Table S1. 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	15.04.2010
	Skagerrak (North Sea)	
SM09	57°33.0'N, 11°31.5'E	12.08 2009
SM10	58°15.2'N, 11°03.5'E	12.10.2009
SM11	58°20.3'N, 11°21.4'E	09.11.2009
SM12	58°15.6'N, 11°25.9'E	07.05.2009
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	07.05.2009
SM16	58°15.6'N, 11°25.9'E	15.05.2009
Alexandrium ostenfeldii (Dinophyceae)	Baltic Sea, Åland, Föglö	
AO01 – AO08	60°05.9'N,20°30.5'E	14.03.2009

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

Treatment	**Temperature (ºC)**	**A_T (µmol kg^{-1})**	**pH_{TS}**	***p*CO_2 (µatm)**
***Skeletonema marinoi* – NW Adriatic**				
Medium: salinity=32psu				
20ºC / 385 ppm	19.20 ± 0.02	2473.65 ± 13.02	8.10 ± 0.01	387.33 ± 7.22
20ºC / 750 ppm	19.07 ± 0.03	2451.88 ± 9.77	7.86 ± 0.01	738.67 ± 9.77
24ºC / 385 ppm	22.50 ± 0.06	2569.76 ± 14.82	8.13 ± 0.01	356.67 ± 14.68
24ºC / 750 ppm	23.57 ± 0.21	2414.86 ± 8.04	7.87 ± 0.01	730.67 ± 6.69
***Skeletonema marinoi* – Skagerrak**				
Medium: salinity=26psu				
20ºC / 385 ppm	18.97 ± 0.07	1862.41 ± 11.46	8.05 ± 0.03	343.00 ± 24.85
20ºC / 750 ppm	19.10 ± 0.06	1854.62 ± 26.02	7.75 ± 0.01	754.33 ± 23.38
24ºC / 385 ppm	23.00 ± 0.15	1791.70 ± 8.83	8.03 ± 0.01	361.03 ± 79.4
24ºC / 750 ppm	23.70 ± 0.17	1860.30 ± 35.72	7.76 ± 0.01	744.12 ± 7.51
Alexandrium ostenfeldii				
Medium: salinity=10psu;				
20ºC / 385 ppm	19.20 ± 0.06	820.59 ± 15.60	7.81 ± 0.02	348.67 ± 12.33
20ºC / 750 ppm	19.20 ± 0.01	808.28 ± 4.34	7.54 ± 0.03	761.06 ± 43.09
24ºC / 385 ppm	22.90 ± 0.17	795.39 ± 6.21	7.82 ± 0.04	344.67 ± 9.84
24ºC / 750 ppm	24.07 ± 0.03	783.14 ± 7.20	7.51 ± 0.02	745.67 ± 29.24

Figure legends

Figure 1. Micrographs of (A-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-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.

Figure 2. Maximum growth rates of 8 strains of *Skeletonema marinoi* from the NW Adriatic under different treatments (means ± SD, n = 3). Treatments that differ significantly from the control are marked with an asterix (*). Labels on the x-axis represent experimental treatments: Contr. (Control, 20°C, 385ppm), +CO_2 (high pCO_2, 20°C, 750ppm), +T (high temperature, 24°C, 385ppm), +CO_2, T (high temperature and pCO_2, 24°C, 750ppm).

Figure 3. Whisker diagrams showing combined replicate growth rates of the 8 Adriatic *Skeletonema marinoi* strains used in this study under different treatments. Whiskers above and below the boxes indicate the 90^{th} and 10^{th} percentiles, dots the respective 95/5 percentiles.

Figure 4. Maximum growth rates of 8 strains of *Skeletonema marinoi* from the Skagerrak (North Sea) under different treatments (means ± SD, n = 3). Treatments that differ significantly from the control are marked with an asterix (*).

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

Figure 6. Maximum growth rates of 8 *Alexandrium ostenfeldii* strains under different treatments (means ± SD, n = 3). Treatments that differ significantly from the control are marked with an asterix (*).

Figure 7. Whisker diagrams showing combined replicate growth rates of 8 *Alexandrium ostenfeldii* strains used in this study under different treatments.

Figure 8. Cellular toxin concentrations (Total PST, STX and GTX2,3) of *Alexandrium ostenfeldii* strains used in this study under different treatments (means ± SD, n = 3).

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

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*

Figure 1

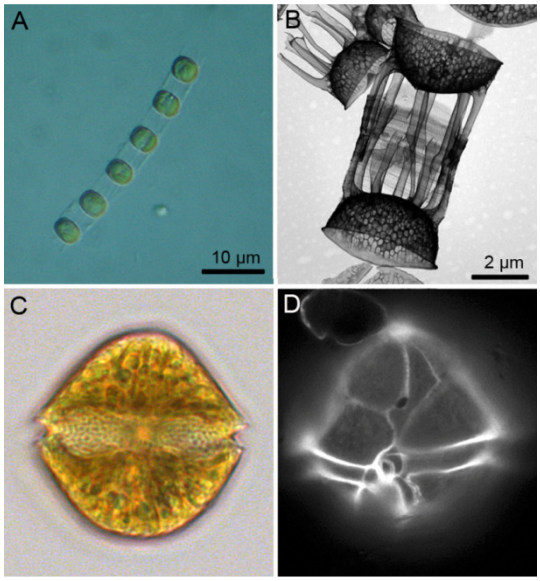
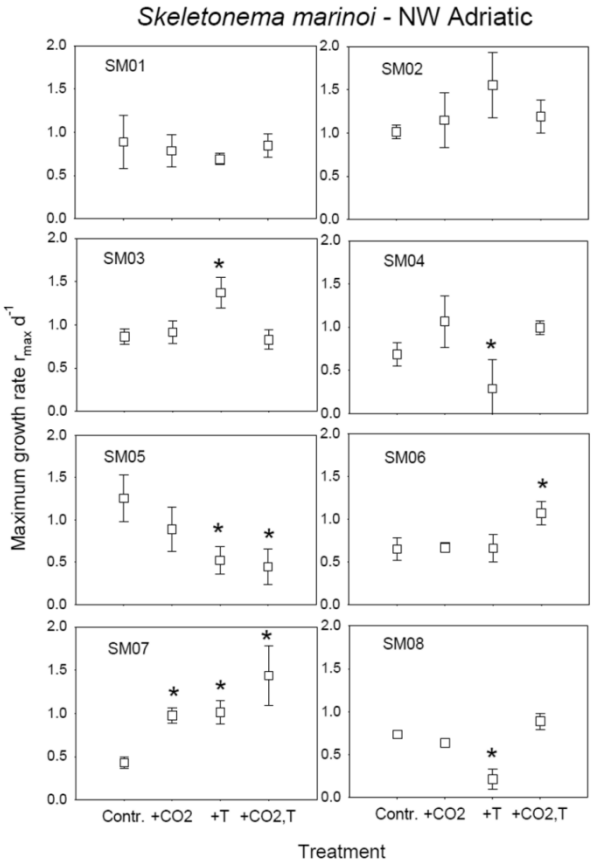


Figure 2



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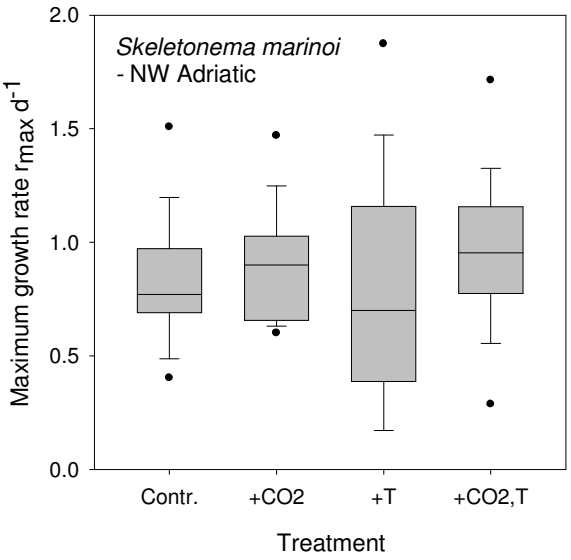

Figure 4

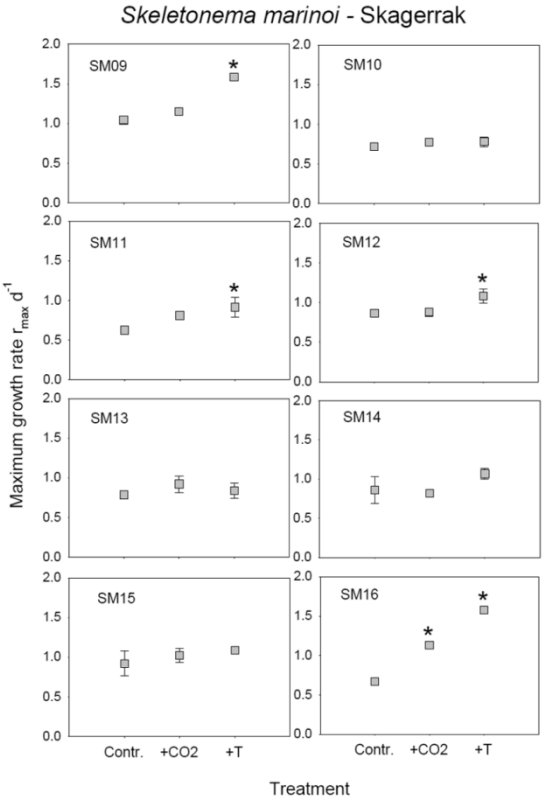

Figure 5

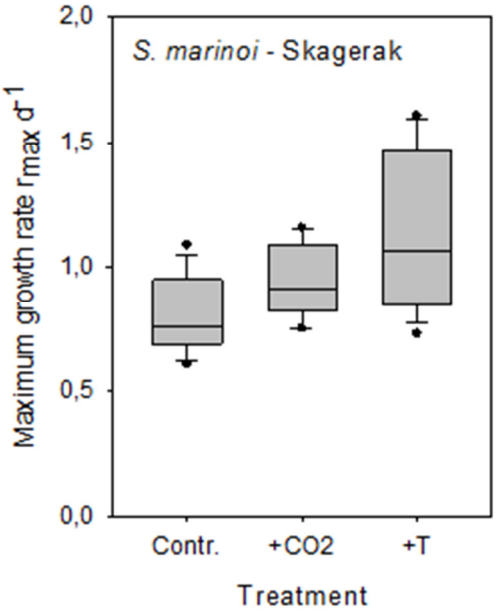

Figure 6

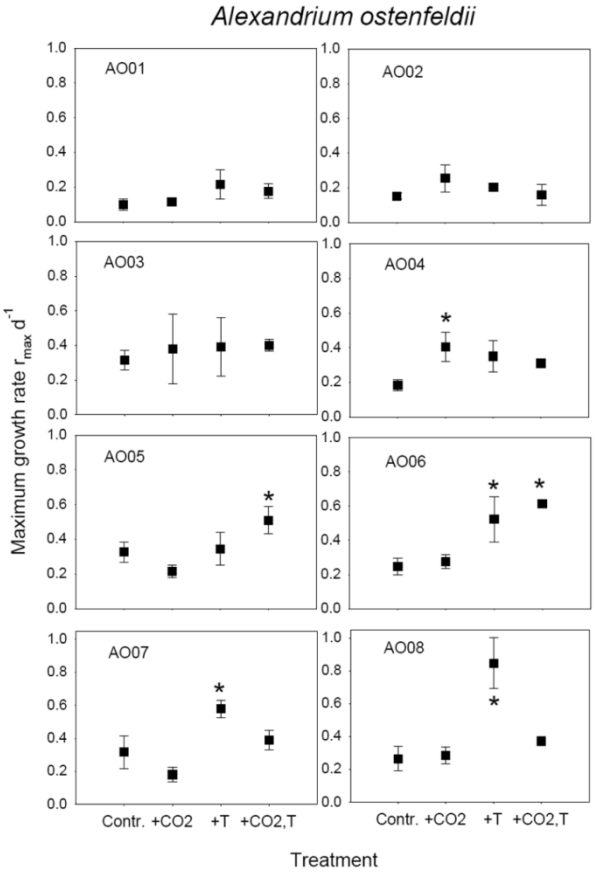

Figure 7

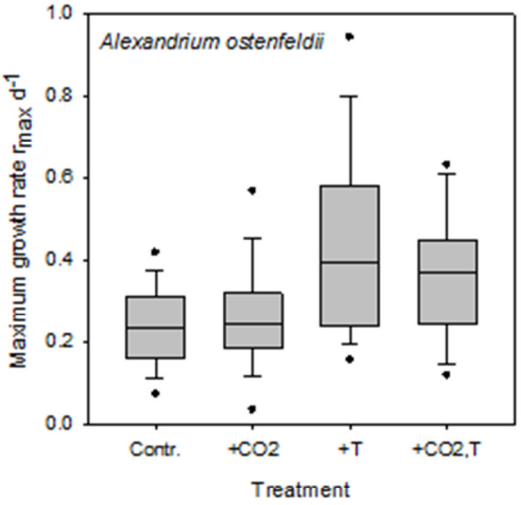

Figure 8

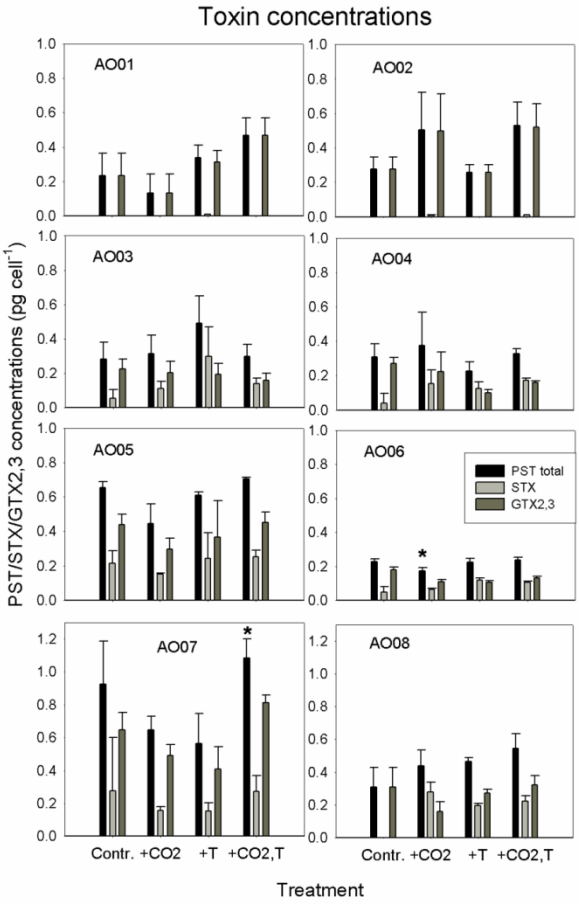

Figure 9

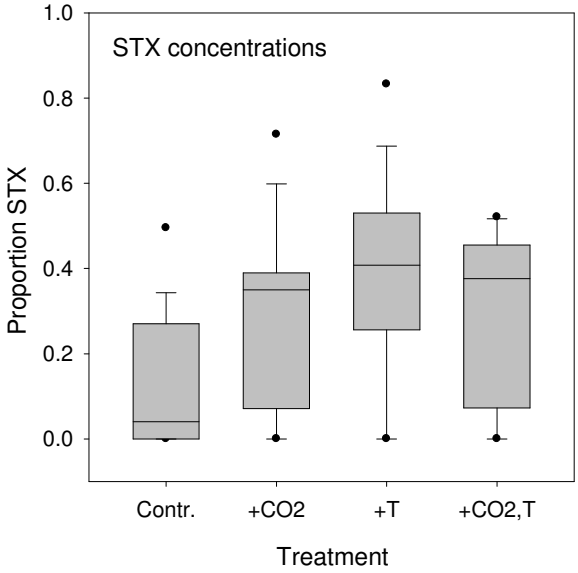

Figure 10