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Utilization of organic nutrients by coccolithophores

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ABSTRACT: Coccolithophores play a prominent role in the marine, and by extension the global, carbon cycle. The ability of coccolithophores to thrive on organic nutrients is assumed to be a key reason for their ecological success, as *Emiliania huxleyi* grows well on a variety of organic nutrients. The ability of other coccolithophores to utilize organic nutrients has, however, not been investigated. We conducted experiments to compare the ability of *E. huxleyi*, *Coccolithus braarudii*, and *Calcidiscus leptoporus* to grow on environmentally common organic nitrogen and phosphorus sources (glycine, L-alanine, L-proline, L-serine, L-glutanic acid, L-histidine, urea, glycerophosphate, adenosine monophosphate, adenosine triphosphate, and β-nicotinamide adenine dinucleotide). The nitrogen and phosphorus additions (200 µM and 14 µM, respectively) were higher than naturally occurring in the ocean, as our goal was to test the ability of coccolithophores to utilize these substances. *E. huxleyi* was able to grow on all tested organic nutrients. *C. braarudii* grew on 7 out of the 10 tested nutrient sources, but did not grow on 3 amino acids. *C. leptoporus* grew on only 3 out of the 7 tested N-sources, with no growth on the 4 amino acids. Similarities in the coccolithophores' ability to utilize specific organic sources suggest common transport systems and enzymes, whereas differences emphasize the presence of species-specific nutrient uptake mechanisms. Such species-specific differences in the ability to utilize certain nutrients may provide explanations for biogeographic distribution patterns and substantiate the suspicion that *E. huxleyi* may not be a good representative of coccolithophores, e.g. for Earth system models.

KEY WORDS: Dissolved organic nitrogen · Dissolved organic phosphorus · *Emiliania huxleyi* · *Coccolithus braarudii* · *Calcidiscus leptoporus* · Coccolithophore distribution

INTRODUCTION

In the context of the global carbon cycle, coccolithophores are one of the important phytoplankton groups (Rost & Riebesell 2004). They are ubiquitous and produce a large amount of organic carbon (some species like *Emiliania huxleyi* form large blooms), and also fix calcium carbonate in the form of calcite plates (so-called coccoliths). *E. huxleyi* is generally chosen to represent coccolithophores in ecosystem and carbon cycle models (Westbroek et al. 1993, Iglesias-Rodriguez et al. 2002, Moore et al. 2002), largely because most laboratory and mesocosm experiments investigating coccolithophores have been conducted with this species (Palenik & Morel 1990a, Engel et al. 2004, Løvdal et al. 2008). However, the question has been raised as to whether *E. huxleyi* is an adequate representative of coccolithophores. Criteria for representativeness include the phylogenetic relationship and the response to environmental conditions. *E. huxleyi* does not meet these criteria either in terms of phylogeny (Sáez et al. 2003) or with regard to calcification physiology (Riebesell et al. 2000, Langer et al. 2006). Moreover, in the northern North Atlantic and the South Atlantic, *Coccolithus pelagicus* and *Calcidiscus leptoporus*, respectively, rather than *E. huxleyi* are the major calcite producers (Baumann et al. 2004, Ziveri et al. 2004).

Emiliania huxleyi is able to grow on a large variety of organic nutrients (Ietswaart et al. 1994, Palenik & Henson 1997, Dyhrman & Palenik 2003), which, if it could be extrapolated to all coccolithophores, would be a central quality in determining interspecific competi-

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tion and succession, e.g. between coccolithophores, dinoflagellates and diatoms. However, a comparison with *Pleurochrysis carterae* suggests that interspecies variability in the ability to utilize organic nutrients may be high for coccolithophores (Palenik & Henson 1997). Intraspecific variability in the ability to utilize specific nutrients is also high within *E. huxleyi* (Palenik & Henson 1997) as is its genetic variation (Iglesias-Rodriguez et al. 2006).

Organic nutrients are the major pool of nitrogen and phosphorus compounds in the ocean. Dissolved organic nitrogen (DON) constitutes 89% of the total dissolved nitrogen (TDN) in the surface water of the open ocean (Berman & Bronk 2003) with urea and amino acids contributing the largest fractions to the labile DON (Antia et al. 1991 and references therein). Urea concentrations range between 0.5 µmol l^{-1} in the open ocean and 8.9 µmol l^{-1} in estuary systems (Antia et al. 1991). Urea concentrations in coastal regions may even increase in the future due to the use of urea fertilizer and poultry production. In Chesapeake Bay (USA) highest urea concentrations were found in areas with most intensive agricultural and poultry operations (Glibert et al. 2005). Amino acids reach concentrations up to 0.5 µmol l^{-1} in the open ocean (Antia et al. 1991) with non-polar amino acids seemingly contributing the largest fraction, followed by polar (amino acids with both acidic and basic side chains), acidic and lastly basic amino acids (Dittmar et al. 2001, Yamashita & Tanoue 2003). Dissolved organic phosphorus (DOP) constitutes up to 75% of the total dissolved phosphorus (TDP) in the ocean and consists mostly of phosphomonoesters (55 to 77%) and nucleotides plus nucleic acids (23 to 45%) (Benitez-Nelson 2000 and references therein). To address the question if *Emiliania huxleyi*'s ability to utilize organic nutrients is representative of ecologically important coccolithophores, we compared the ability of *E. huxleyi*, *Coccolithus braarudii*, and *Calcidiscus leptoporus* to grow on different organic substrates of the natural DON and DOP pools, including urea, amino acids, phosphomonoester, and nucleotides. These experiments were designed to answer the questions, (1) if organic nutrients are able to contribute significantly to the growth of these coccolithophores, (2) if the bioavailability of specific organic compounds is similar for different species of coccolithophores, and (3) if *E. huxleyi* is a good representative for these coccolithophores in terms of nutrient utilization.

MATERIALS AND METHODS

Culturing conditions. Monospecific cultures of the 3 coccolithophores, *Coccolithus braarudii* (strain RCC1200), *Calcidiscus leptoporus* (strain RCC1135, both Roscoff Culture Collection), and *Emiliania huxleyi* (strain PML B92/11) were grown in batch cultures. All cultures were grown at a light intensity of 270 µmol photons m^{-2} s^{-1} in a 16:8 light: dark cycle and a temperature of 17°C. Because *C. braarudii* produced malformed coccoliths in artificial seawater and *C. leptoporus* did not grow at all in artificial seawater, cells were grown in media based on pretreated North Sea water. Cultures of these species could not be made axenic (some coccolithophores do not survive axenic conditions), but bacterial concentrations were kept low via sterile culturing methods.

Media preparation. Media was based on North Sea water in which coccolithophores had been grown during a pretreatment phase to remove nutrients. The North Sea water was first 0.22 µm filtered (Durapore Hydrophilic Cartridge, Millipore), then autoclaved (121°C for 40 min), and inoculated with *Emiliania huxleyi*, except for the phosphorus experiment with *Coccolithus braarudii*. *C. braarudii* was used in the pretreatment phase for that experiment to avoid a false positive result, because *E. huxleyi* releases the ectoenzyme alkaline phosphatase (Riegman et al. 2000), which can persist in seawater and cleave organic phosphorous compounds. During this pretreatment phase the respective cells were grown until all nitrate or phosphate, respectively was utilized (checked by means of nutrient measurements) and then cells were removed by 0.2 µm filtration (PALL Life Sciences Capsule AcroPak*TM* 500). Thereafter, the media was air bubbled for 5 d to restore the pH, which was lowered due to calcification to 8.1. Bubbling the media partially restored dissolved inorganic carbon (DIC), but could not restore alkalinity. However, the specification of the carbonate system was close to that in natural sea water and DIC was most likely not limiting the coccolithophore species (Buitenhuis et al. 1999). After bubbling, the media was sterile filtered (0.2 µm, Nalgene bottle top filters) into sterilized glass bottles and spiked with vitamins, trace metals (f/4 concentrations), selenium (2.66 µmol l^{-1}), and macro-nutrients (either nitrate or organic nitrogen and orthophosphate or organic phosphorus). Nitrogen or phosphorus was added at final concentrations of 200 µmol N l^{-1} and 14 µmol P l^{-1}.

Experimental setup. Growth of all 3 species on nitrogen (N-experiments) was tested in an amino acid experiment (AA-experiment) consisting of 6 treatments with different amino acids, and in an urea experiment (U-experiment). Nitrate was used as a control for both N-experiments. The non-polar amino acids glycine, L-alanine and L-proline, the polar amino acid L-serine, the acidic amino acid L-glutamic acid, or the basic amino acid L-histidine were added into the treatments of the AA-experiment. These substances

are representatives of amino acids in natural marine DON (Antia et al. 1991). Urea treatments were additionally spiked with 100 nM nickel to prevent nitrogen-nickel co-limitation (Price & Morel 1991). Orthophosphate was added to all treatments of the N-experiments as a phosphorus source.

Growth of *Emiliania huxleyi* and *Coccolithus braarudii* on different phosphorus sources was tested in one experiment with 3 treatments (P-experiment). Treatments were spiked either with the phosphomonoester glycerophosphate, the nucleotides adenosine monophosphate (AMP), adenosine triphosphate (ATP), or β-nicotinamide adenine dinucleotide (NAD), which are all representatives of natural marine DOP (Benitez-Nelson 2000). Orthophosphate was used as a control and nitrate was added to all treatments of the P-experiment as a nitrogen source. Growth of *E. huxleyi* on AMP was not tested.

Each of the 3 experiments (AA-, U-, and P-experiment) consisted of 2 phases. During the first 2-wk phase, the acclimatization phase, 50 ml batch cultures were first grown for 1 wk under experimental conditions and then transferred into a second set of 50 ml batch cultures where the cultures were grown for another week under experimental conditions. This procedure allows acclimatization to the respective nutrient source and a dilution of the nutrients from the original culture. The acclimatization phase corresponded to 9 (*Calcidiscus leptoporus* in urea) to 27 (*Emiliania huxleyi* in L-serine) generations depending on nutrient, species, and growth rates. Growth rates were monitored during the second phase, the measuring phase, which lasted for 7 to 10 d, until growth rate decreased. At the beginning of the measuring phase acclimatized cells were inoculated in duplicate 50 ml bottles with initial cell densities of 500 cells ml^{-1} and grown again under experimental conditions. Cell densities were determined every 1 to 3 d.

Measurement of cell density and growth rate. Cells of *Coccolithus braarudii* and *Calcidiscus leptoporus* were counted with an inverted light microscope (Zeiss, Axiovert 135) using a Sedgewick Rafter Cell from PYSER-SGI. Cells of *Emiliania huxleyi* were counted with a Beckman Coulter Counter Multisizer III. Growth rate, μ, was calculated from the regression of cell density versus time during the exponential growth of the measuring phase, and is given as an average of the 2 replicates ± SD. The coefficient of variation was always <9%, if growth was detected. The coefficient of variation is defined as the ratio of the standard deviation σ to the mean μ and compares the degree of variation from 1 data series to another. Growth rates were also normalized to the growth rates on inorganic nutrients (controls) for interspecific comparisons.

RESULTS

N-experiments

Differences in growth rate between species and substrates were prominent. *Emiliania huxleyi* grew on all tested amino acids and growth rates ranged between 0.83 ± 0.01 and 1.35 ± 0.02 d^{-1} (Fig. 1A and Table 1). Growth on glycine, L-alanine, L-serine, and nitrate was statistically the same (ANOVA, Tukey multiple comparison test, $p > 0.05$) (Table 2), and significantly higher than that on L-glutamic acid, L-proline, and L-histidine (ANOVA, Tukey multiple comparison test, $p < 0.001$) (Table 2). The growth on L-histidine was also significantly higher than on L-glutamic acid and L-proline (ANOVA, Tukey multiple comparison test,

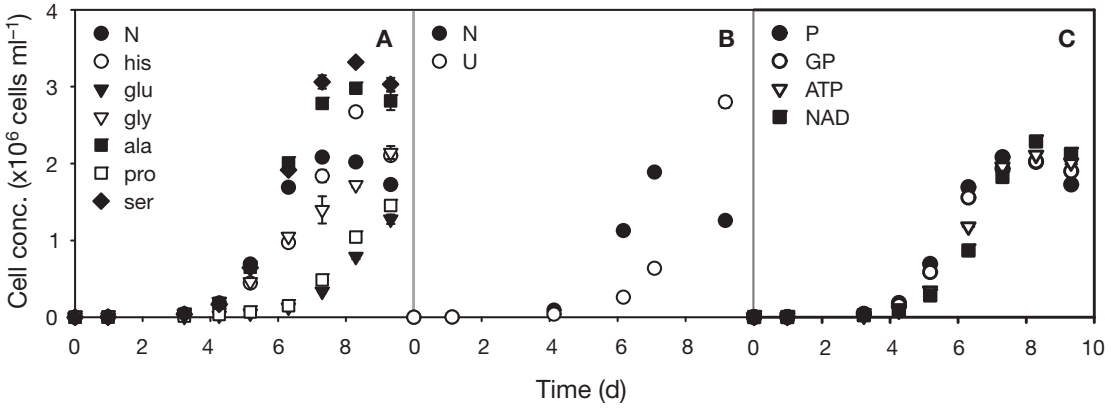

Fig. 1. *Emiliania huxley.* Cell density versus time on different nitrogen (A) and (B), and phosphorus (C) sources. Numbers are the average of 2 replicates, error bars show SD of replicates and not visible error bars indicate a smaller SD than the symbol. N = nitrate, his = histidine, glu = glutamic acid, gly = glycine, ala = alanine, pro = proline, ser = serine, U = urea, P = phosphate, GP = glycerophosphate, ATP = adenosine triphosphate, NAD = β-nicotinamide adenine dinucleotide

Table 1. *Emiliania huxleyi Coccolithus braarudii*, and *Calcidiscus leptoporus.* Growth rates (μ; d^{-1}) in the different nutrient sources. Numbers are the average of 2 replicates ± SD. N = nitrate, his = histidine, glu = glutamic acid, gly = glycine, pro = proline, ala = alanine, ser = serine, U = urea, P = phosphate, GP = glycerophosphate, AMP = adenosine monophosphate, ATP = adenosine triphosphate, NAD = β-nicotinamide adenine dinucleotide, ng = no growth in acclimation phase, nm = treatment not in experiment

Treatment	*Emiliania huxleyi*	*Coccolithus braarudii*	*Calcidiscus leptoporus*
N	1.31 ± 0.01	0.79 ± 0.06	0.46 ± 0.00
his	1.01 ± 0.01	0.70 ± 0.02	0.52 ± 0.03
glu	0.83 ± 0.01	−0.06 ± 0.01	ng
gly	1.33 ± 0.00	0.72 ± 0.04	0.53 ± 0.00
ala	1.32 ± 0.02	0.07 ± 0.03	ng
pro	0.83 ± 0.01	ng	ng
ser	1.35 ± 0.02	0.62 ± 0.02	ng
N	1.17 ± 0.02	0.87 ± 0.02	0.50 ± 0.01
U	0.93 ± 0.00	0.68 ± 0.03	0.44 ± 0.02
P	1.31 ± 0.01	0.89 ± 0.06	nm
GP	1.31 ± 0.00	1.01 ± 0.06	nm
AMP	nm	0.91 ± 0.01	nm
ATP	1.33 ± 0.01	1.00 ± 0.03	nm
NAD	1.13 ± 0.00	ng	nm

$p < 0.001$) (Table 2). *Coccolithus braarudii* did not grow on L-proline during the acclimation phase. No growth was measurable on L-glutamic acid and L-alanine during the measuring phase (*t*-test compared to zero growth rate, $p > 0.05$, $n = 2$), although cells had grown during the acclimation phase (Fig. 2A and Table 1). We have no explanation for the observation that in some cases growth was detected during the acclimatization, but not during the measuring phase. Possibly internal nutrient pools allowed growth for a prolonged period, when no utilizable nutrients were available in the media. Research on the role of internal nutrient pools is needed to solve this enigma. In our discussion we will only consider a test as positive when growth was observed during the measuring phase.

Table 2. *Emiliania huxleyi.* Significance test results (see text) for growth rates in the different nitrogen or phosphorus sources. Results only shown for the nutrients which did provide growth of the species. –: not tested, due to same nutrient source or growth rates from different experiments and therefore not comparable. Level of significance ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, empty cell: $p > 0.05$. N = nitrate, his = histidine, glu = glutamic acid, gly = glycine, ala = alanine, pro = proline, ser = serine, U = urea, P = phosphate, GP = glycerophosphate, ATP = adenosinetriphosphate, NAD = β-nicotinamide adenine dinucleotide

	N	his	glu	gly	ala	pro	ser	U	P	GP	ATP	NAD
N	–	***	***			***	*	***	–	–	–	–
his		–	***	***	***	***	***	–	–	–	–	–
glu			–	***	***		***	–	–	–	–	–
gly				–		***		–	–	–	–	–
ala					–	***		–	–	–	–	–
pro						–	***	–	–	–	–	–
P	–	–	–	–	–	–	–	–	–			***
GP	–	–	–	–	–	–	–	–		–		***
ATP	–	–	–	–	–	–	–	–			–	***

Table 3. *Coccolithus braarudi.* Significance test results (see text) for growth rates in the different nitrogen or phosphorus sources. Results only shown for the nutrients which did provide growth of the species. –: not tested, due to same nutrient source or growth rates from different experiments and therefore not comparable. Level of significance ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, empty cell: $p > 0.05$. N = nitrate, his = histidine, glu = glutamic acid, gly = glycine, ala = alanine, ser = serine, U = urea, P = phosphate, GP = glycerophosphate, AMP = nucleotides adenosine monophosphate, ATP = adenosine triphosphate

	N	his	glu	gly	ala	ser	U	P	GP	AMP	ATP
N	–		***		***	*	*	–	–	–	–
his		–	***		***		–	–	–	–	–
glu			–	***		***	–	–	–	–	–
gly				–	***		–	–	–	–	–
ala					–	***	–	–	–	–	–
P	–	–	–	–	–	–	–	–			
GP	–	–	–	–	–	–	–		–		
AMP	–	–	–	–	–	–	–			–	

Growth rates on the 3 remaining amino acids, glycine, L-serine, and L-histidine were statistically the same as that on nitrate (ANOVA, Tukey multiple comparison test, $p > 0.01$) (Table 3) and ranged between 0.62 ± 0.02 and 0.72 ± 0.04 d^{-1} (Table 1). *Calcidiscus leptoporus* did not grow on L-alanine, L-proline, L-serine, or L-glutamic acid during the acclimation phase. Growth rates on L-histidine and glycine during the measuring phase were 0.52 ± 0.03 and 0.53 ± 0.00 d^{-1}, respectively (Table 1 and Fig. 3A), and statistically not different from the growth rate on nitrate (ANOVA, Tukey multiple comparison test, $p > 0.05$) (Table 4). All 3 cultures grew on urea but growth rates were significantly lower than during growth on nitrate (*t*-test, each $p < 0.01$, $n = 2$) (Figs. 1B, 2B, 3B, and Tables 1, 2, 3, 4).

Table 4. *Calcidiscus leptoporus.* Significance test results (see text) for growth rates in the different nitrogen sources. Results only shown for the nutrients which did provide growth of the species. –: not tested, due to same nutrient source or growth rates from different experiments and therefore not comparable. Level of significance ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, empty cell: $p > 0.05$. N = nitrate, his = histidine, gly = glycine, U = urea

	N	his	gly	U
N	–			**
his		–		–

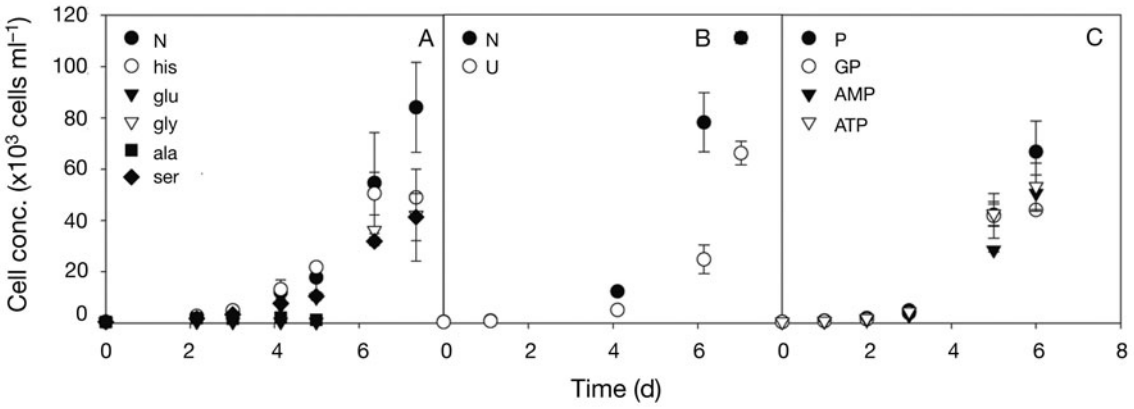

Fig. 2. *Coccolithus braarudii.* Cell density versus time when grown on different nitrogen (A) and (B), and phosphorus (C) sources. Values are the average of 2 replicates; error bars show SD of replicates and not visible error bars indicate a smaller SD than the symbol. N = nitrate, his = histidine, glu = glutamic acid, gly = glycine, ala = alanine, ser = serine, U = urea, P = phosphate, GP = glycerophos-phate, AMP = adenosine monophosphate, ATP = adenosine triphosphate

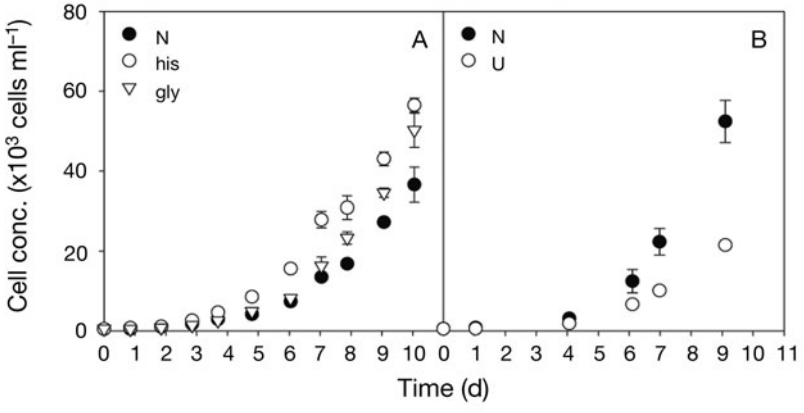

Fig. 3. *Calcidiscus leptoporus.* Cell density versus time when grown on different nitrogen sources. Values are the average of 2 replicates, error bars show SD of replicates and not visible error bars indicate a smaller SD than the symbol. N = nitrate, his = histidine, gly = glycine, U = urea

P-experiment

Emiliania huxleyi grew on all tested organic phosphorus sources, but was not tested on AMP (Fig. 1C and Table 1). Unabated growth of *E. huxleyi* on AMP has, however, been shown (Shaked et al. 2006). Growth rates on glycerophosphate and ATP were statistically equal to those of phosphate (ANOVA, Tukey multiple comparison test, $p > 0.05$) (Table 2), whereas those on NAD were lower (ANOVA, Tukey multiple comparison test, $p < 0.001$) (Table 2). *Coccolithus braarudii* did not grow on NAD during the acclimation phase. Growth rates of *C. braarudii* on glycerophosphate, AMP and ATP were all high and did not differ significantly from each other or from the control (ANOVA, Tukey multiple comparison test, $p > 0.05$) (Fig. 2C and Table 3).

Interspecies comparison

For easier comparison between the species, the growth rates on different nutrient sources were normalized to the respective growth rates on nitrate or phosphate (controls). Normalized growth rates were sometimes higher than 100%, because growth rates in controls were not always the highest (Fig. 4). All 3 species grew well on the basic amino acid L-histidine (Fig. 4A), but while normalized growth of *Emiliania huxleyi* and *Coccolithus braarudii* was 89% and 77%, respectively, the normalized growth rate of *Calcidiscus leptoporus* on L-histidine was 113% (Fig. 4A). Only *E. huxleyi* was able to grow on the acidic amino acid L-glutamic acid (64 ± 1%) (Fig. 4A). Growth on the 3 non-polar amino acids showed large species specific differences (Fig. 4A). All 3 species grew well on glycine, with the growth rate of *C. leptoporus* surpassing that on nitrate (Fig. 4A), but the other non-polar amino acids (Lalanine and L-proline) supported the growth of *E. huxleyi* only (Fig. 4A). The polar amino acid Lserine was bioavailable for *E. huxleyi* and *C. braarudii* (103%± 5% and 78%± 7%, respectively), and relative growth rates were statistically the same (*t*-test, $p > 0.01$, $n = 2$) (Table 5). L-serine did not support growth of *C. leptoporus*. The growth on urea did not differ significantly between the 3 species (ANOVA, Tukey multiple comparison test, $p > 0.05$) (Table 5). *E. huxleyi* and *C. braarudii* grew equally well on glycerophosphate and ATP (*t*-test, each $p > 0.05$, $n = 2$) (Fig. 4C and Table 5). NAD was not utilizable by *C. braarudii*, but *E. huxleyi* was able to grow on NAD.

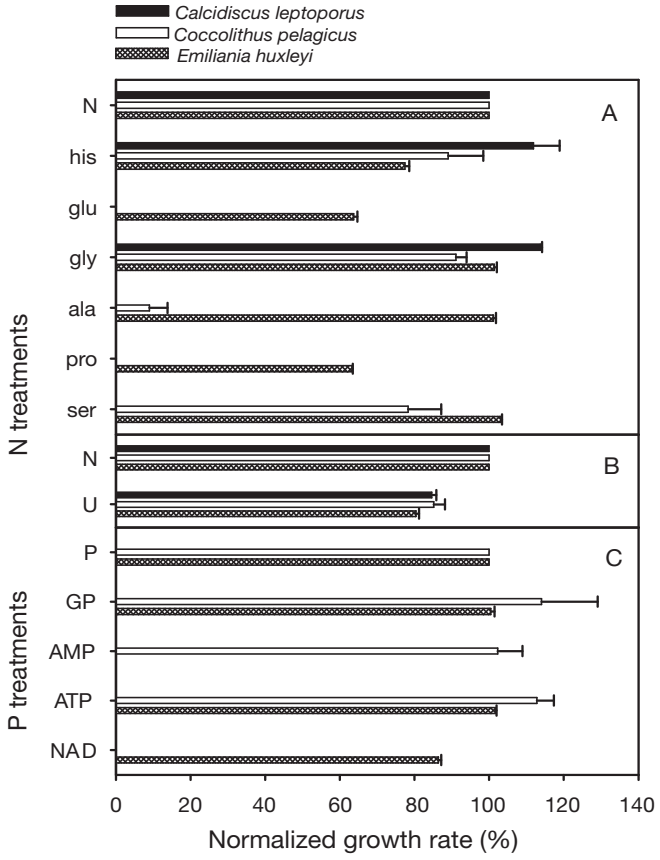

Fig. 4. *Emiliania huxleyi*, *Coccolithus braarudii*, and *Calcidiscus leptoporus.* Growth rates in the different nitrogen (A,B) and phosphorus (C) sources normalized to the growth rates on inorganic nutrients (controls). Values are the average of 2 replicates; error bars show SD of replicates; a not visible error bar indicates a smaller SD than the symbol. N = nitrate, his = histidine, glu = glutamic acid, gly = glycine, ala = alanine, pro = proline, ser = serine, U = urea, P = phosphate, GP = glycerophosphate, AMP = adenosine monophosphate, ATP = adenosine triphosphate, NAD = β-nicotinamide adenine dinucleotide

Table 5. *Emiliania huxleyi*, *Coccolithus braarudii*, and *Calcidiscus leptoporus.* Significance test results (see text) for normalized growth rates in the different nitrogen and phosphorus sources. Results only shown for the nutrients which did provide growth of the species. –: not tested due to same nutrient source or growth rates from different experiments and therefore not comparable. Level of significance ***p < 0.001, **p < 0.01, *p < 0.05, empty cell: p > 0.05. N = nitrate, his = histidine, glu = glutamic acid, gly = glycine, ala = alanine, pro = proline, ser = serine, U = urea, P = phosphate, GP = glycerophosphate, AMP = adenosine monophosphate, ATP = adenosine triphosphate, NAD = β-nicotinamide adenine dinucleotide, *E.hux* = *Emiliania huxleyi*, *C.bra* = *Coccolithus braarudii*, *C.lep* = *Calcidiscus leptoporus*, nd = no data for one or both of the compared species

	N	his	glu	gly	ala	pro	ser	U	P	GP	AMP	ATP	NAD
E.hux vs *C.bra*	–		***		***	nd	*		–		nd		***
E.hux vs *C.lep*	–	*	nd		nd	nd	nd		–	nd	nd	nd	nd
C.bra vs *C.lep*	–		nd		nd	nd	nd		–	nd	nd	nd	nd

DISCUSSION

Nutrient availability is an important factor in the distribution and ecological success of phytoplankton. Our experiments revealed that all 3 species were able to utilize some organic nitrogen and phosphorus sources, but that the ability to grow on specific organic substrates varied between the 3 species. Differences in the ability to utilize organic nutrients may reflect differences in nutrient uptake mechanisms or differences in metabolic utilization. A substrate may be transported into the cell en bloc by specialized transport systems, or, alternatively, the organic substrate may be cleaved by ectoenzymes at the cell surface. In the latter case only the utilizable molecule is transported into the cell. Once inside the cell the molecule has to be metabolized, along either standard or specialized metabolic pathways, to be available for growth. Very little is known about the specific utilization mechanisms of organic nutrients by coccolithophores, but our results allow some speculations.

Three different amino acid transport systems are known: one for basic, one for acidic, and a third for non-polar and polar amino acids (Antia et al. 1991). Alternatively, amino acids are utilized via ectoenzymatic decomposition using amino acid oxidase (Palenik & Morel 1990a). *Emiliania huxleyi* (strain 12-1) produces no amino acid oxidase, but other prymnesiophytes (*Prymnesium parvum* and *Prymnesium calatheiferum*) including a coccolithophore, *Pleurochrysis carterae*, do (Palenik & Morel 1990b). Amino acid oxidase in *P. carterae* cleaves only L-alanine and most C4 or longer L-amino acids, but cannot utilize glycine, or L-serine (Palenik & Morel 1990a). The expression of amino acid oxidase seems to be related to inorganic nitrogen concentrations (Palenik & Morel 1990a, Mulholland et al. 1998): the enzyme activity is enhanced under low inorganic nutrient concentration (Mulholland et al. 1998) as in our N-experiments. In our experiment *E. huxleyi* grew on all tested amino acids including glycine, L-serine, and L-proline (Fig. 4A), suggesting, by extension from results with *P. carterae*, that *E. huxleyi* does not rely on the ectoenzyme amino acid oxidase. Instead we propose that *E. huxleyi* has all 3 transport systems for amino acids. Based on the results that *Coccolithus braarudii* and *Calcidiscus leptoporus* did not grow on L-alanine, but grew on glycine (Fig. 4A), we infer that neither of these species produced amino acid oxidase. Neither *C. braarudii* nor *C. leptoporus* were able to

utilize the acidic amino acid L-glutamic acid (Fig. 4A), but it remains to be tested if they lack the respective transport system or the ability to metabolize L-glutamic acid. The utilization of the non-polar amino acid glycine by all 3 species (Fig. 4A) indicates that the transport system for non-polar/polar amino acids exists in all 3 species. However, the ability to metabolize these polar and non-polar amino acids appears species specific; only *E. huxleyi* grew on all 4 of them (Fig. 4A).

Urea, a small, neutral molecule that can diffuse passively into cells, may be taken up via an active transport system, or may be cleaved by urease (Antia et al. 1991). Urease requires nickel (Price & Morel 1991), and as *Emiliania huxleyi* (strain CCMP374) has been shown to be nickel and nitrogen co-limited while growing on urea (Palenik & Koke 1995), it may be assumed that *E. huxleyi* uses urease. Nothing is known about nickel and nitrogen co-limitation for *Coccolithus braarudii* and *Calcidiscus leptoporus*, but both utilized urea (Fig. 4B), probably using one of the enzymes known to cleave urea, urease or ATP:urea amidolyase (Leftley & Syrett 1973).

Phosphomonoesters and nucleotides are thought to be utilized via ectoenzymatic degradation. Alkaline phosphatase, which is very common in marine algae (Kuenzler & Perras 1965) including *Emiliania huxleyi* (Dyhrman & Palenik 2003, Xu et al. 2006), cleaves phosphomonoesters like glycerophosphate. As both *E. huxleyi* and *Coccolithus braarudii* grew on glycerophosphate (Fig. 4C) it appears likely that both produce alkaline phosphatase. Both also appear to produce the ectoenzyme 5′-nucleotidase, which cleaves nucleotides like ATP (Fig. 4C).

Overall our study suggests that coccolithophores have certain transport systems in common, like those for basic and non-polar/polar amino acids, and that a variety of enzymes, like urease, alkaline phosphatase and 5′-nucleotidase are also present in many or all coccolithophores. However, the utilization of organic nutrients appears species-specific.

Our coccolithophores did not grow under axenic conditions, and bacteria most likely contributed to nutrient cycling during experiments. However, bacteria concentrations were kept low, and the potential microbial impact is estimated to be negligible. A back-of-the-envelope calculation indicates that bacteria could not have been responsible for recycling enough of the organic nutrients to support the observed growth of coccolithophores on inorganic, recycled nutrients. Cultured bacteria from seawater collected in Santa Rosa Sound, Pensacola, Florida released ~10 µg N l^{-1} of inorganic nitrogen compounds in a 72 h period by a bacterial density increase of ~1.7 × 10^6 cells ml^{-1} (Jørgensen et al. 1999). The draw-down of nitrogen in our cultures can be calculated from the increase in cell numbers and the nitrogen content per cell. With a cellular N-content of 1.3 to 1.8 pg N $cell^{-1}$ for *E. huxleyi* (Riegman et al. 2000, Langer & Benner 2009), of 23 to 44 pg N $cell^{-1}$ for *C. braarudii* (unpubl. data), and of 9 to 13 pg N $cell^{-1}$ for *C. leptoporus* (unpubl. data), the minimum amount of nitrogen used by the 3 species can be calculated as 99.9 µg N for *E. huxleyi*, 46.1 µg N for *C. braarudii*, and 16.3 µg N for *C. leptoporus* in the 50 ml experiment bottles. This equals ~2000 µg N l^{-1} for *E. huxleyi*, ~1000 µg N l^{-1} for *C. braarudii*, and ~300 µg N l^{-1} for *C. leptoporus*.

If *Emiliania huxleyi* released a usable amount of DON (Suratman et al. 2008) during the pretreatment of the media (see 'Material and methods'), this could have been utilized by coccolithophores during experiments and the resulting growth should have been visible in all N-experiments. However, we observed no growth of *Coccolithus braarudii* and *Calcidiscus leptoporus* in some AA-experiments.

Concentrations of organic nutrients added were appreciably higher than those found in the ocean (generally <1 µM) and care has to be taken when extrapolating results to *in situ* conditions. Our goal was, however, to investigate the potential ability of these different coccolithophores to utilize organic nutrients. Our results demonstrate interspecific differences in the ability of coccolithophores to grow on specific organic nutrients.

As organic nutrients are not distributed homogenously in the ocean, the difference in the ability to take advantage of organic nutrients helps explain geographic distribution patterns of individual species. Geographical distribution of a species is determined by a suite of environmental factors, including temperature, salinity, mixing depth and stratification, as well as light and nutrient availability. This complexity makes it difficult to relate one of these factors, e.g. bioavailability of nutrients to geographic distribution of a species. Moreover, data on the distribution of specific organic nutrients, e.g. specific dissolved amino acids or ATP in different parts of the ocean are still too rare (but see Azam & Hodson 1977, Antia et al. 1991, Bronk 2002) to investigate the correlation between distribution patterns of coccolithophore species and availability of specific organic nutrients. Nevertheless, some general patterns are apparent. First, *Emiliania huxleyi* was able to grow on all offered nitrogen and phosphorous sources, suggesting that *E. huxleyi* growth is rarely limited by nitrogen or phosphorus. This fits observations that *E. huxleyi* is known as a very cosmopolitan species that may also thrive under low inorganic nutrient concentrations (Andruleit & Rogalla 2002, Hagino & Okada 2006). Second, the difference between *Calcidiscus leptoporus* and *Coccolithus braarudii* in their ability to use the most common organic nitrogen com-

pounds (3 vs. 5 out of 7 tested, respectively) (Fig. 4) suggests a greater dependence of *C. leptoporus* on inorganic nitrogen. This is reflected in a stronger correlation between the concentration of inorganic nutrients in the surface water and the abundance of *C. leptoporus* coccoliths in sediments than between the inorganic nutrient concentrations and the abundance of *C. pelagicus* coccoliths in sediments (Boeckel et al. 2006). Furthermore, in the equatorial and subequatorial Pacific Ocean and the Arabian Sea, *C. leptoporus* does not occur under low inorganic nutrient concentrations, whereas *E. huxleyi* does (Andruleit & Rogalla 2002, Hagino & Okada 2006).

Emiliania huxleyi has often been used as a model organism for carbonate pump models (e.g. Westbroek et al. 1993) and as a representative for the functional group of calcifying marine organisms (Iglesias-Rodriguez et al. 2002, Moore et al. 2002). Our results suggest that while direct utilization of organic nutrients appears to be widespread in coccolithophores, *E. huxleyi* does not fit criteria as a representative of coccolithophores with regard to its ability to utilize organic nutrients. The response to environmental conditions is one criterion for representativeness of a species; another is the phylogenetic relationship of that species. *E. huxleyi* fits neither criterion, as it belongs to an out-group of the coccolithophores (Sáez et al. 2003). This may explain why models that use '*E. huxleyi*' characteristics as a model organism for coccolithophores are not able to reproduce natural coccolithophore distributions well. Discrepancies between models and observations are especially large where *E. huxleyi* is not the dominant coccolithophore (Gregg & Casey 2007).

Finding one representative species for coccolithophores may, however, prove impossible. *Coccolithus pelagicus* and *Calcidiscus leptoporus* have a strong phylogenetic relationship to most other coccolithophores (Sáez et al. 2003), but exhibit clear differences in their response to environmental conditions, not only in the utilization of organic nutrients, but also in their response to changing carbonate chemistry of seawater (Langer et al. 2006). Possibly, interspecific physiological diversity within coccolithophores is too great to appoint a clear representative species. Furthermore, strains of *Emiliania huxleyi* respond different to environmental parameters like temperature, salinity (Fisher & Honjo 1989), and carbonate chemistry (Langer et al. 2009). Characteristics of a model species might have to be chosen depending on characteristics central to the question investigated. Further experiments on the physiology of ecologically prominent coccolithophores are needed to appreciate the functional diversity of coccolithophores.

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LITERATURE CITED

Andruleit H, Rogalla U (2002) Coccolithophores in surface sediments of the Arabian Sea in relation to environmental gradients in surface waters. Mar Geol 186:505–526

Antia NJ, Harrison PJ, Oliveira L (1991) The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia 30:1–89

Azam F, Hodson RE (1977) Dissolved ATP in the sea and its utilization by marine bacteria. Nature 267:696–698

Baumann KH, Böckel B, Frenz M (2004) Coccolith contribution to South Atlantic carbonate sedimentation. In: Thierstein HR, Young JR (eds) Coccolithophores: from molecular processes to global impact. Springer, Berlin, p 367–402

Benitez-Nelson CR (2000) The biogeochemical cycling of phosphorus in marine systems. Earth Sci Rev 51:109–135

Berman T, Bronk DA (2003) Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. Aquat Microb Ecol 31:279–305

Boeckel B, Baumann KH, Henrich R, Kinkel H (2006) Coccolith distribution patterns in South Atlantic and Southern Ocean surface sediments in relation to environmental gradients. Deep-Sea Res I 53:1073–1099

Bronk DA (2002) Dynamics of DON. In: Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter. Academic Press, London, p 153–247

Buitenhuis ET, De Baar HJW, Veldhuis MJW (1999) Photosynthesis and calcification by *Emiliania huxleyi* (Prymnesiophyceae) as a function of inorganic carbon species. J Phycol 35:949–959

Dittmar T, Fitznar HP, Kattner G (2001) Origin and biogeochemical cycling of organic nitrogen in the eastern Arctic Ocean as evident from D- and L-amino acids. Geochim Cosmochim Acta 65:4103–4114

Dyhrman ST, Palenik B (2003) Characterization of ectoenzyme activity and phosphate-regulated proteins in the coccolithophore *Emiliania huxleyi.* J Plankton Res 25: 1215–1225

Engel A, Delille B, Jacquet S, Riebesell U, Rochelle-Newall E, Terbrüggen A, Zondervan I (2004) Transparent exopolymer particles and dissolved organic carbon production by *Emiliania huxleyi* exposed to different CO_2 concentrations: a mesocosm experiments. Aquat Microb Ecol 34: 93–104

Fisher NS, Honjo S (1989) Intraspecific differences in temperature and salinity responses in the coccolithophore *Emiliania huxleyi.* Biol Oceanogr 6:355–361

Glibert PM, Michael B, Trice TM, Lane L (2005) Urea in the tributaries of the chesapeake and coastal bays of Maryland. Water Air Soil Pollut 160:229–243

Gregg WW, Casey NW (2007) Modeling coccolithophores in the global oceans. Deep-Sea Res II 54:447–477

Hagino K, Okada H (2006) Intra- and infra-specific morphological variation in selected coccolithophore species in the equatorial and subequatorial Pacific Ocean. Mar Micropaleontol 58:184–206

Ietswaart T, Schneider PJ, Prins RA (1994) Utilization of organic nitrogen sources by two phytoplankton species and a bacterial isolate in pure and mixed cultures. Appl Environ Microbiol 60:1554–1560

Iglesias-Rodriguez MD, Brown CW, Doney SC, Kleypas J and others (2002) Representing key phytoplanton functional groups in ocean carbon cycle models: coccolithophorids. Global Biogeochem Cycles 16, doi:10.1029/2001GB001454

Iglesias-Rodriguez MD, Schofield OM, Batley J, Medlin LK, Hayes PK (2006) Intraspecific genetic diversity in the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae): the use of microsatellite analysis in marine phytoplankton population studies. J Phycol 42:526–536

Jørgensen NOG, Kroer N, Coffin RB, Hoch MP (1999) Relations between bacterial nitrogen metabolism and growth efficiency in an estuarine and an open-water ecosystem. Aquat Microb Ecol 18:247–261

Kuenzler EJ, Perras JP (1965) Phosphatases of marine algae. Biol Bull 128:271–284

Langer G, Benner I (2009) Effect of elevated nitrate concentration on calcification in *Emiliania huxleyi.* J Nannoplankton Res 30:77–80

Langer G, Young JR, Geisen M, Baumann Kh, Riebesell U, Thoms S (2006) Species-specific responses of calcifying algae to changing seawater carbonate chemistry. Geochem Geophys Geosyst 7:Q09006

Langer G, Nehrke G, Probert I, Ly J, Ziveri P (2009) Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry. Biogeosciences Discuss 6: 4361–4383

Leftley JW, Syrett PJ (1973) Urease and ATP:urea amidolyase activity in unicellular algae. J Gen Microbiol 77:109–115

Løvdal T, Eichner C, Grossart HP, Carbonnel V, Chou L, Martin-Jézéquel V, Thingstad TF (2008) Competition for inorganic and organic forms of nitrogen and phosphorous between phytoplankton and bacteria during an *Emiliania huxleyi* spring bloom. Biogeosciences 5:371–383

Moore JK, Doney SC, Kleypas JA, Glover DM, Fung IY (2002) An intermediate complexity marine ecosystem model for the global domain. Deep-Sea Res II 49:403–462

Mulholland MR, Glibert PM, Berg GM, Van Heukelem L, Pantoja S, Lee C (1998) Extracellular amino acid oxidation by microplankton: a cross-ecosystem comparison. Aquat Microb Ecol 15:141–152

Palenik B, Henson SE (1997) The use of amides and other organic nitrogen sources by the phytoplankton *Emiliania huxleyi.* Limnol Oceanogr 42:1544–1551

Palenik B, Koke JA (1995) Charakterization of a nitrogen-regulated protein identified by cell surface biotinylation of a marine phytoplankton. Appl Environ Microbiol 61: 3311–3315

Palenik B, Morel FMM (1990a) Amino acid utilization by marine phytoplankton: a novel mechanism. Limnol Oceanogr 35:260–269

Palenik B, Morel FMM (1990b) Comparison of cell surface L-amino acid oxidases from several marine phytoplankton. Mar Ecol Prog Ser 59:195–201

Price NM, Morel FMM (1991) Colimitation of phytoplankton growth by nickel and nitrogen. Limnol Oceanogr 36: 1071–1077

Riebesell U, Zondervan I, Rost B, Tortell PD, Zeebe RE, Morel FMM (2000) Reduced calcification of marine plankton in response to increased atmospheric CO_2. Nature 407: 364–367

Riegman R, Stolte W, Noordeloos AAM, Slezak D (2000) Nutrient uptake and alkaline phosphatase (EC 3:1:3:1): activity of *Emiliania huxleyi* (Prymnesiophyceae) during growth under N and P limitation in continuous cultures. J Phycol 36:87–96

Rost B, Riebesell U (2004) Coccolithophores and the biological pump: responses to environmental changes. In: Thierstein HR, Young JR (eds) Coccolithophores: from molecular processes to global impact. Springer Verlag, Berlin

Sáez AG, Probert I, Geisen M, Quinn P, Young JR, Medlin LK (2003) Pseudo-cryptic speciation in coccolithophores. Proc Natl Acad Sci USA 100:7163–7168

Shaked Y, Xu Y, Leblanc K, Morel FMM (2006) Zinc availability and alkaline phosphatase activity in *Emiliania huxleyi*: implications for Zn-P co-limitation in the ocean. Limnol Oceanogr 51:299–309

Suratman S, Weston K, Jickells T, Chance R, Bell T (2008) Dissolved organic matter release by an axenic culture of *Emiliania huxleyi.* J Mar Biol Assoc UK 88:1343–1346

Westbroek P, Brown CW, van Bleijswijk J, Brownlee C and others (1993) A model system approach to biological climate forcing: the example of *Emiliania huxleyi.* Global Planet Change 8:27–46

Xu Y, Wahlund TM, Feng L, Shaked Y, Morel FMM (2006) A novel alkaline phosphatase in the coccolithophore *Emiliania huxleyi* (Prymnesiophyceae) and its regulation by phosphorus. J Phycol 42:835–844

Yamashita Y, Tanoue E (2003) Distribution and alteration of amino acids in bulk DOM along a transect from bay to oceanic waters. Mar Chem 82:145–160

Ziveri P, Baumann KH, Böckel B, Bollmann J, Young JR (2004) Biogeography of selected Holocene coccoliths in the Atlantic Ocean. In: Thierstein HR, Young JR (eds) Coccolithophores from molecular processes to global impact. Springer Verlag, London, p 403–428

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