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Chlorophyll-normalized isoprene production in laboratory cultures of marine microalgae and implications for global models

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Abstract

We used laboratory cultures of marine microalgae to investigate the effects of growth conditions and their taxonomic position on the production of isoprene, a gas that has major effects on atmospheric chemistry and provides stress tolerance to many primary producers. Isoprene was quantified from 21 microalgal strains sampled during exponential growth, using purge-and-trap pre-concentration and gas chromatography with flame-ionization detection. Isoprene production rates varied by two orders of magnitude between strains (0.03–1.34 μmol [g chlorophyll *a*]$^{-1}$ h^{-1}), and were positively correlated with temperature ($r^2 = 0.52$, $p < 0.001$, $n = 59$). Three distinct sea surface temperature (SST)–dependent relationships were found between isoprene and chlorophyll *a* (μmol [g chlorophyll *a*]$^{-1}$ h^{-1}), an improvement in resolution over the single relationship used in previous models: for three polar strains grown at −1°C (slope = 0.03, $R^2 = 0.76$, $p < 0.05$, $n = 9$), nine strains grown at 16°C (slope = 0.24, $R^2 = 0.43$, $p < 0.05$, $n = 27$ with *Dunaliella tertiolecta* excluded), and eight strains grown at 26°C (slope = 0.39, $R^2 = 0.15$, $p < 0.05$, $n = 24$). We then used a simple model that applied the SST-dependent nature of isoprene production to three representative bioregions for the growth temperatures used in this study. This approach yielded an estimate of global marine isoprene production that was 51% higher than previous attempts using an SST-independent single relationship. Taking into account the effect of temperature therefore potentially allows more precise modeling of marine isoprene production, and suggests that increasing the SST-based resolution of data beyond the three groups used here could further improve future modeling simulations.

Isoprene (2-methyl-1,3-butadiene) is globally one of the most important biogenic volatile organic compounds (BVOC) and is emitted into the atmosphere at a rate similar to that of methane (Pacifico et al. 2009). Once in the atmosphere, isoprene drives tropospheric ozone production (Monson and Holland 2001), increases the residence time of gases that contribute to the greenhouse effect (Poisson et al. 2000), and potentially influences secondary organic aerosols, by either stimulating (Claeys et al. 2004) or inhibiting (Kiendler-Scharr et al. 2009) their formation. Isoprene is synthesized by many photoautotrophic organisms, and provides a range of physiological roles; most notably tolerance to thermal stress (Velikova et al. 2006) and acting as an antioxidant (Loreto and Velikova 2001). Additionally, it serves as an "infochemical"; for example, in terrestrial systems to deter herbivorous *Manduca sexta* caterpillars (Laothawornkitkul et al. 2008), and disrupt the chemical signal used in prey location by parasitic wasps (Loivamaki et al. 2008).

Most research on the production and subsequent roles of isoprene in the natural environment is largely confined to plants in terrestrial biomes (Sharkey et al. 2008). Such bias is perhaps not surprising given the higher estimated emission rates from terrestrial (~ 400–750 Tg C yr^{-1}; Müller et al. 2008) compared to marine (~ 0.1–1.9 Tg C yr^{-1}; Milne et al. 1995; Palmer and Shaw 2005; Gantt et al. 2009) biomes. The higher thermal buffering capacity in aquatic ecosystems reduces the requirement for cellular response systems to thermal stress. However, marine emissions have recently been proposed to be as high as 11.6 Tg C yr^{-1} based on a top-down emission model incorporating global chemistry simulations combined with ship-borne measurements (Luo and Yu 2010), suggesting that isoprene may be a more important marine BVOC than previously considered. Such uncertainty in the strength of marine isoprene emissions is due, in part, to inadequate understanding of how taxonomy and environment control isoprene production (Exton et al. 2010, 2012). Filling this knowledge gap is important to enable a full assessment of isoprene emissions from marine compared to terrestrial environments, and to relate these to the emissions of other marine BVOC such as the well-studied marine trace gas dimethyl sulfide.

Almost all marine phototrophs tested to date have been shown to produce isoprene. Isoprene production rates have been determined for 30 strains from 15 species of microalgae (Table 1; Shaw et al. 2003, 2010; Bonsang et al. 2010), 10 species of temperate macroalgae (Broadgate et al. 2004), and temperate intertidal microbial communities along an estuarine gradient (Acuña Alvarez et al. 2009; Exton et al. 2012). Additional studies have identified isoprene production in a further 11 strains from six microalgal species, but without quantification (Moore et al. 1994; McKay et al. 1996; Shaw et al. 2010). While these studies cover a generally broad taxonomic range of algae, the extent to which taxonomic and environmental variability regulates isoprene production is unclear. In situ studies have demonstrated several patterns of marine isoprene production, including seasonal variations (Broadgate et al.

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Table 1. Isoprene production rates in the literature from analysis of laboratory microalgal cultures, normalized either to cell or Chl *a*. Units are as presented by the original authors and data show means ± SE or ranges. "Unquantified" indicates that isoprene production was detected but production rate not presented.

Species (strain No.)	Isoprene production rate (pmol cell^{-1} d^{-1})	Isoprene production rate (μmol [g Chl *a*]$^{-1}$ d^{-1} unless otherwise stated)	Reference
Bacillariophyceae			
Biddulphia mobiliensis	unquantified		Milne et al. 1995
Chaetoceros affinis	1.6–2.2×10^{-6}		Milne et al. 1995
Chaetoceros debilis		0.65±0.20	Bonsang et al. 2010
Chaetoceros neogracilis (CCMP 1318)		28.48 pmol (μg Chl *a*)$^{-1}$*	Colomb et al. 2008
Chaetoceros neogracilis (CCMP 1318)		1.26±1.19	Bonsang et al. 2010
Fragilariopsis kerguelensis		0.56±0.35	Bonsang et al. 2010
Nitzschia sp. (CCMP 580)	unquantified		Moore et al. 1994
Ondontella mobiliensis	unquantified		Moore et al. 1994
Pelagomonas calceolate (CCMP 1214)	5.7±4.4×10^{-8}	1.6±1.6	Shaw et al. 2003
Phaeodactylum tricornutum (Pet Pd)	unquantified		Milne et al. 1995
Phaeodactylum tricornutum (Falkowski)		2.85 pmol (μg Chl *a*)$^{-1}$*	Colomb et al. 2008
Phaeodactylum tricornutum (UTEX 646)		1.12±0.32	Bonsang et al. 2010
Porosira glacialis	unquantified		Moore et al. 1994
Skeletonema costatum	1.1–1.4×10^{-6}		Milne et al. 1995
Skeletonema costatum		1.32±1.21	Bonsang et al. 2010
Skeletonema costatum (CCAP 1077/5)	unquantified		McKay et al. 1996
Skeletonema costatum (CCMP 1332)		1.8	Shaw et al. 2003
Thalassiosira weissflogii (Actin)	unquantified		Milne et al. 1995
Prymnesiophyceae			
Calcidiscus leptoporus (AC365)		5.40 pmol (μg Chl *a*)$^{-1}$*	Colomb et al. 2008
Emiliania huxleyi (CCMP 371)		11.45 pmol (μg Chl *a*)$^{-1}$*	Colomb et al. 2008
Emiliania huxleyi (CCMP 371)		1.0	Bonsang et al. 2010
Emiliania huxleyi (CCMP 373)	3.8±2.1×10^{-7}	1.0±0.5	Shaw et al. 2003
Emiliania huxleyi (MCH)	1.7–2.8×10^{-6}		Milne et al. 1995
Emiliania huxleyi (MCH)	2.3×10^{-6}		Shaw et al. 2003
Emiliania huxleyi (WH 1387)	2.0±2.0×10^{-7}		Shaw et al. 2003
Prymnesium parvum (Prym)	unquantified		Milne et al. 1995
Dinophyceae			
Amphidinium aperculatum (Ahoef)	4.1–6.7×10^{-6}		Milne et al. 1995
Amphidinium sp.	unquantified		Moore et al. 1994
Heterocapsa pygmaea (Gymno)	none detected		Milne et al. 1995
Scrippsiella trochoidea (CCAP 1134/5)	none detected		McKay et al. 1996
Cyanophyceae			
Prochlorococcus sp.		0–22	Shaw et al. 2003
Prochlorococcus sp. (axenic MED4)	1.4±0.8×10^{-9}	1.5±0.9	Shaw et al. 2003
Prochlorococcus sp. (MIT 9401)	1.7±1.3×10^{-9}		Shaw et al. 2003
Prochlorococcus sp. (ss120)	1.1±0.3×10^{-9}		Shaw et al. 2003
Synechococcus sp. (DC2)	unquantified		Milne et al. 1995
Synechococcus sp. (RCC 40)		4.97±2.87	Bonsang et al. 2010
Synechococcus sp. (WH 8103)	4.9±4.7×10^{-9}	1.4	Shaw et al. 2003
Trichodesmium sp. (ISM 101)		3.00±0.52	Bonsang et al. 2010
Trichodesmium sp.		1.6–4.7	Arnold et al. 2009
Chlorophyceae			
Dunaliella tertiolecta	unquantified		Acuña Alvarez et al. 2009
Dunaliella tertiolecta		0.36±0.22	Bonsang et al. 2010
Dunaliella tertiolecta (DUN, Falkowski)		2.85 pmol (μg Chl *a*)$^{-1}$*	Colomb et al. 2008
Prasinophyceae			
Micromonas pusilla (CCMP 489)	2.0±1.0×10^{-8}	1.4±0.8	Shaw et al. 2003

* Original units (pmol L^{-1} Chl *a*$^{-1}$) changed to pmol (μg Chl *a*)$^{-1}$ as per communication with original authors.

1997; Liakakou et al. 2007; Exton et al. 2012), and a positive correlation with chlorophyll *a* (Chl *a*; Bonsang et al. 1992; Milne et al. 1995; Moore and Wang 2006), but these are based on measurements from natural samples containing mixed algal communities. By resolving the uncertainty surrounding the taxonomic and environmental regulation of marine isoprene production, more accurate predictions can be made on how future environmental change may alter the contribution of marine isoprene production to global emissions.

Limited data are currently available to parameterize model simulations of global isoprene emissions from marine biomes; additional measurements of isoprene production from a diverse range of taxa and across environmental conditions have been proposed repeatedly to improve bottom-up modeling (Luo and Yu 2010; Shaw et al. 2010). Therefore, we examined the effect of the taxonomic position and growth temperature of marine algae on isoprene production rates by combining published data with new information from microalgal species isolated from diverse environments. We use these data to explore the potential utility of multiple relationships between isoprene production and Chl *a* concentration, with the aim to improve our ability to constrain and forecast isoprene in future emission models.

Methods

Microalgal culturing—A total of 21 microalgal strains from across seven algal classes were independently isolated or obtained from the National Center for Marine Algae and Microbiota (NCMA, formerly CCMP), the Culture Collection of Algae and Protozoa (CCAP), or The Plymouth Culture Collection of Marine Algae (Table 2). The choice of species included different isolates of the coccolithophore *Emiliania huxleyi* (CCMP 373 and CCMP 1516), as well as multiple species or phylotypes from single genera, including the diatom *Thalassiosira* and the dinoflagellate *Symbiodinium*. Triplicate unialgal cultures of each strain were inoculated in 500 mL sterilized conical glass flasks containing 200 mL of 0.2 μm filtered and autoclaved artificial seawater media supplemented with specific nutrients (Table 2). Batch cultures were grown, without shaking, under temperature conditions optimal for growth. Light was provided by cool-white fluorescent tubes (TL-D 840, Philips) on a 14 : 10 light : dark cycle. Cultures were monitored daily for 2 to 4 weeks through a combination of haemocytometry and fluorometry, and diluted where necessary to maintain cultures in exponential growth. Three days before the measurements, cultures were diluted with fresh media, resulting in a mean final cell density of 4.3 $\times$ 10^5 cells mL^{-1}, ranging from 2.1 $\times$ 10^5 cells mL^{-1} for *Nitzschia* sp. (CCMP 1088) to 9.1 $\times$ 10^5 cells mL^{-1} for *Synechococcus* sp. (CCMP 1334).

Isoprene analysis—Sterile gastight borosilicate glass purge vessels (Fig. 1) were used to measure isoprene production rates as described by Exton et al. (2010), although gas chromatography with flame ionization detection (GC-FID) was chosen as the method of quantification as opposed to chemiluminescence due to the limits of the latter technique when working with low-biomass levels as used here (Exton et al. 2010). Briefly, 150 mL of each culture was transferred to the purge vessels. Each vessel was purged for 30 min with low-hydrocarbon compressed air (British Oxygen Company) scrubbed with a Supelpure HC hydrocarbon trap (Supelco, Sigma-Aldrich) at 275 kPa and a flow rate of 80 mL min^{-1} to remove any preexisting isoprene. Vessels were then incubated under the same temperature and light conditions as used for growth for a consistent 4 h period of the 14 h light cycle, and subsequently subjected to an identical purge (30 min, 275 kPa, 80 mL min^{-1}). Gas from this second purge was concentrated in a cryo-trap held at $-160^{\circ}C$ using a liquid nitrogen boiler. Isoprene concentration was quantified using GC-FID (GC-2010; Shimadzu) fitted with an Alumina Potassium Chloride (Al/KCl) column of 50 m length and 0.53 mm internal diameter (Exton et al. 2010). Ultra-high-purity helium was used as the carrier gas. The effect of purging on biological samples was tested during earlier method development, and demonstrated a linear increase in isoprene production with increasing incubation times after pre-purging, while microscopic examination of cells revealed no obvious damage to the algae.

Calibration was performed using 1–100 μL isoprene standard gas in helium (4.16 μmol L^{-1}; Scientific and Technical Gas) injected directly into the carrier gas flow prior to the purge-and-trap apparatus. The detection limit was below 4.16 pmol or 27.73 pmol L^{-1} in 150 mL of sample (equivalent to the lowest standard gas volume injected during calibration), and tests showed that purge efficiency was $>$ 90% with a 30 min purge. Analytical replicates indicated that the relative variation was below 6% (n = 15). For all samples, "blank" controls comprising of filtered seawater alone were incubated in parallel with the biological samples to identify any background isoprene within the apparatus and seawater. No isoprene was detected in any of the blanks, and so no corrections were applied, and all data reported are thus attributed to biological activity. In contrast to many other BVOC, isoprene has a low Henry's constant (k_H) of $\sim 0.286 \times 10^{-3}$ (mol_{aq}/m^3_{aq})/Pa (0.029 M/atm; Karl et al. 2001), and is characterized by low water solubility and high volatility. This suggests that the effect of temperature on its solubility is small, and we calculated that the difference in solubility between $-1^{\circ}C$ and $26^{\circ}C$ is below 2.3%. Since this is smaller than the relative variation between analytical replicates (6%), no further corrections for differences in incubation temperature were applied.

Data integration—Isoprene production rates for all strains were normalized to the corresponding Chl *a* concentration in order to provide a standardized data set comparable with the majority of existing studies, as well as maximize potential uses for global modeling (Luo and Yu 2010; Shaw et al. 2010). Chl *a* was quantified by filtering 30 mL of culture through 25 mm diameter, 0.7 μm pore-size GF/F glass-fiber filters (MF300, Fisher Scientific). Filters were flash-frozen in liquid nitrogen and stored at $-80^{\circ}C$. Quantification of Chl *a* was performed

Table 2. Isoprene production rates normalized to Chl *a* (iP^{Chl}) for laboratory-cultured microalgae. Provided are strain information and incubation conditions (temperature [Temp.] and light). Each strain was grown in triplicate batch cultures, and isoprene quantified using GC-FID with purge-and-trap analysis after gastight incubation. Data shown are mean ± SE, normalized to Chl *a* (μmol [g Chl *a*]$^{-1}$ h^{-1}).

Species	Strain	Medium	Temp. (°C)	Light (μmol m^{-2} s^{-1})	Isoprene production (iP^{Chl}) (μmol [g Chl *a*]$^{-1}$ h^{-1})
Bacillariophyceae					
Chaetoceros muelleri	CCAP 1010/3	F/2*+Si	16	300	0.39±0.05
Cylindrotheca sp.	University of Essex†	F/2*+Si	16	300	0.11±0.00
Fragilariopsis cylindrus	T. Mock‡	F/2*+Si(2×NO_3^-)	−1	160	0.04±0.01
Nitzschia sp.	CCMP 1088	F/2*+Si(2×NO_3^-)	−1	160	0.04±0.01
Synedropsis sp.	CCMP 2745	F/2*+Si(2×NO_3^-)	−1	160	0.03±0.01
Thalassiosira pseudonana	CCAP 1085/12	F/2*+Si	16	300	0.24±0.01
Thalassiosira weissflogii	CCMP 1051	F/2*+Si	16	300	0.19±0.01
Chlorophyceae					
Dunaliella tertiolecta	CCMP 1320	F/2*	16	180	0.05±0.00
Cryptophyceae					
Rhodomonas lacustris	CCAP 995/3	F/2*	16	300	0.39±0.03
Cyanophyceae					
Synechococcus sp.	CCMP 1334	F/2* Sargasso SW§	26	120	0.49±0.00
Trichodesmium erythraeum	CCMP 1985	YBC II‖	26	300	0.10±0.02
Dinophyceae					
Prorocentrum minimum	Plymouth 18B	F/2*+Si	16	300	0.42±0.06
Symbiodinium sp. (A1¶)	CCMP 2464	ASP-8A#	26	300	0.19±0.13
Symbiodinium sp. (A13¶)	CCMP 2469	ASP-8A#	26	300	0.71±0.35
Symbiodinium sp. (A20¶)	D. Pettay**	ASP-8A#	26	300	0.40±0.12
Symbiodinium sp. (B1¶)	CCMP 2463	ASP-8A#	26	300	1.15±0.07
Prasinophyceae					
Prasinococcus capsulatus	CCMP 1614	F/2*	26	300	1.34±0.24
Tetraselmis sp.	CCMP 965	F/2*	26	300	0.16±0.01
Prymnesiophyceae					
Emiliania huxleyi	CCMP 373	F/2*	16	300	0.12±0.02
Emiliania huxleyi	CCMP 1516	F/2*	16	300	0.47±0.04
Gephyrocapsa oceanica	Plymouth 572	F/2*	16	300	0.64±0.17

* Guillard 1975, made with artificial seawater + MilliQ water + bicarbonate + selenium
† Isolated by G. J. C. Underwood at the University of Essex, United Kingdom
‡ Isolated by T. Mock, University of East Anglia, United Kingdom (Bayer-Giraldi et al. 2010)
§ Natural seawater from the Sargasso Sea (low nutrients)
‖ Chen et al. 1996
¶ ITS2 phylotype
# Provasoli et al. 1957
** Isolated by D. Pettay, maintained by M. Warner, University of Delaware

spectrophotometrically after extraction in 100% methanol, and based on established equations (Ritchie 2006). Final Chl *a*–normalized production rates (termed iP^{Chl}) are in units of μmol (g Chl *a*)$^{-1}$ h^{-1}.

The only deviation from the recommended unit of measurement (Luo and Yu 2010; Shaw et al. 2010) was to report production rates hourly rather than daily. This decision was based on the shorter incubation periods used in this study compared to others (Shaw et al. 2003; Bonsang et al. 2010), and thus the fact that diurnal variation was not accounted for here so that isoprene production measurements reported here are likely to be maximal values. Data from the literature have been converted to hourly production rates for comparison, by dividing by 14 based on the corresponding light cycle used for incubations and the fact that most if not all isoprene is produced during the light period (Shaw et al. 2003; Sharkey et al. 2008).

Statistical analyses—Comparisons of means were performed using one-way analysis of variance, applied to log-transformed data as raw data did not follow a normal distribution. In the case of multiple comparisons, post hoc analysis was also employed using the Tukey test. Pearson's product-moment correlation coefficient was used to measure the strength of association between isoprene production rates and other variables, while linear regression analysis was used to investigate the relationship between isoprene production and Chl *a*. To assess the combined effect of environmental factors (temperature and light), stepwise regression analysis was performed. These analyses were carried out using Minitab version 15 statistical software.

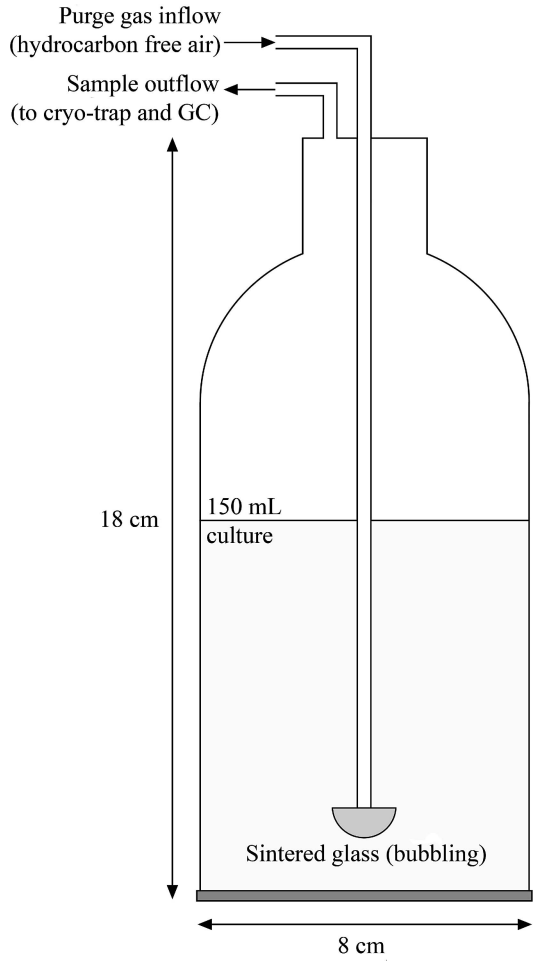

Fig. 1. Schematic diagram of the borosilicate glass purge vessel used in this study to incubate microalgal samples and cryogenically enrich isoprene with purge-and-trap methodology before quantification via gas chromatography using flame-ionization detection (GC-FID).

Results

Laboratory phytoplankton cultures—Mean (± standard error) values of $i\mathrm{P}^{\mathrm{Chl}}$ among all microalgae examined here (Table 2) varied by two orders of magnitude, ranging from 0.03 ± 0.01 in the bacillariophyte *Synedropsis* sp. to 1.34 ± 0.24 μmol (g Chl *a*)$^{-1}$ h^{-1} in the prasinophyte *Prasinococcus capsulatus* (Table 2). Lowest $i\mathrm{P}^{\mathrm{Chl}}$ values were from the three polar strains incubated at −1°C (0.03 ± 0.01 to 0.04 ± 0.01 μmol [g Chl *a*]$^{-1}$ h^{-1}), similar to the chlorophyte *Dunaliella tertiolecta* that had the lowest values for any of the temperate or tropical strains, 0.05 ± 0.00 μmol (g Chl *a*)$^{-1}$ h^{-1}. In addition to *P. capsulatus*, only *Symbiodinium* sp. (phylotype B1) yielded values of $i\mathrm{P}^{\mathrm{Chl}} > 1$ μmol (g Chl *a*)$^{-1}$ h^{-1}, specifically 1.15 ± 0.07; the remaining strains all had rates between 0.03 and 0.71 μmol (g Chl *a*)$^{-1}$ h^{-1}.

Values of $i\mathrm{P}^{\mathrm{Chl}}$ for all strains were initially pooled according to algal class and were lowest for the Chlorophyceae and Bacillariophyceae (~ 0.05–0.15 μmol [g Chl *a*]$^{-1}$ h^{-1}), and highest for Dinophyceae and Prasinophyceae (~ 0.75–0.86 μmol [g Chl *a*]$^{-1}$ h^{-1}; Table 3; $F_{6,58} = 8.22$, $p < 0.001$). However, clear variability was also evident among species within the different classes. For example, in the case of the Bacillariophyceae the three polar strains (*Synedropsis* sp., *Nitzschia* sp., and *Fragilariopsis cylindrus*) yielded $i\mathrm{P}^{\mathrm{Chl}}$ values that were ~ 85% lower than the average $i\mathrm{P}^{\mathrm{Chl}}$ for all other diatom strains ($F_{6,20} = 30.94$, $p < 0.001$; Table 2). Values of $i\mathrm{P}^{\mathrm{Chl}}$ for the two *Thalassiosira* strains (*T. pseudonana* and *T. weissflogii*) were almost the same (0.24 and 0.19 μmol [g Chl *a*]$^{-1}$ h^{-1}, respectively). Similarly, in the case of the Cyanophyceae, $i\mathrm{P}^{\mathrm{Chl}}$ values were 488% higher for *Synechococcus* sp. than for *Trichodesmium erythraeum* ($F_{1,5} = 43.28$, $p < 0.01$). Interestingly, for the Prymnesiophyceae, $i\mathrm{P}^{\mathrm{Chl}}$ values were also significantly different between strains of the same species; $i\mathrm{P}^{\mathrm{Chl}}$ for *E. huxleyi* CCMP 373 was ~ 377% higher than for *E. huxleyi* CCMP 1516 ($F_{2,8} = 18.96$, $p < 0.01$).

Values of $i\mathrm{P}^{\mathrm{Chl}}$ were analyzed according to growth temperature, and overall increased with temperature across all taxonomic groups. Pooling $i\mathrm{P}^{\mathrm{Chl}}$ values for all taxa according to growth temperature yielded mean values of 0.03 ± 0.01 (−1°C), 0.31 ± 0.06 (16°C), and 0.75 ± 0.13 (26°C) μmol (g Chl *a*)$^{-1}$ h^{-1} (Table 3), which were all significantly different from one another ($F_{2,58} = 33.78$, $p < 0.001$); this trend was further supported by a strong positive correlation between isoprene production and growth temperature ($r^2 = 0.52$, $p < 0.001$, $n = 59$), with a resultant linear regression equation of $i\mathrm{P}^{\mathrm{Chl}}$ = (0.027 × growth temperature) − 0.042. It is important to note that $i\mathrm{P}^{\mathrm{Chl}}$ for multiple growth temperatures was examined for two classes only: Bacillariophyceae at −1°C and 16°C, and Dinophyceae at 16°C and 26°C. Both exhibited a significant increase of $i\mathrm{P}^{\mathrm{Chl}}$ by 685% ($F_{1,20} = 86.53$, $p < 0.001$) and 251% ($F_{1,12} = 14.83$, $p < 0.01$), respectively, at the higher temperature (Fig. 2).

A positive correlation was also observed between $i\mathrm{P}^{\mathrm{Chl}}$ and growth light intensity ($r^2 = 0.61$, $p < 0.001$, $n = 59$). However, when all data were pooled based on light intensity, the only significant difference between light levels was the higher isoprene production values obtained at 300 compared to 160 μmol m^{-2} s^{-1} ($F_{3,58} = 3.38$, $p < 0.05$). In fact, performing a stepwise multiple regression with temperature and light relative to $i\mathrm{P}^{\mathrm{Chl}}$ demonstrated that light only provided a 3% increase in model fit (from $R^2_{\mathrm{adj}} = 51.73$ to $R^2_{\mathrm{adj}} = 54.88$), indicating that temperature indeed appears to be the primary driver of variability observed between different algal taxa.

Relationship between $i\mathrm{P}^{\mathrm{Chl}}$ and temperature—Previous studies have suggested that variation in isoprene production (mol L^{-1} h^{-1}) can be explained by variability in Chl *a* concentration, but this notion is based on an SST-independent relationship between isoprene production and Chl *a* from relatively few algal species (Shaw et al. 2003; Palmer and Shaw 2005). Pooling our isoprene production rates for all strains at all temperatures demonstrated a positive relationship with Chl *a* (slope = 0.01, $R^2 = 0.00$; Table 4). This value of 0.01 μmol isoprene (g Chl *a*)$^{-1}$ h^{-1} is over one order of magnitude lower than the SST-independent relationship of 1.8 μmol isoprene (g

Table 3. Isoprene production rates normalized to Chl *a* content for each taxonomic class of microalgal strain tested and each incubation condition. Data shown are the number of tested strains and mean iP^{Chl} values ± SE.

	No. of strains	Isoprene production rate (μmol [g Chl *a*]$^{-1}$ h^{-1})
Taxonomic class		
Bacillariophyceae	7	0.15±0.03
Chlorophyceae	1	0.05±0.00
Cryptophyceae	1	0.39±0.03
Cyanophyceae	2	0.25±0.09
Dinophyceae	5	0.86±0.16
Prasinophyceae	2	0.75±0.29
Prymnesiophyceae	3	0.41±0.09
Incubation temperature (°C)		
−1	3	0.03±0.01
16	10	0.31±0.06
26	8	0.75±0.13
Incubation light (μmol m^{-2} s^{-1})		
120	1	0.49±0.00
160	3	0.03±0.01
180	1	0.05±0.00
300	16	0.51±0.07

Chl *a*)$^{-1}$ d^{-1} (equivalent to 0.13 μmol isoprene [g Chl *a*]$^{-1}$ h^{-1} based on the light regime used in the study) used in existing global models of marine isoprene (Shaw et al. 2003). After analyzing our data based on temperature, the slope of this relationship (μmol isoprene [g Chl *a*]$^{-1}$ h^{-1}) was shown to increase from 0.03 ($R^2 = 0.76$, $p < 0.05$, $n = 9$) at −1°C to 0.39 ($R^2 = 0.15$, $p < 0.05$, $n = 24$) at 26°C (Fig. 3). In the case of microalgae grown at 16°C, an overall slope of 0.04 ($R^2 = 0.08$, $p < 0.05$, $n = 30$) was observed, but the relatively low iP^{Chl} for *D. tertiolecta* greatly influenced this outcome, and after removing *D. tertiolecta* from the data set, the slope increased to 0.24 ($R^2 = 0.43$, $p < 0.05$, $n = 27$; Table 4).

Since temperature was a strong determinant in isoprene production, we explored how the separate quantification of isoprene production in broad latitudinal regions would affect the outcome of previous estimations (Palmer and Shaw 2005) that were based on SST-independent relationships between *i*P and Chl *a* (Shaw et al. 2003). We therefore assigned the three growth temperatures of −1°C, 16°C, and 26°C used here to the broad biogeographical regions of polar (60–90°N and S), temperate (23.5–60°N and S), and tropical (23.5°N–23.5°S) biomes, respectively. Monthly Chl *a* values for each latitude range were produced via the Goddard Earth Sciences Data and Information Services Center (GES-DISC) Interactive Online Visualization and Analysis Infra-

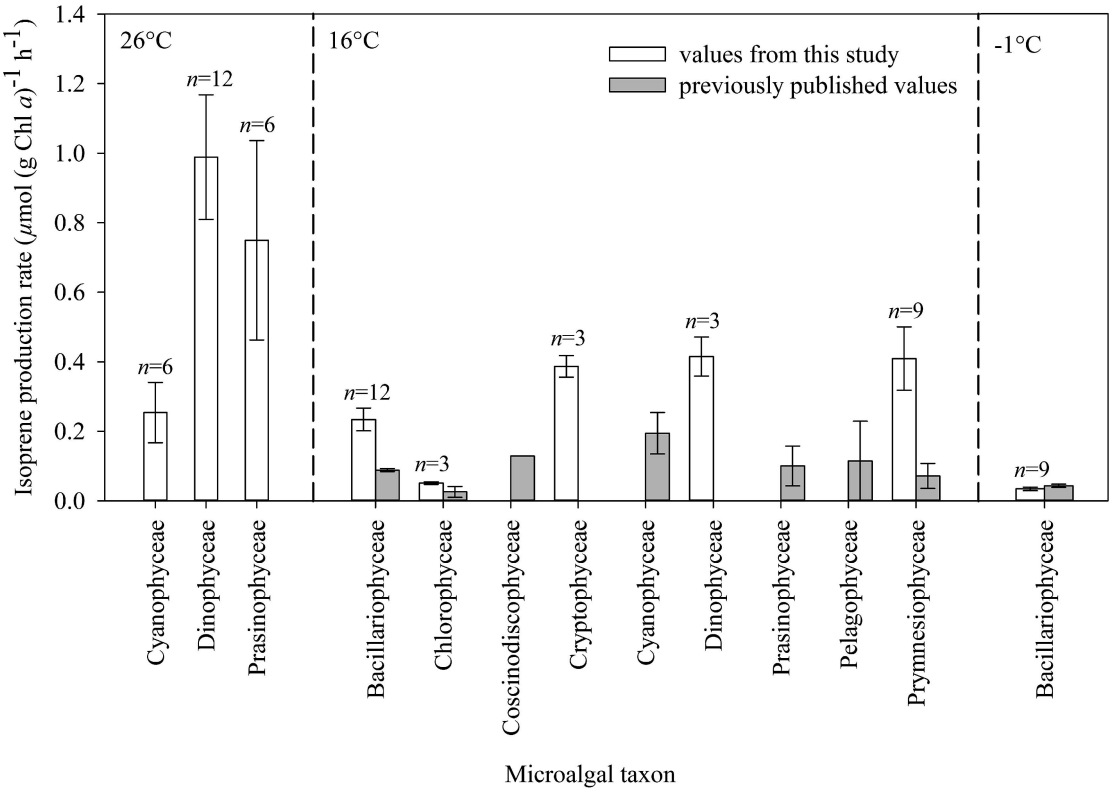

Fig. 2. Isoprene production rates for laboratory-cultured microalgal taxa at a range of growth temperatures. Data shown are the means for all strains analyzed in triplicate during this study (white bars) from each taxonomic group ± standard error (SE) normalized to Chl *a*. Results from previous research are shown in gray bars for comparison (Shaw et al. 2003; Gantt et al. 2009; Bonsang et al. 2010). The numbers of samples are included above each data point, and refer to the number of strains analyzed. Note that exact temperatures used for growth vary between this study and previous ones, but fall into the same broad categories (Bonsang et al. 2010: 4°C and 20°C; Gantt et al. 2009: 22°C; Shaw et al. 2003: 23°C), and hourly production rates were calculated by dividing daily rates in the literature by 14 (based on the light cycle of incubation conditions used in the studies).

Table 4. Regression coefficients for isoprene production rates and Chl *a* concentration in strains of microalgal cultures representing three different growth temperatures. Where applicable, data show regression results including and excluding the outlying results of the chlorophyte *Dunaliella tertiolecta*. All slope and intercept fits were significant at $p < 0.05$, while slope and intercept values are reported as mean (± SE).

	R^2	Slope	Intercept	Sample no. (*n*)
Strains grown at −1°C	0.76	0.03(±0.006)	$0.62\times10^{-6}(\pm0.839\times10^{-6})$	9
Strains grown at 16°C (without *D. tertiolecta*)	0.43	0.24(±0.056)	$0.25\times10^{-5}(\pm0.418\times10^{-5})$	27
Strains grown at 16°C (with *D. tertiolecta*)	0.08	0.04(±0.025)	$0.15\times10^{-4}(\pm0.032\times10^{-4})$	30
Strains grown at 26°C	0.15	0.39(±0.221)	$0.13\times10^{-4}(\pm0.144\times10^{-4})$	24
All strains pooled (without *D. tertiolecta*)	0.01	0.04(±0.060)	$0.20\times10^{-4}(\pm0.054\times10^{-4})$	60
All strains pooled (with *D. tertiolecta*)	0.00	0.01(±0.038)	$0.21\times10^{-4}(\pm0.043\times10^{-4})$	63

structure (GIOVANNI) Ocean Colour Radiometry Sea-viewing Wide Field-of-view Sensor (SeaWiFS) online system, developed and maintained by National Aeronautics and Space Administration: http://disc.sci.gsfc.nasa.gov/giovanni (Ocean Colour Radiometry SeaWiFS products), and averaged over a 5-yr period (January 2006 to December 2010). The total coverage of each region was estimated based on the maximum pixel coverage achieved by SeaWiFS over the 5 yr within the latitudinal ranges (Table 5). The use of remotely sensed marine Chl *a* data represents a similar broad approach to that used in previous models (Palmer and Shaw 2005), although more simplistic calculations are used here that are sufficient to draw comparisons between SST-dependent and -independent approaches. Total Chl *a* content of each region was then applied to: (1) the existing SST-independent relationship from Shaw et al. (2003), (2) the general relationship from all data pooled from our study, and (3) the three individual SST-dependent relationships developed in this study. The results of this excercise indicated a potential overestimation of isoprene production in polar regions, coupled with a large underestimation of temperate and tropical production when using the SST-independent (Shaw et al. 2003) compared to our SST-dependent relationship (Table 5). Overall, use of the geographically dependent SST relationship led to a global isoprene emission rate of 26,902 mol isoprene h^{-1}, which is 51% and 470% higher compared to the values obtained using the SST-independent relationships of Shaw et al. (2003) and this study, respectively.

Discussion

Values of $i\mathrm{P}^{\mathrm{Chl}}$ observed here (0.03 to 1.34 μmol [g Chl *a*]$^{-1}$ h^{-1}) fall within previously published data from phytoplankton monocultures, which range from 0.02 to 2.92 μmol (g Chl *a*)$^{-1}$ h^{-1} (Shaw et al. 2003, 2010; Bonsang et al. 2010), and provide further evidence for the previously published interstrain variation of between one and two orders of magnitude (McKay et al. 1996; Gantt et al. 2009).

Environmental and taxonomic influences on microalgal isoprene production—Of the strains tested here, *D. tertiolecta* CCMP 1320 and the three polar diatoms (*Fragilariopsis cylindrus*, *Nitzschia* sp. CCMP 1088, *Synedropsis* sp. CCMP 2745) exhibited the lowest isoprene production rates, a pattern supported by another recent study in which a total of nine strains were compared (Bonsang et al. 2010). In the case of polar strains, environmental conditions are likely to limit isoprene synthesis. The temperature dependency of isoprene production in marine systems has previously been described, both in microalgal cultures (Shaw et al. 2003) and in temperate macroalgae (Broadgate et al. 2004), and with Antarctic diatoms adapted to low temperatures, the benefits of significant isoprene synthesizing capabilities may not be as important.

An underlying cause for the low production rates in *D. tertiolecta* cultures is less clear. Unlike most algae, the Chlorophyta (including the Chlorophyceae and Prasinophyceae) possess only one of the two distinct pathways for isoprene synthesis. The Acetate/Mevalonate pathway, generally believed to be the dominant pathway of isoprene production, is absent in the Chlorophyta (Lichtenthaler 1999), and therefore $i\mathrm{P}^{\mathrm{Chl}}$ values could be expected to be lower than for other classes (Table 3). That said, the highest isoprene producer in this study, *P. capsulatus* CCMP, is also a member of the Chlorophyta, and similarly Shaw et al. (2003) showed that the chlorophyte *Micromonas putida* CCMP 489 produced relatively high concentrations of isoprene. This suggests that the similarly low production rates measured here and elsewhere for *D. tertiolecta* (Bonsang et al. 2010) appear to be species-specific, rather than a broader taxonomic phenomenon among chlorophytes, which is further supported by the relatively high isoprene production demonstrated by the macroalga *Ulva intestinalis* (Broadgate et al. 2004). Clearly, given the ecological and biogeochemical importance of chlorophytes in aquatic environments, the nature and extent of variability within this class requires further attention.

All five strains of Dinophyceae tested were relatively high producers. One particularly notable pattern was the variability of $i\mathrm{P}^{\mathrm{Chl}}$ among the four *Symbiodinium* phylotypes, where production rates were inversely related to the widely accepted ability of each strain to tolerate high temperature stress: high to low tolerance of A20 > A1 > B1 > A13 (Robison and Warner 2006). Here, $i\mathrm{P}^{\mathrm{Chl}}$ values were highest for A13 and B1, the least stress-tolerant strains, suggesting that isoprene does not play a significant role in thermotolerance in these strains; a pattern that would somewhat contradict the widely accepted role of isoprene in thermotolerance of higher plants (Sharkey et al. 2008), and the positive relationship between temperature stress and production observed previously in algal cultures (Shaw et al. 2003). Therefore, a more plausible explanation

Table 5. Isoprene production rate (mol h^{-1}) estimates for the biogeographical regions broadly representing the three growth temperatures used here, based on the single relationship between isoprene production and Chl *a* concentration (Shaw et al. 2003 and this study) and on multiple relationships as described in this study. Estimates based on this study are shown as mean value with bracketed minimum and maximum ranges based on the SE of regression equations used (see Table 4). Chlorophyll concentrations were obtained from monthly SeaWiFS data averaged over a 5-yr period (January 2006–December 2010), as described in the main text.

Isoprene production estimate (mol isoprene h^{-1})	Marine biogeographical region (representative growth temperature, °C)					
	Northern polar, 90–60°N (−1)	Northern temperate, 60–23.5°N (16)	Tropics, 23.5°N–23.5°S (26)	Southern temperate, 23.5–60°S (16)	Southern polar, 60–90°S (−1)	Global marine total
Single relationship (Shaw et al. 2003)	5096	4289	3573	3749	1105	17,811
Single relationship (this study)	1352(0–3704)	1138(0–3117)	948(0–2597)	995(0–2725)	293(0–803)	4727(0–12,947)
Multiple relationship (this study)	1015(780–1250)	7917(6070–9765)	10,829(4755–16,902)	6920(5306–8535)	220(169–271)	26,902(17,079–36,724)

is that isoprene production rates at ambient growth temperatures do not necessarily relate to their ability to upregulate synthesis under stress conditions, as was also suggested for the stress-dependent production of dimethyl sulfide in *Symbiodinium* sp. (Steinke et al. 2011). Another possibility is the potential for other compounds (e.g., other terpenoids) to afford similar ecophysiological benefits to those provided by isoprene (Yuan et al. 2009). Elevated isoprene production under stress compared to ambient conditions could also explain the lack of difference between coastal and open-ocean phytoplankton strains observed in this study, since phytoplankton in coastal systems will inevitably be exposed to greater environmental fluctuations than in open-ocean systems (Lavaud et al. 2007). Again, further experiments focusing on the effect of transient environmental perturbations among taxa will be an important step in verifying such notions.

Increased isoprene production in response to raised temperature and light levels has previously been established in laboratory microalgal incubations (Shaw et al. 2003; Bonsang et al. 2010). Similarly, seasonal fluctuations in isoprene production in response to changes in light and, more importantly, temperature, has also been shown for temperate estuarine microphytobenthic communities (Exton et al. 2012). However, the relative change from phytoplanktonic community assemblage (adaptation) vs. cellular upregulation (acclimation or stress response) in contributing to isoprene synthesis per unit area is yet to be established. Based on the strong variation in $i\mathrm{P}^{\mathrm{Chl}}$ among taxa demonstrated here and elsewhere (Arnold et al. 2009), the answer to this issue could have important consequences for understanding and, ultimately, predicting finer scale variance of isoprene emissions.

Potential for improved global models of marine isoprene emissions—Importantly, our data have enabled a broader assessment of $i\mathrm{P}^{\mathrm{Chl}}$ variability than previously possible and demonstrated that the relationship between isoprene production and Chl *a* cannot be well described by a single algorithm as used previously (Palmer and Shaw 2005). The data presented here, representing a greatly increased number and range of microalgae for which isoprene production rates are known, provides a lower SST-independent isoprene production rate: 0.04 (Table 4) compared to 0.13 μmol isoprene (g Chl *a*)$^{-1}$ h^{-1} in Shaw et al. (2003; Table 5). However, as with other recent studies (Arnold et al. 2009), our SST-dependent approach suggests that further developing the level of detail incorporated into model simulations is critically dependent upon considering variation in isoprene production rates between different microalgal taxa and growth environments.

Our relationship of 0.12 μmol isoprene (g Chl *a*)$^{-1}$ h^{-1} for the taxa grown at 16°C is consistent with the previously used SST-independent relationship, which is unsurprising, as the latter value was obtained from sampling of temperate strains only (Shaw et al. 2003; Palmer and Shaw 2005). Thus, the increase in global isoprene production observed for our SST-dependent approach is based on contributions from phytoplankton grown under higher temperatures (i.e., tropical) being significantly higher than the overall global mean. Consequently, further improving the accuracy of such models

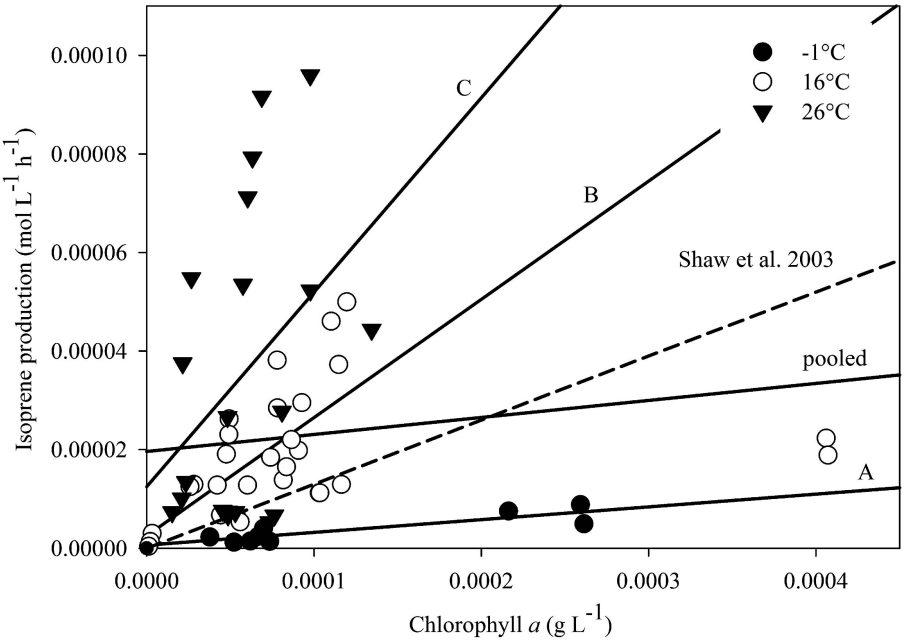

Fig. 3. The relationship between Chl *a* concentration and isoprene production rate in laboratory phytoplankton cultures grown at three different temperatures, showing regressions for (A) strains grown at −1°C (filled circles), (B) strains grown at 16°C (open circles), and (C) strains grown at 26°C (filled triangles; regression equation values are shown in Table 3). Also shown is an overall regression for all strains at all temperatures (''pooled''), and the SST-independent relationship used in previous global models of marine isoprene identified by Shaw et al. (2003; 0.13 μmol isoprene [g Chl *a*]$^{-1}$ h^{-1}). Outlying results for *Dunaliella tertiolecta* are omitted from the regression equations displayed here.

is likely dependent on focusing on representative species from these latitudinal and growth-temperature ranges.

We demonstrate that developing model simulations based on SST-dependent relationships between isoprene production rates and Chl *a* concentrations has the potential to improve precision when estimating global marine isoprene production. Considering the inherent logistical challenges associated with direct measurements of oceanic isoprene in situ, our bottom-up approach of using laboratory measurements of isoprene production forms an important component of assessments of overall production from marine systems. To further develop production models, it is important that a wider range of microalgae from a more diverse range of environments and growth temperatures are screened for their isoprene production rates, and that these are applied to satellite-derived measurements of SST and Chl *a*. To calculate an even more faithful estimate of emission rates, it will also be important to include other processes that contribute to net emission of isoprene from the marine environment, particularly coastal benthic sources (Exton et al. 2012), bacterial consumption (Acuña Alvarez et al. 2009), short-term stress responses, and factors affecting transfer across the marine boundary layer, including wind speed and turbulence. Developing more accurate and robust marine isoprene models will help us fully understand the importance of isoprene from marine systems, while increasing the diversity of organisms for which production rates are known will allow us to better appreciate the physiological processes behind its synthesis in marine algae.

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