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Nitrate:phosphate ratios and *Emiliania huxleyi* blooms

Abstract—It has been hypothesized that phosphate limitation, classically indicated by $\text{NO}_3:\text{PO}_4$ ratios >16 , is one of the critical factors allowing the coccolithophorid *Emiliania huxleyi* to bloom. This hypothesis is based on physiological studies showing that *E. huxleyi* has an exceptionally high affinity for orthophosphate and is able to use organic phosphate. Indeed, *E. huxleyi* has been found to bloom at high $\text{NO}_3:\text{PO}_4$ ratios in some mesocosm studies and in the oceanic northeast North Atlantic. Recent *E. huxleyi* blooms on the southeastern Bering Sea shelf, however, occurred under low $\text{NO}_3:\text{PO}_4$ conditions, which is indicative of nitrogen rather than phosphorus stress. A review of field studies of blooms where nitrate and phosphate were measured indicates that $\text{NO}_3:\text{PO}_4$ was in fact frequently low. A survey of most of the areas of the world ocean where satellite-detected *E. huxleyi* blooms occur also shows that $\text{NO}_3:\text{PO}_4$ ratios are generally low. These observations suggest that *E. huxleyi* is able to exploit situations where either phosphorus or nitrogen is limiting to competing species. They also indicate that attention should be directed to examining organic nitrogen, organic phosphorus, and ammonium during *E. huxleyi* blooms to better understand the role macronutrients play in these blooms.

The coccolithophorid *Emiliania huxleyi* forms extensive and intensive blooms in many cold coastal and oceanic regions (Brown and Yoder 1994). Because of their potential impact on global carbon and sulfur cycles (Holligan et al. 1993), blooms of *E. huxleyi* have received a great deal of attention. However, the particular set of environmental conditions that leads to *E. huxleyi* blooms is not fully understood. Bottom-up factors (shallow mixed layer, high light, and high $\text{NO}_3:\text{PO}_4$ ratios) and top-down factors (reduced grazing) have all been implicated as important for the development of *E. huxleyi* blooms (see Tyrrell and Merico 2004 for a review). One of the key environmental factors thought to favor *E. huxleyi* blooms is phosphate limitation, classically identified as waters with an $\text{NO}_3:\text{PO}_4$ ratio greater than the Redfield ratio 16. We examined whether the evidence supports the universality of high $\text{NO}_3:\text{PO}_4$ ratios as a controlling factor for *E. huxleyi* blooms.

Experimental evidence for exceptional competitive ability of *E. huxleyi* under high $\text{NO}_3:\text{PO}_4$ conditions—In multi-species competition experiments, Riegman et al. (1992) found that a North Sea isolate of *E. huxleyi*, along with the diatom *Chaetoceros socialis*, outcompeted other species when the $\text{NO}_3:\text{PO}_4$ supply ratio was 100. At an $\text{NO}_3:\text{PO}_4$ ratio of 1.5, other species outcompeted *E. huxleyi* (but, notably, *E. huxleyi* still maintained a relatively high population). In a later study, Riegman et al. (2000) demonstrated

that *E. huxleyi* had an unusually high affinity for phosphate under phosphorus stress ($\text{NO}_3:\text{PO}_4 = 300$), with an extremely low half-saturation constant of ca. 1 nmol L^{-1} . Further, Riegman et al. (2000) showed that *E. huxleyi* has two cell surface-bound alkaline phosphatase enzymes enabling it to use organic phosphate at nanomolar levels, in line with findings of an earlier study (Kuenzler and Perras 1965). Therefore, *E. huxleyi* would be expected to be particularly competitive at low phosphate concentrations and high $\text{NO}_3:\text{PO}_4$ ratios.

Support for the idea of phosphorus stress being important for the competitive success of *E. huxleyi* came from mesocosm studies in a Norwegian fjord. In one experiment (May 1992), the most dense *E. huxleyi* blooms occurred in mesocosms receiving the highest $\text{NO}_3:\text{PO}_4$ additions ($16:0.2 \text{ } \mu\text{mol L}^{-1}$, $\text{NO}_3:\text{PO}_4 = 80$) (Egge and Heimdal 1994). Aksnes et al. (1994) developed a simulation model of *E. huxleyi* growth and compared it to the data from this experiment and another (May 1991), where *E. huxleyi* also bloomed most strongly in bags receiving the highest $\text{NO}_3:\text{PO}_4$ additions. They found that they could best reproduce experimental results only by giving *E. huxleyi* an enhanced affinity for phosphate and access to a pool of organic phosphorus that supplemented the phosphate pool.

However, an examination of mesocosm experiments over several years shows that *E. huxleyi* grew well in mesocosms receiving high, low, or Redfield $\text{NO}_3:\text{PO}_4$ nutrient additions (Table 1) (Egge and Heimdal 1994). In many of the experiments, the $\text{NO}_3:\text{PO}_4$ ratio at the beginning of the *E. huxleyi* bloom was lower than the initial ratio, and all except two were lower than the Redfield ratio. Clearly, factors other than high $\text{NO}_3:\text{PO}_4$ ratios were critical to the growth and biomass accumulation of *E. huxleyi* in these experiments. The varied results of these experiments suggest that *E. huxleyi* can take advantage of a wide range of nitrate and phosphate ratios and concentrations in mesocosms where the nutrient environment is artificially altered.

Evidence for high $\text{NO}_3:\text{PO}_4$ ratios in natural blooms of *E. huxleyi*—To our knowledge, there is only one documented *E. huxleyi* bloom that occurred under high $\text{NO}_3:\text{PO}_4$ conditions: the June/July 1991 bloom in the North Atlantic south of Iceland (Holligan et al. 1993). Tyrrell and Taylor (1996) showed that the *E. huxleyi* bloom occurred only in areas where $\text{NO}_3:\text{PO}_4$ was >16 and reached its maximum abundances where $\text{NO}_3:\text{PO}_4$ was >25 . They were successfully able to model *E. huxleyi* population dynamics only when giving it a phosphorus uptake advantage over other algal groups.

Table 1. $\text{NO}_3:\text{PO}_4$ ratios in Norwegian fjord mesocosm experiments that induced *E. huxleyi* blooms.

Date	Nutrient levels ($\mu\text{mol L}^{-1}$)						Max. <i>E. huxleyi</i> (ml^{-1})	Reference
	NO_3	Initial PO_4	$\text{NO}_3:\text{PO}_4$	At initiation of <i>E. huxleyi</i> bloom*				
	NO_3	PO_4	$\text{NO}_3:\text{PO}_4$	NO_3	PO_3	$\text{NO}_3:\text{PO}_4$		
1988	31	6.8	4.6	28	5	5.6	110,000	Bratbak et al. 1993
1989	5	5	1	14	9	1.6	19,000	Bratbak et al. 1993
1991	12.6	1.5	10	<0.1	1.5	<0.05	90,000	Bratbak et al. 1993
	14.7	1.6	10	<0.1	2.5	<0.05	275,000	Bratbak et al. 1993
1990	12.6	0.1	130	2	0.2	10	75,000	Bratbak et al. 1993
	13.9	0.1	130	2	0.2	10	112,000	Bratbak et al. 1993
	15.2	2	7.6	1	0.4	2.5	35,000	Bratbak et al. 1993
1992	15.1	2	7.6	1	0.4	2.5	50,000	Bratbak et al. 1993
	16	5	3.2	0.15	3	0.03	5,000	Egge and Heimdal 1994
	16	1	16	<0.1	<0.1	1	20,000	Egge and Heimdal 1994
1993	16	0.2	80	8	<0.1	80	37,000	Egge and Heimdal 1994
	7.4	3.1	2.4	7.64	3.85	1.98	~3,500	Marañón et al. 1996
1994	9.4	0.2	41	11.92	0.22	54	~5,500	Marañón et al. 1996
	15	1	15	<0.1	0.1	~1	25,000	Egge and Jacobsen 1997
	15	1	15	<0.1	0.1	~1	18,500	Egge and Jacobsen 1997

* Approximated from plots.

High $\text{NO}_3:\text{PO}_4$ ratios, however, appear to be the exception rather than the rule in *E. huxleyi* blooms. In a modeling and data study of the recent *E. huxleyi* blooms in the southeastern Bering Sea, Merico et al. (2004) showed that high $\text{NO}_3:\text{PO}_4$ ratios did not occur in the Bering Sea during the bloom years 1997–2000 (Fig. 1), as might be expected if phosphorus stress were a factor in the appearance of the blooms. This is not surprising given that the Bering Sea is an area of notably low $\text{NO}_3:\text{PO}_4$ ratios (NO_3 scarcity), probably due to intense denitrification in the extensive shallow shelf sediments (Devol et al. 1997). In fact, in most blooms studied to date, $\text{NO}_3:\text{PO}_4$ ratios were low, and nitrate was low or undetectable. Table 2 lists all the field studies of which we are aware of *E. huxleyi* blooms in various parts of the world in which nitrate and phosphate were measured simultaneously. Except for the 1991 North Atlantic bloom, $\text{NO}_3:\text{PO}_4$ ratios are gen-

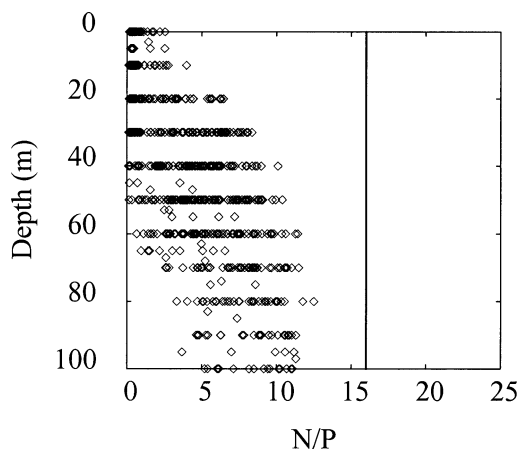


Fig. 1. $\text{NO}_3:\text{PO}_4$ ratios in the upper 100 m of the southeastern Bering Sea shelf in the years 1997–2000, during the occurrence of *E. huxleyi* blooms. The vertical line indicates the Redfield ratio of 16:1. Data collected by the TV *Oshoro Maru*.

erally much lower than 16, indicating nitrogen rather than phosphorus stress. Wider ranges of $\text{NO}_3:\text{PO}_4$ ratios and concentrations were found in an extensive *E. huxleyi* bloom off the northwest U.S./Canada coast (47–49°N) in 1997, but $\text{NO}_3:\text{PO}_4$ ratios were <16 at the majority of stations and depths (248 of 291 samples) where *E. huxleyi* occurred (Horner and Postel pers. comm.). A potential limitation of this analysis is that most of the studies were performed during late stages of bloom development, when the water was visibly colored with shed coccoliths (Table 2). The $\text{NO}_3:\text{PO}_4$ ratios may therefore not reflect nutrient conditions at the initiation of the bloom if there was prior preferential phosphate or nitrate utilization (i.e., relatively high phosphate utilization in the North Atlantic bloom; relatively high nitrate uptake in the other blooms). Of particular importance, then, are the two North Sea studies (Head et al. 1998; Rees et al. 2002) (Table 2), which sampled during the early stages of developing *E. huxleyi* blooms. In both cases, very low $\text{NO}_3:\text{PO}_4$ ratios were observed. Indeed, low $\text{NO}_3:\text{PO}_4$ ratios seem typical of regions where satellites have detected *E. huxleyi* blooms (Table 3; Fig. 1).

It is noteworthy that the one bloom with high $\text{NO}_3:\text{PO}_4$ ratios was in the oceanic North Atlantic, whereas all other blooms were coastal (continental shelves and fjords). In the North Atlantic bloom, nitrate remained in excess (2–5 $\mu\text{mol L}^{-1}$), which is consistent with micronutrient limitation, grazer control, or both. If micronutrients were scarce, the relatively low iron and zinc requirements of *E. huxleyi* (reviewed in Paasche 2002) may have been a factor in its success. It is interesting to note as well that *E. huxleyi* was preferentially grazed in the North Atlantic bloom (Holligan et al. 1993), whereas data (Olson and Strom 2002) and modeling (Merico et al. 2004) of the coastal Bering Sea bloom indicated that reduced grazing was important in bloom initiation and duration. It may well be that factors that govern the dynamics of oceanic blooms of *E. huxleyi* are different from those of coastal blooms.

Table 2. $\text{NO}_3:\text{PO}_4$ ratios measured during *Emiliania huxleyi* blooms. TIN, total inorganic nitrogen; TN, total nitrogen; ND, below detection.

	NO_3 ($\mu\text{mol L}^{-1}$)	PO_4 ($\mu\text{mol L}^{-1}$)	$\text{NO}_3:$ PO_4	NH_4 ($\mu\text{mol L}^{-1}$)	TIN:P	Urea ($\mu\text{mol L}^{-1}$)	TN:P	N uptake	Maximum <i>E.</i> <i>huxleyi</i> Lith: cell (ml^{-1})	Bloom stage*	Reference	
North Atlantic												
Jun/Jul 1991	2–5	0.18–0.2	17–25	—	—	—	—	—	10,000	11–107	Mid–late	Tyrell and Taylor 1996; Fernández et al. 1993
English Channel												
Jun 1992	0.4–0.8	0.04–0.1	8–13	—	—	—	—	—	2,000	175	Late	García-Soto et al. 1995
North Sea												
Jul 1993	0.08–0.15	0.1	0.8–1.5	0.4–1.0	6–11	—	—	—	1,200	—	Late	Van der Wal et al. 1995
Jun/Jul 1994	ND–0.09	0.26–0.37	0.15	—	—	—	—	$\text{NH}_4 > \text{NO}_3 > \text{urea}$	6,000	~10	Early	Head et al. 1998
Jun 1999	<0.13	0.03–0.1	0.2–1.2	<0.1	2.5–7.5	0.1–1.5	9–40	$\text{Urea} > \text{NH}_4 > \text{NO}_3$	2,300	—	Early	Rees et al. 2002
Norwegian fjords												
May 1992	<0.5	0.1	1–5	0.8–1.5	8.5–18	$\ll 0.5$	~10	$\text{NH}_4 > \text{urea} > \text{NO}_3$	7,000	—	—	Kristiansen et al. 1994
May 1993	ND	0.12–0.14	$\ll 1$	0.23–0.54	1.7–4	0.1–0.87	2–5.9	$\text{NH}_4 > \text{urea} > \text{NO}_3$	7,000	60–250	Mid–late	Fernández et al. 1996
May 1993	ND	0.14–0.31	$\ll 1$	0.42–0.54	1.7–3.1	0.13	2.2	$\text{NH}_4 > \text{urea} > \text{NO}_3$	27,000	25	Mid–late	Fernández et al. 1996
Gulf of Maine												
Jun 1989	0.2–0.6	0.02–0.16	~6	0.2–0.3	5.9–6.4	—	—	—	1,500	200	Late	Townsend et al. 1994
Jul 1990	0.1–0.4	0.3–0.8	~2	0.1–0.4	2.1	—	—	—	1,000	—	Late	Townsend et al. 1994
SE Bering Sea												
Jul/Aug 1999	0.23–0.9	ND–0.54	1.5–7†	1.4–6.0	5.6–199†	—	—	—	—	—	Late	Olson and Strom 2002
NW U.S./Canada coast												
July 1997	0.5–25	0.2–1.7	0.1–40	0.07–1.6	0.28–40	—	—	—	20,000	—	—	Horner and Postel un- publ. data

* Stage of bloom based on authors' classification and/or presence of visibly white/high reflectance water and high or low coccolith: cell ratios.

† One value where phosphate below detection not included.

Table 3. Average $\text{NO}_3:\text{PO}_4$ surface ratios at locations in the world ocean where satellite images suggest *E. huxleyi* blooms. Data from the World Ocean Database 1998.

Location	NO_3 ($\mu\text{mol L}^{-1}$)		PO_4 ($\mu\text{mol L}^{-1}$)		$\text{NO}_3:\text{PO}_4$	
	Annual	Summer	Annual	Summer	Annual	Summer
North Atlantic (south of Iceland)	8	4	0.5	0.3	16	13
Southwest Atlantic (off Patagonia)	12	10	1.1	1	11	10
Eastern Bering Sea (shelf region)	10	2	1.2	0.6	8.3	3.3
North Sea (off Norway)	5	2	0.3	0.15	16.6	13.3

Evidence for competitive abilities of E. huxleyi for non-nitrate nitrogen—High $\text{NO}_3:\text{PO}_4$ ratios may not be good indicators of phosphorus stress, of course, if other forms of nitrogen (inorganic and organic) and phosphorus (organic) are available to phytoplankton. Organic nitrogen and organic phosphorus are used by many phytoplankton and may be significant in their nutrition, but data on organic nitrogen and phosphorus concentrations, bioavailability, and species-specific abilities to use them are still limited (e.g., Palenik and Dyrhman 1998; Berman and Bronk 2003). *E. huxleyi* has been found to be able to use some amino acids, amides, urea, and purines such as hypoxanthine (Palenik and Henson 1997). In the few studies that have examined the uptake of nonnitrate nitrogen during *E. huxleyi* blooms, *E. huxleyi* primarily used NH_4 and urea (Table 2). *E. huxleyi* success may at least in part be due to a superior ability to use regenerated nitrogen. It is perhaps the combined abilities of *E. huxleyi* to use nonnitrate nitrogen in addition to its exceptional phosphorus acquisition capacity that provide it a competitive edge in nutrient-depleted waters with shallow mixed layers and high light.

The evidence summarized shows that there is no strong association between high $\text{NO}_3:\text{PO}_4$ ratios (PO_4 more limiting than NO_3) and *E. huxleyi* blooms. In fact, most blooms occurred in NO_3 -limited waters. The recent intense blooms in the eastern Bering Sea offer unequivocal evidence that phosphate limitation is not a prerequisite for *E. huxleyi* bloom formation. We believe that ecosystem modeling that includes *E. huxleyi* should not contain a dependence on high $\text{NO}_3:\text{PO}_4$ ratios as a causative factor of blooms of this species, particularly in coastal waters.

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