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Light-induced growth of phytoplankton collected during the winter from the benthic boundary layer off Oregon, USA

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ABSTRACT: Despite the importance of the spring phytoplankton bloom off Oregon as a food source for zooplankton, little is known about the sources of phytoplankton seed stock for the bloom or its timing. Experiments were conducted in the late winter to determine if the benthic boundary layer (BBL) could be a source of viable phytoplankton and to determine to what extent their growth was limited by light. Water collected on 3 dates from the BBL over the inner-shelf and mid-shelf in January 2003 was used for growth experiments run in shipboard incubators held at 10°C. The water was exposed to *in situ* nutrient concentrations and to 4 light levels and 2 daylengths (9 and 12 h), representative of winter and spring daylengths. Significant increases in chl *a* and particulate organic matter (POM) were observed in high light and medium light treatments at both daylengths. Chl *a* increased minimally (≤0.3 µg l^{-1}) in the low light treatments, while no significant increase in POM occurred. Chl *a* decreased in the control (no light) treatment throughout the experiments. Blooms that developed during these incubations were dominated by *Thalassiosira* spp., *Actinopytchus* sp., *Asterionellopsis glacialis* and other diatoms, consistent with *in situ* phytoplankton community composition during the spring. Growth occurred at light levels that were generally 40 to 50% of that measured in surface waters in January, suggesting that light levels at the surface are conducive to growth, but that mixing prevents significant growth during the winter.

KEY WORDS: Phytoplankton · Benthic boundary layer · Seed stocks · Spring bloom · Light · Mixing · Diatoms

INTRODUCTION

Coastal waters off Oregon often have extremely high levels of primary production during the spring and summer months and can thus support high upper trophic level biomass (reviewed by Botsford et al. 1989). Additionally, the observed high productivity makes the system an important component of both regional and global cycles of carbon and nitrogen (Chavez & Toggweiler 1995). Primary productivity during the summer upwelling season has been intensely studied in the past several decades (e.g. Small & Menzies 1981, Hood et al. 1992). However, relatively little is known about the initiation and evolution of the spring bloom, due in part to difficulties in conducting field sampling in the late winter, when strong storms persist along the coast. The spring phytoplankton bloom off Oregon is an important food source for zooplankton emerging from diapause (Peterson & Miller 1977). Thus, if progress is to be made in our understanding of springtime ecosystem dynamics in coastal upwelling systems, such as off Oregon, fundamental issues regarding sources of phytoplankton seed stock for the bloom and mechanisms behind the timing of the bloom need to be addressed.

During the winter months off Oregon, daylengths are short (ca. 8.5 to 9 h) and photosynthetically available radiation (PAR) reaching the surface of coastal waters is usually <25% of that reaching the surface during the summer (see Fig. 1). Prevailing winds are

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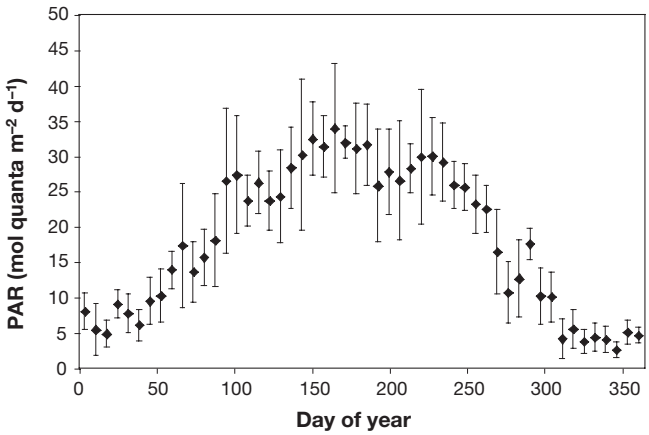

Fig. 1. Annual PAR (averaged weekly from 1997 to 2001) ± SD measured using a 2π PAR sensor located on the roof of the Environmental Protection Agency laboratory in Newport, OR (44.62° N, 124.05° W). Data courtesy of Dr. R. Ozretich (US EPA)

out of the southwest and frequent cyclonic storms cause prolonged coastal downwelling (Huyer et al. 1979, Halliwell & Allen 1987). During downwelling 'events', Ekman transport causes onshore movement of surface waters, which subsequently downwell during development of a bottom front over the mid-shelf in ca. 80 to 120 m of water (Allen & Newberger 1996). Landward of the front, strong vertical mixing can create a nearly homogeneous water column (Allen & Newberger 1996), although in very nearshore waters riverine input may prevent the complete breakdown of stratification in surface waters (Huyer 1977). It has been hypothesized that off Oregon the presence of a downwelling front may enhance the accumulation of plankton and riverine-derived nutrients over the inner shelf, thereby setting the system up for development of a spring bloom once conditions become favorable (NSF-funded CO-OP Coastal Ocean Advances in Shelf Transport program, hypothesis H6).

Concentrations of chl *a* in coastal surface waters off Oregon during the winter are usually <1.0 µg l^{-1} (Small et al. 1989, P. Wheeler unpubl. data). However, elevated concentrations of POC and chl *a* have been observed in the benthic boundary layer (BBL) or bottom nepheloid layer over the shelf (Small et al. 1989). The thickness of the BBL ranges from <5 to >20 m in Oregon coastal water (Perlin et al. unpubl. data). When water collected in winter from the BBL was incubated under continuous light, phytoplankton growth occurred in 5 to 27 d, suggesting a possible benthic source of phytoplankton seed stock for the spring bloom (Small et al. 1989). It has long been hypothesized that some phytoplankton taxa overwinter on the bottom and ultimately serve as seed stock for the next spring bloom once oceanographic conditions become favorable (Gran 1912). Numerous studies have now demonstrated that many types of phytoplankton form resting stages (reviewed by McQuoid & Hobson 1996) that aid in their long term survival under adverse conditions, such as when overwintering on the bottom (Antia & Cheng 1970, Hollibaugh et al. 1981, Lewis et al. 1999, McQuoid et al. 2002).

In order to determine if phytoplankton cells in the BBL off northern Oregon were viable, and to what extent their growth was limited by light, we chose to incubate water from the BBL at *in situ* nutrient concentrations and at light levels that are ecologically relevant to the system (i.e. at both winter and spring daylengths and at varying light levels). By using different photoperiods and light levels and by having 3 wk of *in situ* PAR data from the same time as these experiments were conducted, we were able to determine if daylength or surface light levels could play a role in bloom formation. Additionally, the use of *in situ* nutrient concentrations permitted estimation of a potential C and N biomass yield.

MATERIALS AND METHODS

Three incubations were run from January 21 to February 10, 2003. Water for the first incubation (Fig. 2a) was collected on January 21 from a mid-shelf site off central Oregon (45.00° N, 124.12° W; bottom depth = 80 m). Water for the second incubation (Fig. 2b) was collected on January 23 from an inner-shelf site (45.00° N, 124.04° W; bottom depth = 30 m). Water for the third incubation (Fig. 2c) was collected on January 28 from the same inner-shelf site as the second incubation.

For each incubation, 14 Becton-Dickinson polystyrene tissue culture flasks (1.9 l) were filled with approximately 1.5 l of water collected within the BBL at roughly 1 to 2 m off the bottom. Finely resolved turbidity profiles for each station verified that we did indeed sample within the bottom nepheloid layer. Seawater was pumped directly from depth into a darkened, acid-washed carboy using a winch-controlled pumping and profiling system following the protocol of Hales & Takahashi (2002) and then dispensed into the culture flasks. Prior to being filled, the flasks were vigorously rinsed with 10% HCl followed by multiple rinses with deionized water (DIW). Experiments were conducted in the ship's climate control chamber where the temperature was kept at 10°C. *In situ* temperature throughout the water column at both the inshore and mid-shelf site was near 11°C for the duration of the cruise, consistent with other historical field observations from this time of year (e.g. Huyer 1977). Flasks were either held in homemade incubators or in closed coolers for dark 'control' samples. Water samples from

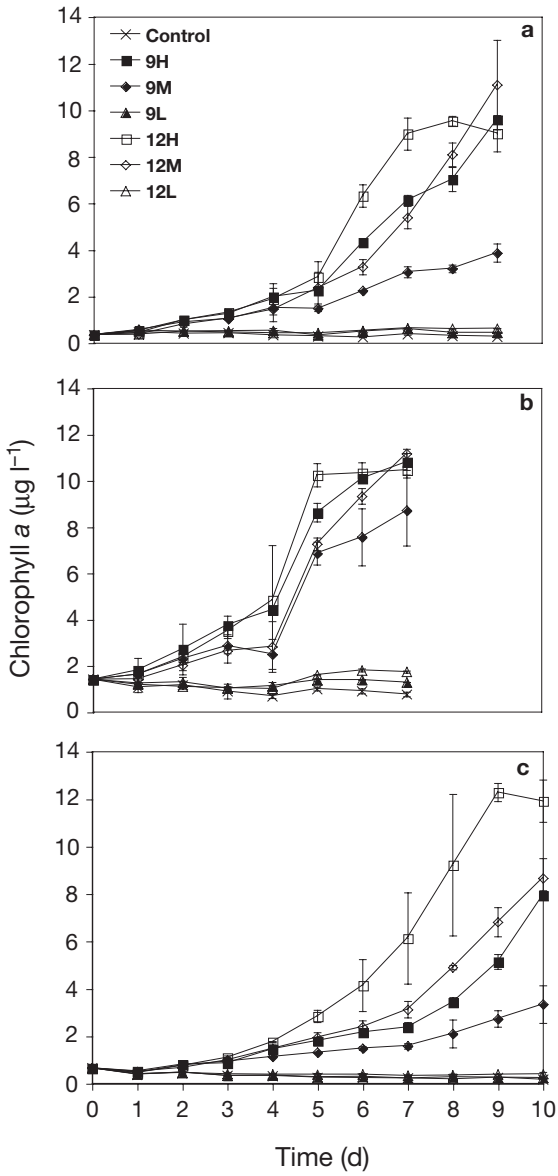

Fig. 2. Daily concentration of chlorophyll *a* (µg l^{-1}) ± SD in (a) the first, (b) the second, and (c) the third incubation. See Table 1 for treatment abbreviations

each site were exposed to 7 different light treatments, as shown in Table 1. Light levels were measured using a Biospherical QSL-2101 Scalar irradiance meter placed inside culture flasks that were filled with DIW and located in each section of the incubators. Two incubators were used, 1 for each daylength treatment. The incubators had two 35 W cool white fluorescent light strips mounted on each side and 1 strip mounted on top running down the middle. Each strip held 2 light bulbs. This arrangement gave the maximum light level seen in Table 1 (140 µmol quanta m^{-2} s^{-1}), and the lower light levels were obtained by placing boards in front of either the lower panel of lights (65 µmol quanta m^{-2} s^{-1}) or in front of both the upper and lower panel of lights on each side of the incubator (19 µmol quanta m^{-2} s^{-1}). Lights were turned on in the incubators at 07:00 h local time and turned off at either 16:00 or 19:00 h local time. Sunrise during the 3 wk cruise occurred from roughly 07:15 to 07:45 h local time. Moderate mixing of samples occurred due to the motion of the boat and the flasks were inverted manually once or twice daily. Flasks were sampled daily in the early morning (07:00 h local time).

Biological analyses. Phytoplankton growth was tracked by measuring *in vivo* fluorescence daily in each sample. In all 3 experiments, *in vivo* fluorescence was positively correlated with phytoplankton carbon (r^2 = 0.88, 0.71 and 0.90, respectively; n = 13), which was estimated by relating total phytoplankton cell biovolume (Hillebrand et al. 1999) to total cell carbon (Menden-Deuer & Lessard 2000). Samples for *in vivo* fluorescence were gently poured into 25 mm round glass test tubes and fluorescence was measured with a Turner 10-au fluorometer. Stability of the fluorometer was tracked by daily measurements of a high and low solid fluorescence standard (Turner Designs). The average coefficient of variation for the high and low standards over the 20 d period was 2.29 and 2.77%, respectively, indicating little systematic variation. During each experiment, samples were collected for extracted chl *a* analysis from each treatment. Samples

Table 1. Description of 7 light treatments and notation used in the text for each treatment. Also, daily integrated PAR measured at the surface over the northern Oregon shelf from January 19 to February 16, 2003

Treatment	Daylength (h)	Light level (µmol quanta m^{-2} s^{-1})	Daily PAR (mol quanta m^{-2} d^{-1})	Daily *in situ* $PAR_{surface}$ (mol quanta m^{-2} d^{-1})
Control	–	0	0	Mean = 9.06 ± 5.55
9L	9	19	0.62	n= 19
9M	9	65	2.11	Max = 20.85
9H	9	140	4.54	Min = 2.21
12L	12	19	0.82	
12M	12	65	2.81	
12H	12	140	6.05	

for chl *a* analysis were vacuum filtered (<200 mm Hg) onto GF/F filters and stored in glass Vacutainers™ at –30°C until laboratory analysis. Chl *a* was extracted from the filters for ≥12 h in the dark at –20°C using 95% methanol and measured with a Turner 10-au fluorometer (Strickland & Parsons 1972). There was a strong positive relationship between extracted chl *a* and *in vivo* fluorescence values in all 3 experiments (r^2 = 0.97, 0.94 and 0.93; n = 23, 24 and 15, respectively) and, thus, all *in vivo* fluorescence values have been converted to, and are reported as, chl *a*. Net growth rates were calculated based on chl *a* measurements while the phytoplankton were in exponential growth. Statistical comparisons of net growth rates between light treatments were made using a Tukey test according to Zar (1996).

Samples for phytoplankton abundance measurements were collected at the beginning (t = 0) and end (t = final) of each experiment. Samples were preserved with Lugol's solution (3 ml Lugol's: 100 ml sample) immediately upon collection, stored in the dark in 250 ml amber glass bottles and analyzed within 3 to 8 mo after collection. For microscopic analyses, the samples were briefly mixed, and 25 to 100 ml of a subsample was allowed to settle for 24 h in Utermöhl settling chambers, with the volume settled dependent on the expected amount of material in the sample. Diatoms and dinoflagellates were then counted using a Wild M40 inverted microscope at 320× magnification. Sample means were considered to be significantly different from zero if they were ≥2 SDs from zero.

Chemical analyses. Nutrient samples were collected in acid-washed 30 ml HDPE bottles and immediately frozen at –30°C until analysis. Samples were analyzed on a Technicon AA-II according to standard wet chemical methods as described by Gordon et al. (1995). The detection limits for NO_3^-, PO_4^- and $Si(OH)_4$ were 0.38 ± 0.27, 0.02 ± 0.01 and 0.83 ± 0.83 µmol l^{-1}, respectively. Particulate organic carbon (POC) and particulate organic nitrogen (PON) were determined from material collected on precombusted GF/F filters. Water samples were pre-filtered through a 202 µm mesh screen to remove zooplankton. Then, 500 or 1000 ml of the filtered water was vacuum filtered (<200 mm Hg) onto precombusted GF/F filters. After filtration, samples were stored in glass Vacutainers™ and immediately frozen at –30°C until laboratory analysis. Samples were processed within 2 to 3 mo of collection. Filters were fumed with concentrated HCl to remove inorganic carbon and dried, followed by analysis using a Control Equipment 440HA CHN elemental analyzer calibrated with acetanilide. During analysis, filter blanks were run after every 12 samples and these values were subtracted from every sample value as a filter blank correction. Filter blanks averaged 28.2 ± 6.56 and 2.22 ± 0.71% of t = 0, POC and PON samples, respectively; 5.53 ± 2.93 and <1% of t = final, high and medium light POC and PON samples, respectively; and 21.57 ± 5.19 and 1.34 ± 0.40% of t = final, low light and control POC and PON samples, respectively.

RESULTS

During the 20 d that we were at sea and conducting these experiments, several periods of strong downwelling-favorable winds were observed (Barth et al. 2003). The intense vertical mixing predicted by Allen & Newberger (1996) was observed inshore of the downwelling front and resuspension of bottom particulate material far up into the water column was also observed (Klymak & Moum 2003, J. Moum & A. Perlin unpubl. data). Distributions of particulate material and chl *a* were nearly homogeneous throughout the water column after downwelling (M. Wetz & P. Wheeler unpubl. data).

Initial nitrate concentrations in the 3 incubations ranged from 6.65 to 7.08 µmol l^{-1}, phosphate from 0.89 to 0.98 µmol l^{-1} and silicate from 7.73 to 11.63 µmol l^{-1} (data not shown). In all 3 incubations, chl *a* increased in the 12H, 12M, 9H and 9M treatments relative to the control treatment (Fig. 2). In the first and second incubations, similar levels of chl *a* were reached in the 12H, 12M and 9H treatments by the end of the experiments, averaging 10.35 ± 1.05 µg l^{-1} (Fig. 2a,b). Highest chl *a* levels in the third experiment were found in the 12H treatment (Fig. 2c), although nutrients were not depleted yet in the 12M or 9H treatments, suggesting that growth might have continued had the experiments not been terminated. It appears that all of the blooms that reached stationary growth were terminated due to depletion of nutrients, as both nitrate and silicate were drawn to below detection limits (data not shown). Net growth rates in the 12H, 12M and 9H treatments averaged 0.41 ± 0.07, 0.33 ± 0.08 and 0.30 ± 0.07 d^{-1}, respectively (Fig. 3). The net growth rate in 12H was significantly greater than in 12M and 9H ($p < 0.01$), while 12M was significantly greater than 9H ($p < 0.01$). Highest levels of POC were observed in the 12H treatment (mean = 100.8 ± 15.8 µmol l^{-1}), followed by the 12M (mean = 53.1 ± 11.2 µmol l^{-1}) and 9H (mean = 49.6 ± 10.8 µmol l^{-1}) treatments (Table 2). In contrast, PON levels at the end of the experiments were similar among these 3 treatments. Growth was also observed in the 9M treatment, both in terms of chl *a*, and POC and PON. However, the maximum level of chl *a* obtained was roughly half of that observed in the 12H, 12M and 9H treatments, and POC and PON levels were lower than in those treatments. Also, net growth rates in the 9M treatment averaged 0.22 ± 0.07 d^{-1} and

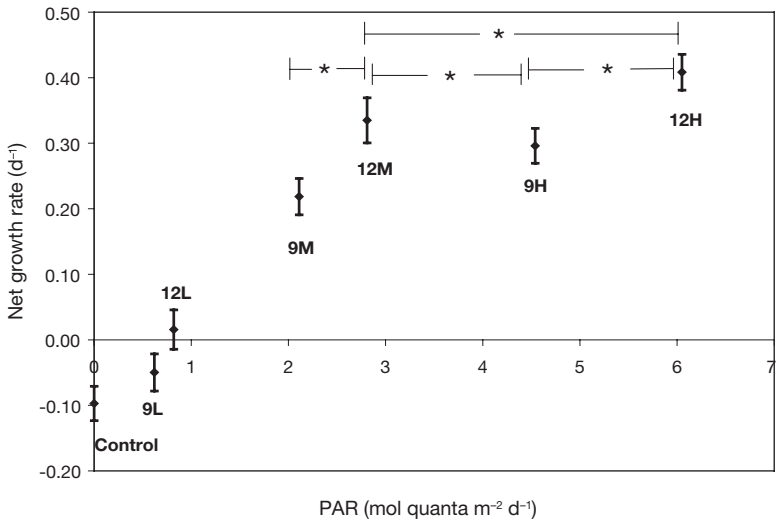

Fig. 3. Average phytoplankton net growth rate (d^{-1}) ± SE for each treatment from all 3 incubations. * Treatments are significantly different ($p < 0.01$) based on Tukey test. See Table 1 for treatment abbreviations

were significantly lower than in the 12H, 12M and 9H treatments. Again, no nutrient appeared depleted, suggesting that growth probably would have continued if the experiments were not terminated. POC in the 12L and 9L treatments did not change relative to the t = 0 sample, although it appears that a slight increase in chl *a* and PON occurred in those treatments. Chl *a* decreased significantly (t-test; $p < 0.05$) in the control treatment relative to the t = 0 sample.

Phytoplankton abundances in the initial water samples for the 3 incubations were low, generally ≤1 cell ml^{-1} (Table 3). Counts from the t = 0 samples may be slight underestimates, as large (ca. 100 to 200 µm diameter) patches of detritus were present and it was difficult to see if phytoplankton cells were associated with them, although some dinoflagellate cysts were observed in the detritus. Furthermore, growth of some genera in the light treatments when they were not detected in the t = 0 sample suggests that they were present, either associated with the detrital material or in low enough abundances to escape our detection. Blooms that occurred in the 12H, 12M, 9H and 9M treatments in the 3 incubations all were composed of high abundances of *Thalassiosira* spp., *Actinopytchus* sp., and *Asterionellopsis glacialis* (Table 3). Genera with abundances greater than 10 cells ml^{-1} in the first incubation also included *Cylindrotheca closterium*, *Pseudo-nitzschia* spp., *Skeletonema costatum*, *Thallasionema* sp., *Navicula* sp. and dinoflagellates, while in the second incubation, *C. closterium*, *Pseudo-nitzschia* spp., *Navicula* spp., dinoflagellates and *Dichtyocha* sp. comprised the rest of the blooms. The third incubation was more diverse than the first 2, with marked increases in the abundance of *Odontella aurita*, *Odontella longicruris*, *Thalassiosira* spp., *Pleurosigma normanii*, *C. closterium*, *Coscinodiscus* sp., *Pseudo-nitzschia* spp., *A. glacialis*, *S. costatum*, *Thallasionema* sp., *Navicula* sp. and dinoflagellates being observed. Vegetative cells of *Chaetoceros* spp. were only observed in 2 treatments among the 3 incubations and resting spores of *Chaetoceros* spp. were also only observed in a few of the treatments. Abundances of phytoplankton at the end of the incubations in the 12L and 9L treatments were similar to the observed abundances in the control and = 0 samples, with the exception of minimal increases in the abundance of several of the diatoms and *Dichtyocha* spp.

Table 2. Concentration of POC and PON (µmol l^{-1}) ± SD at the beginning (t = 0) and end (t = final) of each incubation

Incub. no.	Treatment	POC (µmol l^{-1}) t = 0	POC (µmol l^{-1}) t = final	PON (µmol l^{-1}) t = 0	PON (µmol l^{-1}) t = final
1	Control	9.11 ± 0.63	11.07 ± 0.39	1.14 ± 0.04	1.14 ± 0.06
	9L		10.71 ± 0.12		1.72 ± 0.51
	12L		11.19 ± 3.02		1.66 ± 0.19
	9M		23.34 ± 5.39		4.29 ± 0.28
	9H		49.38 ± 1.07		7.32 ± 0.14
	12M		50.39 ± 5.87		7.51 ± 0.61
	12H		90.90 ± 10.44		7.68 ± 0.14
2	Control	12.23 ± 0.91	14.91 ± 0.19	1.67 ± 0.06	1.89 ± 0.08
	9L		15.69 ± 6.71		2.70 ± 0.75
	12L		16.32 ± 4.74		3.11 ± 0.49
	9M		44.90 ± 6.65		7.98 ± 0.77
	9H		61.54 ± 3.28		7.57 ± 0.43
	12M		65.84 ± 5.95		9.08 ± 0.30
	12H		101.79 ± 1.24		8.05 ± 0.16
3	Control	11.01 ± 0.08	14.37 ± 3.89	1.14 ± 0.08	2.04 ± 0.20
	9L		10.10 ± 0.55		1.78 ± 0.08
	12L		8.73 ± 0.24		1.75 ± 0.12
	9M		23.65 ± 3.13		4.12 ± 0.32
	9H		37.87 ± 2.30		6.62 ± 0.16
	12M		43.09 ± 3.99		7.85 ± 0.68
	12H		109.59 ± 27.92		8.70 ± 0.52

Table 3. Abundance (cells ml^{-1}) ± SD of phytoplankton taxa in incubations. *Mean is significantly different from control mean at $p < 0.05$. nd = not detected, ns = cells present but mean ≤2 SD from zero. For treatment 12L in incubation #1, only 1 sample was available. *Chaeto.*: *Chaetoceros*; see Table 1 for treatment abbreviations

Incub. no.	Genus	t = 0	t = final					
		Control	Control	9L	12L	9M	9H	12M	12H
1	*Chaetoceros* spp.	nd	nd	nd	nd	nd	<1*	nd	nd
	Chaeto. resting	nd	ns	nd	nd	ns	1 ± 0*	ns	nd
	Odontella spp.	nd	nd	ns	nd	ns	ns	ns	ns
	Thalassiosira spp.	1 ± 0	1 ± 0	3 ± 0*	6	51 ± 10*	92 ± 8*	139 ± 36*	88 ± 4*
	Pleurosigma sp.	<1	ns	nd	nd	1 ± 0*	ns	3 ± 0*	ns
	Cylindrotheca sp.	<1	ns	<1*	<1	6 ±2*	13 ± 1*	14 ± 4*	8 ± 1*
	Coscinodiscus sp.	<1	<1	<1	nd	2 ± 1*	ns	6 ± 2*	ns
	Actinopytchus sp.	<1	<1	1 ± 0*	1	ns	30 ± 4*	36 ± 10*	50 ± 19*
	Pseudo-nitzschia spp.	ns	<1	ns	1	10 ± 1*	22 ± 5*	27 ± 1*	ns
	Asterionellopsis sp.	<1	ns	<1	<1	23 ± 9*	82 ± 10*	50 ± 2*	43 ± 0*
	Skeletonema sp.	<1	ns	ns	2	23 ± 2*	16 ± 1*	21 ± 6*	18 ± 6*
	Thalassionema sp.	<1	<1	nd	1	2 ± 1	8 ± 1*	ns	21 ± 2*
	Navicula sp.	<1	<1	ns	1	3 ± 1*	10 ± 3*	3 ± 0*	ns
	Dinoflagellates	ns	ns	<1	1	5 ± 2	11 ± 4*	ns	34 ± 1*
	Dichtyocha spp.	<1	ns	1 ± 0*	<1	ns	nd	nd	nd
2	*Chaetoceros* spp.	nd	nd	nd	nd	nd	nd	nd	nd
	Chaeto. resting	nd	nd	ns	ns	nd	nd	<1	nd
	Odontella spp.	ns	<1	ns	<1*	1 ± 0*	8 ± 2*	ns	nd
	Thalassiosira spp.	1 ± 0	1 ± 0	4 ± 1*	3 ± 1	7 ± 1*	84 ± 39*	67 ± 11*	131 ± 6*
	Pleurosigma sp.	<1	ns	ns	ns	1 ± 0*	3 ± 0*	ns	ns
	Cylindrotheca sp.	<1	<1	ns	ns	12 ± 2*	19 ± 5*	30 ± 1*	ns
	Coscinodiscus sp.	<1	<1	2 ± 0*	<1*	<1*	6 ± 0*	ns	ns
	Actinopytchus sp.	<1	<1	ns	1 ± 0	9 ± 1*	29 ± 2*	ns	54 ± 10*
	Pseudo-nitzschia spp.	ns	ns	<1*	ns	3 ± 0*	7 ± 3*	10 ± 2*	ns
	Asterionellopsis sp.	<1	<1	ns	1 ± 0	15 ± 2*	136 ± 41*	77 ± 24*	70 ± 11*
	Skeletonema sp.	<1	<1	ns	1 ± 0*	1 ± 0*	ns	9 ± 2*	ns
	Thalassionema sp.	ns	<1	ns	ns	1 ± 0*	ns	ns	ns
	Navicula sp.	1 ± 0	<1	2 ± 1	ns	5 ± 1*	ns	13 ± 2*	ns
	Dinoflagellates	1 ± 0	1 ± 0	ns	ns	7 ± 1*	18 ± 8*	ns	63 ± 8*
	Dichtyocha spp.	1 ± 0	2 ± 1	8 ± 1*	7 ± 2	1 ± 0	13 ± 1*	10 ± 2*	11 ± 1*
3	*Chaetoceros* spp.	nd	nd	nd	nd	1 ± 0*	ns	ns	ns
	Chaeto. resting	nd	nd	nd	nd	1 ± 0*	ns	ns	ns
	Odontella spp.	<1	nd	nd	nd	12 ± 3*	42 ± 10*	31 ± 8*	ns
	Thalassiosira spp.	1 ± 0	1 ± 0	2 ± 0*	4 ± 0*	52 ± 16*	42 ± 16*	152 ± 9*	103 ± 3*
	Pleurosigma sp.	<1	ns	nd	<1	ns	7 ± 2*	14 ± 3*	11 ± 3*
	Cylindrotheca sp.	ns	<1	ns	ns	10 ± 1*	42 ± 2*	52 ± 2*	ns
	Coscinodiscus sp.	<1	ns	<1 *	ns	4 ± 2*	6 ± 1*	8 ± 1*	12 ± 4*
	Actinopytchus sp.	<1	<1	<1*	<1	40 ± 15*	67 ± 19*	105 ± 19*	176 ± 36*
	Pseudo-nitzschia spp.	ns	<1	<1*	1 ± 0*	13 ± 2*	23 ± 5*	33 ± 8*	21 ± 3*
	Asterionellopsis sp.	ns	ns	ns	nd	ns	112 ± 27*	56 ± 12*	90 ± 11*
	Skeletonema sp.	<1	<1	nd	nd	27 ± 4*	88 ± 16*	65 ± 14*	ns
	Thalassionema sp.	ns	ns	nd	nd	4 ± 1*	18 ± 2*	14 ± 4*	ns
	Navicula sp.	<1	1 ± 0	1 ± 0*	2 ± 0*	5 ± 2	18 ± 3*	ns	ns
	Dinoflagellates	<1	1 ± 0	2 ± 0	1 ± 0	7 ± 3	15 ± 5*	30 ± 5*	24 ± 9*
	Dichtyocha spp.	ns	2 ± 0	4 ± 0*	4 ± 1	ns	6 ± 3	7 ± 1*	ns

DISCUSSION

Given sufficient light, phytoplankton growth was observed in several of the treatments from water collected from the BBL. Similar levels of chl *a* and PON accumulated in the 12H, 12M and 9H treatments. However, ca. 50% more POC accumulated in the 12H treatment than in the other 2 and the amount of POC that accumulated is well above what would be predicted from Redfield stoichiometry relating C fixation to N assimilation. It appears that the 12H treatment entered stationary growth phase and presumably nutrient depletion several days before the experiments were terminated, while the 12M and 9H treatments were just entering stationary growth at the end of the first 2 incubations. Thus, we suspect that the elevated

POC relative to PON is simply a result of continued C fixation and either intracellular storage or release of the 'excess' fixed C as dissolved organic carbon and conversion of the material to transparent exopolymer particles (i.e. TEP) (Engel et al. 2002, Wetz & Wheeler 2003). Although growth was observed in the 9M treatment, chl *a* and POM concentrations were significantly lower than in the 12H, 12M and 9H treatments, probably as a result of a lower daily integrated PAR from the 9 h light cycle and moderate irradiance.

Diatom abundances generally did not increase significantly in the low light treatments. However, slight increases in chl *a* and PON were observed. Although the light levels in those treatments were not enough for net population growth to occur, the increase in PON suggests that light levels may have been sufficient to provide the cells with enough photosynthetic energy to reduce and assimilate inorganic nitrogen (Turpin 1991). The decreases in chl *a* over the course of the experiments in the control treatments, despite steady abundances, suggests that degradation of the cells' photosynthetic machinery was occurring due to the absence of light and additionally points to a previous history of light exposure.

Several diatom species were predominant in the treatments where blooms took place, including *Thalassiosira* spp., *Skeletonema costatum* and *Actinopytchus* sp. Periodic sampling of Oregon coastal waters in the spring has shown the phytoplankton community to be relatively diverse, with the aforementioned genera making up a portion of the community biomass (Moses & Wheeler 2004). This is in contrast to the summer upwelling blooms in this system, which tend to be dominated by *Chaetoceros* spp. and a few other large centric diatoms (Wetz & Wheeler 2003, Moses & Wheeler 2004). Species from both *Thalassiosira* and *Skeletonema* genera are known to form resting stages (Hargraves & French 1975, Durbin 1978, Syvertsen 1979, reviewed by McQuoid & Hobson 1996) and thus, they might overwinter in the BBL. We are aware of no studies looking at resting stage formation in *Actinoptychus* sp. In contrast, *Chaetoceros* sp. is known to form resting cells and spores (McQuoid & Hobson 1996), but neither resting stages nor vegetative cells were present in most of our BBL and light treatment samples. Nevertheless, *Chaetoceros* spp. have been observed in high abundances during the spring in coastal waters off Oregon (Moses & Wheeler 2004). Thus, it is possible that *Chaetoceros* spp. overwinters in the water column rather than in the BBL. Although our limited spatial coverage of the BBL for the growth experiments precludes us from confirming this, several studies have presented evidence suggesting that seed stocks of upwelling bloom-forming diatoms such as *Chaetoceros* spp. may be entrained in the water column in coastal circulation cells that facilitate their seeding of multiple upwelling 'events' during the upwelling season (e.g. Garrison 1981, Smetacek 1985, Pitcher et al. 1991). In short, it has been argued that in eastern boundary current systems, upwelling-induced diatom blooms are advected offshore and equatorward until nutrients become depleted, after which the diatoms sink into deeper water and are carried back onshore and poleward until some cells are ultimately upwelled again. However, in the wintertime when downwelling conditions predominate, circulation patterns are vastly different. In general, cross-shelf transport is minimal, especially inshore of the downwelling front, and net water movement is poleward (Huyer 1977, Huyer et al. 1978, 1979, Allen & Newberger 1996, Austin & Barth 2002). It is possible that brief periods of northerly (i.e. upwelling-favorable) winds could lead to short-lived equatorward flow and also upwell cells from depth onto the shelf, which would aid in the retention of localized populations of *Chaetoceros* spp. cells. Although upwelling is far less persistent in the winter than during the summer, it nonetheless does occur (Huyer et al. 1978, 1979, Huyer 1983).

The presence of *Pseudo-nitzschia* spp. in our initial BBL samples, a genera that has no species with identified resting stages, suggests that it could have been mixed to the bottom prior to our collecting water for the incubations (e.g. McQuoid 2002). This observation, coupled with the frequency and intensity of mixing over the shelf during the late winter, additionally suggests that the other diatoms, which are known to have benthic resting stages, may be frequently resuspended into the water column. Although prolonged vertical mixing may prevent significant population growth, it may aid in the long term survival of the overwintering phytoplankton, as low respiration rates of the cells coupled with energy derived from brief exposures to minute levels of light have been shown to greatly enhance survival of diatom resting cells (Umebayashi 1972, Smayda & Mitchell-Innes 1974, French & Hargraves 1980). In addition to aiding cell survival, mixing may also be a mechanism by which phytoplankton overwintering on the bottom could be injected into the euphotic zone. Other mechanisms include the brief periods of upwelling or resuspension by internal solitary waves. Periods of upwelling-favorable winds can result in onshore transport of the BBL and presumably its associated seed stocks (Huyer et al. 1978, 1979, Huyer 1983). A study conducted on the same cruise as the experiments in this paper has determined that shoreward propagating internal solitary waves can also result in both resuspension of sediment from the BBL and onshore transport of material in the BBL (Klymak & Moum 2003).

Implications for spring bloom formation off Oregon

The implications of this study are 2-fold. Firstly, Small et al. (1989) and this study both indicate that the BBL can be a source of phytoplankton seed stock for the spring bloom, although we cannot rule out the possibility that some species may overwinter in the pelagic zone. Secondly, it appears that in this region, the daily integrated PAR to which the cells are exposed may actually have more of an influence on phytoplankton population growth than just daylength or solar intensity alone. Growth of phytoplankton from the BBL occurred in our experiments at light levels that were generally ≤67% of the average *in situ* surface PAR during the 20 d cruise. When calculating the weighted spectral distribution of the cool white lights as a function of the characteristic spectral absorption of spring phytoplankton assemblages (Morel 1978) and comparing it with that of the weighted PAR distribution in surface waters and at 80 m depth (the maximum depth of the mixed-layer observed at the mid-shelf site), we derive that the fraction of Photosynthetically Usable Radiation (PUR) of the lights is significantly lower than that of the ambient light (75 and 53% relative to surface and 80 m PUR, respectively). Thus, when taking the spectral distribution into consideration, the average *in situ* PAR required by BBL phytoplankton assemblages to grow is approximately ≤50% of the average surface PAR recorded during the cruise.

Eilertsen et al. (1995) argued that germination of various winter phytoplankton resting stages, and thus the timing of the spring bloom in several Norwegian fjords, was dependent on daylength. In our incubations, it appears that phytoplankton growth rates were a hyperbolic function of light intensity (Fig. 3) with saturating photosynthetic rate values above 2 mol quanta m^{-2} d^{-1} (i.e. in the M and H light treatments). Above 2 mol quanta m^{-2} d^{-1}, growth rates were significantly higher in the 12 h treatments as opposed to the 9 h treatments (*t*-test; $p < 0.05$). Thus, one could argue that at saturating light intensities, the increase in daylength will enhance the net growth rate of the phytoplankton community by extending the period during which maximum photosynthetic rates are achieved. Nonetheless, the fact that significant growth occurred in our 9H and 9M treatments argues against daylength as being a sole preventative factor for spring bloom formation in this system. In support of this, elevated levels of chl *a* (~4 to 5.5 µg l^{-1}) were observed in surface waters over most of the shelf off Newport, Oregon, in mid-February of both 2002 and 2003 (Fleischbein et al. 2003, P. Wheeler unpubl. data), when daylengths were roughly 10 h. Additionally, Peterson & Miller (1977) observed clogging of their zooplankton sampling nets by a dense bloom of the diatom *Thalassiosira* spp. in late February 1970. From Fig. 3, we can estimate the compensation light intensity for the BBL phytoplankton to be approximately 0.75 mol quanta m^{-2} d^{-1}, assuming little heterotrophic activity in the samples. This compensation intensity is very close to that observed in other systems (e.g. Siegel et al. 2002, Letelier et al. 2004). By comparing the derived compensation light intensity to mixed layer PARs, it becomes apparent that mixing causes the phytoplankton to be exposed to irradiances that are generally lower than the compensation intensity (Sverdrup 1953, Smetacek & Passow 1990). For example, we occupied the mid-shelf site at noon local time on 2 dates (January 23 and 25) when downwelling was occurring. On those dates, the water column was mixed completely to the bottom (depth = 80 m). Surface PAR was 10.65 and 3.91 mol quanta m^{-2} d^{-1}, respectively. However, average mixed layer PAR, based on the vertical profile conducted at noon and integrated over the 9 h light period, was only 1.91 and 0.29 mol quanta m^{-2} d^{-1}. On a third date (January 26), following a brief (<1 d) period of relaxed winds, the mixed layer shoaled to ca. 25 m. Surface PAR on this date was 5.96 mol quanta m^{-2} d^{-1} and the average mixed layer PAR, again based on noon solar intensity and integrated over the 9 h light period, was still only 0.64 mol quanta m^{-2} d^{-1}. It should be noted that while the surface PAR values are the total integrated PAR measured during the light period, the mixed layer PARs are estimated from noontime PAR and likely overestimate the average daily mixed layer PAR. Based on our results, the daily integrated PAR at the surface during winter is sufficient for phytoplankton growth. However, the decrease in light availability due to mixing in this coastal region seems to limit phytoplankton growth and ultimately influences the timing of late winter/early spring bloom development.

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