Emission of short-lived halocarbons by three common tropical marine microalgae during batch culture

Yong-Kian Lim[1,2], Siew-Moi Phang[1,3], William T. Sturges[4], Gill Malin[4], Noorsaadah Binti Abdul Rahman[5]

[1] Institute of Ocean and Earth Sciences (IOES), University of Malaya, 50603, Kuala Lumpur, Malaysia.
[2] Institute of Graduate Studies (IPS), University of Malaya, 50603, Kuala Lumpur, Malaysia.
[3] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia.
[4] Centre for Ocean and Atmospheric Sciences, School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom.
[5] Department of Chemistry, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia.

Corresponding author:

Siew-Moi Phang

Email address: phang@um.edu.my

Telephone: (+603) 7967 4640 / 6995

ABSTRACT

Very short-lived halocarbons of marine biogenic origin play an important role in affecting tropospheric and stratospheric chemistry. In recent years, more attention has been paid to tropical regions where the influence of strong convective forces is responsible for rapid uplifting of the volatile organohalogens from the open surface waters into the atmosphere. This laboratory-based study reports on three common tropical marine microalgae capable of emitting a range of short-lived halocarbons, namely CH_3I, $CHBr_3$, CH_2Br_2, $CHBr_2Cl$ and $CHCl_3$. Chlorophyll-a and cell density were highly correlated to the quantity of all five compounds emitted ($p<0.01$). The diatom *Amphora* sp. UMACC 370 had a higher range of CH_3I emission rate (10.55 – 64.18 pmol mg^{-1} day^{-1}, $p<0.01$) than the cyanobacterium *Synechococcus* sp. UMACC 371 and chlorophyte *Parachlorella* sp. UMACC 245 (1.04 – 3.86 pmol mg^{-1} day^{-1} and 0 – 2.16 pmol mg^{-1} day^{-1}, $p<0.01$, respectively). Furthermore, iodine was the dominant halogen emitted in terms of total combined halide mass of all three species. Overall the emissions of short-lived halocarbons were both species- and growth phase-dependent, highlighting the importance of considering cell physiological conditions when determining gas emission rates.

Keywords: Halocarbons; marine microalgae; tropical; batch culture; climate change; algal biotechnology

INTRODUCTION

Biogenic volatile halocarbons are important carriers of halogen radicals to the troposphere and the stratosphere. Very short-lived species (VSLS), such as iodinated (e.g. CH_3I, CH_2BrI, CH_2ClI) and brominated compounds (e.g $CHBr_3$, CH_2Br_2, $CHBr_2Cl$) of oceanic origin, are released into the atmosphere and may be transported to the stratosphere when intense convection occurs in the troposphere (Kritz et al., 1993; Randel & Jensen 2013). These halogen-containing organic compounds might, therefore, contribute to the reactive halogens that account for the catalytic destruction of the ozone layer (WMO, 2014). It is well established that brominated VSLS significantly contribute to stratospheric halogen loading, but the contribution of the shorter-lived iodinated compounds remains controversial (WMO, 2014). Both iodinated and brominated VSLS have the potential to affect tropospheric chemistry (Sherwen et al, 2016).

Global emissions of CH_3I are estimated to be 157-260 Gg I yr^{-1} (Ziska et al., 2013; Stemmler et al., 2014) where some 240 Gg I yr^{-1}, including 60 Gg I yr^{-1} of CH_3I, originates from open seawater and coastlines (Jones et al., 2010). Emission of short-lived brominated compounds such as $CHBr_3$ and CH_2Br_2 from the open oceans has been estimated at 19-255 and 3-62 Gg Br yr^{-1}, respectively (Liang et al., 2010; Liu et al., 2013).

The biological production of halogenated compounds by marine organisms (macroalgae) was first reported by Lovelock *et al*. (1973). The production and emission of halocarbons are well described for some macrophytic algae (seaweeds) from polar and temperate regions (e.g. Manley & Dastoor 1987; Laturnus, Wiencke & Klöser, 1995; Carpenter & Liss 2000; Abrahamsson et al., 2003; Weinberger et al., 2007). Halocarbon emission data for tropical seaweeds have been published more recently (Levine et al., 2008; Keng et al., 2013; Leedham et al., 2013; 2015; Mithoo-Singh et al., 2017). Although seaweeds are recognised as important sources of halocarbons their distribution is mainly in the littoral zones of rocky coastal regions, and these areas represent just 0.3% of the global ocean surface (Moore, 2003). Interest in alternate sources of biogenic halocarbon production has turned attention onto the widely distributed marine microalgae (phytoplankton) that may make a very substantial contribution to ocean-atmosphere fluxes. Leedham et al., (2013) estimated that VSLH originating from the tropics could contribute about 75% of the global halocarbon budget, which suggests that emissions from the open oceans, potentially contributed by marine microalgae, could be highly significant despite the low emission values reported. Sturges et al. (1992), were amongst the first to discover the involvement of microalgae in natural halocarbon production in reporting significant emissions of $CHBr_3$ by Arctic ice microalgae in the field. Subsequently, Tokarczyk & Moore (1993) reported on production of short-lived halocarbons ($CHBr_3$, CH_2Br_2, $CHBr_2Cl$, CH_2ClI) in monospecific phytoplankton cultures isolated from polar and temperate zones. The emissions of volatile halocarbons by microalgae originating from polar and temperate climatic zones have been described in terms of different cell physiological growth stages (Tait & Moore, 1995; Sæmundsdottir & Matrai, 1998; Colomb et al., 2008; Brownell et al., 2010; Hughes, Franking & Malin, 2011), irradiance (Moore et al., 1996; Hughes et al., 2006) and elevated ozone level (Thorenz et al., 2014). A compiled list of studies on halocarbon emissions by microalgae originating from different climatic zones was recently published (Lim et al., 2017). Nonetheless, there is still a distinct lack of data for the emission of short-lived volatile halocarbons by tropical marine microalgae.

Intense tropical convective forcing has been proposed as a vehicle for the fast uplift of volatile compounds into the tropical stratosphere, especially over the oceans (Laube et al., 2008; Fueglistaler et al., 2009; Hossaini et al., 2015). Deep tropical convective heating, particularly the deep overshooting convection which has the potential to increase with climate change, rapidly transports air masses lifting reactive halogen species directly up or above the troposphere. This may further amplify the adverse effect of VSLS on stratospheric chemistry (Pommereau, 2010). Tropical convection, over marine areas where there is high productivity, is reported to be the strongest in south east Asia (SEA) region in the recent years (Sherman & Hempel, 2009; Robinson et al., 2014). Mohd Nazir et al., (2014) used data collected during a research cruise in the Straits of Malacca, South China Sea and Sulu-Sulawesi Sea in 2009, to estimate a regional $CHBr_3$ emission of 63 Gg yr^{-1} for the SEA region. $CHBr_3$ was the most abundant brominated compound, ranging from 5.2 pmol mol^{-1} in the Straits of Malacca to 0.94 pmol mol^{-1} over the open ocean of the South China Sea.

Read et al., (2008) suggested that up to 50% of ozone destruction in the tropical tropopause could be due to halogen chemistry. However, reports on the contribution and impacts of short-lived halocarbon emissions by tropical microalgae remain scarce despite such information being necessary to improve understanding atmospheric and climate change. This paper represents the first report of a detailed batch culture study on halocarbon emission by tropical marine microalgae, with a focus on the relationship between halocarbon emissions and growth phase under controlled laboratory conditions. Microalgae are also seen as potential feedstocks for biofuel production and it is possible that any future establishment of intensive microalgal farming, especially in the sunny tropics, might result in enhanced contributions to the biogenic halocarbon load arising from the oceans.

MATERIALS AND METHODS

2.1 Microalgal cultures

Three local tropical marine algal strains from the University of Malaya Algae Culture Collection (UMACC) were used for this study: the cyanophyte *Synechococcus* sp. UMACC 370 and the bacillariophyte *Amphora* sp. UMACC 370 both isolated from shrimp ponds connected to the

Straits of Malacca in Kuala Selangor, Malaysia, and the chlorophyte *Parachlorella* sp. UMACC 245 isolated from the east-coast waters facing the South China Sea in Terengganu, Malaysia. Stock cultures were grown in Provasoli Medium (Prov50) (CCMP, 1996) under a 12h light:12h dark cycle and at a temperature of 25 ± 1 °C in an incubator shaker set at 100 rpm (PROTECH, model GC-1050). Silicate ($Na_2SiO_3.9H_2O$) was supplemented at 0.01g dm^3 to the culture medium for *Amphora* sp. Irradiance level in the growth chamber was maintained between 30- 40 µmol photons m^{-2} s^{-1} for all the cultures. All cultures were maintained under axenic conditions using standard aseptic techniques; all glassware and growth media were sterilized by autoclaving (15 min at 121°C) before use. Lysogeny broth (LB) (Bertani, 1951) agar plates were used to test and ensure the axenicity of the inoculum cultures.

2.2 Experimental set-up

2.2.1 Starting cell density for the study

A short preliminary study was conducted prior to the growth cycle experiment to determine the suitable cell density to ensure GC-MS detectable levels of a suite of volatile halocarbons. The optical density at 620nm ($OD_{620\ nm}$) of cultures of the three microalgae were adjusted to 0.2, 0.3 and 0.4 at the start of the growth period of four days, prior to measurement of the halocarbons. The cultures were 150 mL in volume and in 250 mL conical flasks. They were incubated with shaking (100 rpm) at 25°C with an irradiance of 40 µmol photons m^{-2} s^{-1} on a 12h light:12h dark cycle. The procedure for halocarbon determination is given below (Section 2.2.2).

2.2.2 Growth cycle experiments.

All three microalgal cultures were grown in batch culture with a starting inoculum size of 10% of a log phase culture, standardized at an OD_{620nm} of 0.4. Triplicate cultures of 150 mL volume were grown in 250 mL conical flasks in an incubator shaker (100 rpm) at 25°C with irradiance of 40 µmol photons m^{-2} s^{-1} on a 12h light:12h dark cycle. Measurements were done every 2 days for 12 days of growth. Triplicate controls consisting of culture medium alone were set up and subsampled in the same way to enable calculation of net production of halocarbons. To calculate emission rates, the net concentration of each halocarbon was normalized to biomass, in terms of chl a (pmol mg^{-1} chl a day^{-1}) and cell density (pmol $cell^{-1}$ day^{-1}). The formula to determine emission rate is as follows:

$$Emission\ rate = \frac{Concentration\ of\ halocarbons}{Biomass\ x\ Incubation\ time}$$

Where:

Emission rate = based on chl a (pmol mg^{-1} day^{-1}) or on cell density (pmol $cell^{-1}$ day^{-1})

Concentration of halocarbon= pmol L^{-1}

Biomass = chl a content (mg L^{-1}) or haemocytometer cell density (cell mL^{-1})

Incubation time = 4 hours

Every two days, 60 mL aliquots of culture were removed from the triplicate flasks and transferred aseptically into centrifuge tubes, centrifuged (3000 rpm or 2415 G-force/rcf for 10 min) and replenished with 60 mL fresh medium. The samples were incubated air-tight for 4 hours in 100 mL glass syringes. This incubation period was set to achieve a sufficient concentration of halocarbons for analysis. To allow normalization of the halocarbon concentration to biomass, an additional 40 mL of each culture was taken at the same time for biomass estimation using the methods described in Section 2.2.4. The state of the cells was determined using PAM Fluorometry (Hughes, Franklin & Malin, 2011; Keng et al., 2013). The value of the maximum quantum efficiency of photosystem II, denoted as F_v/F_m (where F_v is the variable fluorescence measured as the difference between maximum (F_m) and minimum (F_o) fluorescence in dark-adapted culture), was estimated using a Water PAM (Pulmonary Amplitude Modulation) (Walz, Model: WATER-ED, S/N:EDEE0238 Germany) before and after the gas-tight incubation period to indicate the cells' health. Samples from each culture were dark-adapted for 15 minutes prior to F_v/F_m determination.

After 4 hours of incubation, the cultures in the incubation syringes were gently mixed and filtered directly into second 100 mL glass syringe using a two-syringe plus filter system (0.2 µm Merck filter unit) to prevent ingress of air into the syringe. The filtered medium in the second syringe was used for halocarbon analysis.

2.2.3 Analysis of halocarbons

All halocarbon analyses were carried out using a purge-and-trap system developed by the University of East Anglia (UEA), UK (Hughes et al., 2006) equipped with an Agilent Technologies 7890A gas chromatograph (GC). The GC was fitted with a J&W 60 m DB-VRX capillary column

(film thickness 1.40 µm; internal diameter 0.25 mm). The extracted medium subsamples that had been injected into the system were purged for 15 minutes using oxygen-free nitrogen (OFN) at a flow rate of 40 mL min^{-1}. Any aerosols or particles in the bypassing purged gas would be removed through the stuffed glass wool held in a glass tubing. Water vapor in the bypassing of the purged gas was removed through a molecular sieve followed by a counter-flow Nafion dryer (Perma-Pure) using OFN at a rate of 100 mL min^{-1}. The targeted compounds were then trapped and cryogenically focused synchronously purging in a stainless-steel tubing coil immersed in liquid nitrogen at a temperature of -150°C, aided by a thermostated heating device for a total of 15 minutes.

Then to allow sample desorption, liquid nitrogen was quickly swapped with boiling water in a flow of high-purity helium at 1 mL min^{-1} via a heated (95°C) transfer line to the GC. As the run starts, the oven was initially held at 36°C for 5 min, followed by heating up to 200°C at 20°C min^{-1}, and lastly heated up to 240 °C at a rate of 40 °C min^{-1}. The quantification and identification of the compounds were determined by an Agilent 5975C mass-selective detector (MSD), operated in Single Ion Mode. Data was collected between 4 and 18 min. Calibrations for all compounds were done using gravimetrically prepared liquid standards (Sigma-Aldrich) mixed in high-performance liquid chromatography-grade methanol (Fischer Scientific) injected into medium samples. The results of emissions and changes of halocarbon concentration against the phytoplankton-free controls were calculated based on five-point calibration curves. Throughout the experiments, deuterated-iodomethane (CD_3I) (ARMAR chemicals) and deuterated-diiodomethane (CD_2I_2) (Sigma-Aldrich) of constant volume were injected into every medium sample before the halocarbon analysis as a way to monitor and correct for drift in the detector sensitivity. (Hughes et al., 2006). A loss of peak area from the internal standards due to the drift is corrected and equated to the original peak area as initially detected. Peak areas sourced analyte of interest, in this case short-lived volatile halocarbons detected from the samples or controls, were also corrected following the same ratio as the surrogate standards did. The relative response, halocarbon concentration, was then obtained from the calibration that plots concentration against integrated peak area (view supplementary data, Figure S1).

Five halogenated compounds, namely tribromomethane ($CHBr_3$), iodomethane (CH_3I), trichloromethane ($CHCl_3$), dibromochloromethane ($CHBr_2Cl$), and dibromomethane (CH_2Br_2), were detected in the emissions of the three microalgae. Detection limits and precisions of the analyses based on the measurement of standards (Abrahamsson & Pedersén, 2000) were CH_3I, 0.2

pmol L^{-1}, precision, 5.9%; $CHBr_3$, 0.3 pmol L^{-1}, precision 10.3%; $CHCl_3$, 0.5 pmol L^{-1}, precision 7.3%; $CHBr_2Cl$, 0.05 pmol L^{-1}, precision 9.8%; CH_2Br_2, 0.3 pmol L^{-1}, precision 7.9%.

2.2.4 Cell biomass determination

Biomass is estimated using Bright-field Neubauer haemacytometer cell count (Marienfeld-Superior, Germany) under a light microscope (Vejesri et al. 2014). The chlorophyll a content (Chl a) was determined by harvesting the microalgal cells by Millipore filtration using filter paper (Whatmann GF/C, 0.45 µm). The chl-a of the microalgae were extracted using acetone and left overnight 4°C in the dark (Vejeysri et al., 2014; Strickland & Parsons, 1968). The absorption of the extract was measured at 665nm, 645nm and 630nm. Chl-a was calculated using the formula as follows:

$$Chl\ a\ (mg\ m^{-3}) = (Ca\ x\ Va)/Va$$

where, Ca = 11.6 (OD_{665nm}) – 1.31(OD_{645nm}) – 0.14(OD_{630nm})

Va = Volume of acetone (mL) used for extraction

Vc = Volume of culture (L)

$$Chl\ a\ (mg\ L^{-1}) = Chl\ a\ (mg\ m^{-3})/1000$$

The specific growth rate (ų, day^{-1}) for all cultures were based on calculated biomass (chl-a and cell number) using the formula as follows:

$$ų, day^{-1} = \frac{\ln(N_2/N_{1)}}{(t_2 - t_1)}$$

where N_2, is OD_{620nm} at t_2, N_1, is biomass at t_1, and t_2, t_1 are time periods within log phase (Strickland & Parsons, 1968).

2.3 Statistical Analysis

Repeated Measures-ANOVA was used to test the significance (p<0.01) of emission rate of all five compounds by three different microalgae and One-Way ANOVA was used to test the significance (p<0.05) of emission rate amongst the five compounds followed by a post-hoc Tukey test. Pearson Product-Moment correlation coefficient (r) was used to analyze the emission rate from the five detected compounds in term of chl a, cell density and both. Statistical analyses were done using the Statistica 8.0 software.

RESULTS

3.1 Determination of suitable cell density

Only five halocarbons were detected in the emissions from the three microalgae *Synechococcus* sp. UMACC 371, *Amphora* sp. UMACC 370 and *Parachlorella* sp. UMACC 245, namely monoiodomenthane (CH_3I), tribromomethane ($CHBr_3$), dibromomethane (CH_2Br_2), trichloromethane ($CHCl_3$), and dibromochloromethane ($CHBr_2Cl$). After conducting trials with different cell densities, OD_{620nm} of 0.4 was selected for all inoculations. See supplementary data for results comparing OD_{620nm} 0.2, 0.3 and 0.4 (view supplementary data, Table S1).

3.2 Growth curves

The growth curves in terms of chl a (Fig. 1a-c) and cell density (Fig. 2a-c), indicated the exponential and stationary phases for all three taxa (Table 1), and allowed the calculation of the specific growth rates (Table 2).

3.3 Photosynthetic efficiency as cell stress indication

Figure 3a-c show the maximum quantum yield (F_v/F_m) of three tropical marine microalgae across a period of 12 days before and after 4-hour air-tight incubation. F_v/F_m values shown prior to air-tight incubation act as control level. The smallest difference in F_v/F_m before and after air-tight incubation ensured the production of halocarbons trapped during the incubation from cell culture was maximized while cell's health remained unaffected or affected at its minimum by physiological stress created from an air-tight environment. Under ambient laboratory conditions, the healthy range of F_v/F_m for *Synechococcus* sp. UMACC 371, *Parachlorella* sp.245 and *Amphora* sp.370 are within the range of 0.3-0.4, 0.5-0.7 and 0.5-0.7 respectively (Ng et al., 2014; Simis et al., 2012). In general, the cells for all three cultures fall in the healthy F_v/F_m range as mentioned. In other words, the emission of halocarbons were not under the influence of cell stress from air-tight incubation.

3.4 Determination of halocarbon concentration

Figure 4 a-e show the mean concentration of five detected short-lived halocarbons emitted from the culture samples and controls in triplicates. The net concentration of each halocarbon was

obtained by subtracting the concentration of the sample to the control. Such correction yielded positive and negative net concentration of halocarbons, whereby sample concentration that falls below concentration of the control was omitted as loss or consumption of halocarbons by cells. Concentration of the controls that falls below sample concentration was regarded as emission, which in this case is the focus of this study. See supplementary data for the emission ascribed to the microalgal cultures (view supplementary Figure S2). To calculate for emission rate for each compound, the net production of halocarbons was used to normalize with chl a and cell density.

3.5 Emission rate of the halocarbons

Emission of the five detected halocarbon compounds were normalized to chl a (Fig. 5) and cell density (Fig. 6) to determine the emission rate. In general, the trend of emission rates of all five detected compounds for all three taxa across 12-day culture period in terms of chl a and cell density were in good agreement. The emission rates for all five compounds based on chl a and cell density as summarized in Table 3 were highly (p<0.01) correlated. *Amphora* sp. UMACC 370 showed higher emission rate of CH_3I, $CHCl_3$ and CH_2Br_2 in the exponential phase while higher emission rate of $CHBr_3$ and $CHBr_2Cl$ in both exponential and stationary phases. The emission rates of all five compounds for *Synechococcus* sp. UMACC 371 and *Parachlorella* sp. UMACC 245 were lower as compared to *Amphora* sp. UMACC 370. When data for total emission rates for all five compounds were pooled together as shown in Figure 7, *Amphora* sp. UMACC 370 showed higher emission rate percentage as compared to *Synechococcus* sp. UMACC 371 and *Parachlorella* sp. UMACC 245. *Amphora* sp. UMACC 371 showed significantly (p<0.01) higher concentrations of CH_3I emission as compared to *Synechococcus* sp. UMACC 371 and *Parachlorella* sp. UMACC 245. In other words, *Amphora* sp. UMACC 370 was clearly a stronger emitter of the five halogenated compounds, especially CH_3I, as compared to the other two taxa based on chl a and cell density.

3.5.1 Emission rate at exponential and stationary phases

Table 4 shows the estimated (upper and lower limits) emission rate of measured halocarbons under conditions of the experiments by the three tropical marine microalgae. *Amphora* sp. UMACC 370 had the highest emission rates for methyl iodide (CH_3I) in both exponential and stationary phases, reporting 14.18 – 86.79 pmol (mg chla)$^{-1}$ day^{-1} and 10.02 – 18.08 pmol (mg chla)$^{-1}$ day^{-1} respectively when normalized to chl a, and 2.05 – 24.05 pmol (10^9 cell)$^{-1}$ day^{-1} (exponential) and

1.29 – 3.16 pmol (10^9 cell)$^{-1}$ day^{-1} (stationary) when normalized to cell density, as compared to *Synechococccus* sp. UMACC 370 and *Parachlorella sp.* UMACC 245. Estimated emission rate of CH_3I for *Amphora* sp. UMACC 370 based on chl a and cell density in general was higher in exponential phase than in stationary phase. Higher CH_3I emission rate in exponential phase than in stationary phase was also observed for *Synechococcus* sp. UMACC 371 and *Parachlorella* sp. UMACC 245, except in case of *Synechococcus* sp. UMACC 371 where the emission of CH_3I in exponential phase was lower as compared to its stationary phase despite a rise in culture density.

The estimated emission rates of $CHBr_3$, CH_2Br_2, $CHCl_3$, CH_3I and $CHBr_2Cl$ for *Amphora* sp. UMACC 370 were all higher in exponential phase than in stationary phase, except the emission rate based on chl a for $CHBr_2Cl$ was lower in exponential phase (1.84 pmol (mg chla)$^{-1}$ day^{-1}) than in stationary phase (1.89 pmol (mg chla)$^{-1}$ day^{-1}). *Synechococcus* sp. UMACC 370 reported higher range of CH_3I and $CHCl_3$ emission rates in log phase than in stationary phase based on chl a, while higher range of emission rates for CH_3I, $CHBr_3$, $CHCl_3$ and CH_2Br_2 based on cell density. *Parachlorella* sp. UMACC 245 reported lower estimated emission rates for $CHBr_3$, $CHCl_3$, CH_2Br_2 and $CHBr_2Cl$ during exponential phase than in stationary phase.

Estimated emission rate of $CHCl_3$ was higher in exponential phase as compared to stationary phase; reporting 30.96 pmol (mg chla)$^{-1}$ day^{-1} and 0.37 pmol (mg chla)$^{-1}$ day^{-1} respectively for *Synechococcus* sp. UMACC 371, 48.51 pmol (mg chla)$^{-1}$ day^{-1} and 1.27 pmol (mg chla)$^{-1}$ day^{-1}, respectively for *Amphora* sp. UMACC 370. Similar trend of higher $CHCl_3$ emission rate in exponential phase than in stationary phase was also observed based on cell density. *Parachlorella* sp. UMACC 245 had higher emission rates during the exponential phase as compared to its stationary phase based on chl a and cell density.

Out of the three brominated compounds, estimated emission rates for $CHBr_3$ was higher than CH_2Br_2 and $CHBr_2Cl$ based on chl a in stationary phase across all three tropical marine microalgae. The estimated emission rates of $CHBr_3$, CH_2Br_2 and $CHBr_2Cl$ by *Synechococcus* sp. UMACC 371 based on chl a and cell density were higher in exponential phase as compared to their stationary phase. Higher estimated emission rates based on chl a during stationary phase than in exponential phase was observed for $CHBr_3$ and $CHBr_2Cl$ both by *Parachlorella* sp. UMACC 245 and *Amphora* sp. UMACC 370, except for CH_2Br_2 where emission rate during exponential phase was higher than its stationary phase. *Amphora* sp. UMACC 370 had at least approximately

two times higher of CH_2Br_2, $CHBr_3$ and $CHBr_2Cl$ emission rates during both exponential and stationary phases based on chl a. *Chlorella* sp. UMACC 245 showed the least emission rates of all three brominated compounds during exponential phase as compared to *Synechococcus* sp. UMACC 371. Higher emission rates in stationary phase than exponential phase based on chl a for the three brominated compounds was observed more obvious for *Parachlorella* sp. UMACC 245 as compared to *Synechococcus* sp. UMACC 371.

In this study, the estimated range of emission rates of each halocarbon that varied amongst the three microalgae suggested that the emission rates of each halogenated compound were species- dependent due to the different algal growth physiology at exponential and stationary phases. Higher emission rate for all five halocarbons during exponential phase than in stationary phase for *Synechococcus* sp. UMACC 371, *Amphora* sp. UMACC 370 and *Parachlorella* sp. UMACC 245 when normalized to chl a (except $CHBr_3$ and CH_2Br_2 for the *Parachlorella*) and cell density, suggests that the emissions of these halocarbons over 12 days of culturing were growth phase-dependent. None of the five halocarbons was found to be emitted in the same amount and concentration from the same microalgal species over the culture period, suggesting that the emissions of halocarbon may be strain-specific despite originating from the same microalgal species.

3.6 Axenicity of culture

All cultures were checked by culture on nutrient agar prior to start of experiment, and shown to be free of bacterial contamination, hence the net production of halocarbons observed relative to the subtraction of the controls are ascribed to the microalgal cultures.

DISCUSSION

The VSLH detected in the microalgae were $CHBr_3$, CH_3I, $CHCl_3$, $CHBr_2Cl$ and CH_2Br_2. *Amphora* sp. UMACC 371 emitted higher concentrations of halogenated compounds, especially CH_3I ($p<0.01$) as compared to *Synechococcus* sp. UMACC 371 and *Parachlorella* sp. UMACC 245. The emission of CH_3I was significantly ($p<0.05$) higher compared to other detected compounds, $CHBr_3$, $CHCl_3$, $CHBr_2Cl$ and CH_2Br_2.

In the present study, halocarbon emission rates were higher at exponential phase in general for the three microalgae. Exponential phase cells are actively growing and in a healthy state. As the culture proceed to stationary phase, the cell growth slows down and eventually stops due to chemical and physical changes such as nutrients, irradiance and increase in inhibitory compounds in the medium (Becker, 1994). pH increase in the medium (view supplementary data, Figure S3), which may be due to consumption of the inorganic carbon source, would influence algal activity (Ying, Gilmour & Zimmerman, 2014; Azov, 1982). While it is often assumed that physiological stress does occur when microalgal cells transit from exponential to stationary phase due to limiting conditions and the stress would trigger haloperoxidase mechanism to produce more halocarbons (Moore, Webb & Tokarczyk, 1996), the present study indicates otherwise. All five halocarbons detected by the three tropical microalgae were found to emit at higher rates at exponential phases, with exception of two brominated compounds, $CHBr_3$ and $CHBr_2Cl$ by *Amphora* sp. UMACC 370. Manley & de la Cuesta (1997) reported consistency of higher emission rates of CH_3I at exponential for *Navicula* sp., *Nitzschia* sp., and *Porosira glacialis* from Bacillariophyta and *Phaeocystis* sp., a Chrysophyta. The higher emission rates at exponential phase may be explained as follows: i) the tropical microalgal species used in the present study may be more tolerant to the stress of an aging culture, and the condition did not lead to increased production of the halocarbons. This might have to do with the low "leakage" of hydrogen peroxide from the algal cells into the medium (Palenik, Zafiriou & Morel, 1987; Wong et al., 2003) ii) the exponential phase cells are actively metabolizing, allowing higher rate of methylation of haloperoxidase for halocarbon production, as compared to the cells that experience limiting conditions in stationary phase. The halo-enzymes at healthy state may be less susceptible to inhibition at its active site that allow higher chance of methylation to occur. This suggests that a more detailed research has to be done on relating the change in physiological cell state with varying nutrient composition such as sulfur, nitrogen, phosphate, that may affect the haloperoxidase-mechanism iii) higher concentration of cells in stationary phase produced less superoxide per cell than those with lower density (Marshall, 2002). As oxidative radicals produced in the cells mediate the oxidation of halides present in the medium (Neidleman & Geigert, 1986), this suggests a possibility that lower algal cell density as measured by chl a and cell density during the exponential in this study enhances the production of halocarbons and ultimately the emission rates. It has been reported that algal cells at exponential growth can be more toxic than those in stationary or late exponential phase (Tang & Gobler 2009).

The toxicity is caused by production of peroxidase and catalase that react with multiple compounds including organic hydroperoxides and lipid peroxides in cells. The enzymes can increase the rates of dismutation and decomposition reaction of other highly reactive oxidative species into hydrogen peroxide (H_2O_2). Thus, H_2O_2 surge in the cells from these reactions may be the cause to trigger halocarbon production (Tang & Gobler 2009).

In case of exception observed for $CHBr_3$, $CHBr_2Cl$ where emission rates were higher at stationary phase, these brominated compounds may be more prone to be produced due to the physiological cell stress created from the limiting conditions during growth transition. Previous studies have shown an overall higher emission at stationary phase for iodomathane, CH_3I (Scarratt & Moore, 1999; Smythe-Wright et al., 2006; Brownell, Moore and Cullen, 2010; Hughes, Franklin & Malin, 2011) and brominated compounds, $CHBr_3$, CH_2Br_2 and $CHBr_2Cl$ (Tokarczyk & Moore, 1994; Moore, Webb & Tokarczyk, 1996) and each of these emissions was strain-specific. Nonetheless, the discrepancies of higher emission at exponential over stationary as compared to the present study may largely due to: 1) non-normalized biomass emission. Emission for the brominated compounds and biomass such as algal cell density were calculated separately but not normalized which makes it difficult to compare with to the emission rates in this study. Emission rates were calculated in some of the previous studies but was not possible to make comparison in term of different growth phase, and another study compare lag and exponential phases but not stationary phase. 2) the difference in method used, such as gas-phase using head-space were used in many previous study while water-phase using purge-and -trap system was used, 3) it could just be that the emission rates of halogenated compounds were strain-specific.

Brownell, Moore & Cullen (2010) reported CH_3I emission by temperate *Synechoccocus* sp. CCMP 2370 (clone WH 8102) over the course of 27 days. The emission peaked at approximately 22-25 pmol L^{-1} on Day 15 during its late stationary phase, with chl a of 0.5-1.0 µg L^{-1}. In comparison to the present study, the emission of CH_3I by our tropical *Synechococcus* sp. UMACC 371 peaked at 0.53 pmol L^{-1} on Day 10 during its mid-stationary phase, with chl a at approximately 2.0 mg L^{-1}. While there is a consistency of CH_3I emissions peak during the stationary phase for both cyanobacteria strains, the emission by *Synechococcus* sp. CCMP 2370 was at an order of five times higher than that from UMACC 371. The difference may be due to: i) incubation conditions where experiments done were under lower controlled temperature of 20-21°C, higher irradiance

at 60-70 µmol photons m^{-2} s^{-1} and at nutrient-repleted condition as compared to this study. It is assumed that biological processes affected by constant environmental factors such as differences in temperature, irradiance and nutrients (Brownell, Moore & Cullen, 2010) were responsible for the lower emission of CH_3I by *Synechococcus* sp. UMACC 371. ii) resultant physiological condition of the two cyanobacterial strains. The difference in starting cell density of inocula as well as chl a obtained during the same phase when maximum CH_3I emission was achieved for both studies may contribute to the variance in emission. Hughes, Franklin & Malin (2011) made a similar report on CH_3I emission by the temperate *Synechococcus* sp. CCMP 2370 grown at 22°C under light intensity of 40 µE m^{-1} d^{-1} for over a total of 24 days, with exponential phase starting from Day 4 to 16. The CH_3I concentration measured throughout the experiment range from 2-4 pmol L^{-1} which are close to the medium-only control, suggesting relatively low emission of the CH_3I compound despite a long exponential phase. Table 5 summarizes CH_3I emission by *Synechococcus* sp. from different climatic zones. As observed, the emission of this iodomethane is clearly strain-specific.

In the present study, *Amphora* sp. UMACC 370, a Bacillariophyta had higher emission and emission rates, particularly CH_3I ($p<0.01$) as compared to the other two taxa from Cyanophyta and Chlorophyta. Manley & de la Cuesta (1997) also reported higher CH_3I emission in both exponential and stationary phases from Bacillariophyta, as compared to species from Chlorophyta, Chrysophyta, Cyanophyta and Dinophyta, which further supports results from the present study of higher CH_3I emission from the Bacillariophyta than Chlorophyta and Cyanophyta. *Synechococcus*, a Cyanophyta from present and previous studies (Hughes, Franklin & Malin, 2011; Brownell et al., 2010, Sæmundsdottir & Matrai, 1998; Manley & de la Cuesta, 1997) has consistently been shown as a weak emitter of CH_3I; showing either low (close to control level) or no emission and brominated compound such as CH_3Br with no emission.

From the total halogen mass emitted as halocarbons calculated in percentage over the course of 12 day growth period as summarized in Table 6, the emission contribution from iodine dominates over bromine and chlorine for the taxa that emit the highest (*Amphora*) and second highest (*Synechococcus*) total combined halide mass. Calvert & Lindberg (2004) reported the potential influence of iodine-containing compound on tropospheric chemistry, where small amount of iodinated compounds that present in polar air mass containing representative of Br_2-

437 BrCl- trace gas mixtures do significantly enhance ozone depletion. With significant concentration
438 of CH_3I observed in oceanic atmospheres (Calvert & Lindberg., 2004; Yamamoto et al., 2001;
439 Blake et al., 1997), it is possible that the contribution of iodine from biogenic sources like *Amphora*
440 and *Synechococcus* may be significant over the tropical region. This encourages the local
441 measurement of IO and precursor iodine-containing compounds as well as their interaction with
442 currently acknowledged important trace gases like O_3 and BrO in the tropics for future studies and
443 understanding.

444 In order to assess the importance of the source of $CHBr_3$, CH_3I, CH_2Br_2 and $CHBr_2Cl$ from
445 tropical region, a comparison was made between the emission rates found in this study and those
446 reported from tropical marine macroalgae by Keng et al., 2013. For brown seaweeds they reported
447 a range of 4.7 to $6.5x10^3$ pmol g DW^{-1} hr^{-1} of $CHBr_3$, 11.6 to 34.7 pmol g DW^{-1} hr^{-1} for CH_3I, 15.1
448 to 620 pmol g DW^{-1} hr^{-1} for CH_2Br_2 and 21.1 to 175 pmol g DW^{-1} hr^{-1} for CHBr2Cl. Our results,
449 using dry-weight (DW) and converted to the same units give emission rates of $CHBr_3$ between
450 0.28 to 0.83 pmol g DW^{-1} hr^{-1}, 0.85 to 2.72 pmol g DW^{-1} hr^{-1} for CH_3I, 0.01 to 0.24 pmol g DW^{-1}
451 hr^{-1} for CH_2Br_2 and 0.01 to 0.2 pmol g DW^{-1} hr^{-1} for $CHBr_2Cl$ from all three tropical microalgae.
452 Whilst our halocarbon emission rate per unit mass range from 3 to 30000 times lower than
453 emissions from seaweeds reported by Keng et al., 2013, the importance of marine microalgae is
454 potentially greater on account of the fact that they inhabit more than 70% of the earth water
455 surfaces and possibly a significant vertical column of ocean. Nonetheless, these results represent
456 a significant contribution to understanding the region (tropical) significance of the marine
457 microalgae as source of volatile halocarbons although caution has to be taken when extrapolating
458 laboratory derived data to the natural population.

459 It should be noted that this study reports the emissions of short-lived halocarbons by a
460 limited number of marine tropical microalgae under a limited range of conditions. Eight
461 compounds (others include CH_2BrI, $CHBrCl_2$, CH_2I_2) were screened while only five compounds
462 (CH_3I, $CHBr_3$, $CHCl_3$, CH_2Br_2, $CHBr_2Cl$) were detected above the detection limit by GCMS to
463 calculate for the emission (rates). More data should be collected by studies on a wide array of
464 marine tropical microalgae and further screened for a more complete regional data of short-lived
465 halocarbons contributed by marine microalgae from the tropics. Our results provide the first report
466 of halocarbon emission by monospecific marine microalgal cultures from the tropics. This

contributes to the library of existing reports on halocarbon emission by phytoplankton from polar and temperate regions. Controlled studies where the algae are subjected to environmental stress either in the laboratory or on-site, should be done for more accurate global scale normalization. Satellite-based modeling to obtain regional phytoplankton biomass such as chl a to normalize with extrapolated data from controlled studies will be helpful to establish a direct link of exact source to the emission of the halocarbons. Work is now under way to determine how much environmental stress such as varying irradiance levels, salinity and temperature would affect the emission of halocarbons for the tropical marine microalgae.

CONCLUSIONS

The compounds CH_3I, $CHBr_3$, $CHCl_3$, CH_2Br_2 and $CHBr_2Cl$ were shown to be emitted by tropical marine microalgae, *Synechoccocus* sp. UMACC 371, *Parachlorella* sp. UMACC 245 and *Amphora* sp. UMACC 370. *Amphora* was found to have higher emission and emission rates of the five short-lived halocarbons, especially CH_3I ($p<0.01$). The emission rates for the three tropical microalgae differ between the exponential and stationary phases, with higher emission rates at exponential phase. Results show that emission and emission rate of volatile short-lived halogenated compounds by the three tropical microalgae strains are not only strain-specific but also growth phase-dependent, which implies the significant role of cell growth physiological state when determining the emission rates.

ACKNOWLEDGEMENTS

The research for this paper is funded by the Higher Institution Centre of Excellence (HICoE) Fund, Ministry of Higher Education (IOES-2014 & IOES-2014F), University of Malaya Research Grant RU009-2015 & RU012-2016 (IOES), University of Malaya Postgraduate Research Fund, PPP (PG302-2016A) and the Fundamental Research Grant Scheme (FP018-2012A), Ministry of Higher Education.

REFERENCES

Abrahamsson K, Choo KS, Pedersén M, Johansson G, Snoeijis P (2003) Effects of temperature on the production of hydrogen peroxide and volatile halocarbons by brackish water algae. Phytochemistry 64: 725-734

Azov Y (1982) Effect of pH on inorganic carbon uptake in algal cultures. Appl Environ Microbiol 43(6): 1300-1306

Becker EW (1994) Microalgae: biotechnology and microbiology. Cambridge University Press. pp. 54-55

Bertani G (1951) Studies on lysogenesis. I. The mode of phage liberation by lysogenic Escherichia coli. J Bacteriol 62(3): 293-300

Blake NJ, Blake DR, Chen T-Y, Collins Jr. JE, Sachse GW, Anderson BE, Rowland FS (1997) Distribution and seasonality of selected hydrocarbons and halocarbons over the western Pacific basin during PEM-West A and PEM-West B. J Geophys Res - Atmos 102: 28315–28331

Brownell DK, Moore RW, Cullen JJ (2010). Production of methyl halides by *Prochlorococcus marinus* and *Synechococcus*. Global Biogeochem Cy 24: GB2002. doi:10.1029/2009GB003671

Butler JH, King DB, Lobert JM, Montzka SA, Yvon-Lewis SA, Hall BD, Warwick NJ, Mondeel DJ, Aydin M, Elkins JW (2007) Oceanic distributions and emissions of short-lived halocarbons. Global Biogeochem Cy 21(1): GB1023. doi:10.1029/2006GB002732

Calvet JG, Lindberg SE (2004) Potential influence of iodine-containing compounds on the chemistry of the troposphere in the polar spring. I. Ozone depletion. Atmos Environ 38: 5087-5104.

Carpenter LJ, Liss PS (2000) On temperate sources of bromoform and other reactive organic bromine gases. J Geophys Res - Atmos 105(D16): 20539-20547

CCMP (Provasoli-Guillard Center for Culture of Marine Phytoplankton) (1996) Bigelow Laboratory for Ocean Sciences, Mc Kown Point, West Boothbay Harbor, Maine 04575, USA. Website: http://ccmp.bigelow.org/. Accessed: April 4, 2016

520 Colomb A, Yassaa N, Williams J, Peeken I, Lochte K (2008) Screening volatile organic
521 compounds (VOCs) emissions from five marine phytoplankton species by head space gas
522 chromatography/mass spectrometry (HS-GC/MS). J Environ Monitor 10: 325-330. doi:
523 10.1039/b715312k

524 Elliott S, Rowland FS (1993) Nucleophilic substitution rates and solubilities for methyl halides in
525 seawater. Geophys Res Lett 20: 1043-1046

526 Fueglistaler S, Dessler AE, Dunkerton TJ, Folkins I, Fu Q, Mote PW (2009) Tropical
527 Tropopause Layer. Rev Geophys 47: RG1004. doi:10.1029/2008RG000267

528 Hill VL, Manley SL (2009) Release of reactive bromine and iodine from diatoms and its possible
529 role in halogen transfer in polar and tropical oceans. Limnol Oceanogr 54(3): 812-822

530 Hossaini R, Chipperfield MP, Montzka SA, Rap A, Dhomse S, Feng W (2015) Efficiency of short-
531 lived halogens at influencing climate through depletion of stratospheric ozone. Nat Geosci 8:186-
532 190

533 Hughes C, Franklin DJ, Malin G (2011) Iodomethane production by two important marine
534 cyanobacteria: *Prochlorococcus marinus* (CCMP 2389) and *Synechococcus* sp. (CCMP 2370).
535 Mar Chem 125: 19 –25

536 Hughes C, Malin G, Nightingale PD, Liss PS (2006). The effect of light stress on the release of
537 volatile iodocarbons by three species of mainre microalgae. Limnol Oceanogr 51(6): 2849 – 2854

538 Jones CE, Hornsby KE, Sommariva R, Dunk RM, von Glasow R, McFiggans GB, Carpenter LJ
539 (2010). Quantifying the contribution of marine organic gases to atmospheric iodine. Geophys Res
540 Lett 37(18): L18804. doi: 10.1029/2010GL043990

541 Keng FS-L, Phang S-M, Rahman NA, Leedham EC, Robinson AD, Harris NRP, Pyle JA, Sturges
542 WT (2013). Volatile halocarbon emissions by three tropical brown seaweeds under different
543 irradiances. J Appl Phycol 25: 1377. doi: 10.1007/s10811-013-9990-x

544 Kritz MA, Rosner SW, Kelly KK, Loewenstein M, Chan KR (1993) Radon measurements in the
545 lower tropical stratosphere: evidence for rapid vertical transport and dehydration of tropospheric
546 air. J Geophys Res 98: 8725-8736

547 Laube JC, Engel A, Bonisch H, Mobius T, Worton DR, Sturges WT, Grunow K, Schmidt U (2008)
548 Contribution of very short-lived organic substances to stratospheric chlorine and bromine in the
549 tropics – a case study. Atmos Chem Phys 8 (23): 7325 – 7334

550 Laturnus F, Wiencke C, Klöser H. (1995) Antarctic macroalgae – sources of volatile halogenated
551 organic compounds. Mar Environ Res 14(2): 169-181

552 Leedham EC, Hughes C, Keng FS-L, Phang S-M, Malin,G, Sturges WT (2013) Emission of
553 atmospherically significant halocarbons by naturally occurring and farmed tropical macroalgae.
554 Biogeosciences 10: 3615–3633

555 Leedham Elvidge EC, Phang S-M, Sturges WT, Malin G (2015) The effect of dessication on the
556 emission of volatile halocarbons from two common temperate macroalgae. Biogeosciences 12:
557 387–398

558 Levine JG, Braesicke P, Harris NRP, Savage NH, Pyle JA (2007) Pathways and timescales for
559 troposphere-to-stratosphere transport via the tropical tropopause layer and their relevance for very
560 short lived substances. J Geophys Res 112: D04308. doi:10.1029/2005Jd006940

561 Liang Q, Stolarski RS, Kawa SR, Nielsen JE, Douglass A R, Rodriguez JM, Blake DR, Atlas E L,
562 Ott LE (2010) Finding the missing stratospheric Bry: a global modeling study of $CHBr_3$ and
563 CH_2Br_2. . Atmos Chem Phys 10: 2269-2286

564 Lim Y-K, Phang S-M, Abdul Rahman N, Sturges WT, Malin G (2017) Halocarbon emissions from
565 marine phytoplankton and climate change. Int J Environ Sci Tech 1-16. doi: 10.1007/s13762-016-
566 1219-5

567 Liu Y, Yvon-Lewis SA, Thornton DCO, Butler JH, Bianchi TS, Campbell L, Hu L, Smith WR
568 (2013) Spatial and temporal distributions of bromoform and dibromomethane in the Atlantic
569 Ocean and their relationship with photosynthetic biomass J Geophys Res 118(8): 3950–3965

570 Lovelock JE, Maggs RJ (1973) Halogenated Hydrocarbons in and over the Atlantic. Nature 241:
571 194-196

572 Manley SL, Dastoor MS (1987) Methyl halide (CH3X) production from giant kelp, *Macrocystis*,
573 and estimates of global production by kelp. Limnol Oceanogr 32: 709 –715

574 Manley SL, de la Cuesta JL (1997) Methyl iodide production from marine phytoplankton cultures.
575 Limnol Oceanogr 42(1): 142-147

576 Marshall JA (2002). Photosynthesis does influence superoxide production in the ichthyotoxic alga
577 Chattonella marina (Raphidophyceae). J Plankton Res 24(11): 1231–36

578 Mithoo-Singh PK, Keng FS-L, Phang SM, Leedham-Elvidge EC, Sturges WT, Malin G, Abd
579 Rahman N (2017) Halocarbon emissions by selected tropical seaweeds: species-specific and
580 compound-specific responses under changing pH. *PeerJ* 5: e2918. doi: 10.7717/peerj.2918

581 Mohd Nadzir MS, Phang S-M, Abas MR, Abdul Rahman N, Abu Samah A, Sturges WT, Oram
582 DE, Mills GP, Leedham EC, Pyle JA, Harris NRP, Robinson AD, Ashfold MJ, Mead MI. Latif
583 MT, Mohd Hanafiah M, Khan MF. Amiruddin AM (2014) Bromocarbons in the tropical coastal
584 and open ocean atmosphere during the Prime Expedition Scientific Cruise 2009 (PESC 09). Atmos
585 Chem Phys 14(15): 8137-8148

586 Moore RM (2003) Marine Sources of Volatile Organohalogens. Handb Environ Chem 3(P): 85-
587 101

588 Moore RM, Tokarczyk R (1993) Volatile biogenic halocarbons in the northwest Atlantic. Global
589 Biogeochem Cy 7: 195 – 210

590 Moore RM, Webb M, Tokarczyk R, Wever R (1996) Bromoperoxidase and iodoperoxidase
591 enzymes and production of halogenated methanes in marine diatom cultures. J Geophys Res-
592 Oceans 101: 20899-20908

593 Neidleman SL, Geigert J (1986) Biohalogenation - principles, basic roles and applications; Ellis
594 Horwood Ltd Publishers; Chichester, UK. pp 113-130.

595 Ng F-L, Phang S-M, Periasamy V, Yunus K, Fisher AC (2014) Evaluation of algal biofilm on
596 Indium Tin Oxide (ITO) for use in biophotovoltaic platforms based on photosynthetic performance.
597 *PLOS ONE* 9(5): e97643. doi: 10.1371/journal.pone.0097643

598 Palenik B, Zafiriou OC, Morel FMM (1987) Hydrogen peroxide production by a marine
599 phytoplankter". *Limnology and Oceanography*. American Society of Limnol Oceanogr 32(6):
600 1365–1369

601 Pommereau J-P (2010) Troposphere-to-stratosphere transport in the tropics. C R Geosci 342 (4-
602 5): 331-338

603 Randel WJ, Jensen EJ (2013) Physical processes in the tropical tropopause layer and their roles in
604 a changing climate, Nat Geosci 6(3): 169-176

605 Read KA, Mahajan AS, Carpenter LJ, Evans ME, Faria BVE, Heard DE, Hopkins JR, Lee JD,
606 Moller SJ, Lewis AC, Mendes L, Mcquaid JB, Oetjen H, Saiz-Lopez A, Pilling MJ, Plane JMC
607 (2008) Extensive halogen-mediated ozone destruction over the tropical Atlantic Ocean. Nature
608 453: 1232-1235

609 Robinson AD, Harris NRP, Ashfold MJ, Gostlow B, Warwick NJ, O'Brien LM, Beardmore EJ,
610 Nadzir MSM, Phang S-M, Samah AA, Ong S, Ung HE, Peng LK, Yong SE, Mohamad M, Pyle
611 JA (2014) Long term halocarbon observations from a coastal and an inland site in Sabah,
612 Malaysian Borneo. Atmos Chem Phys 14(16): 8369- 8388

613 Sæmundsdottir S, Matrai PA (1998) Biological production of methyl bromide by cultures of
614 marine phytoplankton. Limnol Oceanogr 43(1), 1998, 81--87

615 Sherman K, Hempel G (eds.) (2009) The UNEP Large Marine Ecosystem Report. Perspective on
616 Changing Conditions in LMEs of the World's Regional Seas. UNEP Regional Seas Report and
617 Studies No. 182. UNEP, Nairobi, Kenya. pp 872

618 Sherwen T, Schmidt JA, Evans MJ, Carpenter LJ, Großmann K, Eastham SD, Jacob DJ, Dix B,
619 Koenig TK, Sinreich R, Ortega I, Volkamer R, Saiz-Lopez A, Prados-Roman C, Mahajan AS,
620 Ordóónez C (2016) Global impacts of tropospheric chemistry (Cl, Br, I) on oxidants and
621 composition on GEOS-Chem. Atmos Chem Phys 16: 12239-12271

622 Simis SGH, Huot Y, Babin M, Seppälä, Metsamaa L (2012) Optimization of variable fluorescence
623 measurements of phytoplankton communities with cyanobateria. Photosynth Res 112: 13-30

624 Smythe-Wright D, Boswell SM, Breithaupt P, Davidson RD, Dimmer CH, Eiras Diaz LB (2006)
625 Methyl iodide production in the ocean: Implications for climate change. Global Biogeochem Cy
626 20: GB3003

627 Stemmler I, Hense I, Quack B, Maier-Reimer E (2014) Methyl iodide production in the open ocean.
628 Biogeosciences 11: 4459–4476

Strickland JDH, Parsons TR (1968) A Practical Handbook of Seawater Analysis. Ottawa: Fisheries Research Board of Canada, Bulletin 167, pp 293

Sturges WT, Cota GF, Buckley PT (1992) Bromoform emission from Arctic ice algae, Nature 358: 660 – 662

Tang YZ, Gobler CJ (2009) Characterization of the toxicity of Cochlodinium polykrikoides isolates from Northeast US estuaries to finfish and shellfish. Harmful Algae 8(3): 454–62

Tait VK, Moore RM (1995) Methyl chloride (CH_3Cl) production in phytoplankton culture Limnol Oceanogr 40(1), 189 – 195

Thorenz UR, Carpenter LJ, Huang RJ, Kundel M, Bosie J, Hoffmann T (2014) Emission of iodine containing volatiles by selected microalgae species. Atmos Chem Phys 14: 14575 – 14598

Tokarczyk R, Moore RM (1993) Production of volatile organohalogens by phytoplankton cultures. Geophy Res Lett 21(4): 285 – 288

Weinberger F, Coquempot B, Forner S, Morin P, Kloareq P (2007) Different regulation of haloperoxidation during agar oligosaccharide-activated defence mechanisms in two related red algae, Gracilaria sp. And Gracilaria chilensis. J Exp Bot 58(15/16): 4365-4372

WMO (World Meteorological Organization), 2014. Scientific Assessment of Ozone Depletion: 2014, World Meteorological Organization, Global Ozone Research and Monitoring Project Report No. 55, 416 pp., Geneva, Switzerland

Wong GTF, Dunstan WM, Kim D-B (2003) The decomposition of hydrogen peroxide by marine phytoplankton. Oceanol Acta 26(2): 191–198

Yamamoto H, Yokouchi Y, Otsuki A, Itoh H (2001) Depth profiles of volatile halogenated hydrocarbons in seawater in the Bay of Bengal. Chemosphere 45: 371–377.

Ying K, Gilmour DJ, Zimmerman WB (2014) Effects of CO_2 and pH on growth of the microalga Dunalliella salina. J Microb Biochem Tech 6: 167-173

Ziska F, Quack B, Abrahamsson K, Archer SD, Atlas E, Bell T, Butler JH, Carpenter LJ, Jones CE, Harris NRP, Hepach H, Heumann KG, Hughes C, Kuss J, Krüger J, Liss P, Moore RM, Orlikowska A, Raimund S, Reeves CE, Reifenhäuser W, Robinson AD, Schall C, Tanhua T,

Tegtmeier S, Turner S, Wang L, Wallace D, Williams J, Yamamoto H, Yvon-Lewis S, Yokouchi, Y (2013) Global sea-to-air flux climatology for bromoform, dibromomethane and methyl iodide, Atmos Chem Phys 13(17): 8915-8934

Figure caption

Fig. 1 Growth curves based on chlorophyll-a. Cell growth phases of three tropical marine microalgae, (a) *Synechococcus* sp. UMACC 371; (b*) Parachlorella* sp. UMACC 245; (c) *Amphora* sp. UMACC 370 based on biomass, chlorophyll-a (mg L^{-1}) over 12 day culture period. n = 3

Fig. 2 Growth curves based on cell density. Cell growth phases of three tropical marine microalgae, (a) *Synechococcus* sp. UMACC 371; (b*) Parachlorella* sp. UMACC 245; (c) *Amphora* sp. UMACC 370 based on biomass, cell number (cell mL^{-1}) over 12 day culture period. n = 3

Fig. 3 Maximum quantum efficiency, Fv/Fm. The mean of Fv/Fm for (a) *Synechococcus* sp. UMACC 371; (b*) Parachlorella* sp. UMACC 245; (c) *Amphora* sp. UMACC 370 before and after incubation over 12 day culture period. n = 3

Fig. 4 Concentration of short-lived halocarbons. The mean concentration of halocarbon emitted by the three tropical marine microalgae against the controls over 12 day growth period for compound (a) $CHBr_3$, (b) CH_3I, (c) $CHCl_3$, (d) $CHBr_2Cl$ and (e) CH_2Br_2. n = 3

Fig. 5 Emission rate normalized to chlorophyll-a. Concentration of compound (a) $CHBr_3$, (b) CH_3I, (c) $CHCl_3$, (d) $CHBr_2Cl$ and (e) CH_2Br_2 normalized to chlorophyll-a for the three tropical microalgae throughout 12 day growth period. n = 3

Fig. 6 Emission rate normalized to cell density. Concentration of compound (a) $CHBr_3$, (b) CH_3I, (c) $CHCl_3$, (d) $CHBr_2Cl$ and (e) CH_2Br_2 normalized to cell number for the three tropical microalgae throughout 12 day growth period. n = 3.

Fig. 7 Total emission rate in percentage. Total rate of emission (%) of all five halocarbons in comparison amongst the three tropical marine microalgae based on (a) cell number and (b) chlorophyll-a.

Fig.1

Click here to download line figure Fig.1.pdf

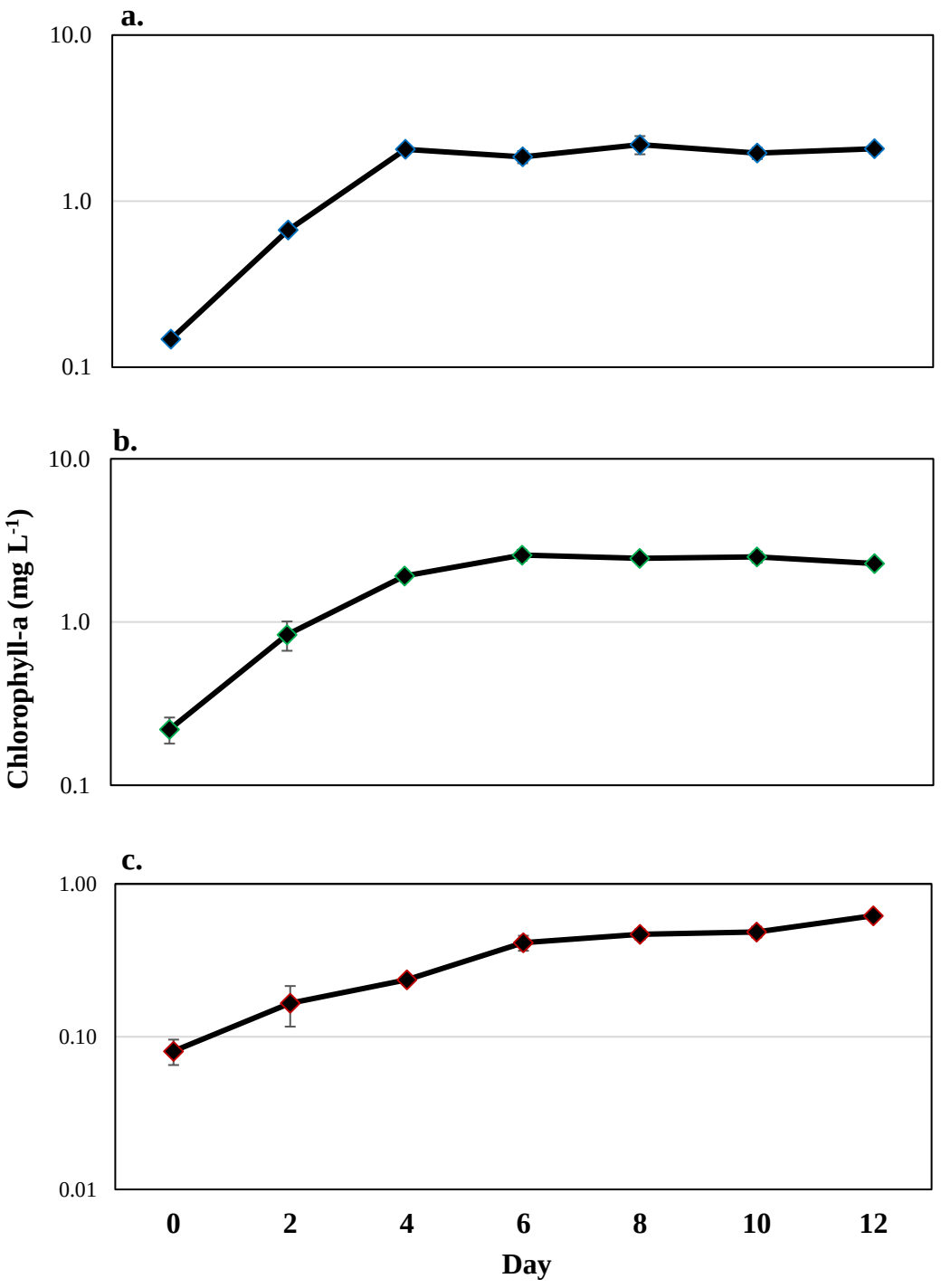


Fig.2

Click here to download line figure Fig.2.pdf

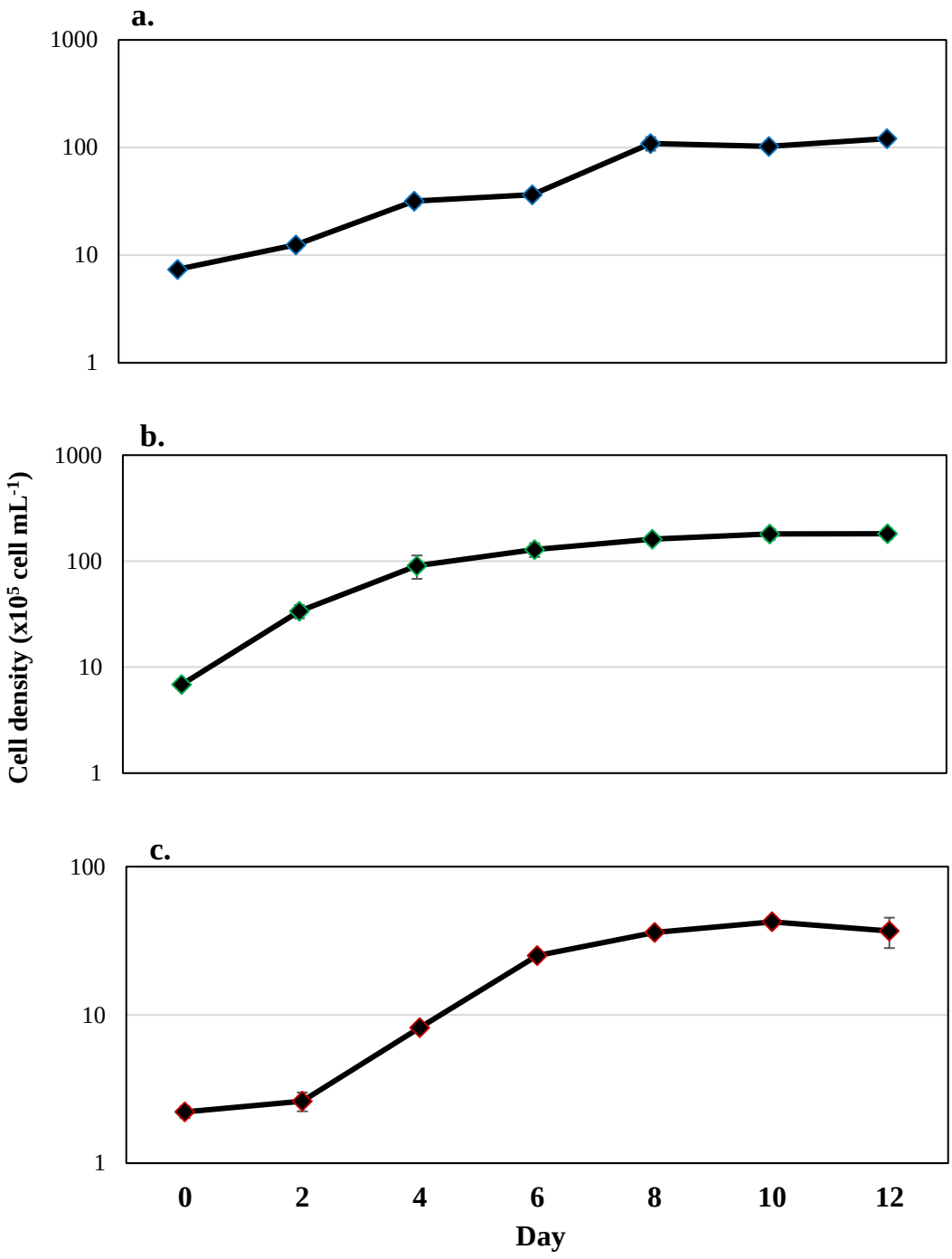


Fig3

Click here to download colour figure Fig.3.pdf

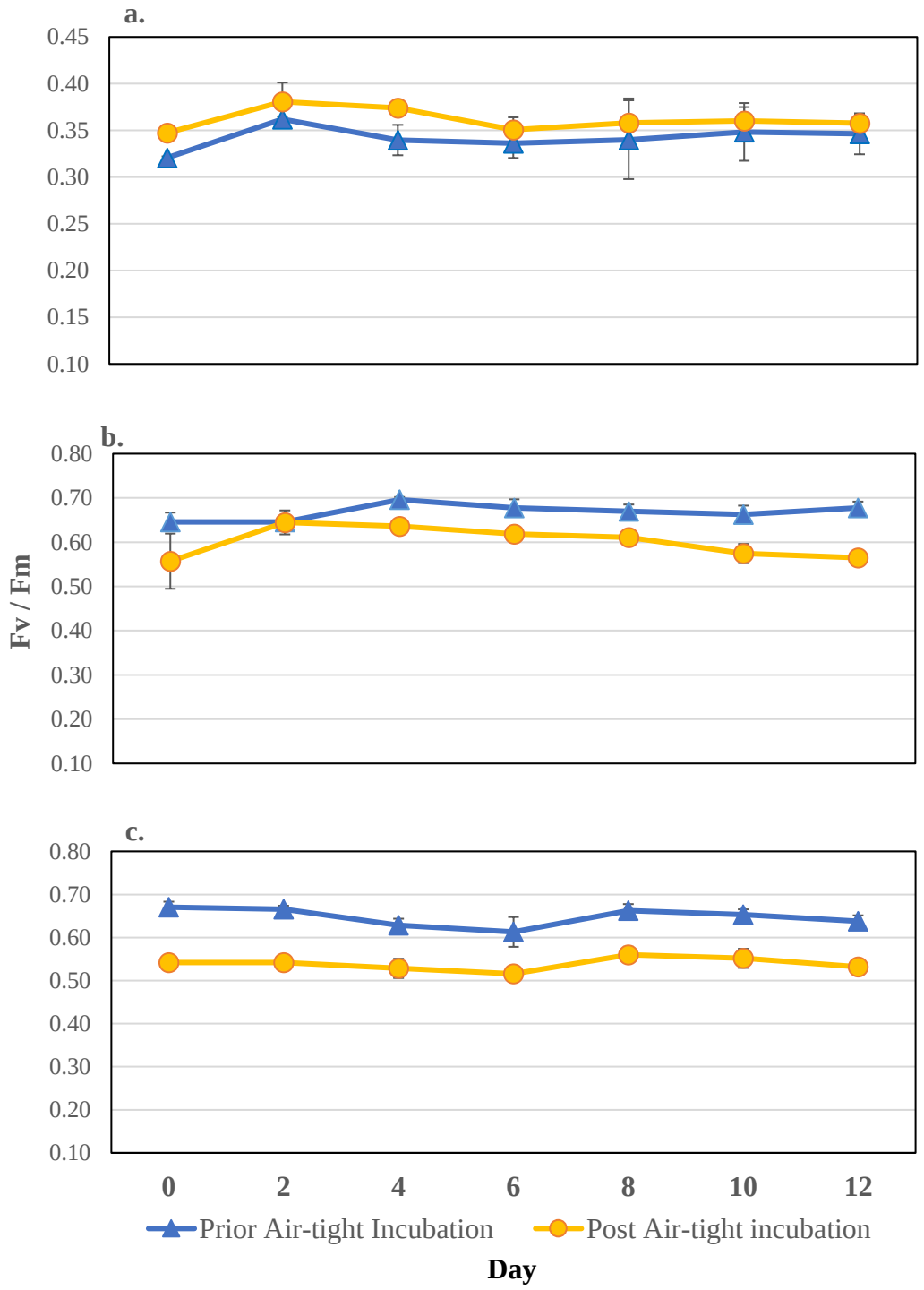


Fig.4

Click here to download colour figure Fig.4.pdf

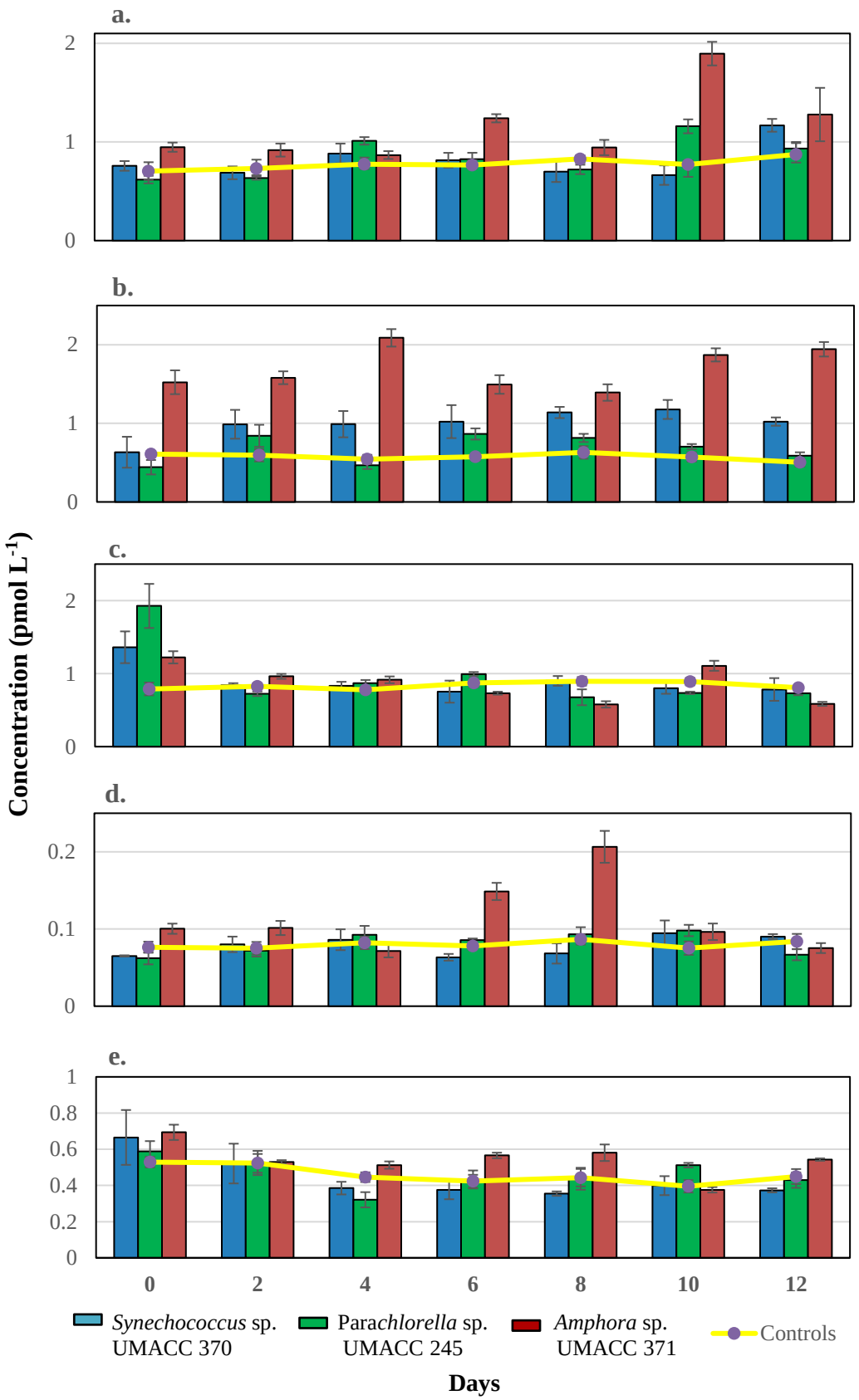


Fig.5

Click here to download colour figure Fig.5.pdf

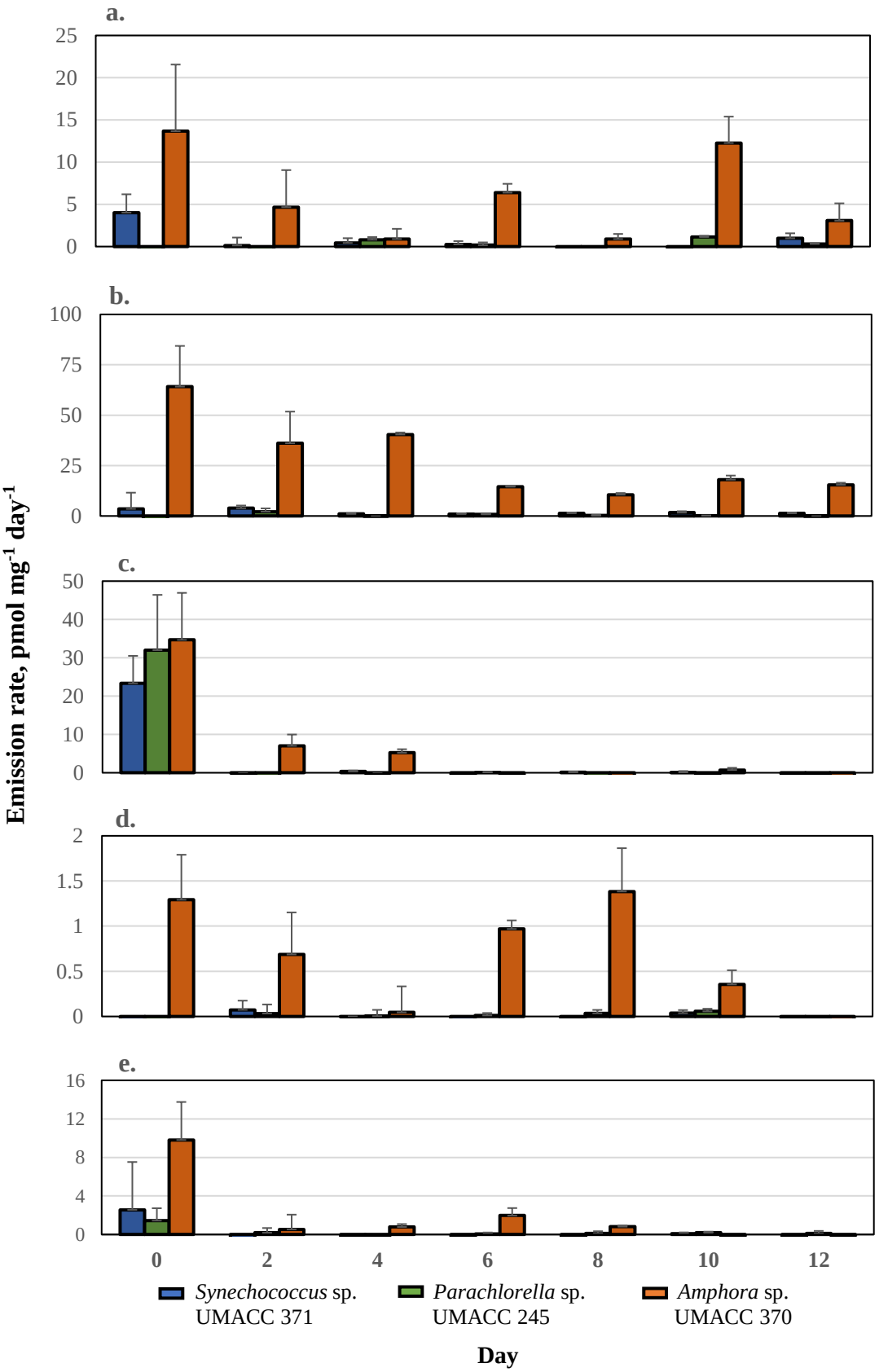


Fig.6

Click here to download colour figure Fig.6.pdf

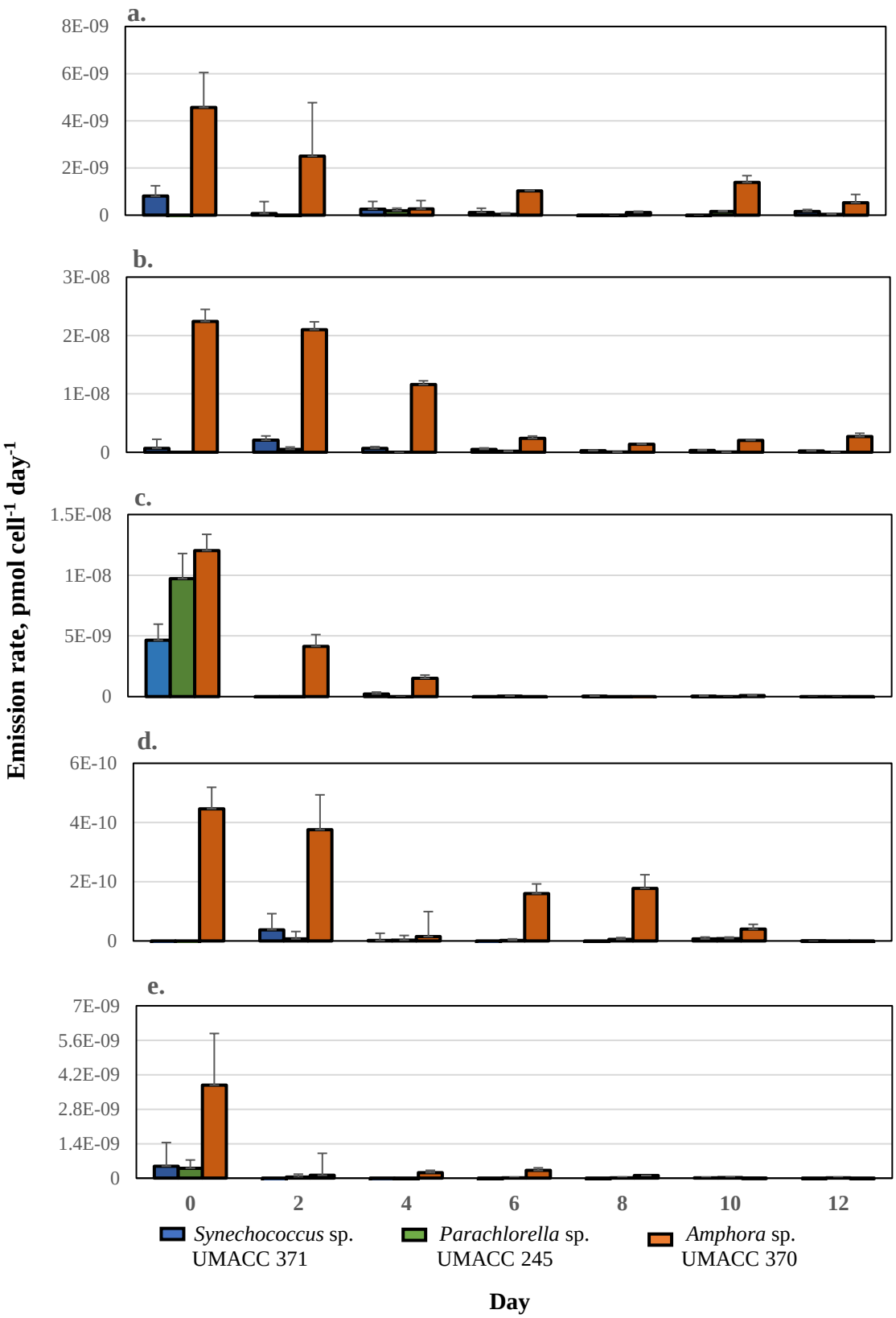
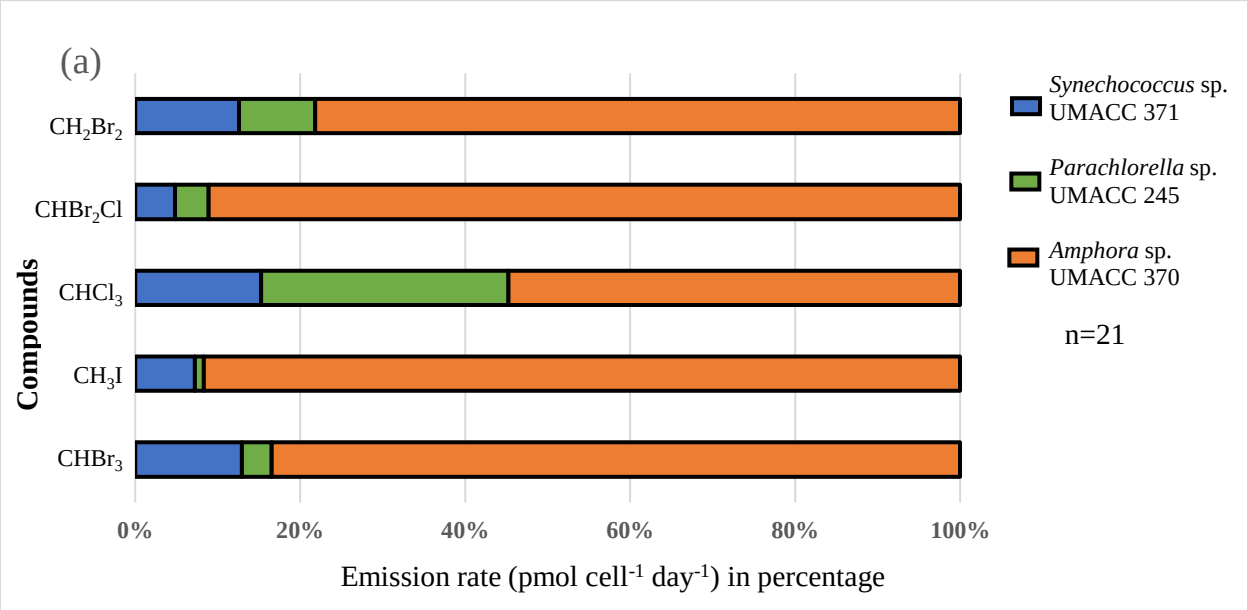


Fig7

Click here to download colour figure Fig.7.pdf



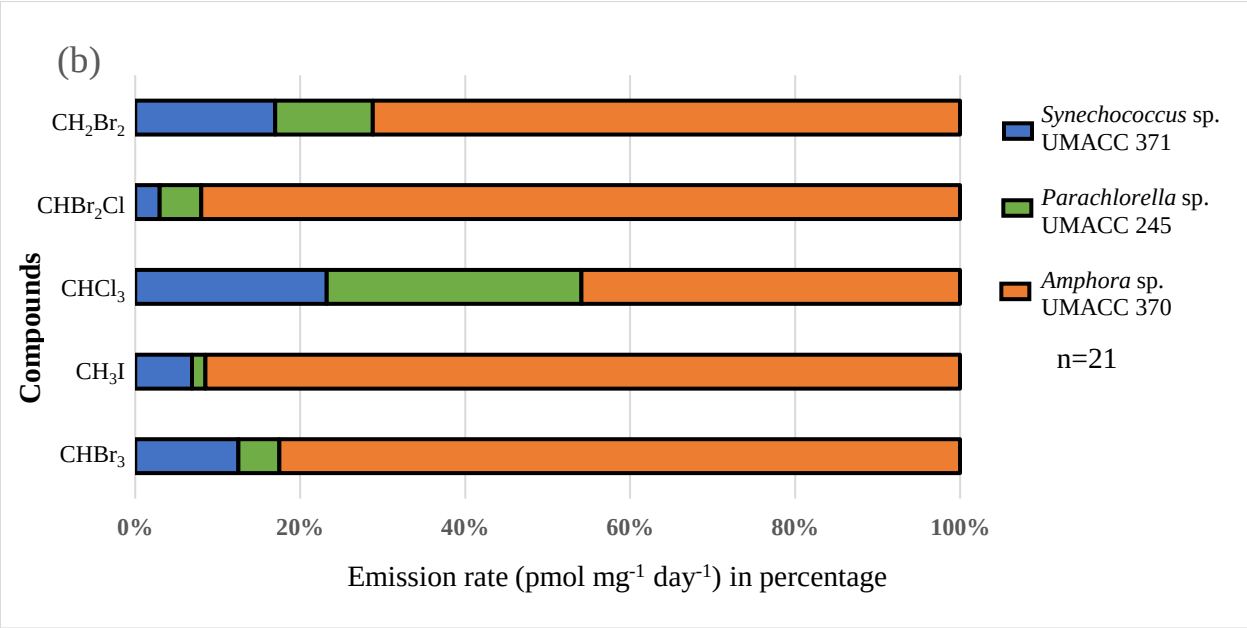


Table 1 Algal growth stages determined by chlorophyll-a and cell density. Selected range and representative points of exponential and stationary phases for the three tropical marine microalgae are shown

Taxa	Exponential phase		Stationary phase	
	Phase range	Representative point	Phase range	Representative point
Synechococcus sp. UMACC 371	Day 0—4	Day 4	Day 4—12	Day 8
Parachlorella sp. UMACC 245	Day 0—4		Day 4—12	
Amphora sp. UMACC 370	Day 0—6 Day 2 – 6#		Day 6—12	

For Amphora, the exponential phase ranged from day 2 to day 6.

Table 2 Specific growth rate. The mean of specific growth rate (ų) of the three tropical marine microalgae based on their exponential growth phase of chlorophyll-a and cell number. n = 3

Taxa	Specific Growth Rate (ų), n = 3	
	Chlorophyll-a	Cell number
Synechococcus sp. UMACC 371	0.66 (±0.0118)	0.36 (±0.0376)
Parachlorella sp. UMACC 245	0.54 (±0.0609)	0.64 (±0.0658)
Amphora sp. UMACC 370	0.27 (±0.0388)	0.74 (±0.0507)

Table 3 Correlation of the halocarbons. Pearson Product-Moment correlation coefficient (r) of the emission rate from the five detected compounds in term of (a) chlorophyll-a, (b) cell number, (c) chlorophyll-a and cell number

(a)	$CHBr_3$	CH_3I	$CHCl_3$	$CHBr_2Cl$	CH_2Br_2
$CHBr_3$	1.0000	0.7122**	0.4224**	0.6016**	0.4642**
CH_3I	0.7122**	1.0000	0.4828**	0.6390**	0.6195**
$CHCl_3$	0.4224**	0.4828**	1.0000	0.3081*	0.6543**
$CHBr_2Cl$	0.6016**	0.6390**	0.3081*	1.0000	0.4659**
CH_2Br_2	0.4642**	0.6195*	0.6543**	0.4659**	1.0000

Number of replicates (n) = 63, ** indicates significance level (p) < 0.01; * = (p) < 0.05.

(b)	$CHBr_3$	CH_3I	$CHCl_3$	$CHBr_2Cl$	CH_2Br_2
$CHBr_3$	1.0000	0.7864**	0.6176**	0.8391**	0.6266**
CH_3I	0.7864**	1.0000	0.5964**	0.8489**	0.6430**
$CHCl_3$	0.6176**	0.5964**	1.000	0.5872**	0.6872**
$CHBr_2Cl$	0.8391**	0.8489**	0.5872**	1.0000	0.6070**
CH_2Br_2	0.6266**	0.6430**	0.6872**	0.6070**	1.0000

Number of replicates (n) = 63, ** indicates significance level (p) < 0.01

(c)	$CHBr_3$ [a]	CH_3I [a]	$CHCl_3$ [a]	$CHBr_2Cl$ [a]	CH_2Br_2 [a]
$CHBr_3$ [b]	0.8390**	0.5278**	0.4296**	0.6061**	0.4269**
CH_3I [b]	0.8018**	0.8969**	0.5593**	0.7816**	0.6087**
$CHCl_3$ [b]	0.5228**	0.4419**	0.9511**	0.4412**	0.5715**
$CHBr_2Cl$ [b]	0.6152**	0.5217**	0.3628**	0.8200**	0.4114**
CH_2Br_2 [b]	0.6254**	0.6003**	0.7117**	0.5977**	0.9610**

Number of replicates (n) = 126, ** indicates significance level (p) < 0.01, [a] denotes chlorophyll a-normalized compounds; [b] denotes cell density-normalized compounds

Table 4 Emission rate at different growth phases. Concentrations of five halocarbons normalized to chlorophyll-a (pmol (mg chla)$^{-1}$ day^{-1}) and cell number (pmol (10^9 cell)$^{-1}$ day^{-1}) at exponential and stationary phase for (a) *Synechococcus* sp. UMACC 371, (b) *Parachlorella* sp. UMACC 245 and (c) *Amphora* sp. UMACC 370

(a)				
Compounds	Exponential phase		Stationary Phase	
	pmol (mg chla)$^{-1}$ day^{-1}	pmol (10^9 cell)$^{-1}$ day^{-1}	pmol (mg chla)$^{-1}$ day^{-1}	pmol (10^9 cell)$^{-1}$ day^{-1}
$CHBr_3$	0.00 – 5.97	0.00 – 1.18	0.00 – 1.58	0.00 - 0.32
CH_3I	0.00 – 12.27	0.00 – 2.70	0.74 – 2.23	0.16 – 0.79
$CHCl_3$	0.00 – 30.96	0.00 – 5.95	0.00 – 0.37	0.00 - 0.07
$CHBr_2Cl$	0.00 -- 0.13	0.00 - 0.07	0.00 - 0.07	0.00 - 0.01
CH_2Br_2	0.00 – 8.23	0.00 – 1.58	0.00 - 0.21	0.00 - 0.04

(b)				
Compounds	Exponential phase		Stationary Phase	
	pmol (mg chla)$^{-1}$ day^{-1}	pmol (10^9 cell)$^{-1}$ day^{-1}	pmol (mg chla)$^{-1}$ day^{-1}	pmol (10^9 cell)$^{-1}$ day^{-1}
$CHBr_3$	0.00 – 1.16	0.00 – 0.30	0.00 – 1.28	0.00 - 0.19
CH_3I	0.00 – 3.36	0.00 – 0.83	0.00 – 1.02	0.00 - 0.23
$CHCl_3$	0.00 – 48.68	0.00 – 12.11	0.00 – 0.26	0.00 – 0.05
$CHBr_2Cl$	0.00 - 0.22	0.00 - 0.05	0.00 - 0.08	0.00 - 0.01
CH_2Br_2	0.00 – 2.63	0.00 - 0.66	0.00 - 0.33	0.00 - 0.04

(c)				
Compounds	Exponential phase		Stationary Phase	
	pmol (mg chla)$^{-1}$ day^{-1}	pmol (10^9 cell)$^{-1}$ day^{-1}	pmol (mg chla)$^{-1}$ day^{-1}	pmol (10^9 cell)$^{-1}$ day^{-1}
$CHBr_3$	0.00 – 22.46	0.00 – 5.97	0.45 – 8.81	0.09 – 1.59
CH_3I	14.18 – 86.79	2.05 – 24.05	10.02 – 18.08	1.29 – 3.16
$CHCl_3$	0.00 – 48.51	0.00 – 12.90	0.00 – 1.27	0.00 – 0.15
$CHBr_2Cl$	0.00 – 1.84	0.00 - 0.49	0.00 – 1.89	0.00 - 0.21
CH_2Br_2	0.00 – 14.04	0.00 – 5.85	0.00 – 2.77	0.00 – 0.44

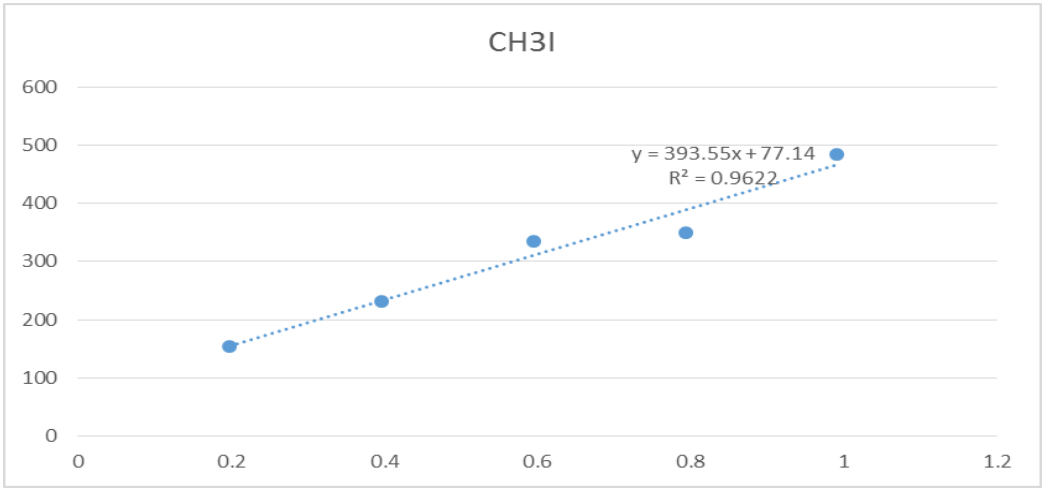
Table 5 Comparison of CH_3I emission by *Synechococcus* sp. from different climatic zones. The emission (pmol L^{-1}) of CH_3I and biomass from *Synechococcus* strains at different laboratory conditions

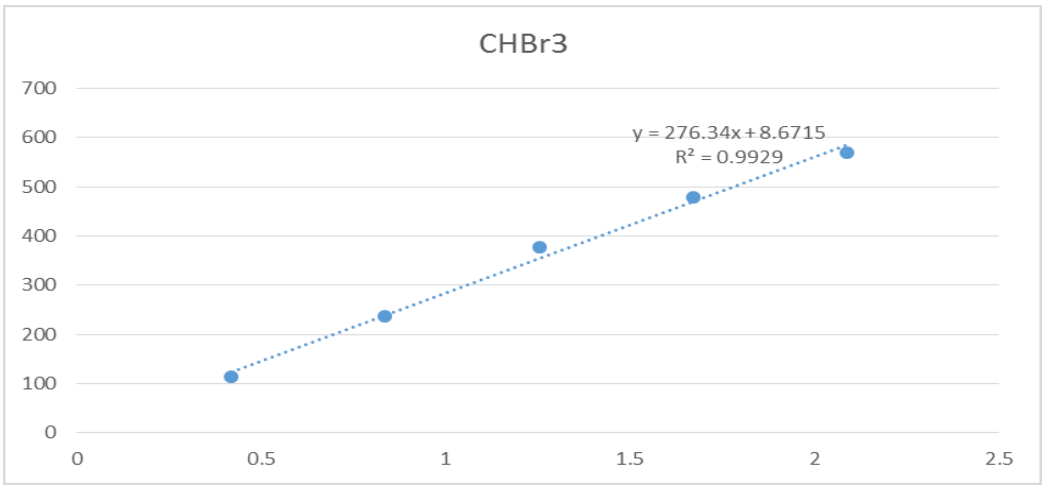
Taxa (Cyanophyta)	**Laboratory experimental condition and results**			**Reference**
	Incubation condition	CH_3I Emission	Biomass	
Synechococcus sp. CCMP 1334	• 18°C, • f/2 medium, • 20 µmol photons m^{-2} s^{-1}	No emission	Not reported	Manley & de la Cuesta, 1997
Synechococccus sp. CCMP 2370	• 21°C, • aged coastal seawater+PRO99, • 60µmol photons m^{-2} s^{-1}	Up to 20 pmol L^{-1}	0.5-1.0 µg L^{-1}	Brownell et al., 2010
Synechococcus sp. CCMP 2370	• 22°C, • artificial seawater+PRO99, • 40µmol photons m^{-2} s^{-1}	Up to 40 pmol L^{-1}	0-225 in vivo fluorescence	Hughes, Franklin & Malin, 2011
Synechococcus sp. UMACC 371	• 25°C, • prov50, • 40µmol photons m^{-2} s^{-1}	Up to 0.528 pmol L^{-1}	2 mg L^{-1}	Present study

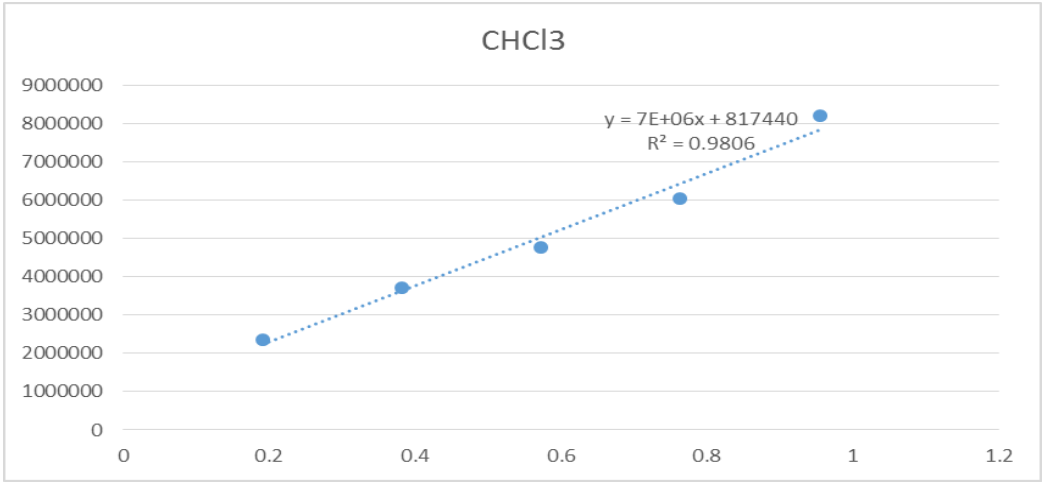
Table 6 Total mass of emitted halides. Total halogen mass emitted as halocarbons and percentage contribution to the total from bromine, chlorine and iodine. Taxa are arranged in decreasing total mass halogens emitted order

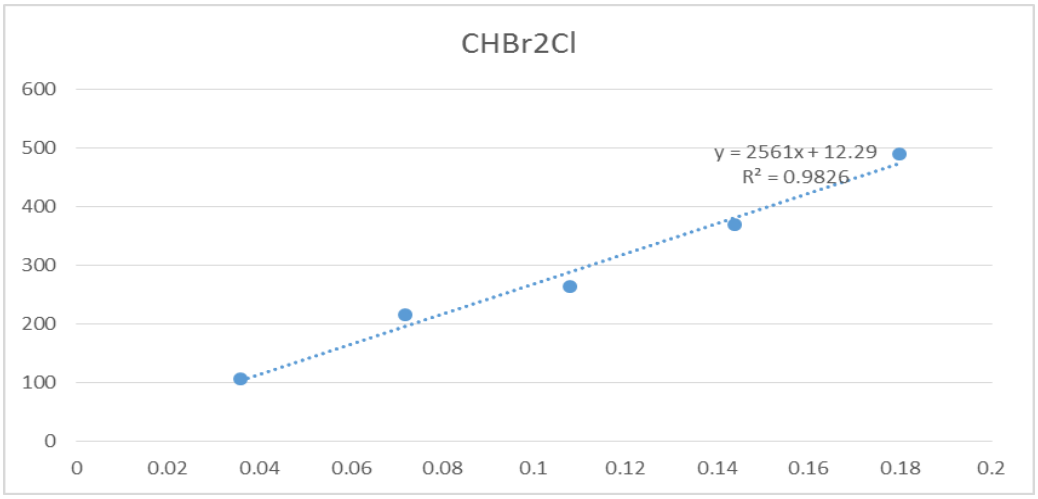
Taxa	**Total halogens emitted (pg)**	**% Br**	**% Cl**	**% I**
Amphora sp. UMACC 370	5223.6	34.39	5.93	59.7
Synechococcus sp. UMACC 371	2033.9	35.43	13.40	51.17
Parachlorella sp. UMACC 245	1573.8	32.29	47.01	21.02

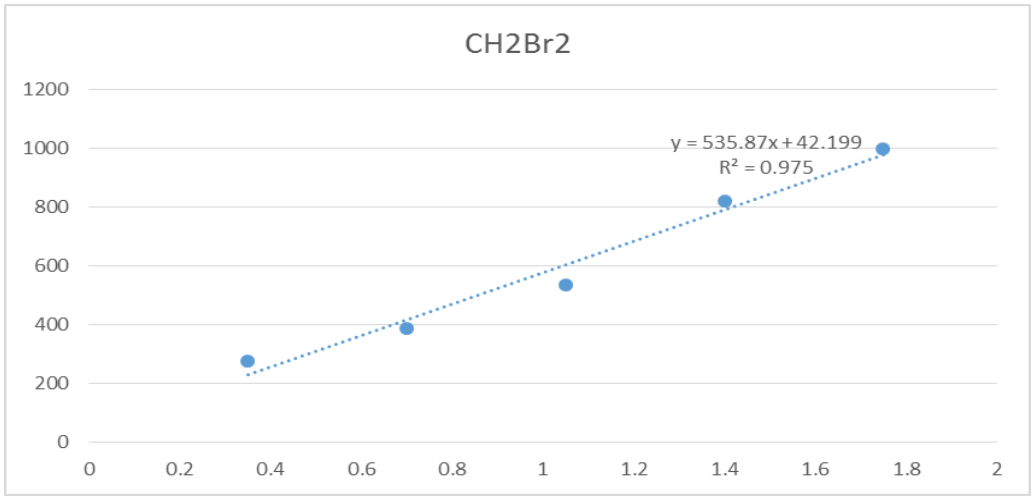
Calibration curves plotting integrated peak area against concentration (p mol^{-1}) for all five halocarbons with their respective linear regression (R^2).



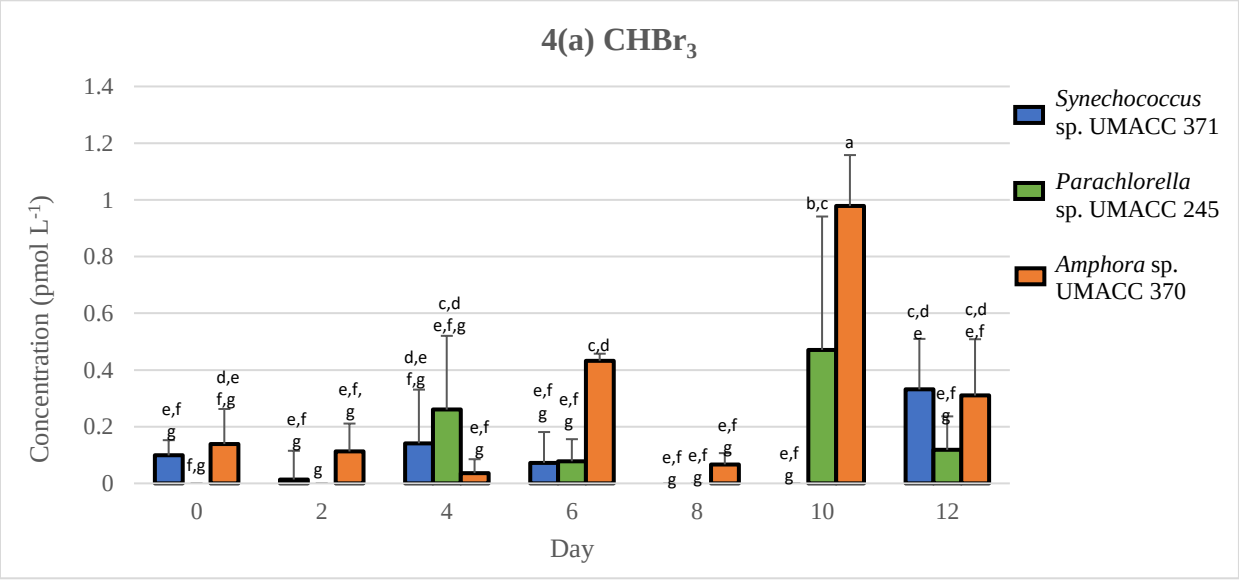


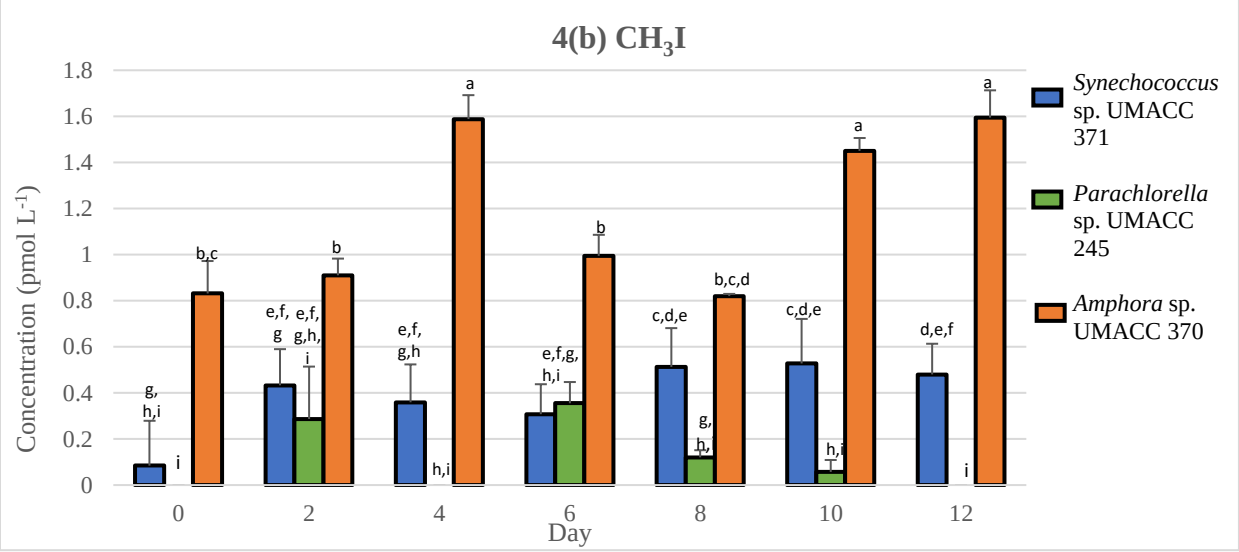


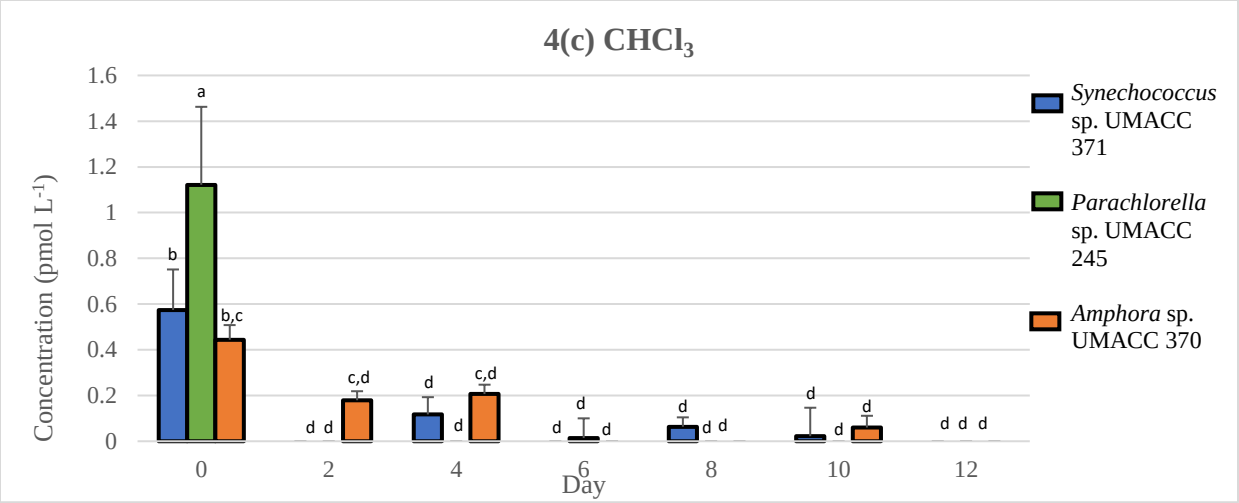

CHBr2Cl
600
500
400
300
200
100
0
y = 2561x + 12.29
R² = 0.9826
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2

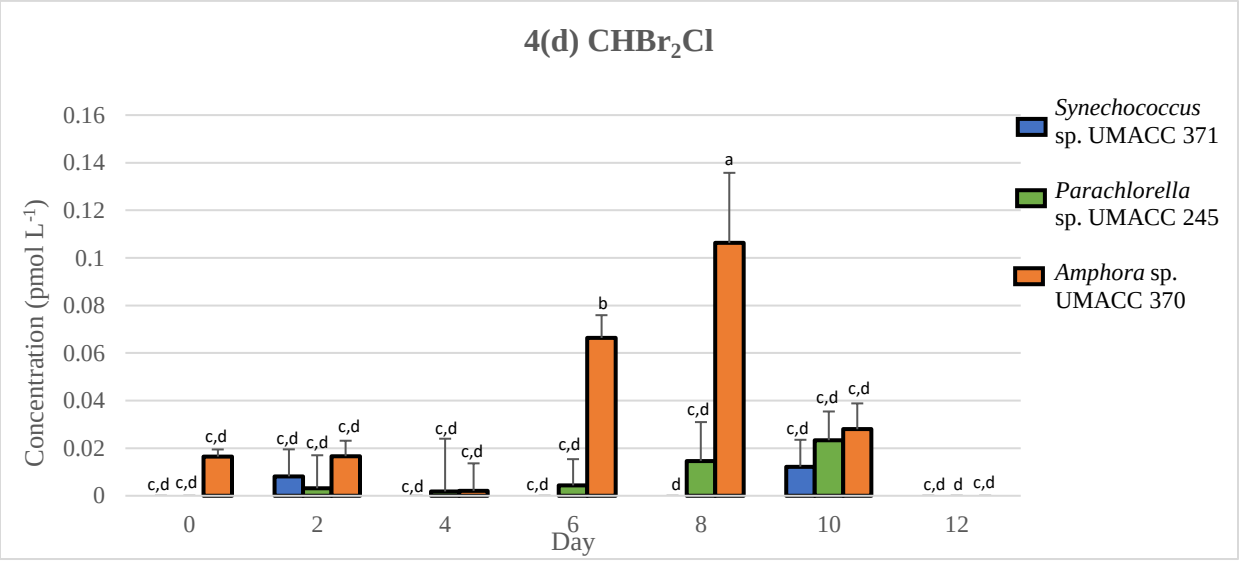

CH2Br2
1200
1000
800
600
400
200
0
y = 535.87x + 42.199
R² = 0.975
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2

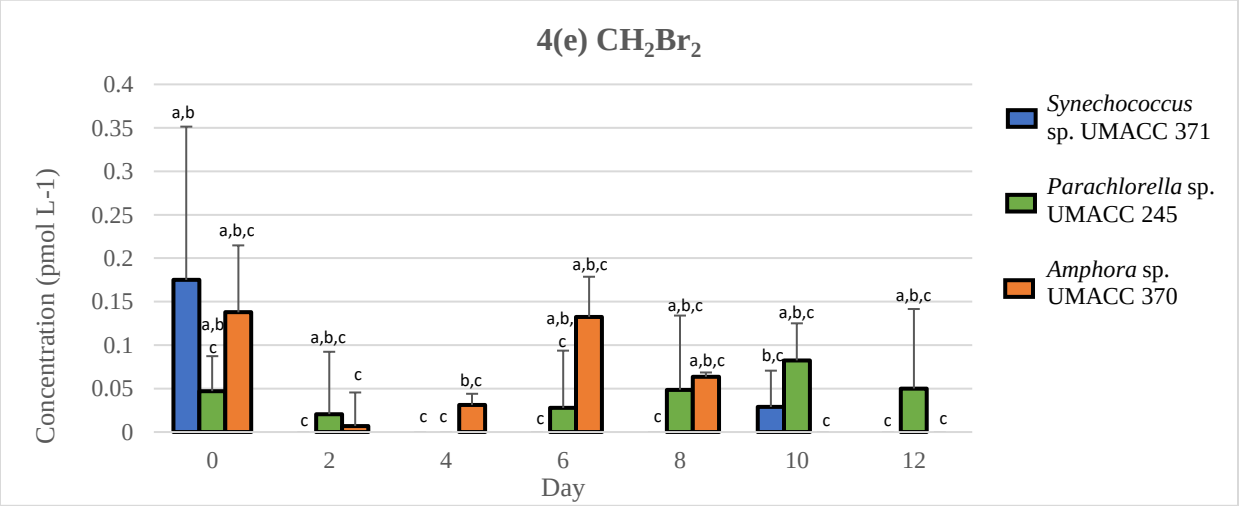
Net concentration (emission) of halocarbons. Concentration of halocarbons emitted by the three tropical marine microalgae over 12 day culture period for compound (a) $CHBr_3$, (b) CH_3I, (c) $CHCl_3$, (d) $CHBr_2Cl$ and (e) CH_2Br_2.



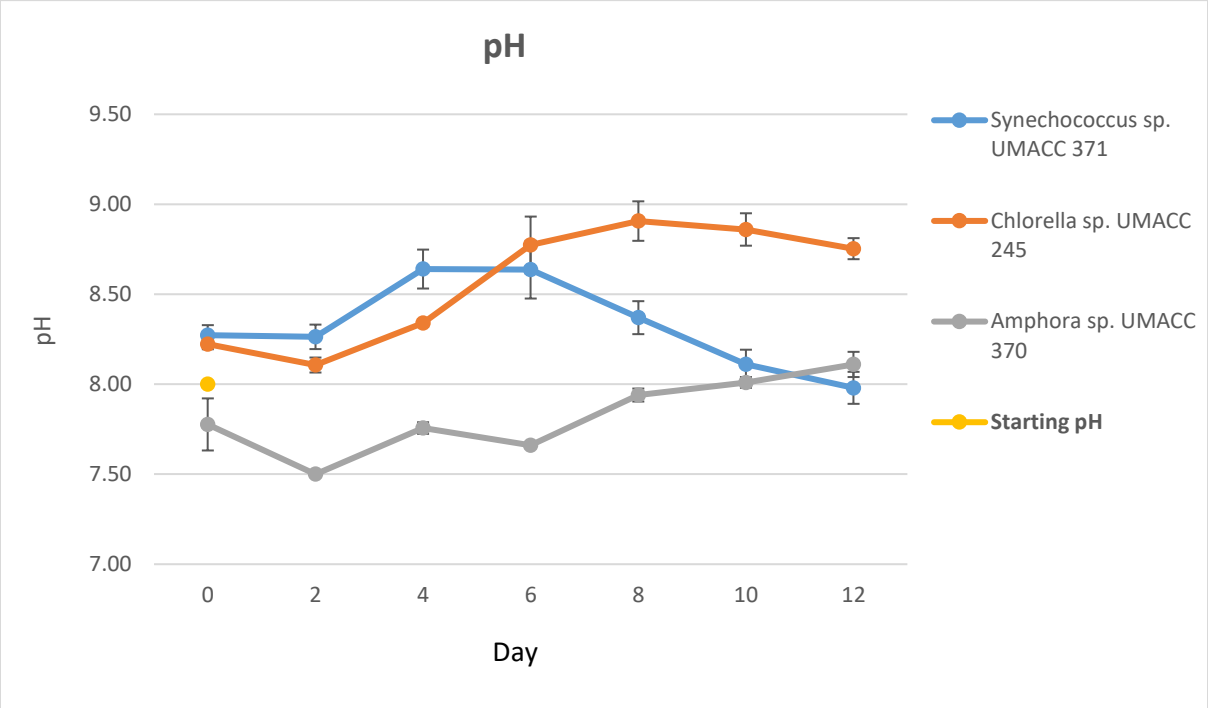



4(c) CHCl3
Concentration (pmol L-1)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
Day
Synechococcus sp. UMACC 371
Parachlorella sp. UMACC 245
Amphora sp. UMACC 370


4(d) CHBr2Cl
Concentration (pmol L-1)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
2
4
6
8
10
12
Day
Synechococcus sp. UMACC 371
Parachlorella sp. UMACC 245
Amphora sp. UMACC 370


4(e) CH2Br2
Concentration (pmol L-1)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
2
4
6
8
10
12
Day
Synechococcus sp. UMACC 371
Parachlorella sp. UMACC 245
Amphora sp. UMACC 370

pH. Responding changes of pH over 12 day culture period for the three taxa. Prior to start of inoculation, pH of medium was standardized to a starting pH of 8.0.



Concentration (pmol L-1)

Supplementary Table 1 (a-c): Concentration of five VSLH emitted by three selected tropical r

Table 1 (a): Synechoc

					0.2			
	Day	Rep	Sample	Control	Net	Average	SD	Sample
		1	0.584	0.532	0.052	NE	NIL	0.724
	0	2	0.562	0.581	NE			0.743
		3	0.496	0.513	NE			0.738
CH3I		1	0.512	0.533	NE	NE	NIL	0.551
	2	2	0.508	0.529	NE			0.495
		3	0.517	0.497	0.02			0.576
		1	0.825	0.515	0.31	0.200333	0.097377	0.687
	4	2	0.69	0.523	0.167			0.604
		3	0.666	0.542	0.124			0.59
					0.2			
	Day	Rep	Sample	Control	Net	Average	SD	Sample
		1	0.621	0.785	NE	NE	NIL	0.621
	0	2	0.619	0.726	NE			0.657
		3	0.641	0.814	NE			0.639
CHCl3		1	0.668	0.745	NE	NE	NIL	0.715
	2	2	0.647	0.774	NE			0.623
		3	0.672	0.683	NE			0.656
		1	0.711	0.788	NE	NE	NIL	0.669
	4	2	0.798	0.719	0.079			0.684
		3	0.772	0.793	NE			0.689
					0.2			
	Day	Rep	Sample	Control	Net	Average	SD	Sample
		1	0.675	0.717	NE	NE	NIL	0.734
	0	2	0.682	0.829	NE			0.673
		3	0.713	0.764	NE			0.712
CHBr3		1	0.746	0.815	NE	NE	NIL	0.748
	2	2	0.716	0.729	NE			0.717
		3	0.739	0.708	NE			0.669
		1	0.804	0.771	0.033	0.026	0.007	0.745
	4	2	0.77	0.751	0.019			0.8
		3	0.828	0.802	0.026			0.766
					0.2			
	Day	Rep	Sample	Control	Net	Average	SD	Sample
		1	0.486	0.525	NE	NE	NIL	0.522
	0	2	0.476	0.479	NE			0.521
		3	0.519	0.524	NE			0.522
CH2Br2		1	0.466	0.484	NE	NE	NIL	0.476
	2	2	0.464	0.492	NE			0.462
		3	0.472	0.521	NE			0.513
		1	0.536	0.587	NE	NE	NIL	0.472
	4	2	0.534	0.523	0.011			0.517
		3	0.413	0.563	NE			0.518

								0.2
	Day	Rep	Sample	Control	Net	Average	SD	Sample
		1	0.0743	0.0792	NE	NE	NIL	0.0694
	0	2	0.0718	0.0713	0.0004			0.0733
		3	0.0679	0.0743	NE			0.0712
CHBr2Cl		1	0.0778	0.0826	NE	NE	NIL	0.0752
	2	2	0.0742	0.0818	NE			0.0793
		3	0.0736	0.0769	NE			0.0818
		1	0.0788	0.0844	NE	NE	NIL	0.0715
	4	2	0.0743	0.0829	NE			0.0788
		3	0.0796	0.0854	NE			0.0736

NE = No Emission

marine microalgae at OD620NM 0.2, 0.3 and 0.4. n = 3

occus sp. UMACC 371

		0.3					0.4		
Control	Net	Average	SD	Sample	Control	Net	Average	SD	
0.514	0.21	0.18	0.03	1.822	0.572	1.25	1.021333	0.234182	
0.563	0.18			1.578	0.546	1.032			
0.588	0.15			1.341	0.559	0.782			
0.511	0.04	0.039	0.028513	0.654	0.529	0.125	0.112333	0.020232	
0.485	0.01			0.605	0.516	0.089			
0.509	0.067			0.645	0.522	0.123			
0.584	0.103	0.072	0.027622	0.762	0.538	0.224	0.178333	0.060285	
0.554	0.05			0.774	0.573	0.201			
0.527	0.063			0.672	0.562	0.11			
		0.3					0.4		
Control	Net	Average	SD	Sample	Control	Net	Average	SD	
0.719	NE	NE	NIL	0.683	0.742	NE	NE	NIL	
0.747	NE			0.704	0.711	NE			
0.688	NE			0.689	0.758	NE			
0.714	0.001	NE	NIL	0.728	0.752	NE	NE	NIL	
0.721	NE			0.713	0.726	NE			
0.741	NE			0.694	0.718	NE			
0.698	NE	NE	NIL	1.995	0.745	1.25	1.034333	0.193221	
0.714	NE			1.657	0.681	0.976			
0.631	0.058			1.603	0.726	0.877			
		0.3					0.4		
Control	Net	Average	SD	Sample	Control	Net	Average	SD	
0.786	NE	NE	NIL	0.794	0.771	0.023	0.022	0.009539	
0.745	NE			0.747	0.735	0.012			
0.772	NE			0.799	0.768	0.031			
0.821	NE	NE	NIL	0.74	0.725	0.015	0.0195	0.006364	
0.819	NE			0.786	0.794	NE			
0.789	NE			0.756	0.732	0.024			
0.724	0.021	0.021667	0.007024	0.839	0.805	0.034	0.046667	0.014189	
0.785	0.015			0.825	0.763	0.062			
0.737	0.029			0.828	0.784	0.044			
		0.3					0.4		
Control	Net	Average	SD	Sample	Control	Net	Average	SD	
0.518	0.004		NIL	0.489	0.557	NE	NE	NIL	
0.542	NE	NE		0.472	0.513	NE			
0.546	NE			0.529	0.582	NE			
0.517	NE		NIL	0.61	0.542	0.068	0.036333	0.028711	
0.486	NE	NE		0.53	0.518	0.012			
0.533	NE			0.572	0.543	0.029			
0.519	NE		NIL	0.512	0.515	NE	NE	NIL	
0.497	0.02	NE		0.424	0.489	NE			
0.529	NE			0.467	0.525	NE			

0.3					0.4				
Control	Net	Average	SD	Sample	Control	Net	Average	SD	
0.0814	NE	NE	NIL	0.0731	0.0786	NE	NE	NIL	
0.0789	NE			0.0696	0.0783	NE			
0.0811	NE			0.0729	0.0824	NE			
0.0819	NE	NE	NIL	0.0725	0.0815	NE	NE	NIL	
0.0823	NE			0.0736	0.0822	NE			
0.0832	NE			0.0742	0.0775	NE			
0.0807	NE	NE	NIL	0.1023	0.0843	0.018	0.012873	0.004493	
0.0829	NE			0.0917	0.0821	0.00962			
0.0844	NE			0.0949	0.0839	0.011			

Table 1

					0.2		
	Day	Rep	Sample	Control	Net	Average	SD
		1	0.587	0.532	0.055	0.091333	0.038109
	0	2	0.712	0.581	0.131		
		3	0.601	0.513	0.088		
CH3I		1	0.635	0.533	0.102	0.120133	0.038179
	2	2	0.6234	0.529	0.0944		
		3	0.661	0.497	0.164		
		1	0.749	0.515	0.234	0.242	0.035679
	4	2	0.804	0.523	0.281		
		3	0.753	0.542	0.211		
					0.2		
	Day	Rep	Sample	Control	Net	Average	SD
		1	0.623	0.785	NE	NE	NIL
	0	2	0.688	0.726	NE		
		3	0.712	0.814	NE		
CHCl3		1	0.724	0.745	NE	NE	NIL
	2	2	0.765	0.774	NE		
		3	0.705	0.683	0.022		
		1	0.729	0.788	NE	NE	NIL
	4	2	0.718	0.719	NE		
		3	0.736	0.793	NE		
					0.2		
	Day	Rep	Sample	Control	Net	Average	SD
		1	0.703	0.717	NE	NE	NIL
	0	2	0.743	0.829	NE		
		3	0.732	0.764	NE		
CHBr3		1	0.683	0.815	NE	NE	NIL
	2	2	0.714	0.729	NE		
		3	0.713	0.708	0.005		
		1	0.693	0.771	NE	NE	NIL
	4	2	0.724	0.751	NE		
		3	0.942	0.802	NE		
					0.2		
	Day	Rep	Sample	Control	Net	Average	SD
		1	0.478	0.525	NE	NE	NIL
	0	2	0.4374	0.479	NE		
		3	0.4882	0.524	NE		
CH2Br2		1	0.4837	0.484	NE	NE	NIL
	2	2	0.492	0.492	NE		
		3	0.513	0.521	NE		
		1	0.5494	0.587	NE	NE	NIL
	4	2	0.5275	0.523	0.0045		
		3	0.5288	0.563	NE		

					0.2		
	Day	Rep	Sample	Control	Net	Average	SD
		1	0.0637	0.0792	NE	NE	NIL
	0	2	0.0664	0.0713	NE		
		3	0.0638	0.0743	NE		
CHBr2Cl		1	0.0726	0.0826	NE	NE	NIL
	2	2	0.0752	0.0818	NE		
		3	0.0736	0.0769	NE		
		1	0.0759	0.0844	NE	NE	NIL
	4	2	0.0732	0.0829	NE		
		3	0.0738	0.0854	NE		

(b): Amphora sp. UMACC 370

		0.3					0.4	
Sample	Control	Net	Average	SD	Sample	Control	Net	Average
0.759	0.514	0.245	0.283667	0.034429	1.357	0.572	0.785	0.666333
0.858	0.563	0.295			1.139	0.546	0.593	
0.899	0.588	0.311			1.18	0.559	0.621	
0.705	0.511	0.194	0.268333	0.064702	1.322	0.529	0.793	0.864667
0.797	0.485	0.312			1.405	0.516	0.889	
0.808	0.509	0.299			1.434	0.522	0.912	
1.128	0.584	0.544	0.555	0.12287	1.517	0.538	0.979	1.056
0.992	0.554	0.438			1.718	0.573	1.145	
1.21	0.527	0.683			1.606	0.562	1.044	
		0.3					0.4	
Sample	Control	Net	Average	SD	Sample	Control	Net	Average
0.884	0.719	0.165	0.278333	0.134526	1.385	0.742	0.643	0.775667
0.99	0.747	0.243			1.482	0.711	0.771	
1.115	0.688	0.427			1.671	0.758	0.913	
0.673	0.714	NE	NE	NIL	1.205	0.752	0.453	0.367667
0.683	0.721	NE			1.011	0.726	0.285	
0.712	0.741	NE			1.083	0.718	0.365	
0.683	0.698	NE	NE	NIL	0.86	0.745	0.115	0.107467
0.689	0.714	NE			0.7594	0.681	0.0784	
0.694	0.631	0.063			0.855	0.726	0.129	
		0.3					0.4	
Sample	Control	Net	Average	SD	Sample	Control	Net	Average
0.721	0.786	NE	NE	NIL	0.948	0.771	0.177	0.152667
0.717	0.745	NE			0.913	0.735	0.178	
0.743	0.772	NE			0.871	0.768	0.103	
0.694	0.821	NE	NE	NIL	0.8145	0.725	0.0895	0.089367
0.713	0.819	NE			0.896	0.794	0.102	
0.728	0.789	NE			0.8086	0.732	0.0766	
0.7369	0.724	0.0129	0.019267	0.007213	1.016	0.805	0.211	0.224333
0.8028	0.785	0.0178			0.938	0.763	0.175	
0.7641	0.737	0.0271			1.071	0.784	0.287	
		0.3					0.4	
Sample	Control	Net	Average	SD	Sample	Control	Net	Average
0.511	0.518	NE	NE	NIL	0.5991	0.557	0.0421	0.0285
0.498	0.542	NE			0.5244	0.513	0.0114	
0.521	0.546	NE			0.614	0.582	0.032	
0.52287	0.517	0.00587	0.008233	0.002716	0.687	0.542	0.145	0.141533
0.49363	0.486	0.00763			0.6016	0.518	0.0836	
0.5442	0.533	0.0112			0.739	0.543	0.196	
0.52646	0.519	0.00746	0.015487	0.007181	0.5576	0.515	0.0426	0.042833
0.5147	0.497	0.0177			0.51	0.489	0.021	
0.5503	0.529	0.0213			0.5899	0.525	0.0649	

	0.3					0.4		
Sample	Control	Net	Average	SD	Sample	Control	Net	Average
0.0763	0.0814	NE	NE	NIL	0.0733	0.0786	NE	NE
0.0722	0.0789	NE			0.0747	0.0783	NE	
0.0787	0.0811	NE			0.0789	0.0824	NE	
0.0829	0.0819	0.01	NE	NIL	0.1	0.0815	0.0184	0.018867
0.0813	0.0823	NE			0.106	0.0822	0.0238	
0.0816	0.0832	NE			0.0919	0.0775	0.0144	
0.09018	0.0807	0.00948	0.011973	0.004883	0.0928	0.0843	0.0085	0.010957
0.1005	0.0829	0.0176			0.09167	0.0821	0.00957	
0.09324	0.0844	0.00884			0.0987	0.0839	0.0148	

							0.2	
SD		Day	Rep	Sample	Control	Net		Average
0.103718			1	0.523	0.532	NE		NE
		0	2	0.535	0.581	NE		
			3	0.524	0.513		0.011	
0.063122	CH3I		1	0.528	0.533	NE		NE
		2	2	0.513	0.529	NE		
			3	0.521	0.497		0.024	
0.083648			1	0.508	0.515	NE		NE
		4	2	0.511	0.523	NE		
			3	0.514	0.542	NE		
							0.2	
SD		Day	Rep	Sample	Control	Net		Average
0.13506			1	0.734	0.785	NE		NE
		0	2	0.757	0.726		0.031	
			3	0.738	0.814	NE		
0.084032	CHCl3		1	0.683	0.745	NE		NE
		2	2	0.743	0.774	NE		
			3	0.712	0.683		0.029	
0.026128			1	0.753	0.788	NE		NE
		4	2	0.724	0.719		0.003	
			3	0.732	0.793	NE		
							0.2	
SD		Day	Rep	Sample	Control	Net		Average
0.043016			1	0.733	0.717		0.016	NE
		0	2	0.784	0.829	NE		
			3	0.722	0.764	NE		
0.012701	CHBr3		1	0.736	0.815	NE		NE
		2	2	0.713	0.729	NE		
			3	0.719	0.708		0.011	
0.057178			1	0.753	0.771	NE		NE
		4	2	0.748	0.751	NE		
			3	0.773	0.802	NE		
							0.2	
SD		Day	Rep	Sample	Control	Net		Average
0.015646			1	0.516	0.525	NE		NE
		0	2	0.524	0.479		0.045	
			3	0.503	0.524	NE		
0.05628	CH2Br2		1	0.481	0.484	NE		NE
		2	2	0.522	0.492		0.03	
			3	0.497	0.521	NE		
0.021951			1	0.534	0.587	NE		NE
		4	2	0.516	0.523	NE		
			3	0.523	0.563	NE		

SD			Day	Rep	Sample	Control	Net	0.2	Average
NIL				1	0.0753	0.0792	NE		NE
			0	2	0.0739	0.0713		0.026	
				3	0.0725	0.0743	NE		
0.004717		CHBr2Cl		1	0.0758	0.0826	NE		NE
			2	2	0.0753	0.0818	NE		
				3	0.0754	0.0769	NE		
0.003371				1	0.0812	0.0844	NE		NE
			4	2	0.0781	0.0829	NE		
				3	0.0827	0.0854	NE		

1(c): Parachlorella sp. UMACC 245

			0.3					0.4
SD	Sample	Control	Net	Average	SD	Sample	Control	Net
NIL	0.521	0.514	0.007	NE	NIL	0.7052	0.572	0.1332
	0.519	0.563	NE			0.688	0.546	0.142
	0.532	0.588	NE			0.6489	0.559	0.0899
NIL	0.435	0.511	NE	NE	NIL	0.5434	0.529	0.0144
	0.479	0.485	NE			0.5938	0.516	0.0778
	0.494	0.509	NE			0.5899	0.522	0.0679
NIL	0.512	0.584	NE	NE	NIL	0.516	0.538	NE
	0.525	0.554	NE			0.543	0.573	NE
	0.517	0.527	NE			0.553	0.562	NE
			0.3					0.4
SD	Sample	Control	Net	Average	SD	Sample	Control	Net
NIL	0.673	0.719	NE	NE	NIL	1.285	0.742	0.543
	0.711	0.747	NE			1.043	0.711	0.332
	0.703	0.688	0.015			0.962	0.758	0.204
NIL	0.624	0.714	NE	NE	NIL	0.684	0.752	NE
	0.718	0.721	NE			0.694	0.726	NE
	0.723	0.741	NE			0.715	0.718	NE
NIL	0.688	0.698	NE	NE	NIL	0.721	0.745	NE
	0.667	0.714	NE			0.675	0.681	NE
	0.621	0.631	NE			0.632	0.726	NE
			0.3					0.4
SD	Sample	Control	Net	Average	SD	Sample	Control	Net
NIL	0.752	0.786	NE	NE	NIL	0.684	0.771	NE
	0.728	0.745	NE			0.722	0.735	NE
	0.736	0.772	NE			0.713	0.768	NE
NIL	0.8256	0.821	0.00455	0.004853	0.002887	0.714	0.725	NE
	0.8211	0.819	0.00213			0.755	0.794	NE
	0.7969	0.789	0.00788			0.737	0.732	NE
NIL	0.6834	0.724	NE	NE	NIL	0.8193	0.805	0.0143
	0.723	0.785	NE			0.77243	0.763	0.00943
	0.729	0.737	NE			0.7995	0.784	0.0155
			0.3					0.4
SD	Sample	Control	Net	Average	SD	Sample	Control	Net
NIL	0.525	0.518	0.007	NE	NIL	0.532	0.557	NE
	0.538	0.542	NE			0.505	0.513	NE
	0.526	0.546	NE			0.542	0.582	NE
NIL	0.513	0.517	NE	NE	NIL	0.5632	0.542	0.0212
	0.519	0.486	NE			0.5424	0.518	0.0244
	0.524	0.533	NE			0.5796	0.543	0.0366
NIL	0.5334	0.519	0.0144	0.013757	0.003905	0.5347	0.515	0.0197
	0.50657	0.497	0.00957			0.49721	0.489	0.00821
	0.5463	0.529	0.0173			0.53243	0.525	0.00743

			0.3					0.4
SD	Sample	Control	Net	Average	SD	Sample	Control	Net
NIL	0.0784	0.0814	NE	NE	NIL	0.0694	0.0786	NE
	0.0738	0.0789	NE			0.0732	0.0783	NE
	0.0748	0.0811	NE			0.0784	0.0824	NE
NIL	0.0775	0.0819	NE	NE	NIL	0.0798	0.0815	NE
	0.0831	0.0823	0.0008			0.0756	0.0822	NE
	0.0765	0.0832	NE			0.0732	0.0775	NE
NIL	0.0813	0.0807	0.0006	NE	NIL	0.08663	0.0843	0.00233
	0.0823	0.0829	NE			0.08451	0.0821	0.00241
	0.0834	0.0844	NE			0.08743	0.0839	0.00353

Average SD
0.1217 0.027889
0.053367 0.034107
NE NIL
Average SD
0.359667 0.171185
NE NIL
NE NIL
Average SD
NE NIL
NE NIL
0.013077 0.003215
Average SD
NE NIL
0.0274 0.008126
0.01178 0.00687

Average	SD
NE	NIL
NE	NIL
0.002757	0.000671

Supplementary data Table S2

Click here to download attachment to manuscript Supplementary data, TableS2.xlsx

Click here to view linked References

Emission rate of the five compounds by 3 taxa based on chlorophyll a normalization, p<0.01, Tuke

Cell No.	Taxa	Time effect	1	2	3	4	5	6
1	Syne	CHBr3_Chla		0.999999	0.994676	1.000000	1.000000	1.000000
2	Syne	CH3I_Chla	0.999999		0.999998	0.999207	0.999957	0.999986
3	Syne	CHCl3_Chla	0.994676	0.999998		0.924368	0.977267	0.992675
4	Syne	CHBr2Cl_Chla	1.000000	0.999207	0.924368		1.000000	1.000000
5	Syne	CH2Br2_Chla	1.000000	0.999957	0.977267	1.000000		1.000000
6	Chlorella	CHBr3_Chla	1.000000	0.999986	0.992675	1.000000	1.000000	
7	Chlorella	CH3I_Chla	1.000000	0.999995	0.995334	1.000000	1.000000	1.000000
8	Chlorella	CHCl3_Chla	0.963095	0.999393	1.000000	0.821914	0.913816	0.712052
9	Chlorella	CHBr2Cl_Chla	1.000000	0.999889	0.981137	1.000000	1.000000	1.000000
10	Chlorella	CH2Br2_Chla	1.000000	0.999983	0.992128	1.000000	1.000000	1.000000
11	Amphora	CHBr3_Chla	0.675055	0.946576	0.999003	0.393237	0.540566	0.499606
12	Amphora	CH3I_Chla	0.000026	0.000026	0.000026	0.000026	0.000026	0.000026
13	Amphora	CHCl3_Chla	0.413974	0.796605	0.983151	0.188644	0.294719	0.262992
14	Amphora	CHBr2Cl_Chla	1.000000	0.999999	0.997709	1.000000	1.000000	1.000000
15	Amphora	CH2Br2_Chla	1.000000	1.000000	0.999999	0.999932	0.999997	0.999993

ey HSD test, Approximate probabilities for Post Hoc tests, Repeated-Measured ANOVA. n = 3

7	8	9	10	11	12	13	14	15
1.000000	0.963095	1.000000	1.000000	0.675055	0.000026	0.413974	1.000000	1.000000
0.999995	0.999393	0.999889	0.999983	0.946576	0.000026	0.796605	0.999999	1.000000
0.995334	1.000000	0.981137	0.992128	0.999003	0.000026	0.983151	0.997709	0.999999
1.000000	0.821914	1.000000	1.000000	0.393237	0.000026	0.188644	1.000000	0.999932
1.000000	0.913816	1.000000	1.000000	0.540566	0.000026	0.294719	1.000000	0.999997
1.000000	0.712052	1.000000	1.000000	0.499606	0.000026	0.262992	1.000000	0.999993
	0.758157	1.000000	1.000000	0.543709	0.000026	0.297232	1.000000	0.999998
0.758157		0.592459	0.704065	0.999999	0.000026	0.999789	0.941571	0.999044
1.000000	0.592459		1.000000	0.397960	0.000026	0.191719	1.000000	0.999939
1.000000	0.704065	1.000000		0.492297	0.000026	0.257523	1.000000	0.999992
0.543709	0.999999	0.397960	0.492297		0.000026	1.000000	0.317421	0.801273
0.000026	0.000026	0.000026	0.000026	0.000026		0.000026	0.000026	0.000026
0.297232	0.999789	0.191719	0.257523	1.000000	0.000026		0.120528	0.508726
1.000000	0.941571	1.000000	1.000000	0.317421	0.000026	0.120528		0.999997
0.999998	0.999044	0.999939	0.999992	0.801273	0.000026	0.508726	0.999997	

Emission rate of the five compounds by 3 taxa based on cell number normalization, p<0.01, Tuk

Cell No.	Taxa	Time effect	1	2	3	4	5	6
1	Syne	CHBr3_Cell		0.999998	0.999998	1.000000	1.000000	1.000000
2	Syne	CH3I_Cell	0.999998		1.000000	0.999778	0.999966	0.999989
3	Syne	CHCl3_Cell	0.999998	1.000000		0.999803	0.999971	0.999990
4	Syne	CHBr2Cl_Cell	1.000000	0.999778	0.999803		1.000000	1.000000
5	Syne	CH2Br2_Cell	1.000000	0.999966	0.999971	1.000000		1.000000
6	Chlorella	CHBr3_Cell	1.000000	0.999989	0.999990	1.000000	1.000000	
7	Chlorella	CH3I_Cell	1.000000	0.999996	0.999996	1.000000	1.000000	1.000000
8	Chlorella	CHCl3_Cell	0.992000	0.999982	0.999980	0.960817	0.979905	0.896970
9	Chlorella	CHBr2Cl_Cell	1.000000	0.999969	0.999973	1.000000	1.000000	1.000000
10	Chlorella	CH2Br2_Cell	1.000000	0.999992	0.999993	1.000000	1.000000	1.000000
11	Amphora	CHBr3_Cell	0.982580	0.999906	0.999895	0.931765	0.961533	0.949313
12	Amphora	CH3I_Cell	0.000026	0.000026	0.000026	0.000026	0.000026	0.000026
13	Amphora	CHCl3_Cell	0.330464	0.734685	0.728966	0.189468	0.248505	0.219922
14	Amphora	CHBr2Cl_Cell	1.000000	0.999999	0.999999	1.000000	1.000000	1.000000
15	Amphora	CH2Br2_Cell	1.000000	1.000000	1.000000	0.999979	0.999997	0.999993

ɛey HSD test, Approximate probabilities for Post Hoc tests, Repeated-Measure ANOVA. n = 3

7	8	9	10	11	12	13	14	15
1.000000	0.992000	1.000000	1.000000	0.982580	0.000026	0.330464	1.000000	1.000000
0.999996	0.999982	0.999969	0.999992	0.999906	0.000026	0.734685	0.999999	1.000000
0.999996	0.999980	0.999973	0.999993	0.999895	0.000026	0.728966	0.999999	1.000000
1.000000	0.960817	1.000000	1.000000	0.931765	0.000026	0.189468	1.000000	0.999979
1.000000	0.979905	1.000000	1.000000	0.961533	0.000026	0.248505	1.000000	0.999997
1.000000	0.896970	1.000000	1.000000	0.949313	0.000026	0.219922	1.000000	0.999993
	0.919834	1.000000	1.000000	0.961041	0.000026	0.247185	1.000000	0.999997
0.919834		0.862937	0.906224	1.000000	0.000026	0.992794	0.987653	0.999974
1.000000	0.862937		1.000000	0.931259	0.000026	0.188718	1.000000	0.999979
1.000000	0.906224	1.000000		0.954099	0.000026	0.230175	1.000000	0.999995
0.961041	1.000000	0.931259	0.954099		0.000026	0.985126	0.904301	0.999123
0.000026	0.000026	0.000026	0.000026	0.000026		0.000026	0.000026	0.000026
0.247185	0.992794	0.188718	0.230175	0.985126	0.000026		0.086043	0.433654
1.000000	0.987653	1.000000	1.000000	0.904301	0.000026	0.086043		0.999995
0.999997	0.999974	0.999979	0.999995	0.999123	0.000026	0.433654	0.999995	

Comparison of emission rate rate amongst the five compounds based on chlorophyll a normalization, p<0.05, based on Tukey HSD test, Approximate Probabilities for Post Hoc tests, ONE-WAY ANOVA. n = 21						
Cell No.	Compound	1	2	3	4	5
1	CHBr3		0.000042	0.567810	0.702598	0.909870
2	CH3I	0.000042		0.011796	0.000017	0.000017
3	CHCl3	0.567810	0.011796		0.044062	0.127529
4	CHBr2Cl	0.702598	0.000017	0.044062		0.993673
5	CH2Br2	0.909870	0.000017	0.127529	0.993673	

Comparison of emission rate rate amongst the five compounds based on cell number normalization, p<0.05, based on Tukey HSD test, Approximate Probabilities for Post Hoc tests, ONE-WAY ANOVA. n = 21						
Cell No.	Compound	1	2	3	4	5
1	CHBr3		0.000074	0.504514	0.902098	0.987369
2	CH3I	0.000074		0.027560	0.000018	0.000022
3	CHCl3	0.504514	0.027560		0.096284	0.224701
4	CHBr2Cl	0.902098	0.000018	0.096284		0.995305
5	CH2Br2	0.987369	0.000022	0.224701	0.995305	