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Differing responses of three Southern Ocean *Emiliania huxleyi* ecotypes to changing seawater carbonate chemistry

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ABSTRACT: The invasion of anthropogenic carbon dioxide into the surface ocean is altering seawater carbonate speciation, a process commonly called ocean acidification. The high latitude waters of the Southern Ocean are one of the primary and most severely affected regions. Coccolithophores are an important phytoplankton group, responsible for the majority of pelagic calcium carbonate production in the world's oceans, with a distribution that ranges from tropical to polar waters. *Emiliania huxleyi* is numerically the most abundant coccolithophore species and appears in several different ecotypes. We tested the effects of ocean acidification on 3 carefully selected *E. huxleyi* ecotypes isolated from the Southern Ocean. Their responses were measured in terms of growth, photosynthesis, calcification, cellular geometry, and stoichiometry. The 3 ecotypes exhibited differing sensitivities in regards to seawater carbonate chemistry when cultured at the same temperature (14°C) and continuous light (110 µmol photons m^{-2} s^{-1}). Under future ocean acidification scenarios, particulate inorganic to organic carbon ratios (PIC:POC) decreased by 38–44, 47–51 and 71–98% in morphotype A 'over-calcified' (A o/c), A and B/C, respectively. All ecotypes reduced their rate of calcification, but the cold-water adapted ecotype (morphotype B/C) was by far the most sensitive, and almost ceased calcification at partial pressure of carbon dioxide (pCO_2) levels above 1000 µatm. We recommend that future surveys for *E. huxleyi* cells in the Southern Ocean should include the capability of recognising 'naked cells' by molecular and microscopic tools. The distinct differences in the physiological responses of these 3 dominant Southern Ocean coccolithophore ecotypes are likely to have consequences for future coccolithophore community structures and thereby the Southern Ocean carbon cycle.

KEY WORDS: Southern Ocean · Coccolithophores · Ocean acidification · *Emiliania huxleyi*

INTRODUCTION

The Southern Ocean is one of the world's regions affected most severely by anthropogenic climate change (Orr et al. 2005, Gille 2008), but it still remains under-studied compared to low-latitude oceans. The invasion of anthropogenic carbon dioxide into the ocean is causing a decrease in pH (ocean acidification) and an increase in the availability of carbon dioxide (ocean carbonation). Coccolithophores are an important part of the oceanic planktonic community and are capable of fixing carbon in the form of organic material via photosynthesis, and additionally, in the form of cal-

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cium carbonate by intracellular calcification. Changing seawater carbonate speciation can affect the processes of photosynthesis and calcification in coccolithophores and other phytoplankton species (Riebesell & Tortell 2011) with consequences for the cycling of carbon in the ocean. Numerically, the most abundant coccolithophore is *Emiliania huxleyi,* which is categorized into several different morphotypes based on coccolith structure and geometry (Young et al. 2003, Cook et al. 2011, Hagino et al. 2011). In the Southern Ocean, *E. huxleyi* is predominantly present in 3 morphotypes: A 'over-calcified' (A o/c), A and B/C, each of which have distinct coccolith morphology, light harvesting pigments and genes (Cook 2010, Cook et al. 2011, 2013, Krueger-Hadfield et al. 2014). Interestingly, these 3 morphotypes exhibit a clear north-to-south distribution gradient with morphotype A o/c mainly distributed north of 48°S, morphotype A ranging between 43 and 53°S and morphotype B/C distributed between 43 and 65°S (Cubillos et al. 2007). The distribution of these 3 morphotypes appears to be mainly determined by temperature and the Antarctic Polar Front (Findlay & Giraudeau 2000, Mohan et al. 2008, Winter et al. 2014) but a possible influence of carbonate chemistry has also been considered (Cubillos et al. 2007). Morphotype B/C exhibits the most southward distribution and produces (in contrast to morphotypes A o/c and A) very delicate coccoliths with relatively low calcium carbonate content (Poulton et al. 2013). Experimental studies on Southern Ocean coccolithophores and especially the B/C morphotype (termed *E. huxleyi* var. *aurorae* by Cook et al. 2011) are scarce in comparison to the well-studied morphotype A from the Northern Hemisphere. The geographical location of the Southern Ocean and its Polar Front generate an isolated ecosystem for phytoplankton in terms of gene transfer and recombination. Thus, a diverging response of Southern Ocean coccolithophores in comparison to their Northern Hemisphere counterparts is expected, and it is correspondingly necessary to investigate Southern Ocean species when determining potential anthropogenically induced changes in this region's marine ecosystems and biological carbon uptake mechanisms.

Here, we present results on the physiological response (in terms of growth, photosynthesis and calcification) of the 3 dominant Southern Ocean *E. huxleyi* eco-morphotypes over a broad range of carbonate chemistry scenarios in controlled laboratory experiments.

MATERIALS AND METHODS

Culture conditions

The strains used for this study were carefully selected from over 400 single cell isolates, based on the results of previous investigations that identified eco-physiological and/or genetic differences between the 3 investigated morphotypes (Cubillos et al. 2007, Cook et al. 2011, 2013, Krueger-Hadfield et al. 2014). Strains of *Emiliania huxleyi* (Table 1) originating from the coast of Tasmania (morphotype A o/c) and the Southern Ocean (morphotypes A and B/C) were grown as asexual diploids at 14°C in 0.2 µm filtrated natural seawater (collected offshore of Bruny-Island, Tasmania) with a salinity of 35 and a continuous photon flux density of 100 to 115 µmol photons m^{-2} s^{-1}. The exposure to continuous light desynchronizes the cellular division cycle of *E. huxleyi* (Müller et al. 2008), which results in an independence of sampling time and cellular volume (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m531p081_supp.pdf). Macro- and micronutrients were added according to f/20 and f/80, respectively, corresponding to 88 µmol l^{-1} nitrate and 3.6 µmol l^{-1} phosphate (Guillard 1975). This 4-fold diluted application of micronutrients (compared to macronutrients) resulted in better

Table 1. *Emiliania huxleyi* eco/morphotypes investigated, with corresponding strain codes from the Institute for Marine and Antarctic Studies (IMAS) Algae Culture Collection

Strain code	Morphotype	Origin	Isolator and date
EHTB 11.15	A over-calcified	Trumpeter Bay, Tasmania, 43° S, 147° E	M. de Salas, Apr 2006
EHBH 13.28	A over-calcified	Bicheno, Tasmania, 42° S, 148° E	S. Cook, Jun 2006
EHSO 5.14	A	Southern Ocean, 50° S, 149° E	S. Cook, Feb 2007
EHSO 5.30	A	Southern Ocean, 50° S, 149° E	S. Cook, Feb 2007
EHSO 5.11	B/C	Southern Ocean, 50° S, 149° E	S. Cook, Feb 2007
EHSO 8.15	B/C	Southern Ocean, 54° S, 146° E	S. Cook, Feb 2007

growth behaviour of the A and B/C morphotypes. Exponentially growing cultures were kept in semi-continuous dilute batch conditions, assuring cell conditioning to nutrient repletion.

Experimental setup

Experimental incubations were carried out in triplicate under dilute batch culture conditions (as described above) in 500 ml autoclaved borosilicate flasks. Carbonate chemistry speciation was adjusted by additions of HCl, NaOH and $NaHCO_3$ to the media resulting in a partial pressure of carbon dioxide (pCO_2) range from 240 to 1750 µatm. Exponentially growing cultures were acclimated to experimental conditions for ca. 10 generations and allowed to grow for ca. 5 to 11 generations corresponding to a dissolved inorganic carbon (C_T) maximal consumption of 5%. On average, incubations were terminated at cell densities between 25 000 and 62 000 cells ml^{-1}. Samples for C_T and total alkalinity (A_T) were taken at the beginning and end of the experimental incubations. At the end of the incubation, samples were taken for cell number and coccosphere/cell volume, coccolith volume, total particulate carbon (TPC), particulate organic carbon/nitrogen (POC/PON) and scanning electron microscopy (SEM). Experiments were conducted at 14°C, which represents the average annual temperature in regions of the Southern Ocean where all 3 morphotypes co-occur (Cubillos et al. 2007, Dong et al. 2008).

Carbonate chemistry

The carbonate system was monitored via C_T and A_T measurements at the start and the end of the experiments. C_T and A_T were analysed as the mean of triplicate measurements with the infrared detection method using an Apollo SciTech DIC-Analyzer (Model AS-C3) and the potentiometric titration method (Dickson et al. 2003), respectively. Data were corrected to Certified Reference Materials (CRM; Scripps Institution of Oceanography). Consecutive measurements of the Dickson standard resulted in an average precision of >99.8% for both C_T and A_T. Carbonate system parameters were calculated from temperature, salinity, C_T and A_T (mean values from the start and end of experiments) using CO2SYS (v.1.05 by E. Lewis and D. W. R. Wallace), with the stoichiometric equilibrium constants for carbonic acid given in Roy et al. (1993).

Cell numbers, growth rate, coccosphere and cell volumes

Samples for cell number and coccosphere/cell volume were processed directly after sampling and each measured 3 times with a Coulter Multisizer™ 4. Afterwards, the samples were acidified with HCl (0.1 mM) to dissolve all free and attached coccoliths and subsequently measured again to determine the cell volume of *E. huxleyi* (Müller et al. 2012). The mean cell number was used to calculate the growth rate, µ (d^{-1}), during the culture experiments as:

$$\mu = (\ln c_1 - \ln c_0) / (t_1 - t_0) \quad (1)$$

where c_0 and c_1 are the cell concentrations at the beginning (t_0) and end of the incubation period (t_1), expressed in days. Cell numbers were measured before and after sampling to account for the increase in cell number during the 1 to 2 h procedure.

Elemental analyses

For each experiment, 3 sub-samples were filtered onto pre-combusted quartz filters (450°C for 4 h) and frozen at –20°C. TPC and POC were measured at the Central Science Laboratory of the University of Tasmania (CSL-UTAS) on separate filters using a Thermo Finnigan EA 1112 Series Flash Elemental Analyser. The filter for POC analysis was treated with fuming HCl (~10 h) to remove all inorganic carbon (Hedges & Stern 1984). Particulate inorganic carbon (PIC) was calculated from the difference of TPC and POC. PON was analysed simultaneously with the POC measurements. Cell quota of particulate matter, PM (PM ≡ PIC, POC and PON) was calculated as:

$$\text{PM/cell} = \text{PM}_{\text{filter}} / (V \times N) \quad (2)$$

where PM_{filter} is the mass (pg) of particulate matter $filter^{-1}$, V is the volume (ml) filtrated and N is the number of cells ml^{-1}. Cell quota estimates are associated with an error of <5%. Production rates (PIC_{prod}, POC_{prod} and PON_{prod}) were calculated by multiplying the cell quota by µ.

Scanning electron microscopy

Samples for SEM were filtered onto polycarbonate filters (0.8 µm pore size) and then dried at 60°C pending analyses. Sputter coated (Gold-Palladium) filter portions were observed on a Hitachi SU-70 field emission SEM at CSL-UTAS.

RESULTS

Carbonate chemistry

Manipulation of the seawater carbonate chemistry resulted in a significant change in C_T concentrations, whereas A_T remained constant (average variation of <0.6%) in each strain specific experiment. Detailed information on the carbonate parameters of each experiment can be found in Tables S1–S3 in the Supplement at www.int-res.com/articles/suppl/m531p081_supp.pdf.

Growth and cellular production rates

The highest growth rates were measured at ambient pCO_2 for morphotypes A o/c, A and B/C at 0.64, 0.48 and 0.34 d^{-1}, respectively, whereas the lowest growth rates (0.20 d^{-1} in morphotype A and B/C) were detected at high pCO_2 (Fig. 1a,d,g). All strains showed significantly decreased growth rates at their highest individually tested pCO_2 levels compared to ambient conditions (1-way ANOVA, $p < 0.05$) with morphotype-specific sensitivities (reduction of 5–6, 53–56 and 28–41% in morphotypes A o/c, A and B/C, respectively). Over the tested pCO_2 range, POC_{prod} increased with pCO_2 to a certain threshold, and a further pCO_2 elevation led to reduced POC_{prod} rates. Morphotype-specific pCO_2 ($CO_{2aquatic}$) optima ranges were identified as ~1000 to 1100 µatm (37 to 41 µmol kg^{-1}), ~800 to 900 µatm (30 to 34 µmol kg^{-1}) and ~600 to 700 µatm (22 to 26 µmol kg^{-1}) for morphotypes A o/c, A and B/C, respectively (Fig. 1b,e,h). PIC_{prod} decreased when elevating pCO_2 from ambient to high conditions by 29–35, 54–59 and 81–99% in morphotypes A o/c, A and B/C, respectively (Fig. 1c,f,i).

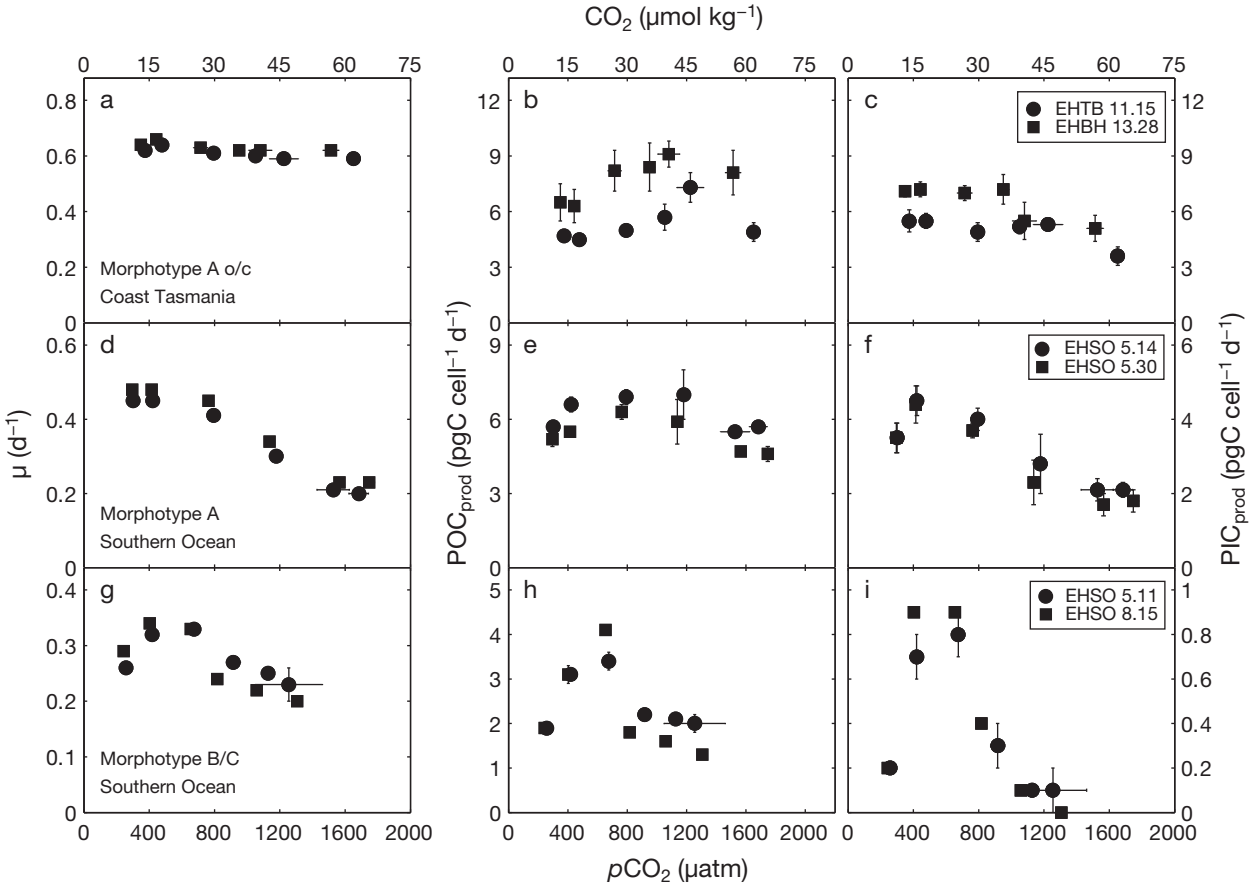

Fig. 1. Growth rate (µ) and production rates of particulate organic and inorganic carbon (POC_{prod} and PIC_{prod}) of 3 *Emiliania huxleyi* morphotypes in response to changing carbonate chemistry (pCO_2 and CO_2). (a–c) Morphotype A 'over-calcified' (A o/c; strains EHTB 11.15 and EHBH 13.28), (d–f) morphotype A (strains EHSO 5.14 and EHSO 5.30), and (g–i) morphotype B/C (strains EHSO 5.11 and EHSO 8.15). All data points represent the mean (±SD) of triplicate treatments

Note: Figs 1,2 & 4 were amended after publication. See Corrigendum.

Cellular geometry, stoichiometry and morphology

Coccosphere and cell volume of morphotype A o/c were not influenced by changes in carbonate chemistry, whereas the cell volume of morphotype A increased from low to high pCO_2 by 55%. Coccosphere volumes of morphotype A reached maximum values at ~800 µatm and remained unchanged at further elevated pCO_2 conditions. Morphotype B/C exhibited the highest cell and coccosphere volumes at ~650 µatm pCO_2; further elevation decreased the coccosphere volume to values approaching those for cell volume (Fig. 2a,d,g, closed and open symbols, respectively). PIC:POC ratios decreased in all morphotypes from ambient towards increased pCO_2 conditions by 38–44, 47–51 and 71–98% in morphotypes A o/c, A and B/C, respectively (Fig. 2b,e,h). POC:PON remained unchanged in morphotype A o/c and increased with pCO_2 in morphotypes A and B/C (Fig. 2c,f,i). Metrics for the degree of malformation of coccoliths were not quantified in this study, and thus the extent of malformation based on SEM surveys are qualitative in nature. Coccoliths of morphotype A o/c showed minor malformations when precipitated at pCO_2 values above 1500 µatm. An increased number of malformed coccoliths of morphotype A were visible at above 1100 µatm. Morphotype B/C exhibited no increase in the number of malformed coccoliths at elevated pCO_2, but above 1000 µatm an increased number of cells were naked (i.e. not covered by any coccoliths) (Fig. 3).

DISCUSSION

This study investigated the response of 3 Southern Ocean morphotypes of *Emiliania huxleyi* to changes in carbonate chemistry meant to simulate future ocean acidification scenarios. Distinct physiological responses between the 3 morphotypes were evident

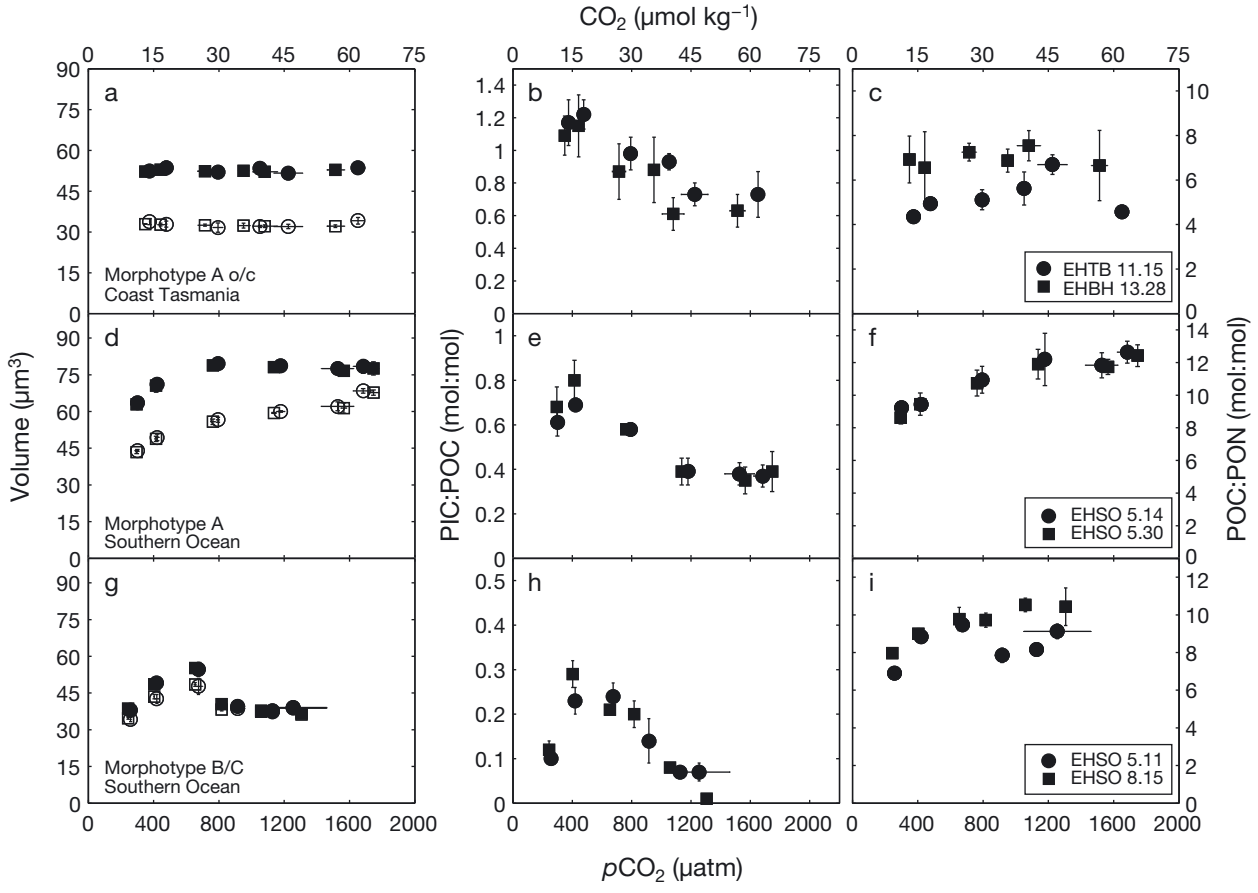

Fig. 2. Cellular geometry and stoichiometry of 3 *Emiliania huxleyi* morphotypes in response to changing carbonate chemistry (pCO_2 and CO_2). (a–c) Morphotype A 'over-calcified' (A o/c) (strains EHTB 11.15 and EHBH 13.28), (d–f) morphotype A (strains EHSO 5.14 and EHSO 5.30), and (g–i) morphotype B/C (strains EHSO 5.11 and EHSO 8.15). Coccosphere and cell volumes are represented by closed and open symbols, respectively, in (a), (d) and (g). All data points represent the mean (±SD) of triplicate treatments. PIC: particulate inorganic carbon; POC: particulate organic carbon; PON: particulate organic nitrogen

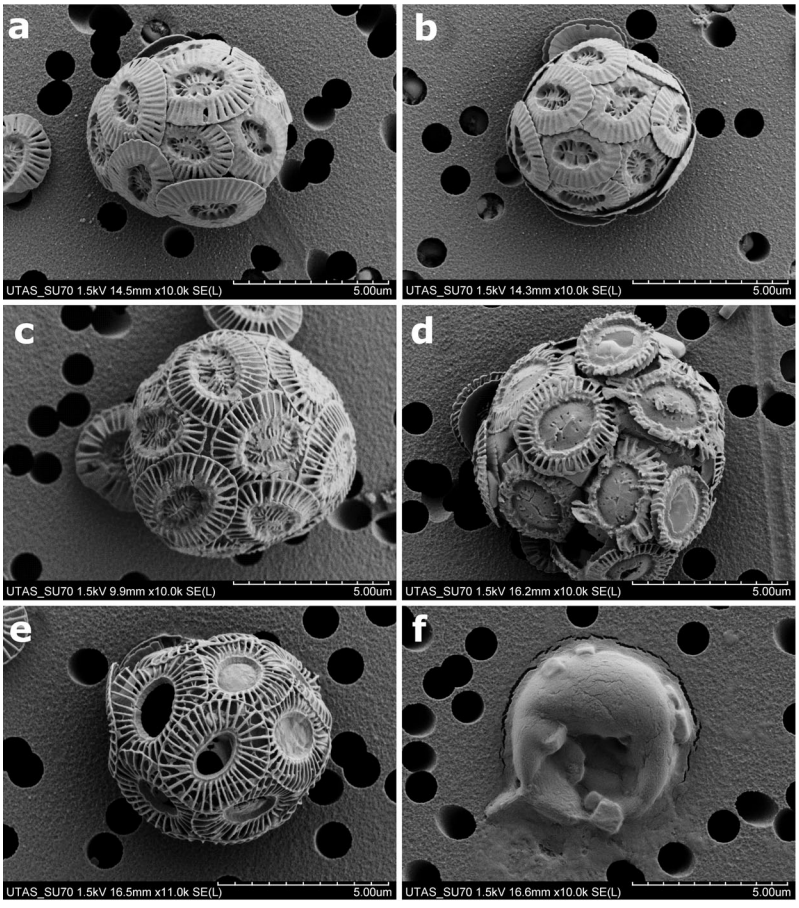

Fig. 3. Representative scanning electron microscopy (SEM) pictures of *Emiliania huxleyi* morphotypes — (a,b) A 'over-calcified' (A o/c), (c,d) A and (e,f) B/C — under ambient and high pCO_2 (left and right panels, respectively)

in µ, POC_{prod}, PIC_{prod}, cellular geometry and stoichiometry (Figs. 1 & 2). In general, morphotype B/C displayed higher sensitivity to changes in seawater carbonate chemistry, which became more explicit by plotting the relative change in µ, POC_{prod}, PIC_{prod} and PIC:POC from ambient to elevated pCO_2 conditions (Fig. 4). Following the Intergovernmental Panel on Climate Change scenario RCP8.5 for the end of this century (IPCC 2013), morphotype A o/c would be expected to display no change in growth rate, whereas morphotypes A and B/C could exhibit a ~20% reduction (Fig. 4, grey area). Such robustness of the over-calcified morphotype A was previously suggested by Beaufort et al. (2011), in the context of documenting its ecological fitness in the relatively acidic upwelling-waters of the Patagonian Shelf. On the other hand, the reduction in growth rate of about 20% exhibited in our work on Southern Ocean morphotypes A and B/C at pCO_2 values is higher than the reduction in growth rate of ~10% observed for the Northern Hemisphere morphotype A (Barcelos e Ramos et al. 2010, Müller et al. 2010, Bach et al. 2011). The measured reduction of ~10 to 20% in PIC_{prod} of morphotype A and A o/c is similar to observations of the Northern Hemisphere types (Riebesell et al. 2000, Barcelos e Ramos et al. 2010, Bach et al. 2011). Morphotype B/C, however, displayed a severe reduction in PIC_{prod} of −60% at pCO_2 values of ~900 µatm. Furthermore, B/C exhibited a reduced POC_{prod} at pCO_2 values of 800 µatm, whereas morphotypes A o/c and A were only affected above 1500 µatm. In contrast, Langer et al. (2009) reported a reduced POC_{prod} of morphotype A at pCO_2 levels of 900 µatm. This might be explained by the higher experimental temperature (14 vs. 17 to 20°C) and higher light intensity (100 to 115 vs. 400 µmol photons m^{-2} s^{-1}) used in the European study, as temperature and light can significantly alter the physiological sensitivity of *E. huxleyi* to ocean acidification (Zondervan et al. 2002, Feng et al. 2008, De Bodt et al. 2010, Sett et al. 2014). All morphotypes showed a clear reduction in PIC:POC ratio from ambient to elevated pCO_2 levels (Fig. 2) which is in line with the majority of previous research (Raven & Crawfurd 2012). Most notably, morphotype B/C nearly ceased the production of coccoliths at pCO_2 values above 1050 µatm (Fig. 1), conditions under which it was mostly present in its 'naked' form (Fig. 3f). The life cycle of *E. huxleyi* is characterized by 3 distinct stages: (1) the coccolith-carrying non-motile diploid form (C-cell), (2) the naked non-motile diploid form (N-cell) and (3) the scaly motile haploid form (S-cell). The latter haploid stage possesses organic body scales covering the cell and 2 flagella that enable motion (Paasche 2001, von Dassow et al. 2009). Light and electron microscopy indicated that the non-calcified 'naked' cells of morphotype B/C were N-cells because no signs of organic scales, flagella or motion were detected. At pre-industrial levels of less than 280 µatm, morphotype B/C only produced coccoliths in small numbers. The observation of morphotype B/C reducing or ceasing the production of coccoliths when growing under suboptimal conditions has not been made

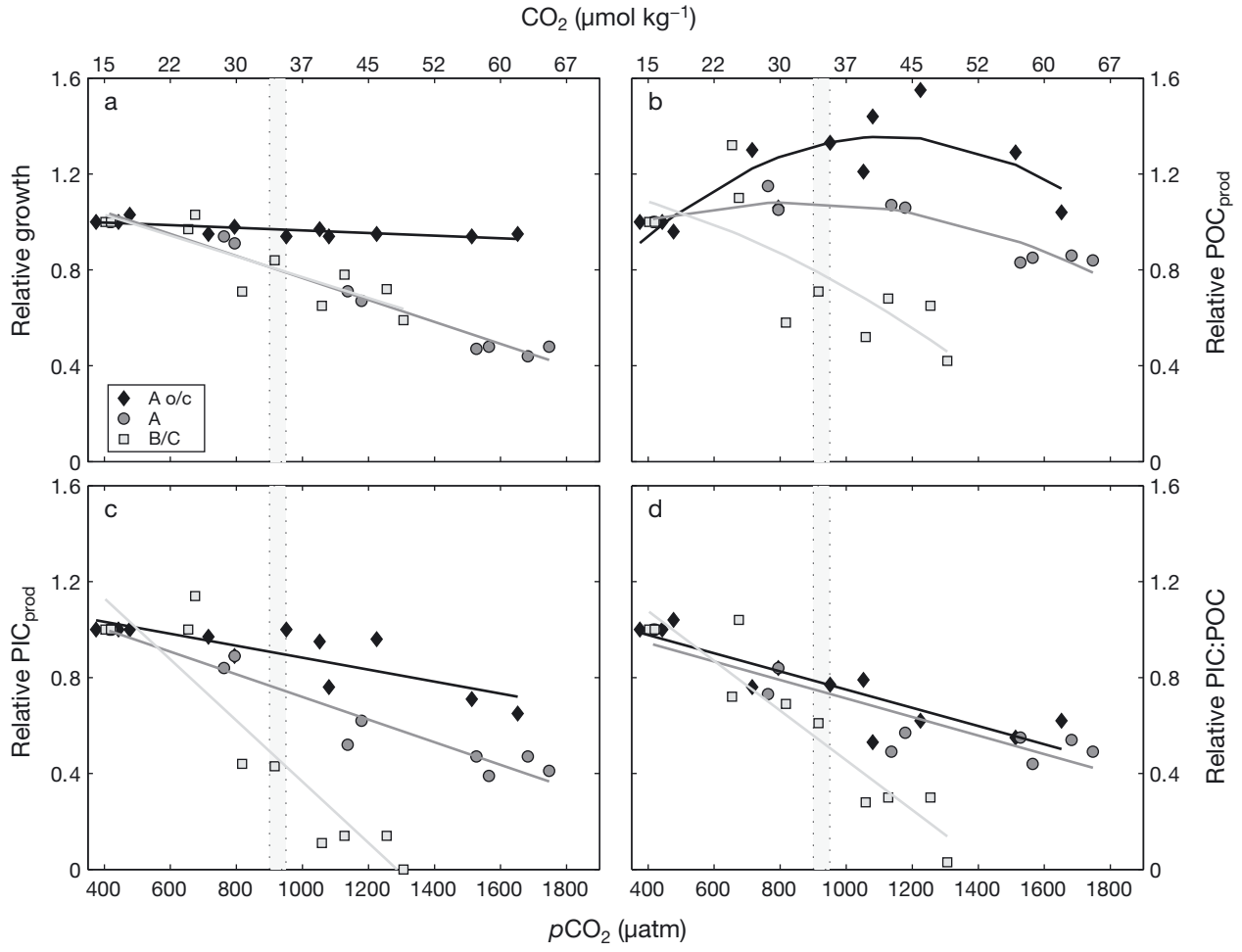

Fig. 4. Relative change in (a) growth, production rate of (b) particulate organic carbon (POC_{prod}) and (c) particulate inorganic carbon (PIC_{prod}), and (d) PIC:POC ratio in response to pCO_2 and CO_2. Gray shaded areas correspond to the projected pCO_2 values for the end of this century in the Southern Ocean according to Intergovernmental Panel on Climate Change (IPCC) scenario RCP8.5. Solid lines represent the best fit using Matlab tool 'polyfit'

before in laboratory or field observations. Non-calcified N-cells of morphotype A have been mainly observed in laboratory cultures and have been described as a mutant appearance after extended culturing periods (Paasche 2001). In natural populations, N-cells can easily be overlooked since differentiation between other spherical nanoplankton of similar size is nearly impossible. Carefully conducted light and/or electron microscopy is necessary to detect N-cells in natural populations, which is quite labour and time intensive. Recently, new molecular methods have been developed to identify non-calcified S-cells in natural populations (Frada et al. 2012); however, a fast and feasible method to differentiate and quantify S- and N-cells in natural population has not yet been applied. Nevertheless, the question arises whether this underproduction of coccoliths by morphotype B/C is only caused by altered carbonate chemistry or whether other environmental stressors could trigger a similar response. Observations from our culture collection indicate that even under 'satisfactory conditions', some morphotype B/C strains only produce a small number of coccoliths (less than 3 $cell^{-1}$) while showing normal growth behaviour. The occurrence of healthy growing non-calcified N- and S- cells and our own observations seem to indicate that the process of calcification is not crucial for survival and reproduction in *E. huxleyi*. Therefore, it is conceivable that the energy consuming process of calcification can be down-regulated during unfavourable conditions and severe physiological stress. On the contrary, 'over-production' of coccoliths by *E. huxleyi* is commonly observed under phosphorus limitation (Paasche 2001). However, phosphorus limitation mainly interferes with the cell cycle by stopping cell division, while photosynthesis

and calcification continue to function at reasonable rates which causes an increase in cell size and the number of coccoliths per cell (Müller et al. 2008).

Cell volume and the cell surface to volume ratio can influence physiological rates and ecological processes, such as nutrient diffusion and uptake, light absorption mechanisms, growth, photosynthesis, sinking and grazing rates (see Finkel et al. 2010 and references therein). Over the tested pCO_2 range, each morphotype exhibited a differing behaviour of cell volume in regards to pCO_2. Whereas A o/c showed no change, morphotype A increased cell volume with pCO_2 and morphotype B/C showed an optimum peak at pCO_2 values of around 650 to 680 µatm (Fig. 2). It should be noted that the observed decrease in PIC_{prod} with increasing pCO_2 (Fig. 1) would be steeper when normalised to cellular volume. This is partly reflected in the steep decrease of PIC:POC (Fig. 2) because cell volume (x) was linearly correlated with cellular POC quota (y) in morphotypes, A and B/C ($y = 0.56x - 13.72$; $r^2 = 0.89$, $p < 0.0001$, $n = 24$) whereas no correlation was found in morphotype A o/c. Additionally, this might indicate that the observed increase in POC_{prod} of morphotype A o/c (Fig. 1) is mostly associated with organic exudates that, in the form of transparent exopolymer particles (TEP), are measured together with the intra-cellular POC on the sample filter. With regard to metabolic rates as influenced by cell volume, we might therefore expect differences in the eco-physiological performance and competitive fitness (e.g. nutrient uptake efficiency) of these 3 ecotypes in a future acidified ocean.

All 3 *E. huxleyi* morphotypes are a common component of the Southern Ocean plankton, appearing in surface waters from 43 to 65°S (Cubillos et al. 2007). An increasing body of morphological, physiological and genetic studies indicates that the 3 morphotypes selected in this study occupy distinct ecological niches (Cubillos et al. 2007, Cook et al. 2011, 2013, Krueger-Hadfield et al. 2014). Morphotypes A o/c and A are found in the coastal regions of Tasmania and as far as 52°S, whereas morphotype B/C is the dominant form in the open Southern Ocean waters from 45 to 65°S (Cubillos et al. 2007). Morphotype B/C (*E. huxleyi* var. *aurorae*) is a Southern Ocean specialist, with slower growth rates but survival strategies enabling growth at 4°C. It is low-light adapted (Cook 2010) and produces about 50% less coccolith calcite (Poulton et al. 2013). The previously mentioned differences between the 3 morphotypes and their contrasting physiological responses to changing seawater carbonate chemistry indicate that these 3 Southern Ocean morphotypes each represent a distinct ecotype.

Since the late 1990s, a poleward expansion of *E. huxleyi* has been observed (Cubillos et al. 2007, Winter et al. 2014). In the Southern Ocean, morphotype B/C appears to be the expanding ecotype, while in the Arctic (e.g. Bering Sea), morphotype A is increasingly successful (Merico et al. 2003). These shifts in distribution are the result of physico-chemical alterations of the environment, with temperature probably being the dominant driver (Winter et al. 2014). Being a cold water ecotype, the poleward expansion of morphotype B/C can be interpreted as an escape from increasing water temperatures at lower latitudes and/or an extension of habitat with waters above 4°C at high latitudes. Our findings demonstrate that morphotype B/C is highly sensitive to changes in seawater pCO_2/pH and, therefore, its poleward expansion could be problematic by the end of this century when high latitude cold waters are expected to experience drastic changes in surface water carbonate chemistry (McNeil & Matear 2008). In other words, *E. huxleyi* eco-morphotype B/C might find itself caught between ocean warming (expanding southwards) and ocean acidification (expanding northwards) without any known escape strategies. Admittedly, this scenario does not account for adaptation, as demonstrated in a North Atlantic morphotype A strain (Lohbeck et al. 2012) but which has not yet been investigated for morphotype B/C.

Temperature adaptation in *E. huxleyi* occurs independently of ocean acidification levels (Schlüter et al. 2014). We also recognise that our experimental study was conducted at a water temperature of 14°C, which is not expected for the Southern Ocean within the next century. It would be reasonable to argue that a seawater temperature of 14°C might be above the optimal growth conditions for morphotype B/C, which is dominant in waters with surface temperature below 12°C (Cubillos et al. 2007, Dong et al. 2008). This possible temperature stress, together with changing carbonate speciation could be a 'double-stress' and thus potentially responsible for the high sensitivity observed in this study. However, the measured growth rates of morphotype B/C ($\mu = 0.34\ d^{-1}$) at 14°C and ambient pCO_2 conditions are well below reported growth rates in the literature. Cook et al. (2011) reported growth rates for morphotype B/C (strains EHSO 5.11 and 8.15) of $0.59\ d^{-1}$ at 16°C and ambient pCO_2 conditions. Additionally, Sett et al. (2014) demonstrated that under optimum CO_2 concentrations for growth rate, PIC_{prod} and POC_{prod} is modulated by temperature, and further-

more, that the sensitivity (in terms of growth rate) to *p*CO2/pH was not influenced by increasing the experimental temperature from 15 to 20°C. Therefore, we conclude that the high sensitivity of morphotype B/C is specific to changing carbonate chemistry speciation.

Understanding the biological response to single environmental factors as well as their synergistic effects is fundamental to reliably predict future alterations of community structures and the resulting ecosystem effects. Interestingly, in our work, we discovered morphotype B/C cells growing without producing coccoliths, which makes it nearly impossible to identify these cells in the field with routine microscopic equipment. Future surveys for *E. huxleyi*, in the Southern Ocean in particular should therefore include the capability to recognise non-calcified cells (haploid and diploid) by molecular tools and/or microscopy.

CONCLUSIONS

The 3 dominant Southern Ocean ecotypes of *Emiliania huxleyi* exhibited distinctive differences in their physiological response to simulated ocean acidification scenarios, with ecotype B/C being the most sensitive in terms of reduced growth, calcification, POC production and PIC:POC ratio. These differences should be taken into account when exploring future changes in carbon dynamics of the Southern Ocean, as well as the distribution and community composition of coccolithophores in this region. Most notably, we observed a near cessation of calcification with elevated pCO_2 levels for the most sensitive ecotype (B/C), which is dominant in the Southern Ocean south of 53°S.

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