



RESEARCH ARTICLE

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Effects of Temperature and Light on Methane Production of Widespread Marine Phytoplankton

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Key Points:

- Temperature, daylength, and light intensity influenced methane production from marine algae
- Light intensity showed a very pronounced effect on methane formation rates from all investigated species
- Global change will likely increase methane release rates of marine phytoplankton

Supporting Information:

- Supporting Information S1

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Abstract Methane (CH₄) production in the ocean surface mixed layer is a widespread but still largely unexplained phenomenon. In this context marine algae have recently been described as a possible source of CH₄ in surface waters. In the present study we investigated the effects of temperature and light intensity (including daylength) on CH₄ formation from three widespread marine algal species *Emiliania huxleyi*, *Phaeocystis globosa*, and *Chrysochromulina* sp. Rates of *E. huxleyi* increased by 210% when temperature increased in a range from 10°C to 21.5°C, while a further increase in temperature (up to 23.8°C) showed reduction of CH₄ production rates. Our results clearly showed that CH₄ formation of *E. huxleyi* is controlled by light: When light intensity increased from 30 to 2,670 μmol m⁻² s⁻¹, CH₄ emission rates increased continuously by almost 1 order of magnitude and was more than 1 order of magnitude higher when the daylength (light period) was extended from 6/18 hr light-dark cycle to continuous light. Furthermore, light intensity is also an important factor controlling CH₄ emissions of *Chrysochromulina* sp. and *P. globosa* and could therefore be a species-independent regulator of phytoplankton CH₄ production. Based on our results, we might conclude that extensive blooms of *E. huxleyi* could act as a main regional source of CH₄ in surface water, since blooming of *E. huxleyi* is related to the seasonal increase in both light and temperature, which also stimulate CH₄ production. Under typical global change scenarios, *E. huxleyi* will increase its CH₄ production in the future.

Plain Language Summary Methane is a gas that affects the Earth's climate and is typically produced by microbes in the absence of oxygen or through geological processes. Surprisingly, methane is also produced in oceanic surface waters that are well oxygenated, known as the ocean-methane paradox. Marine phytoplankton has recently been discovered as a methane source, which might help to explain the paradox. Environmental factors such as light and temperature might be important for controlling methane production from marine algae. In order to understand how environmental factors affect methane formation from phytoplankton, we performed several experiments under laboratory conditions. We find that temperature, light intensity, and day length strongly control methane production of phytoplankton. The field blooms of marine algae, which are often strongly related to the seasonal increase of light and temperature, could act as an important regional source of methane in oceanic surface waters. Under typical global change scenarios, marine algae might increase their methane production in the 21st century.

1. Introduction

Huge amounts of methane (CH₄) are formed in the oceans, but only a small proportion is released to the atmosphere (Weber et al., 2019). In this context the biogeochemical cycle of CH₄ in the oceans is of great interest, and in particular, the frequently observed CH₄ production within the ocean surface mixed layer is challenging our previous understanding of biogeochemical CH₄ formation processes. Traditionally, it is thought that CH₄ in the oceans is either produced by geological processes (abiotic) or by methanogenic archaea (biotic). Because methanogenic archaea are strict anaerobic microorganism, their CH₄ production is limited to anoxic environments (Kirschke et al., 2013; Saunio et al., 2016; Thauer et al., 2008). However, there is growing evidence that CH₄ is also produced by organisms such as cyanobacteria (Bižić et al., 2020) and eukaryotes including plants (Keppler et al., 2006), fungi (Lenhart et al., 2012), lichens

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(Lenhart et al., 2015) and algae (Klitzsch et al., 2019; Lenhart et al., 2016), animals (Ghyczy et al., 2008), and humans (Keppler et al., 2016) and even in the presence of oxygen.

The observation of CH₄ in freshwater and saline surface waters (often described as methane paradox) has recently received much attention although some studies already conducted four decades ago (Scranton, 1977; Scranton & Brewer, 1977; Scranton & Farrington, 1977) have reported about CH₄ supersaturation in the ocean mixed layer. Furthermore, many recent studies (Grossart et al., 2011; Günthel et al., 2019; Hartmann et al., 2020; Tang et al., 2016) have shown that CH₄ formation is not limited to salt-water but also occurs in freshwater lakes. Several hypotheses exist to explain CH₄ formation in oxygenated waters, and some of them will be discussed briefly. Methanogenic archaea living in anoxic environments of particles or fish and zooplankton guts might form CH₄ (de Angelis & Lee, 1994; Karl & Tilbrook, 1994; Schmale et al., 2018; Stawiarski et al., 2019; Zindler et al., 2013). The algal metabolite dimethylsulfoniopropionate (DMSP) and its degradation products dimethyl sulfide (DMS) or dimethyl sulfoxide (DMSO) could be precursors of both archaeal (Damm et al., 2008; Florez-Leiva et al., 2013) and bacterial produced CH₄, when bacteria suffer under nitrogen deficiency (Damm et al., 2010). Moreover, photochemical degradation of DMS and acetone has been shown to produce CH₄, but the reaction is limited to anoxic waters (Bange & Uher, 2005; Zhang, Xie, et al., 2015). In oligotrophic Pacific waters CH₄ formation might mainly related to the bacterial cleavage of methylphosphonates when supply of phosphorous is limited (del Valle & Karl, 2014; Karl et al., 2008; Metcalf et al., 2012; Repeta et al., 2016).

Phytoplankton might contribute to CH₄ production in both oxic marine and freshwater environments. The first indication of CH₄ production from phytoplankton was provided by culture experiments of the diatom species *Thalassiosira pseudonana* and the haptophyte species *E. huxleyi* (Scranton, 1977; Scranton & Brewer, 1977; Scranton & Farrington, 1977). Later on, many field studies have reported a relationship between CH₄ supersaturation and the occurrence of phytoplankton in lakes and oceans (e.g., Bogard et al., 2014; Conrad & Seiler, 1988; Damm et al., 2008; Grossart et al., 2011; Oudot et al., 2002; Owens et al., 1991; Rakowski et al., 2015; Tang et al., 2014; Weller et al., 2013; Zindler et al., 2013). Although a good statistical correlation was not observed in all previous studies (e.g., Brooks et al., 1981; Burke et al., 1983; Forster et al., 2009; Lamontagne et al., 1975; Watanabe et al., 1995), it was suggested that phytoplankton is one of the likely CH₄ sources. However, clear evidence of CH₄ formation from marine algae—examined in cultures of marine haptophytes—was only provided recently when Lenhart et al. (2016) and Klitzsch et al. (2019) applied stable isotope labeling experiments to unambiguously show that the three widespread marine algae such as *E. huxleyi*, *Chrysochromulina* sp., and *Phaeocystis globosa* indeed produce CH₄ per se and without the help of methanogenic archaea. Very recently, when using stable isotope labeling experiments and concentration measurements, it could be also shown that several freshwater algal species, including diatoms, cryptophytes, and green algae (Hartmann et al., 2020), but also several species of marine and limnic cyanobacteria (Bižić et al., 2020) emit CH₄. Thus, both marine algae and cyanobacteria could significantly contribute to the commonly observed oceanic CH₄ supersaturation (Bižić et al., 2020; Klitzsch et al., 2019; Scranton, 1977). In summary, previous investigations mainly focused on explaining the sources for CH₄ in oxic surface waters; however, the effects of environmental parameters such as temperature, light intensity, or nutrient availability on CH₄ production from phytoplankton are still unknown.

In the present study we investigated the effects of temperature and light intensity (including daylength) on CH₄ formation from the three widespread marine algal species *E. huxleyi*, *P. globosa*, and *Chrysochromulina* sp. *Emiliania huxleyi* occurs in ocean worldwide except in the polar regions (McIntyre et al., 1970). The algal species develops large populations (blooms) in subpolar to temperate areas usually in summer time, especially under highly stratified conditions, when the mixed layer depth shallows due to increasing temperature. Blooming of *E. huxleyi* is then supported by high light intensity caused by shallow mixed layer depth and incidence light (Nanninga & Tyrrell, 1996; Raitsoos et al., 2006; Tyrrell & Merico, 2004; Tyrrell & Taylor, 1996). Therefore, we have studied in detail CH₄ formation in relation to temperature (range from 10.1°C to 23.8°C), light intensity (30 to 2,670 μmol m⁻² s⁻¹), and daylength (period of light irradiation) during growth of *E. huxleyi*. We furthermore investigated the effect of light intensity on CH₄ formation by the two other widespread marine, but noncalcifying haptophytes *P. globosa* and *Chrysochromulina* sp. These two species can also form large blooms and are often found as main members in marine phytoplankton communities (Brown & Yoder, 1994; Schoemann et al., 2005; Thomsen, 1994). The results of *E. huxleyi* will be

discussed with regard to their potential importance in marine environments during blooming. Finally, the observed CH₄ formation patterns of the three algal species will be evaluated on the basis of the CH₄ production potential (CH₄-PP), which expresses differences in growth rates and thus the success of a species at the community level.

2. Materials and Methods

2.1. Experimental Setup

Emiliana huxleyi RCC1216 provided from the Roscoff Culture Collection (<http://roscoff-culture-collection.org/>, last access: 11 April 2020) were used to investigate the effect of temperature, light intensity, and day-length on CH₄ production rates. We performed an additional experiment to study the effect of light intensity on different algae species. Therefore, *E. huxleyi* and two other haptophytes *P. globosa* PLY 575 and *Chrysochromulina* sp. PLY 307 obtained from the Marine Biological Association of the United Kingdom (<https://www.mba.ac.uk/facilities/culture-collection>, last access: 11 April 2020) were studied. The cultures were maintained in quasi-exponential growth by frequent dilution with medium in order to keep them largely free of bacteria. All culture experiments were conducted under the use of sterile techniques. For a more detailed discussion about the potential interplay between algae and bacteria, we would like to refer the reader to the manuscript by Klintzsch et al. (2019). Briefly, Klintzsch et al. concluded that CH₄ production is clearly dependent on algal growth and that it is highly unlikely that bacteria alone are responsible for CH₄ production in the studied cultures. Each sample was taken at the end of the light period. Cultures were grown in batch mode (Langer et al., 2013). We used F/2 growth medium (Guillard & Ryther, 1962) that was based on sterile filtered (0.2 μm Ø pore size) North Sea seawater (sampled off Helgoland, Germany, 32 PSU). Cells were grown in crimped serum bottles (160 ml) filled with 140 ml medium and 20 ml headspace. Culture experiments were carried out with four independent repetitions. For determination of the CH₄ mixing ratio samples of 10 ml of headspace gas was sampled. The amount of produced CH₄ in culture group vials was calculated in respect to control groups. The culture and control group flasks were simultaneously sealed under ambient air and thus contained the same CH₄ background concentration. Please note that the produced CH₄ has been determined for the entire incubation flask—dissolved in the medium plus CH₄ of the headspace volume. For details on determination of CH₄ formation, please refer to section 2.2. For all experiments performed with algae and F/2 medium, the average CH₄ content in the cultures group at the end of incubation was higher than that found in the algae-free blanks as shown in Tables S1–S4. All growth rates and initial and final cell densities are given in the supporting information (Tables S1–S4).

Cultures were illuminated by cold white LED bulbs (LED Base Classic A100, Osram, Germany). The light spectrum of the LED bulbs is provided in Figure S1. The photosynthetic active radiation (PAR) was measured inside each incubation jar by using a light meter (ULM-500 Universal, WALZ, Germany) with a spherical quantum PAR sensor (US-SQS/L, WALZ, Germany). Temperature was logged by (UX120-006 M, HOBO, Germany).

2.2. Determination of CH₄ Mass

The CH₄ mass was determined at the end of the incubation period. In order to determine the CH₄ mass of the whole incubation flask (dissolved plus the CH₄ of the headspace volume), an aliquot (10 ml) of head space gas was taken from the incubation vials using a gastight syringe. In order to maintain headspace pressure when taking the headspace gas sample, an equivalent volume of seawater was injected into the flasks by syringe. The added volume was taken into account when determining the cell density (section 2.5). The sample gas was separated by gas chromatography using a GC-14B (Shimadzu, Japan) equipped with a 2 m column (Ø = 3.175 mm inner diameter), packed with Molecular Sieve 5A 60/80 mesh from Supelco. Methane was recorded by a flame ionization detector (FID) and quantified (mixing ratio) by using two reference standards containing 9,837 and 2,192 parts per billion by volume (p.p.b.v) CH₄. Mixing ratios were corrected for head space pressure. The latter was measured inside the incubation flask before gas sampling using a pressure meter (GMSD 1,3 BA, Greisinger). The CH₄ mass (m_{CH_4}) was determined by its mixing ratio (x_{CH_4}) and the ideal gas law (Equation 1),

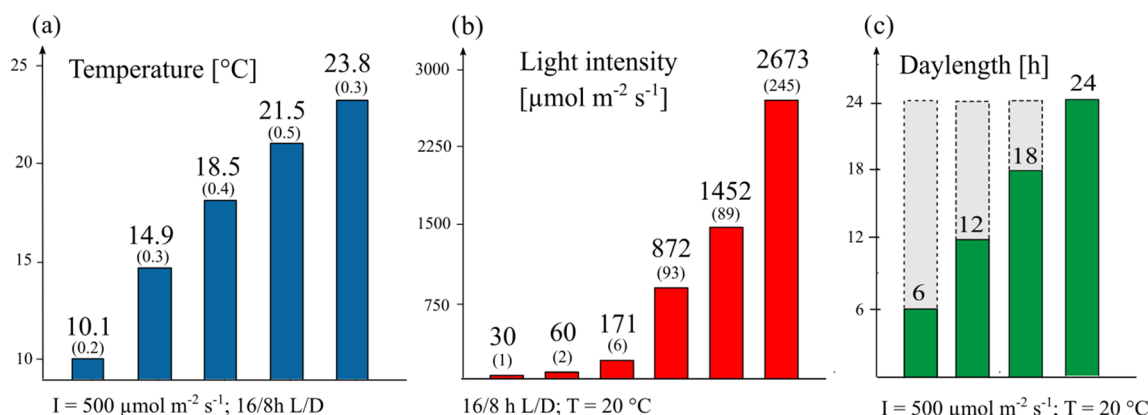


Figure 1. Treatment conditions of temperature (a), light intensity (b), and daylength (c) experiment. All treatments were carried out with four independent replications. L/D = light/dark; I = light intensity. (a) Mean values of logged temperature during the test period. (b) Mean light intensity values that were measured inside each incubation jar. The standard deviation is given in brackets.

$$m_{CH_4} = M_{CH_4} \times x_{CH_4} \frac{p \times V}{R \times T}, \quad (1)$$

where M_{CH_4} = molar mass, p = pressure, T = temperature, R = ideal gas constant, and V = volume.

The dissolved CH_4 concentration was calculated by using the equation of Wiesenburg and Guinasso (1979).

2.3. Treatments of Alternating Temperature, Light Intensity, or Daylength at Cultures of *E. huxleyi*

To investigate the effect of temperature, light intensity, and daylength (daylength refers to the light period within a 24-hr light-dark cycle), three independent experiments with *E. huxleyi* were carried out in which one of the three parameters was varied as described in Figure 1. Within each experiment, the other two parameters were kept constant with 16/8-hr light-dark cycle; $\sim 500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and 20°C , respectively. All treatments were carried out with four independent repetitions. Control groups contained F/2 medium only. Cultures were acclimated (≈ 10 generations) to the environmental conditions prior to the experiment. Cell density at inoculation varied between treatments depending on the growth rates under the given environmental conditions. Cultures of *E. huxleyi* were allowed to grow not more than $0.4 \times 10^6 \text{ cells ml}^{-1}$ (exponential phase) before they were harvested. The majority ($>95\%$) of culture replicates reached final cell densities between 0.1×10^6 and $0.3 \times 10^6 \text{ cells ml}^{-1}$ (Tables S1–S3). Possible culture artifacts of CH_4 production rates, which could result from a cell density effect (Langer et al., 2013), were excluded for each investigated parameter by correlating the CH_4 production rates with the cell density on the harvest day (Figure S2).

2.4. Treatments of Alternating, Light Intensity on Cultures of *P. globosa*, *Chrysochromulina* Sp., and *E. huxleyi*

The effect of light intensity was studied in two further haptophytes: *P. globosa* and *Chrysochromulina* sp. in addition to *E. huxleyi*. Two light intensities ($427 \pm 12 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and $1,165 \pm 42 \mu\text{mol m}^{-2} \text{ s}^{-1}$) with four replicates were applied, respectively. Cultures were grown under a 16/8 hr light-dark cycle and 20°C . Cultures were preadapted (≈ 10 generations) to light intensities before the experiment was started. The initial and final cell densities correspond to the exponential phase for each species (Klintzsch et al., 2019) and are given in the supporting information (Table S4). Control groups contained F/2 medium only.

2.5. Determination of Cell Density

For the determination of cell densities either a Fuschs-Rosenthal or Neubauer counting chamber (depending on cell density) was used. At least minimum of four aliquots of each culture sample were counted.

2.6. Determination of Growth and CH₄ Production Rates

All production rates were measured at exponentially growing cultures. For further information of measuring production rates from batch culture experiments, we refer to Klintzsch et al. (2019) and Langer et al. (2012, 2013).

We calculated the growth rate (μ) from cell densities (N) of the beginning (t_0, N_0) and end (t_1, N_1) of the experiment (Equation 2).

$$\mu = \frac{\ln(N_1) - \ln(N_0)}{(t_1 - t_0)} \quad (2)$$

The POC-based CH₄ production rates were calculated from the cellular organic carbon content (POC_{cell}). The latter was obtained from cell volume (V_{cell}) by using the carbon to volume relationship in Equation 3 according to Menden-Deuer and Lessard (2000):

$$POC_{cell} = 0.216 \times V_{cell}^{0.939}. \quad (3)$$

The cell volume was calculated from the cell diameter in light micrographs, which was measured by using the program ImageJ (Schindelin et al., 2012). We followed the recommendation of Olenina et al. (2006) and assumed a ball shape for calculating the cell volume for the three species investigated here.

The carbon-specific growth rate was calculated from the product of POC and growth rate μ (Equation 4):

$$\mu_{POC} = \mu \times POC_{cell}. \quad (4)$$

The CH₄ production rates were calculated by multiplying the growth rate μ with the corresponding cellular or POC-CH₄ quota, which was measured at the end of the experiment. The daily cellular CH₄ production rates (CH_4P_{cell} , ag CH₄ cell⁻¹ day⁻¹, ag = 10⁻¹⁸ g) were calculated according to Equation 5:

$$CH_4P_{cell} = \mu \times \frac{m(CH_4)}{cell}, \quad (5)$$

where $m(CH_4)$ is the amount of CH₄ that was produced at the end of the experiment.

The daily cellular CH₄ production rates (CH_4P_{POC} , $\mu\text{g CH}_4 \text{g}^{-1} \text{POC day}^{-1}$) were calculated from growth rate and CH₄-POC quotas at the end of the experiment according to Equation 6.

$$CH_4P_{POC} = \mu \times \frac{m(CH_4)}{POC}. \quad (6)$$

The CH₄ production potential (CH₄-PP) was calculated to scale variations in cellular production rates to community level. Detailed explanations for calculating the production potential (PP, which is not confined to CH₄) have been provided by Gafar et al. (2018) and Gafar and Schulz (2018). Please note that these authors have calculated the PP for CaCO₃ but the concept is the same for CH₄. In accordance to the authors, the CH₄-PP can be calculated for different growth periods, when a cellular standing stock for each time period is calculated from a given starting cell density (N_0). The related amount of produced CH₄ (CH₄-PP) for each period of growth and respectively standing stock is the product of the cellular standing stock and CH₄ quota (Equation 7).

$$CH_4PP = N_0 \times e^{\mu \times t} \times \frac{m(CH_4)}{cell} \quad (7)$$

In the present study and in accordance to Klintzsch et al. (2019) the CH₄-PP was calculated for a standing stock that is obtained after 7 days of growth starting with a single cell.

The sensitivity of growth, POC production and the rate of CH₄ formation to temperature were quantified by their activation energy (E_a), which is derived from the Arrhenius equation (Equation 8).

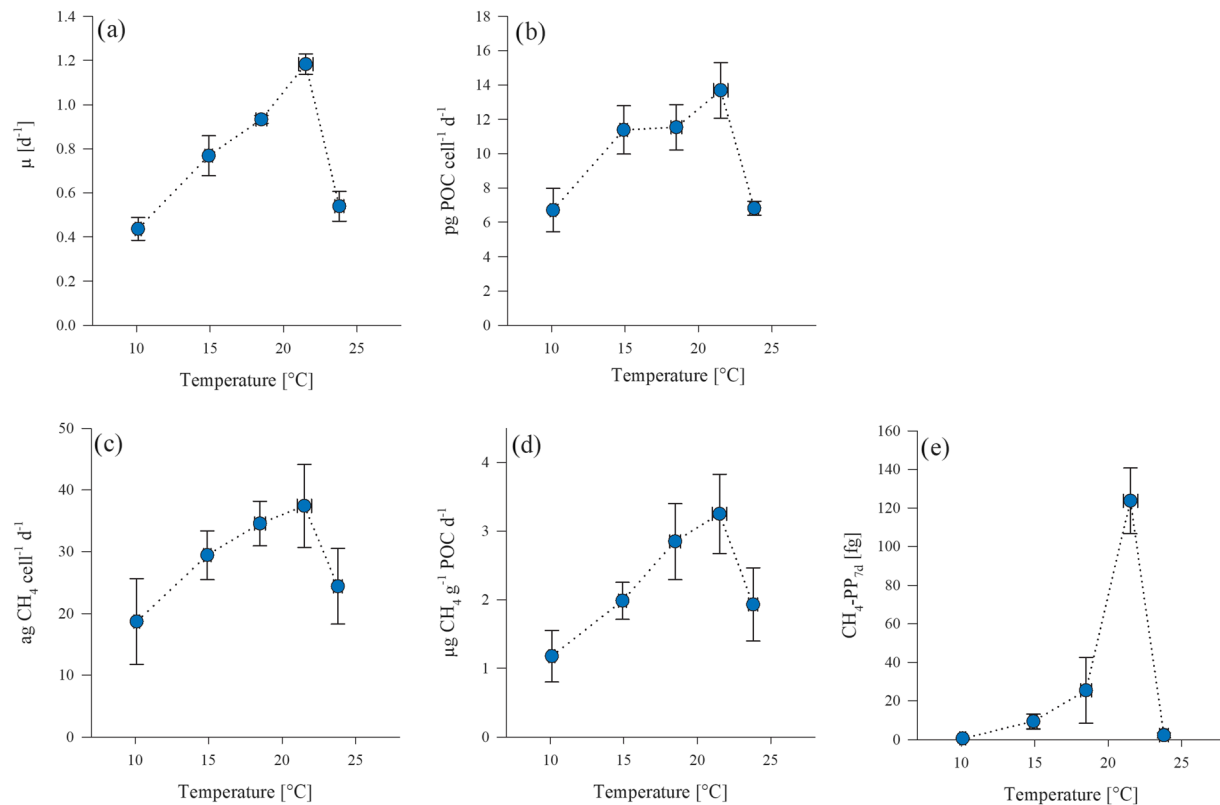


Figure 2. Relationship between temperature and growth rate (a), POC production rate (b), cellular (c), and POC normalized CH₄ production rate (d) and CH₄-PP (e) of *E. huxleyi*. Values are the mean of four replicated culture experiments with standard deviation (SD).

$$k(T) = A_{exp} \left(\frac{-E_a}{RT} \right), \quad (8)$$

where k = reaction rate constant (here for growth, POC, or CH₄ production rate), A = pre-exponential factor, R = gas constant, and T = temperature. The activation energies (E_a) of the rate can then be calculated by multiply the slope of the Arrhenius plot by $-R$, using a plot of $\ln(k)$ as function of T^{-1} .

2.7. Statistics

For each environmental factor (sections 3.1–3.3) the total data set of cellular and POC normalized CH₄ production was analyzed for statistical differences in the mean values among the treatment groups by using a one-way analysis of variance (ANOVA). Furthermore, within the individual culture experiments of *E. huxleyi*, *P. globosa*, and *Chrysochromulina* sp. (section 3.4), the mean values (cellular and POC normalized CH₄ production) of the two light intensities treatments (medium and high light) were compared by t tests.

3. Results

3.1. Temperature Effect

Growth and POC production rates have more than doubled when increasing temperatures from 10.5°C to 21.5°C (Figures 2a and 2b). The optimum of growth and POC production was reached at 21.5°C (1.18 day⁻¹; 13.7 ± 1.6 pg POC cell⁻¹ day⁻¹), while a further increase in temperature to 23.8°C led to a drastic reduction of about 50% for both growth and POC production rates. A similar pattern was observed for CH₄ production rates (cellular and POC normalized) as shown in Figures 2c and 2d. The POC normalized and cellular CH₄ production increased by 2.8- and 2.0-fold, respectively, when temperature increased by 11.4°C (from 10.1°C to 21.5°C). At 21.5°C the optimum of CH₄ production rates was reached (3.2 ± 0.6 μg CH₄ g⁻¹ POC day⁻¹; 37.4 ± 6.7 ag CH₄ cell⁻¹ day⁻¹). Further increase in temperature from 21.5°C to 23.8°C

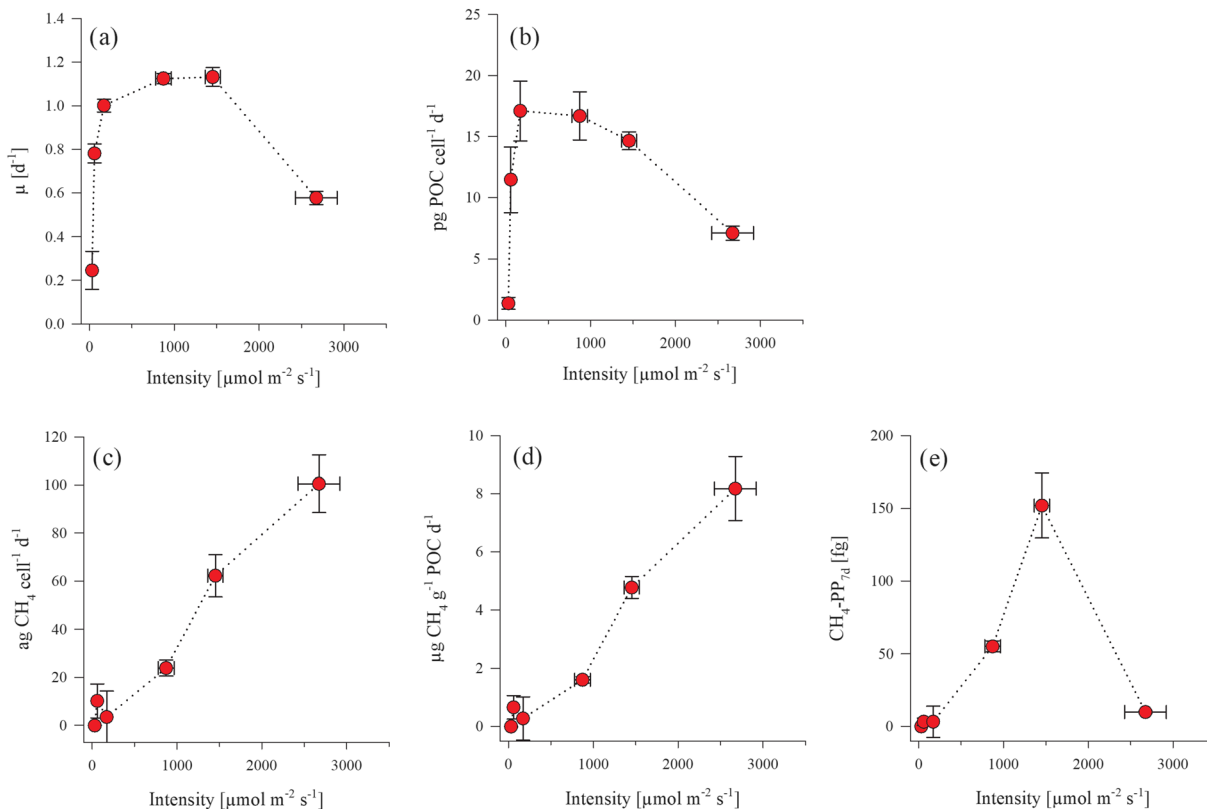


Figure 3. Relationship between light intensity and growth rate (a), POC production rate (b), cellular (c), and POC normalized CH_4 production rate (d) and CH_4 -PP e of *E. huxleyi*. Values are the mean of four replicated culture experiments with SD.

showed a reduction of CH_4 production by 40% and 35%, for POC normalized and cellular CH_4 production, respectively. Statistical analysis (ANOVA) confirmed the temperature dependence of CH_4 production with p values of 0.002 and <0.001 for POC normalized and cellular CH_4 production rates, respectively. After 1 week of growth the total amount of generated CH_4 is specified by the CH_4 -PP (Figure 2e). With increasing temperature (from 10.1°C to 21.5°C) the CH_4 -PP raised by 2 orders of magnitude (from 0.7 ± 0.2 to 124 ± 17.1 fg CH_4) before it declined drastically at 23.8°C (Figure 2e). Consequently, the optimum temperature (21.5°C) was identical for the five investigated parameters (Figure 2).

3.2. Light Intensity Effect

The growth and POC production rates of *E. huxleyi* increased drastically when light intensity increased from 30 to $171 \mu\text{mol m}^{-2} \text{s}^{-1}$, and values remained relatively constant at higher light intensities in the range of 171 – $1,450 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figures 3a and 3b). However, increasing the light intensity to $2,670 \mu\text{mol m}^{-2} \text{s}^{-1}$ caused a clear reduction of both growth and POC production rates. From 30 to $171 \mu\text{mol m}^{-2} \text{s}^{-1}$ the growth rate increased fourfold (from 0.25 ± 0.09 to $1.00 \pm 0.03 \text{ day}^{-1}$) and POC production rates by over 1 order of magnitude (from 1.4 ± 0.48 to $17.1 \pm 2.5 \text{ pg POC cell}^{-1} \text{ day}^{-1}$). The POC production was highest at $171 \mu\text{mol m}^{-2} \text{s}^{-1}$ and was similar at $872 \mu\text{mol m}^{-2} \text{s}^{-1}$ while rates were slightly smaller at $1,452 \mu\text{mol m}^{-2} \text{s}^{-1}$. The growth rate increased by 10% between 171 and $872 \mu\text{mol m}^{-2} \text{s}^{-1}$ but did not further change when reaching values of $1,450 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($1.13 \pm 0.03 \text{ day}^{-1}$). Thus, the optimum growth rate is reached at higher light intensities compared to the POC production rates. However, a further increase in light intensity up to $2,673 \mu\text{mol m}^{-2} \text{s}^{-1}$ led to a significant reduction ($\approx 45\%$) of both POC production and growth rates. In contrast, the cellular and POC normalized CH_4 production rates increased steadily with increasing light intensity (Figures 3c and 3d). Methane formation (on a cell basis) was below the detection limit at $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ but measurable ($6.9 \pm 9.5 \text{ ag CH}_4 \text{ cell}^{-1} \text{ day}^{-1}$; $0.47 \pm 0.63 \text{ pg POC cell}^{-1} \text{ day}^{-1}$ on average) between 60 and $171 \mu\text{mol m}^{-2} \text{s}^{-1}$ while at $872 \mu\text{mol m}^{-2} \text{s}^{-1}$ production rates strongly increased ($23.9 \pm 3.3 \text{ ag CH}_4 \text{ cell}^{-1}$

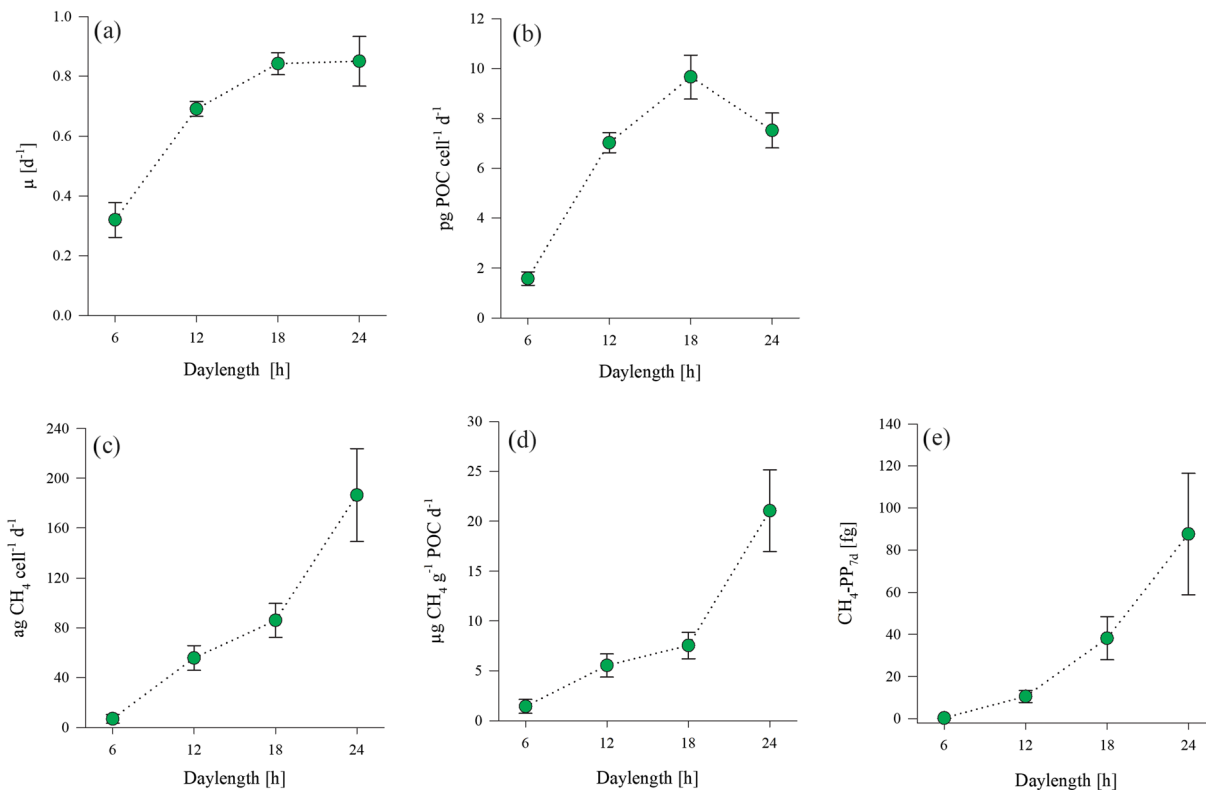


Figure 4. Relationship between day length and growth rate (a), POC production rate (b), cellular (c), and POC normalized CH₄ production rate (d) and CH₄-PP (e) of *E. huxleyi*. Values are the mean of four replicated culture experiments with SD.

day⁻¹; $1.6 \pm 0.1 \mu g$ CH₄ g⁻¹ POC day⁻¹). From 872 to 2,670 μmol m⁻² s⁻¹ cellular and POC normalized CH₄ production increased by 4.2- and 5.1-fold up to 100 ± 12 ag CH₄ cell⁻¹ day⁻¹ and $6.8 \pm 0.9 \mu g$ CH₄ g⁻¹ POC day⁻¹, respectively. The light dependence of both cellular and POC-normalized CH₄ production was also indicated by statistical analysis (ANOVA; $p < 0.001$). The CH₄-PP (Figure 3e) increased with increasing light intensities by 2 orders of magnitude up to 152 ± 22 fg CH₄ at $1450 \mu mol$ m⁻² s⁻¹ and sharply decreased by one order of magnitude (to 9.9 ± 0.9 fg CH₄) at higher light intensity ($2,670 \mu mol$ m⁻² s⁻¹). The optimum of CH₄-PP is therefore in accordance with the optimum of growth rate.

3.3. Daylength Effect

The extension of the daylength (period of light irradiation) from 6 to 18 hr increased the growth rates 2.6-fold (from 0.32 ± 0.06 to 0.84 ± 0.04 day⁻¹), while the growth rates remained constant when a period of continuous light (24 hr) was set (Figure 4a). In contrast to the growth rate, an optimum of POC production rates was observed at 18 hr daylength and decreasing with longer irradiation period of 24 hr (Figure 4b). POC production rates increased between 6 and 18 hr daylength by 6.1-fold (from 1.6 ± 0.3 to 9.7 ± 0.9 pg POC cell⁻¹ day⁻¹) and declined by 22% at continuous light. Cellular and POC normalized CH₄ production rates increased from 6 hr daylength to continuous light period by 2 and 1 order of magnitude from 6.9 ± 3.4 to 186 ± 37 ag CH₄ cell⁻¹ day⁻¹ and 1.1 ± 0.6 to $21.0 \pm 4.1 \mu g$ CH₄ g POC⁻¹ day⁻¹, respectively (Figures 4c and 4d). The dependence of CH₄ production on temperature was verified by statistical analysis (ANOVA), with cellular and POC normalized CH₄ production rates showing p values of $p < 0.001$ and $p = 0.004$, respectively. The cellular and POC normalized CH₄ production was particularly enhanced by the 6 hr extension of the daylength between 18 hr and continuous light that accounted for 56% and 69% of the total increase in cellular and POC normalized CH₄ production, correspondingly. The CH₄-PP increased constantly by over 2 orders of magnitude with longer light irradiation periods (from 0.19 ± 0.12 fg CH₄ at 6 hr light to 70.4 ± 23.1 fg CH₄ at continuous light, Figure 4e).

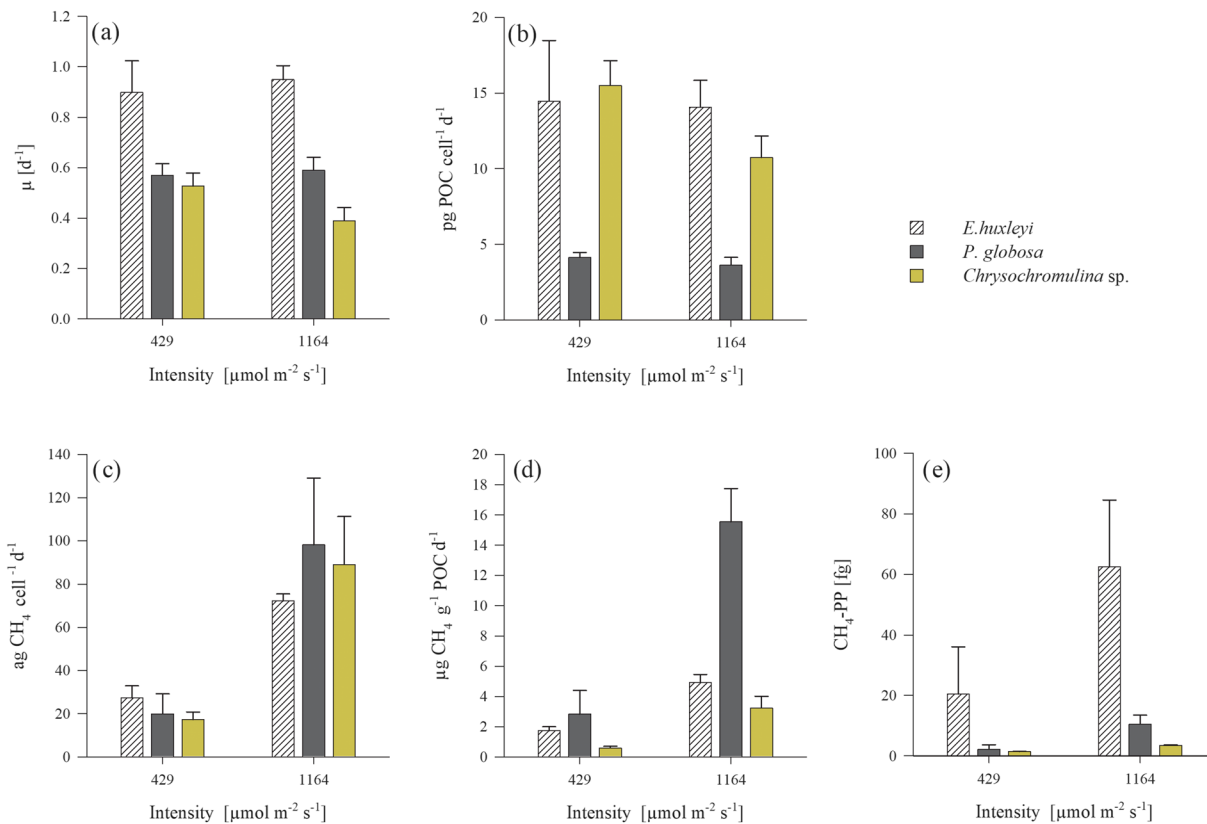


Figure 5. Relationship between light intensity and growth rate (a), POC production rate (b), cellular (c), and POC normalized CH $_4$ production rate (d) and CH $_4$ -PP (e) of *E. huxleyi*, *P. globosa*, and *Chrysochromulina* sp. by moderate (429 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and high light intensity (1,164 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Values are the mean of four replicated culture experiments with SD.

3.4. Comparison of Light Intensity Effects of *E. huxleyi*, *Chrysochromulina* Sp., and *P. globosa*

We compared growth and CH $_4$ formation patterns of the three algal species *E. huxleyi*, *P. globosa*, and *Chrysochromulina* sp. at moderate and high light intensities (429 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 1,164 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The growth rates at both light intensities are shown in Figure 5a. At moderate light intensity the exponential growth rate μ was highest for *E. huxleyi* ($0.90 \pm 0.13 \text{ day}^{-1}$) followed by *P. globosa* and *Chrysochromulina* sp. (with $0.57 \pm 0.05 \text{ day}^{-1}$ and $0.55 \pm 0.04 \text{ day}^{-1}$, respectively). Growth rates of *E. huxleyi* and *P. globosa* remained constant at higher intensity, while growth rate of *Chrysochromulina* sp. declined by 29%. The POC production rates are shown in Figure 5b. At moderate light intensity the POC production rates of *E. huxleyi* and *Chrysochromulina* sp. were in the same range with 14.5 ± 0.5 and $15.5 \pm 1.6 \text{ pg POC cell}^{-1} \text{ day}^{-1}$, respectively, and were about three times higher than for *P. globosa* ($4.1 \pm 0.3 \text{ pg POC cell}^{-1} \text{ day}^{-1}$). The exposure to high light intensity led to a 31% lower POC production rate of *Chrysochromulina* sp. while rates of *E. huxleyi* and *P. globosa* remained constant. Thus, an increase in light intensity declined growth rate and POC production of *Chrysochromulina* sp., while that of *E. huxleyi* and *P. globosa* remained constant. Cellular CH $_4$ production rates of all investigated species were enhanced by increasing light intensities (Figure 5c). Cellular CH $_4$ production rates ranged from 17 ± 3.6 (*Chrysochromulina* sp.) to $27 \pm 5.6 \text{ ag CH}_4 \text{ cell}^{-1} \text{ day}^{-1}$ (*E. huxleyi*) at medium light. In response to higher light intensity the cellular CH $_4$ production rates increased by 2.6-fold (*E. huxleyi*) and about fivefold (*P. globosa* and *Chrysochromulina* sp.) resulting in a cellular CH $_4$ production rates ranged from 72.1 ± 3.3 (*E. huxleyi*) to $98.2 \pm 30.8 \text{ ag CH}_4 \text{ cell}^{-1} \text{ day}^{-1}$ (*P. globosa*). The response in cellular production rates to higher light intensity was also displayed by *t* test with *p* values of <0.001, 0.006, and 0.005 for *E. huxleyi*, *P. globosa*, and *Chrysochromulina* sp., respectively. The POC normalized CH $_4$ production rates increased with increasing light intensity (Figure 5d). Within the medium and high light intensities, the variation of POC normalized CH $_4$ production rates between

species was greater than that of cellular CH₄ production rates. When CH₄ production rates were normalized to POC, rates of moderate light intensities were in a range of 0.58 ± 0.12 to $2.8 \pm 1.6 \mu\text{g CH}_4 \text{g}^{-1} \text{POC day}^{-1}$ with *Chrysochromulina* sp. and *P. globosa* showing the lowest and highest rates, respectively. At high light intensity rates were about 2.8-fold (*E. huxleyi*) and 5.5-fold (*P. globosa* and *Chrysochromulina* sp.) greater than for those observed at moderate light intensity. These differences were also shown by *t* test with *p* values <0.001, 0.004, and <0.001 for *E. huxleyi*, *P. globosa*, and *Chrysochromulina* sp., respectively. The respective rates ranged from 3.24 ± 0.78 (*Chrysochromulina* sp.) to $15.6 \pm 2.2 \mu\text{g CH}_4 \text{g}^{-1} \text{POC day}^{-1}$ (*P. globosa*). All three species showed enhanced CH₄-PP with the higher light intensity (Figure 5e). The increase from moderate to high light ranged between 2.4-fold (*Chrysochromulina* sp.) and 4.9-fold (*P. globosa*). However, the variation of the CH₄-PP within the species is greater than that resulting from the different light treatments. The CH₄-PP was 1 order of magnitude higher for *E. huxleyi* in comparison to the other two species (Figure 5e). This is in line with the higher growth rate of *E. huxleyi*.

4. Discussion

Previous studies indicated that several marine algae produce CH₄ (Klitzsch et al., 2019; Lenhart et al., 2016; Scranton, 1977; Scranton & Brewer, 1977; Scranton & Farrington, 1977), while the modulating influence of environmental parameters is unknown. Our results clearly show that CH₄ formation by *E. huxleyi* is influenced by temperature, light intensity, and the length of irradiation period. Furthermore, light intensity is also an important factor controlling emission rates of the two other marine algae *Chrysochromulina* sp. and *P. globosa*. We will first discuss the effects of environmental parameters on growth and POC normalized CH₄ production from a physiological perspective. Afterward, the effects of environmental parameters on laboratory CH₄ production rates of *E. huxleyi* are discussed in relation to their possible importance on populations (blooms) in marine environments. Finally, we discuss the impact of environmental parameters on CH₄ production in biogeochemical terms using the well-established but rarely applied concept of the PP (see Klitzsch et al., 2019, and references therein).

4.1. Temperature Effect on Growth and CH₄ Formation of *E. huxleyi* From a Physiological Perspective

Emiliania huxleyi occurs, except for the polar regions, in oceans worldwide and has the largest known temperature growth range (1–31°C) compared to other coccolithophores (McIntyre et al., 1970). The temperature response of growth rate is strain specific (Brand, 1982; Langer et al., 2009), and the optimum temperature for strain RCC1216 in this study tallies well with the published value (Langer et al., 2009). The growth curve (Figure 2a) exhibits the asymmetry typical for a temperature response. The ascending, shallow sloped, part of the curve is characterized by an accelerating effect of temperature on all biochemical reactions, whereas the descending, steep sloped, part is characterized by inactivation of enzymes, and denaturation of proteins and membranes (DeLong et al., 2017; Grimaud et al., 2017; Kingsolver, 2009). In accordance with this general concept of temperature effects on physiological processes, we observe a positive correlation of all analyzed physiological parameters with temperature up to 21.5°C (the optimum) followed by a negative correlation above this temperature. We conclude that CH₄ production is a normal physiological process as opposed to a heat stress response stemming from structural damage to cellular architecture. Please note that CH₄ production trends are identical, regardless of the normalization, that is, normalization to cell or POC (Figures 2c and 2d).

The ascending part of the temperature curve can be further analyzed using the Arrhenius equation. According to this equation (Equation 8), the thermal sensitivity of a chemical reaction is proportional to its activation energy. While the Arrhenius equation was originally used to describe chemical reactions, the equation might be also applied to describe the thermal sensitivity of biochemical reactions and biological growth rates, whereby high activation energies indicate high sensitivity to temperature (Gillooly et al., 2001; Grimaud et al., 2017). The calculated activation energies of growth rate, POC, and CH₄ production were 59, 41, and 63 kJ mol⁻¹, respectively. The growth rate and CH₄ production are therefore somewhat more sensitive to temperature than POC production is. The activation energy of CH₄ production is in the range of basic metabolic processes, indicating that CH₄ production in algae is not an abiotic process. For example, the average activation energy of respiration for a wide range of organisms, including microbes, plants, and animals,

is between 40 and 71 kJ mol⁻¹ (Gillooly et al., 2001). In addition, activation energies of most enzymatic reactions are in the range of 21 to 63 kJ mol⁻¹ (Segel, 1993). By contrast abiotic CH₄ formation from thermal degradation experiments as described from dried soils usually showed higher activation energies above 70 kJ mol⁻¹ (Jugold et al., 2012; Liu et al., 2019).

4.2. Light Intensity and Daylength Effects on Growth and CH₄ Formation of *E. huxleyi* From a Physiological Perspective

We grew *E. huxleyi* under a wide range of light intensities and daylengths.

4.2.1. Light Intensity

Our results demonstrate that CH₄ formation and growth rate of *E. huxleyi* is sensitive to light intensity. Under light-limited condition, growth rate increased sharply with increasing light intensity, leveled off at saturated light, and decreased at inhibiting light intensities (Figures 3a and 3b). This pattern of light intensity response (Figure 3a) is typical of phytoplankton cultures (Edwards et al., 2015, and reference inside). The optimum light intensity for POC production is lower than the one for growth rate, which was also observed by Trimborn et al. (2007). The growth rate of *E. huxleyi* was remarkably tolerant against high light intensities ($\geq 1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$), a phenomenon well documented in the literature (Balch et al., 1992; Gafar & Schulz, 2018; Harris et al., 2005; Loebel et al., 2010; Nanninga & Tyrrell, 1996; Nielsen, 1997; Trimborn et al., 2007). Interestingly CH₄ production was even more tolerant to high light, so much so that we could not determine the optimum light intensity. This is in notable contrast to the temperature response patterns described above. While we do not know the chain of events leading to this light intensity response, the response patterns suggest that, first, CH₄ production is a light dependent process and, second, photo-inhibition of growth rate and POC production do not impair CH₄ production. The latter is particularly intriguing because it seems to suggest a decoupling of CH₄ production from photosynthetic production of both energy equivalents and putative CH₄ precursors originating in the POC pool. This warrants further, more detailed, physiological studies into the nature of the light dependency of CH₄ production.

4.2.2. Daylength

The CH₄ formation and growth rate were furthermore controlled by daylength. The growth rate showed a saturation curve (Figure 4a). This pattern is similar to results on *E. huxleyi* reported by Paasche (1967) and even other phytoplankton species (e.g., Bouterfas et al., 2006). By contrast, growth rates of *E. huxleyi* have been reported to be independent of daylength (Nielsen, 1997) or to be inhibited by continuous light (Van Rijssel & Gieskes, 2002). The response to daylength has been suggested to be strain specific (Bretherton et al., 2019) and is dependent on other environmental parameters, for example, on seawater CO₂ concentration (Bretherton et al., 2019; Zhang, Bach, et al., 2015) and light quality (Glover et al., 1987). This could be one reason why the response of growth in relation to daylength differs between studies. In our study POC production decreased at continuous light while growth rate did not (Figure 4b). While growth rate might be inhibited by a lack of a dark period rather than by photoinhibition (Brand & Guillard, 1981), the decline of POC production at continuous light could partly be due to photoinhibition. Please note (see also above) that the response pattern of CH₄ production is independent of the normalization (cell or POC). Interestingly, it is again CH₄ production that neither levels off nor shows inhibition at continuous light. This observation reinforces the pattern described above, namely, POC production declines while CH₄ production increases further. This “double dependency” of CH₄ production on light, that is, both light intensity and daylength, renders light-dependent processes the prime target for further elucidating the mechanism of CH₄ production in *E. huxleyi*.

4.3. Light Intensity Effects on Growth and CH₄ Formation of *E. huxleyi*, *Chrysochromulin* Sp., and *P. globosa*

Methane production was light dependent in *P. globosa* and *Chrysochromulina* sp. too. *Emiliania huxleyi* and *P. globosa* showed a greater light tolerance with respect to growth rate and POC production than *Chrysochromulin* sp. (Figures 5a and 5b). In contrast to POC production and growth rate of *Chrysochromulina* sp., CH₄ production was not inhibited and once again confirms the remarkable light dependency of CH₄ production. The increase in CH₄ production with light intensity in *P. globosa* and *Chrysochromulina* sp. was even higher than that of *E. huxleyi*. However, in each case there was a positive correlation of CH₄ production and light intensity, which could therefore be a common feature of different

phytoplankton taxa. This hypothesis is supported by recent findings of Bižić et al. (2020), who investigated different cyanobacterial species that are found in phytoplankton communities of ocean and lakes. While cyanobacteria produce CH₄ in light and dark phase, the CH₄ production rates elevated during the light phase.

4.4. Potential Relevance for CH₄ Production of *E. huxleyi* Populations in the Field

Large blooms of *E. huxleyi* typically occur at subpolar to temperate areas in the summer months, when the water is highly stratified, due to the seasonal increase in temperature and light intensity (Iglesias-Rodriguez et al., 2002; Nanninga & Tyrrell, 1996; Raitso et al., 2006; Tyrrell & Merico, 2004). The occurrence of *E. huxleyi* in field is therefore correlated with high solar radiation, shallow mixed layer depth, and increased sea surface temperature (SST; Raitso et al., 2006). We compare the reported environmental conditions that support *E. huxleyi* growth in the field with those that stimulate CH₄ production in our laboratory grown cultures to assess whether CH₄ formation by *E. huxleyi* could be of ecological relevance.

Emiliana huxleyi grows at water temperatures between 1°C and 31°C in field and has the largest temperature growth range of all *coccolithophores* (McIntyre et al., 1970). The wide temperature range results from the adaptation of individual strains to narrower temperature ranges in cold or warm water masses (Brand, 1982; Langer et al., 2009). The temperature range of the incubation experiments includes the range of the seasonal variation in the SST of the Tasman Sea off New Zealand where the investigated strain (RCC1216) has been isolated and is therefore of ecological relevance. The monthly mean SST of the Tasman Sea off New Zealand ranges from 13.5°C to 18.7°C between coldest and warmest month (time period 2007–2017, https://statisticsnz.shinyapps.io/sea_surface_temperature_oct19/, last access: 11 April 2020). With a temperature increase from 10.1°C to 21.5°C cellular CH₄ production rates would double according to laboratory derived rates (section 3.1). Inhibition of growth rate and CH₄ production due to heat stress is less likely in the field, since the mean SST of the warmest month is below the optimum temperature of the investigated strain. This is in line with literature data showing that most strains grow below their optimal growth condition in field (Langer et al., 2009; Rosas-Navarro et al., 2016).

Our laboratory data also suggest that longer light irradiation periods during summer could have a stimulating effect on CH₄ production, especially on *E. huxleyi* populations in subpolar regions, where daylength changes dramatically between winter and summer. For example, the CH₄ production would increase by a factor of 5 due to a daylength increase from 6 hr in winter to 18 hr in summer (section 3.3). *Emiliana huxleyi* usually blooms in the North Atlantic in June and July at high light intensity in the surface layer, which is caused by strong sunlight and shallow mixed layer depth (10–20 m; Nanninga & Tyrrell, 1996; Raitso et al., 2006; Tyrrell & Merico, 2004; Tyrrell & Taylor, 1996). For instance, light intensities of 935 and 1,140 μmol m⁻² s⁻¹ were measured in *E. huxleyi* blooms in the field. In addition, long-term observations in a mesocosm in a Norwegian fjord have shown that *E. huxleyi* blooms at light intensities between >530 and 1,200 μmol m⁻² s⁻¹, whereas the mean light intensities in the surface layer are ~63% and 43% of the incident light intensity at 10 and 20 m mixed layer depth, respectively (Nanninga & Tyrrell, 1996, and reference inside). Thus, the *E. huxleyi* cells would have been exposed to light intensities between 228 and 756 μmol m⁻² s⁻¹ in the mixed layer, which falls within the light intensity range of our incubation experiments (section 3.2). Since the CH₄ production of *E. huxleyi* increased linearly with the light intensities in culture experiments, the high light intensities in the surface layer could also support the CH₄ formation of *E. huxleyi* in the field. Judging from our laboratory CH₄ production and the reported light intensity range where blooms typically occur, the CH₄ production could vary by a factor of 4. It can be concluded that light intensity will considerably affect sea surface water CH₄ production of *E. huxleyi* in field.

4.5. The Biogeochemical Perspective: Methane PP (CH₄-PP)

While the considerations in section 4.4 apply to the behavior of field populations on the cellular level, they are not appropriate for assessing the biogeochemical significance of this behavior. Several recent studies have emphasized that the PP (see section 2 for calculation), as opposed to the cellular production, is the relevant parameter for biogeochemical assessments (Gafar & Schulz, 2018; Gafar et al., 2018; Klintzsch et al., 2019; Kottmeier et al., 2016; Marra, 2002; Schlüter et al., 2014). We calculated the CH₄-PP of *E. huxleyi* for different temperature, light intensity, and daylength conditions. For all three parameters, the CH₄-PP increases toward the optimum, as does the cellular CH₄ production, but the increase in CH₄-PP was by 1 order of magnitude higher than for the cellular CH₄ production (Figures 2c, 3c, and 4c and Figures 2e, 3e,

and 4e). This illustrates the importance of using the PP when considering the biogeochemical impact of changing environmental conditions. Another such illustration is the strong contrast between the light intensity response patterns of cellular, or POC normalized, CH₄ production, and the CH₄-PP. The sharp decline in CH₄-PP at the highest light intensity is not reflected in the cellular CH₄ production curve. However, in the field this difference is of minor importance because the highest light intensity used here, ~2,700 μmol m⁻² s⁻¹, is considerably higher than even peak light intensities observed in typical *E. huxleyi* blooms (~1,200 μmol m⁻² s⁻¹; see references above). It is noteworthy that the decline in CH₄-PP at the highest temperature tested here is also of little relevance in the field because *E. huxleyi* usually grows at suboptimal temperatures in the field, a situation that will also not change in the foreseeable future, despite global warming (Rosas-Navarro et al., 2016). It is concluded first that the CH₄-PP of *E. huxleyi* in the field will be maximal in midsummer when *E. huxleyi* typically blooms. Second, global change will increase the CH₄-PP of *E. huxleyi* through both warming and increased stratification entailing higher light intensities in the surface layer. Compared to the other two tested haptophytes, *E. huxleyi* has the highest CH₄-PP, a difference not mirrored in cellular CH₄ production: This is yet another example of the importance of using the PP when considering the biogeochemical impact of CH₄ formation by phytoplankton. As a general caveat it should be noted that the above conclusions are confined to our experimental conditions. Conditions in the field will include other factors such as grazing. This inevitable limitation of experimental data is the price one has to pay for discovering relationships between environmental parameters and the performance of an organism. However, this does not mean that our data are never directly applicable to the field situation as illustrated by the good match of satellite data and *E. huxleyi* calcite production potential reported by Gafar et al. (2018).

5. Conclusions

We have determined the CH₄ production of three haptophytes under varying environmental conditions and conclude the following:

1. Temperature, light intensity, and daylength influence CH₄ production.
2. CH₄ production is strongly light dependent; even increasing with light intensity when growth rate and POC production are photoinhibited.
3. The biogeochemically relevant parameter CH₄-PP increased with temperature, light intensity, and daylength over the range typical for present-day seasonality and global change predictions for the coming century.
4. *E. huxleyi* has a considerably higher CH₄-PP than *P. globosa* and *Chrysochromulina* sp.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement

We provide the data in heiDATA, which is an institutional repository for research data of Heidelberg University (<https://doi.org/10.11588/data/AGKWSG>).

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