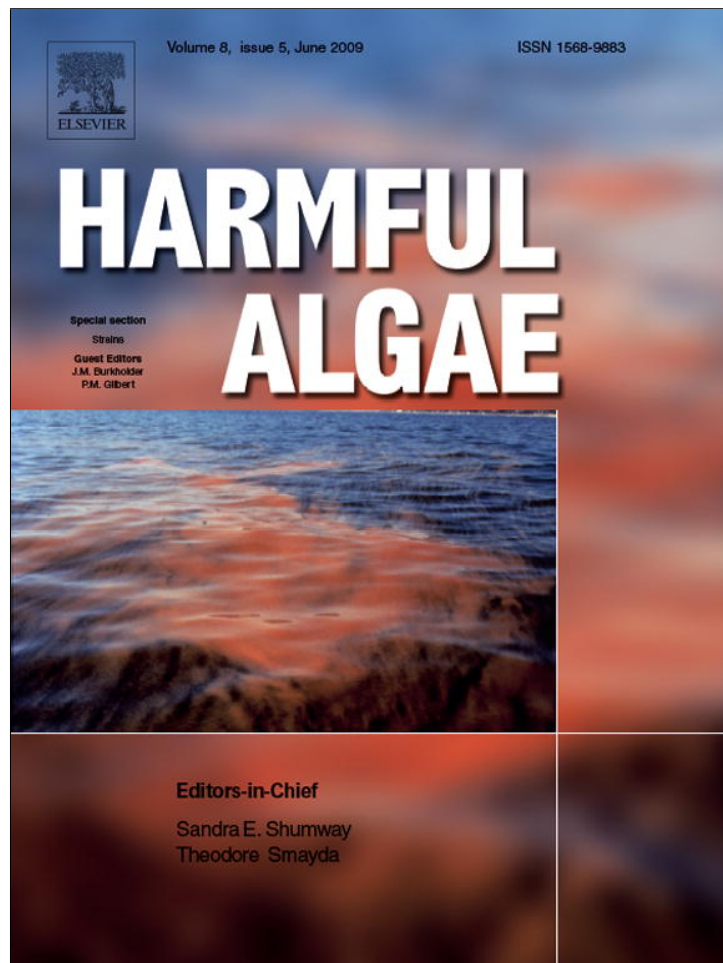


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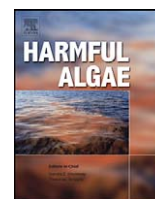
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Inter-strain differences in nitrogen use by the coccolithophore *Emiliana huxleyi*, and consequences for predation by a planktonic ciliate

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ABSTRACT

Four strains of the coccolithophore *Emiliana huxleyi* (CCMP strains 370, 373, 374, 379) were tested for their ability to grow on various nitrogen sources. All strains grew on ammonium, nitrate, and urea, although growth of CCMP379 on urea was low. Responses to other dissolved organic nitrogen (DON) sources varied. CCMP379 did not grow on any DON source other than urea. All other strains grew on one of the two tested amino acids: CCMP370 and CCMP373 on glutamine, and CCMP374 on alanine. All three of these strains also grew on hypoxanthine; in addition, two grew well on acetamide and one on ethanolamine. *E. huxleyi* strains also differed in their susceptibility to predation by the ciliate *Strobilidium* sp. CCMP374 was ingested at substantially higher rates than CCMP373 regardless of *E. huxleyi* growth condition. Ciliate feeding rates also depended on *E. huxleyi* growth condition. For CCMP374, feeding rates were $2\times$ higher on growing *E. huxleyi* cells than on non-growing cells (average 27.5 versus 15.6 cells ciliate⁻¹ h⁻¹, respectively). For CCMP373, a relationship between *E. huxleyi* growth rate and ciliate feeding rate was not evident, but *E. huxleyi* grown on some N sources (ammonium, nitrate, urea) were ingested at consistently higher rates than *E. huxleyi* grown on other sources (ethanolamine, glutamine). Interstrain differences in the ability to utilize DON and resist predation may contribute to maintenance of high genetic diversity within this cosmopolitan, bloom-forming species.

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1. Introduction

The haptophyte *Emiliana huxleyi* is the dominant bloom-forming coccolithophore in the present-day ocean. The importance of the species to ocean carbon cycling and calcium carbonate deposition, as well as to climate regulation through the production of dimethylsulfide, has been investigated intensively (e.g. Harris, 1996; Holligan et al., 1993). *E. huxleyi* is sometimes considered a harmful algal bloom (HAB) species because persistent biomass accumulations can disrupt ecosystem structure and function (Napp and Hunt, 2001). Blooms of *E. huxleyi* are often associated with stratified surface waters; considerable research has focused on the species' adaptation to high light and low nutrient conditions (reviewed by Paasche, 2002). Reduced zooplankton grazing has been implicated in bloom formation and persistence (Merico et al., 2004; Olson and Strom, 2002), while viral lysis may be important in bloom demise (Wilson et al., 2002 and references therein).

E. huxleyi has long been known to harbor a high degree of intraspecific variability. The species comprises several morphotypes that have discrete geographic distributions (reviewed by Paasche, 2002). A high degree of inter-strain diversity has been

found for numerous biochemical and physiological properties, including pigment, alkenone and thiol composition (Conte et al., 1998; Dupont et al., 2004; Stolte et al., 2000), constitutive and induced enzyme activity (Dyhrman and Palenik, 2003; Steinke et al., 1998), and growth response to temperature (Brand, 1982). *E. huxleyi* does not contain sufficient small subunit ribosomal RNA diversity to be considered a multi-species complex (Medlin et al., 1996). However, diversity within more rapidly evolving genes is high, and relates to morphological distinctions that historically have been used to group different strains (Medlin et al., 1996; Schroeder et al., 2005). High microsatellite diversity within bloom populations indicates active sexual reproduction and an ability to adapt rapidly to environmental variation (Iglesias-Rodríguez et al., 2006).

A fundamental question for species such as *E. huxleyi* is how high inter-strain diversity contributes to the range of ecological niches that the species can occupy. We chose to investigate two aspects of this question. First, we examined the use of dissolved organic nitrogen (DON) for growth by four *E. huxleyi* strains. Use of DON for growth by HAB species is thought to be an adaptation that contributes to their success (Glibert et al., 2005; Smayda, 1997). Growth of *E. huxleyi* on DON is well known (Palenik and Henson, 1997 and references therein) and could contribute to the species' ability to thrive in stratified, low-nitrate waters where regeneration of DON probably constitutes much of the N supply (Lessard

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Table 1
Isolation date and collection location for *Emiliana huxleyi* strains used in the present study.

Strain	Isolation date	Collection location	Strain synonyms
CCMP370	1959	59.50°N 10.60°E Oslo Fjord, Norway	451B, F451
CCMP373	1960	32.17°N 64.50°W Sargasso Sea	BT6, CSIRO-CS-57
CCMP374	1990	42.50°N 69.00°W ^a Gulf of Maine	89E, CCMP1949
CCMP379	1957	50.17°N 4.25°W ^a English Channel	92A, P-92A, UTEX1061, CCAP/1A, Plymouth#2

^a Approximate.

et al., 2005). However, a systematic investigation of inter-strain variability in DON use has not been conducted. Second, we studied the relationship between use of different N sources for growth and susceptibility to predation by a planktonic ciliate. This work builds on the observation that different *E. huxleyi* strains are not grazed equivalently by planktonic protists (Strom et al., 2003a). Interactions between strain identity, growth conditions, and predation pressure will have important consequences for the range of ecological niches occupied by *E. huxleyi*.

2. Materials and methods

2.1. Culture methods

Four axenic strains of *E. huxleyi* (CCMP370, CCMP373, CCMP374, CCMP379) were obtained from the Center for the Culture of Marine Phytoplankton, Bigelow Laboratories, Boothbay Harbor, ME, USA (Table 1). Phytoplankton were maintained on f/2 medium without added silicate, prepared from local seawater (salinity = 30). Cultures were grown at 15 °C in moderate light (45–60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) on a 12L:12D cycle. All growth and feeding experiments were conducted under these light and temperature conditions unless otherwise noted. Sterile transfer methods were used to maintain *E. huxleyi* cultures in a bacteria-free state. Periodic checks for bacteria using 4'6'-diamidino-2-phenylindole (DAPI) staining and epifluorescence microscopy confirmed that cultures were bacteria-free throughout the study. All strains were predominantly non-calcifying under our culture conditions.

The naked choreotrich ciliate *Strobilidium* sp. was isolated from northern Puget Sound waters and maintained on a mixed phytoplankton diet. Ciliates were cultured on 'ciliate medium' (Gifford, 1985) prepared from local seawater, and grown at 15 °C in dim light (<5 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) on a 12L:12D cycle. Stocks of ciliates for feeding experiments were grown on *Isochrysis galbana* plus *Mantoniella squamata*.

2.2. N source-growth experiments

N-limited batch cultures of *E. huxleyi* were produced by growing cells in f/10 without added nitrate. Growth in these 1-l cultures was supported only by nitrogen occurring naturally in local seawater. Nitrate concentrations in the source seawater used for media preparation ranged from 10 to 23 μM during the period of these and the following experiments (January through June; Shannon Point Marine Center water quality database). In vivo fluorescence was measured each day at noon ± 2 h by sterile removal of a small volume of culture, which was placed in a Turner 10-AU fluorometer. When cultures reached early stationary phase (2–3 d after cessation of exponential growth), 29.5-ml aliquots from each 1-l culture were transferred to a series of eighteen 35-ml glass tubes. Cell densities at this time ranged from 3.5 to 7.9×10^5 cells ml^{-1} . Different nitrogen sources were then added to duplicate tubes in 0.5-ml volumes of ultrapure ('Nanopure') water for a concentration of 20 μM . Nitrogen sources were inorganic (ammonium chloride, sodium nitrate), amino acids (l -alanine, l -glutamine), and other

organic compounds (urea, acetamide, ethanolamine, hypoxanthine). Control (no N) tubes received 0.5 ml ultrapure water. Growth on the various N sources was monitored daily by in vivo fluorescence; tubes were placed directly in the fluorometer after inverting several times to mix cells. Specific growth rates (d^{-1}) were calculated as the slopes of $\ln(\text{fluorescence})$ versus time. Slopes were determined from least-squares regression; only the linearly increasing portion of each relationship (i.e. the period of exponential growth) was used in the analysis. Growth rates indistinguishable from zero were identified by computing 95% confidence intervals around means for each N source treatment and strain.

2.3. Ciliate feeding experiments

N-limited, 1-l batch cultures of *E. huxleyi* CCMP373 and CCMP374 were grown as above until cultures reached early stationary phase. Aliquots (29.9 ml) from each 1-l culture were placed in glass tubes; N sources (in quadruplicate for each strain) were added in 0.1-ml volumes of ultrapure water for a concentration of 20 μM . N sources were chosen to encompass a range of *E. huxleyi* growth responses as determined in the N source-growth experiments described above. Both strains also had a no-N control treatment. Fluorescence in the N-enriched tubes was monitored daily as above. After several days of growth (3 d: CCMP374; 5 d: CCMP373), the cell density in each tube was determined by haemocytometer counting and ciliate feeding rate measurements were made. For CCMP374, all cultures were growing exponentially at the 3-d sampling except the no-N and l -alanine treatments, neither of which grew at all over the full 7-d test period. For CCMP373 at 5 d, the no-N control showed no growth, the ammonium and nitrate treatments were 1 d into stationary phase, the ethanolamine and l -glutamine treatments were in early exponential phase, and the urea treatment was in mid-exponential phase. Growth rates over the 2-d period preceding the feeding experiments were calculated from in vivo fluorescence as described above.

Stock cultures of *Strobilidium* sp. were gently reverse-concentrated through a 10- μm mesh sieve, then resuspended in clean ciliate medium to reduce background prey levels and to allow ciliates to digest any residual prey in their food vacuoles. This procedure was done at 9 am; feeding rate measurements began at approximately 3 pm. Resuspended ciliate stock culture (20 ml) was placed in 60-ml polycarbonate bottles and *E. huxleyi* culture was added to achieve a concentration of approximately 4×10^5 cells ml^{-1} ; we used one 20-ml ciliate sample per replicate tube of *E. huxleyi*. Ciliates were allowed to feed for 9 min (CCMP374) or 15 min (CCMP373). These time periods were chosen based on preliminary experiments to allow measurable feeding but not complete filling of food vacuoles (e.g. Strom et al., 2003b).

Experiments were terminated by fixing the entire contents of each experimental bottle using a procedure modified from Sherr and Sherr (1993). Contents were added to 200 μl alkaline Lugol's solution, immediately post-fixed by addition of 500 μl borate-buffered formalin, then destained by addition of 700 μl 3% sodium thiosulfate solution. Slides were prepared within 2 h of sample fixation by filtering fixed samples through 1.0 μm pore-size

polycarbonate filters under gentle vacuum; cells were stained with DAPI during slide preparation. Slides were stored at -20°C until analysis by epifluorescence microscopy within 6 weeks of slide preparation. Ciliates on each slide were located using UV excitation (bandpass 340–389 nm; long-pass 430 nm) to visualize DAPI-stained nuclei. *E. huxleyi* cells in food vacuoles of individual ciliates were then enumerated under blue light excitation (bandpass 450–490 nm; long-pass 520 nm), which elicits bright red fluorescence in ingested *E. huxleyi* cells. Food vacuole contents of at least 100 ciliates per slide were enumerated. Feeding rates were calculated from average food vacuole contents per ciliate/incubation time, and were corrected for residual vacuole contents from maintenance diets. Residual vacuole contents were estimated from samples taken from reuspended ciliate stock cultures just before the feeding rate measurements were made. Residual prey levels in food vacuoles were 28% of the lowest experimental feeding level for CCMP374, and 43% of the lowest feeding level for CCMP373. Significant effects of *E. huxleyi* N source on ciliate feeding rates were evaluated with one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) post hoc test to identify homogeneous subsets.

3. Results

Three of the four studied *E. huxleyi* strains grew at similar rates in 1-l batch cultures of f/10 without added nitrate (CCMP370: 0.67 d^{-1} ; CCMP374: 0.72 d^{-1} ; CCMP379: 0.66 d^{-1}), while CCMP373 grew more slowly (0.43 d^{-1}). Rates of growth in 30-ml tubes during N source assays were lower (Table 2), probably due to the high initial cell densities in these tubes combined with the moderate ($20\ \mu\text{M}$) N addition levels. Ability to grow on different N sources varied among *E. huxleyi* strains (Fig. 1). All grew on ammonium, nitrate, and urea, although the growth rate of CCMP379 on urea was low and a reduced cell yield was obtained relative to ammonium and nitrate (Fig. 1D, Table 2). Furthermore, CCMP379 grew very little or not at all on other organic nitrogen sources (Fig. 1D). We observed that CCMP379 cells tended to clump upon reaching stationary phase, leading to sharp reductions in fluorescence (Fig. 1D). All other strains grew on one of the two tested amino acids: CCMP370 and CCMP373 on glutamine and CCMP374 on alanine. No strain grew on both. Hypoxanthine supported relatively high growth in all strains except CCMP379. Acetamide supported relatively high growth in CCMP370 and CCMP374; growth on ethanolamine was high only for CCMP374.

In preparation for the ciliate feeding rate measurements, two strains (CCMP373, CCMP374) were grown a second time on a subset of the original range of N sources. Agreement with the first N source assay was good (Table 2). Similar growth rates were observed for CCMP374 on all N sources except alanine. While growth on alanine was moderate during the first assay, almost no growth occurred during the second experiment. For CCMP373, rates of growth were

Table 2
Growth rates (d^{-1}) of 4 *Emiliania huxleyi* strains on different nitrogen sources.

N source	<i>Emiliania huxleyi</i> CCMP strain designation					
	370	374 Exp. 1	374 Exp. 2	373 Exp. 1	373 Exp. 2	379
Ammonium	0.15 (0.003)	0.23 (0.010)	0.25 (0.003)	0.05 (0.022)	0.29 (0.025)	0.15 (0.015)
Nitrate	0.13 (0.001)	0.21 (0.011)	0.23 (0.006)	0.10 (0.005)	0.28 (0.033)	0.18 (0.013)
Urea	0.19 (0.003)	0.33 (0.010)	0.32 (0.008)	0.09 (0.005)	0.05 (0.004)	0.07 (0.007)
L-glutamine	0.07 (0.001)	0.00 (0.006)*		0.18 (0.002)	0.57 (0.016)	-0.01 (0.020)*
L-alanine	0.01 (0.019)*	0.17 (0.029)	0.02 (0.005)	0.03 (0.001)		-0.01 (0.026)*
Ethanolamine	0.03 (0.014)	0.18 (0.018)		0.06 (0.041)*	0.10 (0.019)	-0.06 (0.050)*
Acetamide	0.12 (0.004)	0.18 (0.004)	0.23 (0.005)	0.05 (0.013)		0.03 (0.014)
Hypoxanthine	0.17 (0.018)	0.26 (0.038)		0.11 (0.016)		0.01 (0.001)

For strains 374 and 373, data from two sets of experiments are shown. Growth rates with no added nitrogen (see Section 3) were subtracted from tabulated values. Rates are averages ($n = 2$ for experiment set 1; $n = 4$ for experiment set 2) with 1 SD in parentheses. Starred values are not significantly different from zero.

Table 3

Feeding rates (cells ciliate $^{-1}\text{ h}^{-1}$) of *Strobilidium* sp. on two *Emiliania huxleyi* strains grown on different nitrogen sources.

N source	<i>E. huxleyi</i> strain	
	373	374
Ammonium	13.0 (2.5)a	28.4 (4.1)a
Nitrate	13.0 (1.6)a	28.5 (6.5)ab
Urea	12.0 (0.7)ab	26.5 (4.4)ab
L-glutamine	9.4 (2.1)bc	nd
L-alanine	nd	17.8 (0.7)bc
Ethanolamine	7.2 (2.6)c	nd
Acetamide	nd	26.5 (6.5)ab
no N	7.6 (2.2)bc	13.4 (1.4)c

CCMP strain designations are shown. Rates are averages ($n = 4$) with 1 SD in parentheses. Letters to the right of rates show homogeneous subsets within each strain, according to Tukey's HSD post hoc test. nd = not determined.

generally higher during the second experiment (Table 2), especially on ammonium, nitrate, and glutamine. During both experiments, CCMP373 required a 2–5 d induction period before growth on any organic N source commenced.

Feeding rates of the ciliate *Strobilidium* sp. were uniformly higher on *E. huxleyi* CCMP374 than on CCMP373 (Table 3). Average feeding rates ranged from 13.4 to 28.5 cells ciliate $^{-1}\text{ h}^{-1}$ on CCMP374, compared to a range of 7.2 to 13.0 cells ciliate $^{-1}\text{ h}^{-1}$ on CCMP373. For both strains, the no-N control treatments sustained the lowest or near-lowest feeding rates. For CCMP374, ciliate feeding was also low on cells from the alanine treatment; *E. huxleyi* cells in this treatment did not grow over the 7-d incubation period of this experiment. *E. huxleyi* cells from all other CCMP374 N treatments (ammonium, nitrate, urea, acetamide) were fed on at high and statistically indistinguishable rates (Table 3). Ciliate feeding rates were compared with *E. huxleyi* growth rates during the 48 h preceding the feeding experiment. Measurements of alkaline phosphatase activity (Dyhrman and Palenik, 2003) and lectin binding (Wohlford, 2006) have shown that *E. huxleyi* cell surface properties respond to changes in nutrient availability over approximately a 48-h time period. For CCMP374, there was a positive relationship between ciliate feeding rate and *E. huxleyi* growth rate during the 48 h preceding the feeding experiment (Fig. 2A). Non-growing cells were ingested at low rates while growing cells were ingested at rates nearly $2\times$ higher.

When CCMP373 was offered as prey, ciliates fed at low rates on cells from no-N treatments as well as on cells grown with either ethanolamine or glutamine (Table 3). At least some replicates in both ethanolamine and glutamine treatments had commenced growth during the 48 h preceding the ciliate feeding experiment. Thus CCMP373 did not show the same clear relationship between *E. huxleyi* growth rate and ciliate feeding rate as was evident for CCMP374 (Fig. 2). Highest feeding rates on CCMP373 were observed for cells grown on ammonium, nitrate, and urea (Table 3).

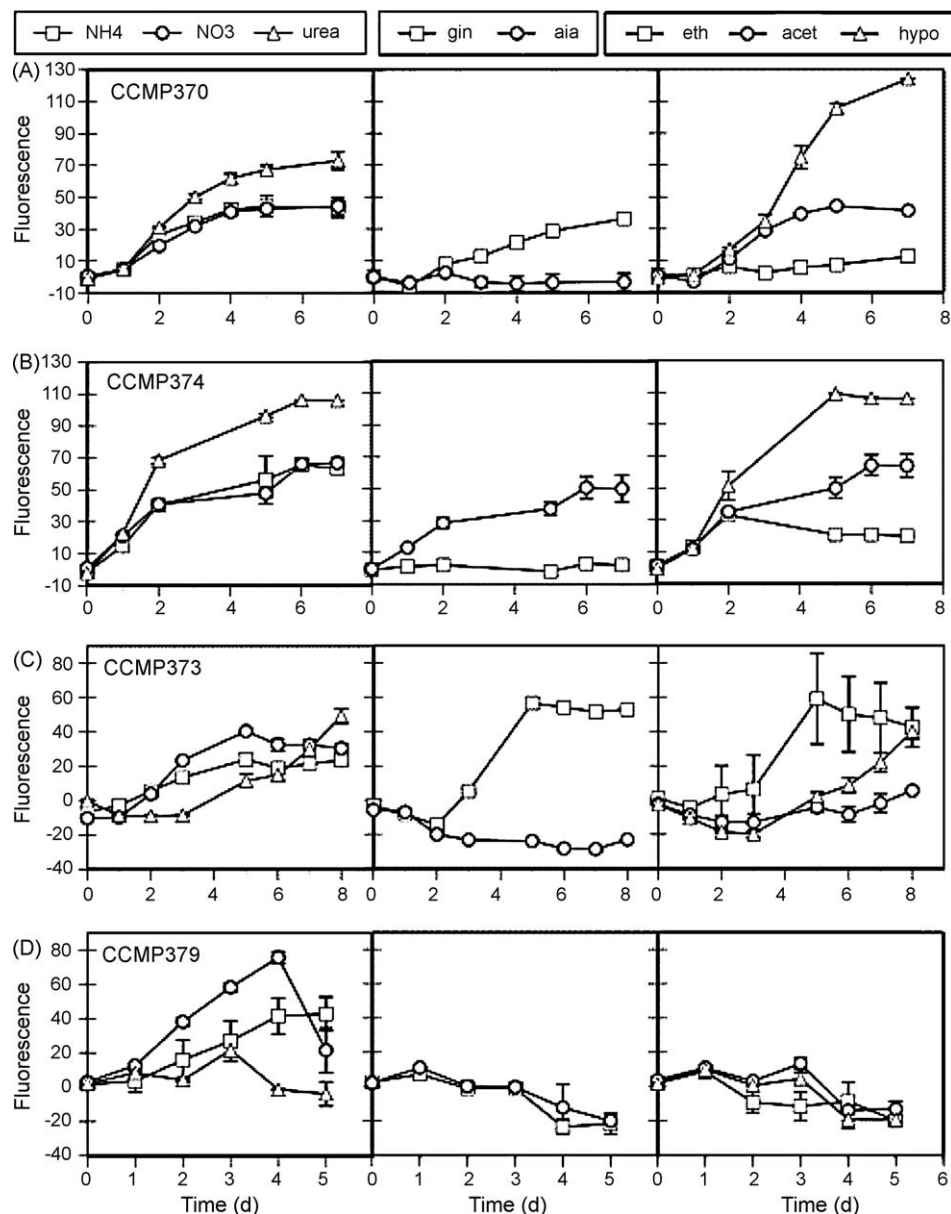


Fig. 1. Growth curves (in vivo fluorescence over time) for growth on various N sources by *Emiliania huxleyi* strains CCMP370 (A), CCMP374 (B), CCMP 373 (C), and CCMP379 (D). Fluorescence in no-N control treatments was subtracted from plotted values. Data points are averages; error bars show range of observations ($n = 2$). N source abbreviations: ala = L-alanine; gln = L-glutamine; eth = ethanolamine; acet = acetamide; hypo = hypoxanthine.

4. Discussion

Our results demonstrate extensive variability in the ability of four *E. huxleyi* strains to grow on different organic N sources. In particular, CCMP374 grew well on alanine but not glutamine, while CCMP370 and CCMP373 showed the opposite pattern. Only CCMP374 grew well on ethanolamine. CCMP379 contrasted strongly with all other strains in terms of N use, growing poorly or not at all on any organic N source, including urea.

Although a systematic survey of strain N use has not previously been conducted, *E. huxleyi* is well known to grow on DON. *E. huxleyi* blooms in two Norwegian fjords were supported by substantial uptake of urea relative to ammonium and nitrate (Fernandez et al., 1996). In laboratory tests, *E. huxleyi* strain L grew on alanine and leucine, with only slight or no growth on a number of other amino acids (Ietswaart et al., 1994). Palenik and Henson (1997) demonstrated that numerous *E. huxleyi* strains could grow on the amides acetamide and formamide and on the purine

hypoxanthine, though at varying rates depending on strain. In agreement with our results, they found good growth of CCMP373 and CCMP374 on acetamide and hypoxanthine. They also observed very slow growth of CCMP379 on acetamide. In contrast to our findings, however, they observed no growth of CCMP379 on urea and no growth of CCMP374 on any amino acid, including alanine, which supported good growth in the first of our two experiments (Table 2).

As in most studies, our strains were preconditioned by growth on nitrate (in f/2 medium, and as the dominant inorganic N species in local seawater during growth of 1-l batch cultures). Previous work (Berland et al., 1979) has shown that very long induction times (>2 weeks) can be required for *E. huxleyi* growth on amino acids to commence. Presumably this is due to the need to upregulate and synthesize new proteins for membrane transport and catabolism, although transformation of organic to inorganic (and thus usable) N sources over time by contaminating bacteria cannot be ruled out. Variations in N exposure history (due to

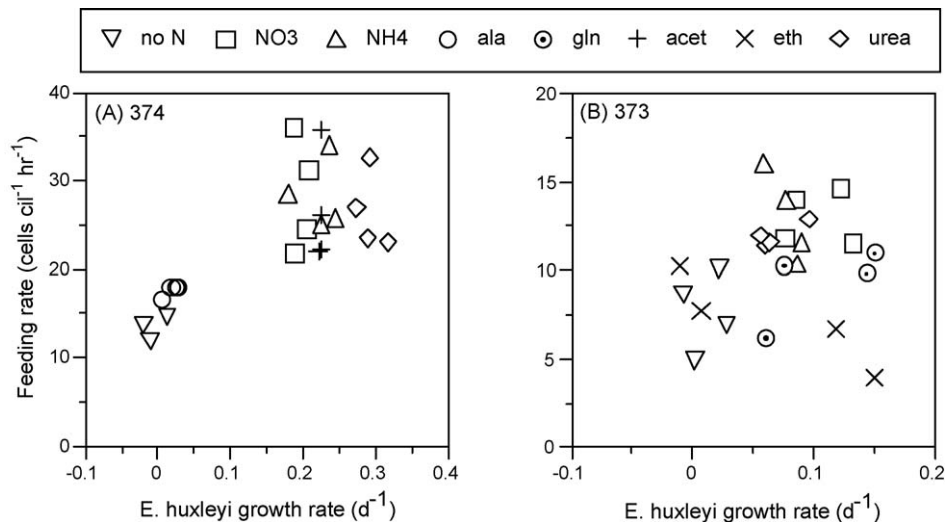


Fig. 2. Feeding rates of ciliate *Strobilidium* sp. as a function of prey growth rate. Prey were *Emiliania huxleyi* strains CCMP374 (A) and CCMP373 (B). *E. huxleyi* were grown on a variety of N sources (abbreviations as in Fig. 1); growth rates plotted here were calculated over the 48-h period preceding the feeding rate measurements. Comparisons to 24-h growth rates showed similar patterns. Note axis scale differences.

changing composition of the total available N pool in local seawater used for media preparation) could explain the differences in amino acid use that we saw between the first and second experiments for CCMP373 and CCMP374 (Table 2), as well as the differences between our results and those of Palenik and Henson (1997). In addition, the time period for induction depends on the concentration of N supplied. Our additions of 20 μ M were relatively low compared to the mM levels required for induction of glycine use in *E. huxleyi* strain BT-6 (Berland et al., 1979). Thus our findings should not be taken as proof that certain *E. huxleyi* strains are incapable of using a given N source under any set of conditions. Rather, our results compare the range of strain responses to preconditioning and organic N supply representative of a pre-bloom (high nitrate) – bloom (N depletion) – post-bloom (moderate DON supply) sequence.

The pattern of strain N use was not clearly related to isolation location (Table 1). Strains from both an oligotrophic, open ocean site (CCMP373) and from seasonally productive coastal sites (CCMP370, CCMP374) could grow on a variety of N sources. There is no obvious geographic explanation for the inability of CCMP379 to grow on DON. This strain was collected from the English Channel in summer, in a general location known to experience low summer nitrate levels (Jordan and Joint, 1998). The explanation may lie in the high degree of genetic polymorphism that can be found in a single *E. huxleyi* population. For example, Iglesias-Rodríguez et al. (2006) found that nearly all *E. huxleyi* individuals collected from one Norwegian fjord in a given year were genetically distinct. Given this polymorphism, perhaps more surprising is the strong similarity between CCMP370 and CCMP374, collected 31 years apart from different coasts of the North Atlantic (Table 1). This similarity is evident in N use (this study), DMSP lyase activity (Steinke et al., 1998), palatability to protist grazers (Strom et al., 2003b) and in the 3' untranslated region of a coccolith calcification gene (Schroeder et al., 2005). In addition to genetic polymorphism, some *E. huxleyi* genotypes may be cosmopolitan, with decadal-scale persistence in nature.

Large interstrain differences were also seen in susceptibility to predation by the ciliate *Strobilidium* sp. Feeding rates were approximately twice as high on CCMP374 as on CCMP373 when identical N sources were compared (Table 3). Secondly, growth rate (Fig. 2A) and N source for growth (Fig. 2B) affected feeding rates on a given strain. Our finding that CCMP374 was ingested at a higher rate than CCMP373 is consistent with previous results

(Strom et al., 2003a). Three ciliate and two heterotrophic dinoflagellate grazers all fed at higher rates on CCMP370 and CCMP374 than on CCMP373 and CCMP379. Only the dinoflagellate *Oxyrrhis marina* did not show strain-dependent differences in feeding. Strom et al. (2003a) argued that such effects are likely due to cell surface differences among strains. In support of this, a recent study (Wootton et al., 2007) showed that feeding rates on *Isochrysis galbana* by *O. marina* were reduced when prey cell surface characteristics were altered by lectin binding. Size differences between strains are small (Strom et al., 2003a), especially in comparison to the range of prey sizes readily consumed by *Strobilidium* sp. (at least 3–15 μ m), so size variations are unlikely to be the cause of the 2-fold differences in feeding rates that we observed.

Cell surface cues could also signal nutritional differences, leading to the observed relationships between *E. huxleyi* growth rate or N source and ciliate feeding. For example, some of the genes expressed by *E. huxleyi* during N- or P-limitation are related to cell surface processes (Dyhrman et al., 2006). Dyhrman and Palenik (2003) found three cell-surface proteins associated with P-stressed *E. huxleyi* CCMP374; these proteins were potentially associated with use of dissolved organic P for growth. Even more intriguing is their observation that CCMP373 had only two of these three cell surface P-stress proteins, plus an additional novel protein, while CCMP379 had none. N stress also resulted in the appearance of novel cell surface proteins in some *E. huxleyi* strains (CCMP374, CCMP1516) but not others (CCMP373, CCMP379) (Dyhrman and Palenik, 2003; Palenik and Koke, 1995). Taken together, these studies suggest a mechanism whereby inherent strain differences, coupled with expression of cell-surface proteins related to growth state and nutrient source, lead to cell surface attributes that can be perceived by protist grazers. Variation in such attributes evidently leads to differences in feeding rate that could have substantial effects on top-down regulation of *E. huxleyi* populations. Reduced grazing by microzooplankton has been implicated as one of the most important factors allowing the development and persistence of *E. huxleyi* blooms in nature (Merico et al., 2004; Olson and Strom, 2002). Cell surface characteristics could also affect susceptibility to viral infection, thought to be an important cause of *E. huxleyi* bloom termination (Bratbak et al., 1993; Wilson et al., 2002).

Our data demonstrate that inter-strain differences in ability to use DON for growth likely contribute to the range of ecological niches occupied by *E. huxleyi*. Most tested strains could use DON

from various compound classes; this may contribute to the species' ability to bloom in stratified waters where N supply is largely from regenerative processes. CCMP379, however, showed limited ability to grow on DON. In agreement with previous findings, feeding by a common planktonic ciliate was also dependent on *E. huxleyi* strain identity. Thus variable predation may also contribute to strain diversity in nature. The finding that *E. huxleyi* growth rate and N source were related to predation pressure shows that understanding *E. huxleyi* bloom dynamics and strain diversity in nature will require an understanding of the relationships between resource use and predation.

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