EMISSION OF VOLATILE HALOCARBONS FROM SELECTED SPECIES OF TROPICAL SEAWEEDS

FIONA KENG SEH LIN

INSTITUTE FOR ADVANCED STUDIES
UNIVERSITY OF MALAYA
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EMISSION OF VOLATILE HALOCARBONS FROM SELECTED SPECIES OF TROPICAL SEAWEEDS

ABSTRACT

Marine emissions of short-lived biogenic halocarbons affect atmospheric composition and chemistry. Research has shown that seaweeds emit these halocarbons into the seawater and air. The presence of these gasses could affect local weather while contributing to the global halocarbon load, possibly causing stratospheric ozone loss. This is especially evident in the tropics due to the existence of vertical transport that could affect the global circulation of these compounds in the atmosphere. There is a lack of information on the contribution of tropical seaweeds to the global halocarbon budget. This is of concern especially under a changing climate. This study was formulated to investigate the response of tropical seaweeds to variable environmental conditions, in terms of halocarbon emissions. In addition, seaweeds cultivated using three different systems were studied. Laboratory studies were conducted for investigating the effect of temperature (20–40 °C) on the halocarbon emission by *Gracilaria manilaensis, Ulva reticulata, Kappaphycus alvarezii* and *Turbinaria conoides* and the combined effect of temperature (25–34 °C) and irradiance (0–177 μmol photons m^{-2} s^{-1}) on the halocarbon emission by *K. alvarezii*. Higher temperature results in decreased emissions of $CHBr_3$, CH_2Br_2 and CH_2I_2 (r = (−0.69)–(−0.95); p< 0.01) in most seaweeds. The combined effect of irradiance and temperature significantly affect the emission of halocarbons by *K. alvarezii*, with stronger association to temperature. Of the three cultivation systems, the amount of halocarbon released by *G. manilaensis* especially CH_2Br_2 was found to be the highest in the cage culture in the river mouth, followed by the offshore platform and tank culture. Daylight emissions by *G. manilaensis* from both onshore and offshore cultivation systems was 10 to 52 times greater than in the dark, while emissions by *K. alvarezii* were between two and five times greater during the day. Halocarbon emissions by *G.*

manilaensis was positively affected by temperature, irradiance and seawater phosphate level during cultivation. Negative correlations were found between ammoniacal nitrogen, nitrate and nitrite levels with the CH_2Br_2 emitted by *K. alvarezii*. Estimation from this study showed that the harvesting activity of *K. alvarezii* in year 2017 in Malaysia could release 72–360 mol Br hr^{-1}; a value that is slightly higher than the estimated value derived from previously predicted flux rate estimated from wild Malaysian seaweeds. Under laboratory-controlled conditions, *K. alvarezii* releases higher amount of halocarbon. However, *in situ* studies revealed that *G. manilaensis* emits higher amount of halocarbon due to the fluctuations in the environment. In light of the impending climate change with a likely rise in temperature, seaweed farming has the potential to be expanded to the subtropical waters which could bring about increased emissions of halocarbons. In summary, halocarbons including the highly emitted $CHBr_3$ and CH_2Br_2 was found to be emitted by the selected wild and farmed tropical seaweeds. Although the emissions were species-dependent, the emission of these compounds were found to be closely associated to changes in environmental parameters such as temperature, irradiance, and seawater nutrients.

Keywords: Halocarbons; Bromoform; Air-Sea Gas Exchange; Environmental Change; Seaweed

PEMBEBASAN HALOKARBON MUDAH MERUAP DARIPADA RUMPAI LAUT TROPIKA YANG TERPILIH

ABSTRAK

Pembebasan halokarbon biogenik berjangka hayat pendek mempengaruhi komposisi dan kimia atmosfera. Kajian telah membuktikan kemampuan rumpai laut untuk membebaskan halokarbon ini ke dalam air laut dan udara. Kehadiran gas ini boleh mempengaruhi cuaca tempatan sambil menyumbang kepada beban halokarbon global, dan berkemungkinan untuk menyebabkan kehilangan ozon stratosfera. Kejadian ini amat ketara di kawasan tropika disebabkan oleh kewujudan pergerakan menegak yang mungkin mempengaruhi peredaran global sebatian tersebut di atmosfera. Maklumat mengenai sumbangan rumpai laut tropika terhadap anggaran halokarbon global amat terhad. Ini amat membimbangkan memandangkan iklim kian berubah. Kajian ini dilakukan untuk mengkaji tindak balas rumpai laut tropika terhadap perubahan persekitaran, dari segi pembebasan halokarbon. Rumpai laut yang ditanam menggunakan tiga sistem yang berbeza juga telah dikaji. Kajian makmal dilakukan untuk menyiasat pengaruh suhu (20–40 °C) terhadap pembebasan halokarbon oleh *Gracilaria manilaensis*, *Ulva reticulata*, *Kappaphycus alvarezii* dan *Turbinaria conoides* dan kesan gabungan suhu (25–34 °C) dan sinaran (0–177 μmol foton m^{-2} s^{-1}) ke atas pembebasan halokarbon oleh *K. alvarezii*. Suhu yang lebih tinggi menyebabkan kekurangan pembebasan $CHBr_3$, CH_2Br_2 dan CH_2I_2 (r = (−0.69)–(−0.95); p<0.01) oleh kebanyakan rumpai laut. Kesan gabungan cahaya dan suhu mempengaruhi pembebasan halokarbon oleh *K. alvarezii* secara ketara, dan mempunyai perkaitan yang lebih kukuh dengan suhu. Dari tiga sistem penternakan, jumlah halokarbon yang paling tinggi dibebaskan oleh *G. manilaensis* terutamanya CH_2Br_2 adalah dalam sistem sangkar di muara sungai, diikuti oleh pelantar luar pesisir dan sistem kultur dalam tangki. Kajian *in situ* menunjukkan pembebasan halokarbon oleh *G. manilaensis* yang dikultur di dalam tangki dan luar

pesisir mencapai 10–52 kali lebih tinggi pada waktu siang berbanding dengan waktu malam, sementara pembebasan halokarbon oleh *K. alvarezii* didapati 2–5 kali lebih tinggi pada siang hari. Pembebasan halokarbon oleh *G. manilaensis* dipengaruhi secara positif oleh suhu, penyinaran dan tahap fosfat air laut semasa penanaman. Hubungan negatif didapati antara tahap nitrogen, nitrat dan nitrit amonia dengan pembebasan CH_2Br_2 oleh *K. alvarezii*. Anggaran dari kajian ini menunjukkan bahawa aktiviti penuaian *K. alvarezii* pada tahun 2017 di Malaysia dapat melepaskan 72–360 mol Br hr^{-1}; nilai yang lebih tinggi sedikit daripada nilai anggaran dari kadar fluks yang diramalkan sebelumnya, yang dianggarkan berdasarkan pembebasan oleh rumpai laut liar Malaysia. Di bawah keadaan terkawal makmal, *K. alvarezii* membebaskan jumlah halokarbon yang lebih tinggi. Walau bagaimanapun, kajian *in situ* menunjukkan bahawa *G. manilaensis* mengeluarkan jumlah halokarbon yang lebih tinggi disebabkan oleh perubahan faktor persekitaran. Berdasarkan perubahan iklim dan kenaikan suhu yang mungkin berlaku, penternakan rumpai laut berpotensi untuk diperluaskan ke perairan subtropika yang boleh meningkatkan pembebasan halokarbon. Secara rumusan, halokarbon seperti $CHBr_3$ dan CH_2Br_2 telah dibebaskan oleh rumpai laut liar dan ternakan tropika yang dipilih. Walaupun pembebasan adalah bergantung kepada species, namun pembebasan sebatian-sebatian ini didapati berkait rapat dengan perubahan dalam parameter alam sekitar seperti suhu, sinaran, dan nutrien air laut.

Kata kunci: Halokarbon; Bromoform; Penukaran Gas Udara-Laut; Perubahan Persekitaran; Rumpai Laut

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I thank God for His abundant blessings, for the good health of my family and for watching over me this far. Thank you, my beloved parents, for staying strong and holding on. Love you All.

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$CHBr_3$	:	Bromoform
CH_2Br_2	:	Dibromomethane
$CHBr_2Cl$	:	Dibromochloromethane
$CHBrCl_2$	:	Dichlorobromomethane
CH_3I	:	Iodomethane
CH_2I_2	:	Diiodomethane
GCMS	:	Gas Chromatography – Mass Spectrometry
P&T	:	Purge-and-Trap
ppbv	:	Parts-per-billion by volume
FW	:	Fresh Weight
DW	:	Dry Weight
Hr	:	hour
F_v/F_m	:	Maximal quantum yield
L:D	:	Day(light) to dark
LB	:	Lower bound
UB	:	Upper bound

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

The discovery of the ozone hole over the Antarctic in 1985 brought attention to the ozone-depleting potential of halocarbons in the stratosphere. For many years it was thought that the long-lived anthropogenic chlorofluorocarbons (CFCs) were responsible for depleting the ozone layer. However, the recent discovery of the presence of reactive bromine species in the upper troposphere/lower stratosphere spurred interest in the short-lived biogenic halocarbons. These compounds could potentially contribute to the stratospheric halogen budget, leading to more catalytic destruction in the stratosphere.

Efforts to quantify the biogenic sources of these compounds (particularly $CHBr_3$), especially those of marine origin, e.g. seaweeds, phytoplankton and seagrass meadows, are often complicated by inherent biological variability as well as spatial and temporal changes in emissions. The contribution of the coastal regions and the oceans to the stratospheric load of halocarbons has been widely debated. This highlights the need to understand the factors affecting the release of these compounds from marine sources, for which data for modelling purposes are generally lacking.

As the seaweeds are also subjected to changing environmental conditions, investigating the effects of these on the emission of very short-lived halocarbons (VSLH) by the seaweeds could help towards better estimations of halocarbon emissions. This is especially important in light of global changes in both climate and the environment, the expansion of the seaweed cultivation industry, and the interactions between halocarbon emissions and their environment.

This study provides information on the emission of volatile short-lived halocarbons by selected seaweeds from the tropical region, where seaweed cultivation is practised widely.

This is one of the earliest studies to investigate the effect of environmental parameters on the emission of halocarbons by tropical seaweeds, both from the natural habitat as well as farmed *Kappaphycus* and *Gracilaria* species. In addition, *in situ* measurements of halocarbons released during the onshore and offshore cultivation of these economically important seaweeds in the tropics, will also contribute to better management of mass cultivation activities.

1.1 Research questions

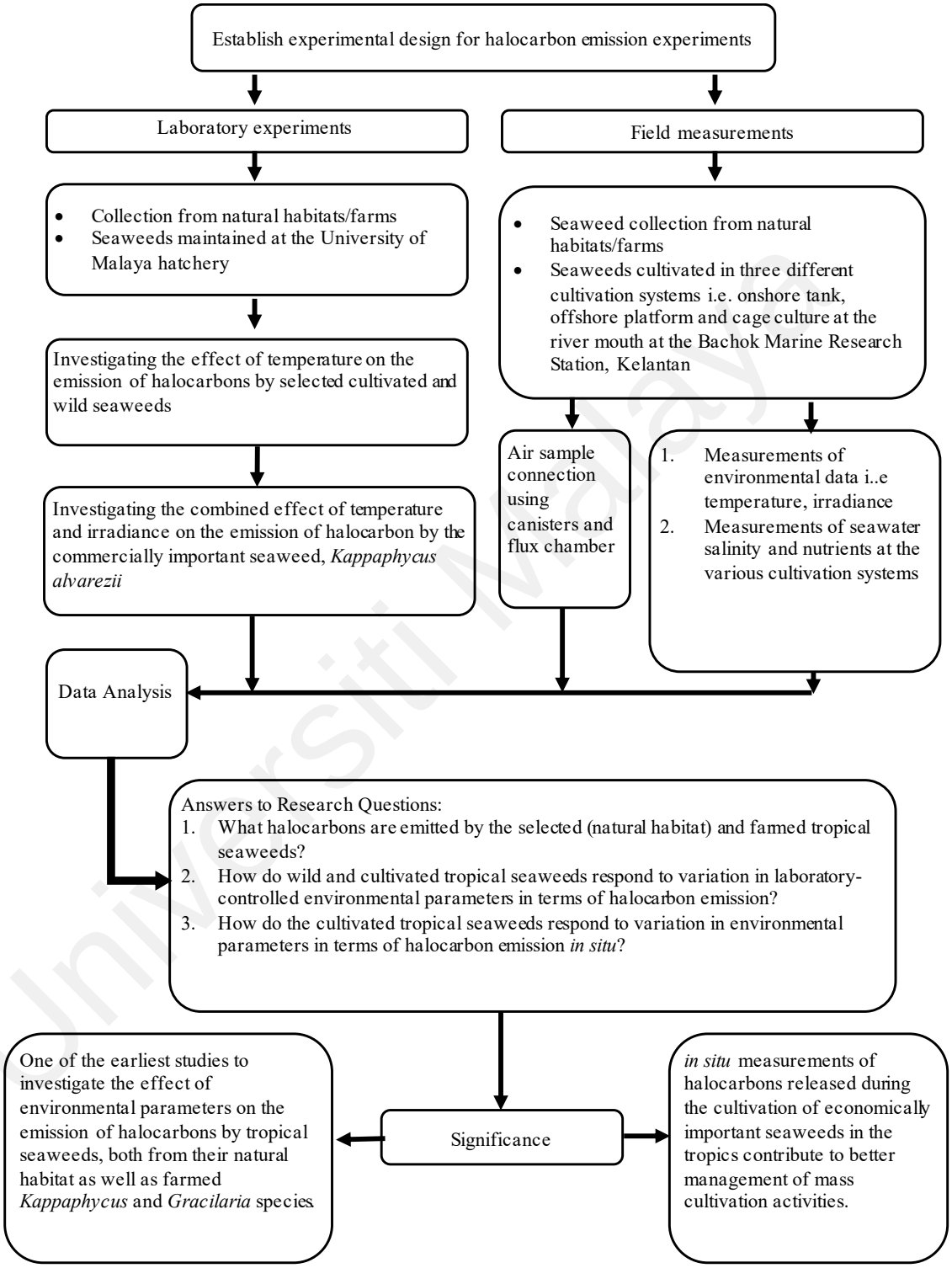
i. What halocarbons are emitted by the selected wild (natural habitat) and farmed tropical seaweeds?
ii. How do the wild and cultivated tropical seaweeds respond to variation in laboratory-controlled environmental parameters in terms of halocarbon emission?
iii. How do the cultivated tropical seaweeds respond to variation in environmental parameters in terms of halocarbon emission *in situ*?

1.2 Objectives

i. To investigate the effect of varying temperature levels on the emissions of halocarbons by cultivated and wild seaweeds, *Gracilaria manilaensis* Yamamoto & Trono*, Ulva reticulata* Forsskål*, Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva and *Turbinaria conoides* (J. Agardh) through laboratory-based studies.
ii. To further determine the combined effect of temperature and irradiance on the emissions of halocarbons by the commercially important seaweed, *Kappaphycus alvarezii.*

iii. To characterise the halocarbons released by *Gracilaria manilaensis* and *Kappaphycus alvarezii in situ*, during cultivation and relate this to the environmental measurements.

1.3 Research Approach



CHAPTER 2: LITERATURE REVIEW

2.1 Introduction to halocarbon

Halocarbons are carbon compounds containing one or more halogen atoms. While long-lived halocarbons (lifetime more than six months) are commonly associated with anthropogenic sources, very short-lived halocarbons (VSLH; lifetime less than six months) e.g. bromoform ($CHBr_3$) and dibromomethane (CH_2Br_2), are mostly of biogenic origin. VSLH have been linked to climate change through their potential to deplete the protective stratospheric ozone layer, influence atmospheric chemistry, and contribute to local weather change and radiative forcing via cloud nuclei formation. Reactive bromine constituted around 5 (3-7) ppt, or 25%, of the total stratosphere bromine recorded in 2016 (WMO, 2018). This originates from short-lived bromocarbons i.e. $CHBr_3$ and CH_2Br_2 (Liang *et al.*, 2014). Bromine and iodine are more efficient than chlorine in depleting the ozone layer (Chipperfield & Pyle, 1998; Daniel *et al.*, 1999), therefore making it important to account for the sources of emission.

The ocean contributes a large amount of these very short-lived brominated and iodinated halocarbons especially in the tropical and subtropical regions (Ziska *et al.*, 2013; WMO, 2018). The most prominent compounds are $CHBr_3$, CH_2Br_2 and methyl iodide (CH_3I). Global fluxes of $CHBr_3$ and CH_2Br_2 range between 120 – 820 and 57 – 100 Gg Br yr^{-1} (WMO, 2018). Besides the shorter lifetime of the compounds, the wide range in values likely reflects the high variability in seaweed emissions compared to open ocean emissions (Butler *et al.*, 2007; Leedham *et al.*, 2013; Liang *et al.*, 2010; WMO, 2018). Variabilities in the current global estimates of oceanic halogen load, derived from top-down and bottom-up modelling, are commonly attributed to a lack of data for oceanic inputs and under-representation of coastal emissions (Ziska *et al.*, 2013; Fuhlbrügge *et*

al., 2016; Ziska *et al.*, 2017). Uncertainties in estimation and prediction due to changes in environmental factors altering the source emission of halocarbons have been reported (Ziska *et al.,* 2013; Hepach *et al.*, 2014; Ziska *et al.,* 2017). Quack and Wallace (2013) discussed the role of $CHBr_3$ in atmospheric chemistry, its distribution (based on a detailed compilation of mixing ratios and seawater $CHBr_3$ measurements) and reflected on the possible reasons behind the high variability in $CHBr_3$ emission estimations. The production of halocarbons, their role, impact on the environment and halocarbon destruction mechanisms have also been reviewed previously (Paul & Pohnert, 2011; Carpenter & Archer 2012; Wever & van der Horst, 2013). Nonetheless, in the light of impending climate and environmental change, and the interactions between halocarbon emissions by seaweeds and climate change, it would be timely to investigate how environmental factors affect the release of halocarbons by seaweeds.

Seaweeds are known to be emitters of the short-lived brominated compounds including $CHBr_3$ and CH_2Br_2 and could contribute to significantly higher concentrations (up to three-fold) of $CHBr_3$, $CHBr_2Cl$, CH_2Br_2, $CHIBr_2$, CH_2IBr and CH_2I_2 in coastal areas compared to areas further offshore (Keng *et al.*, 2013; Leedham *et al.*, 2013; Leedham Elvidge *et al.*, 2015). Extrapolation of data from small-scale studies on tropical seaweeds in South-East Asia indicates a contribution of 6–224 Mmol Br yr^{-1} (Leedham *et al.*, 2013). However, such estimations involve multiple assumptions and errors might arise from uncertainties concerning seaweed distribution, as well as community and ecosystem level interactions (Fordham, 2015). The global seaweed industry is expanding rapidly and production doubled to 30.4 million tonnes from 2005 to 2015 (FAO, 2018). About 29.4 million tonnes is cultivated and this is dominated by the red seaweeds. The top cultivated seaweeds in the world in terms of biomass include the red carrageenophytes *Eucheuma denticulatum* and *Kappaphycus alvarezii* in tropical and subtropical waters, with a combined yield of 12 million tonnes in 2015 (FAO 2018). Thus, it is important to

be able to estimate and predict the increased contribution of halocarbons from the seaweed industry, and especially in the Asian region (Phang *et al.*, 2015).

Involvement of algal methyl transferase and haloperoxidases has been reported in relation to the production of methyl halide and polyhalogenated compounds respectively (Almeida *et al.,* 2001; Manley, 2002; Toda & Itoh, 2011; Yokouchi *et al.*, 2014). The activities of methyl transferases yield monohalogenated compounds including CH_3Br, CH_3I, di- and polyhalogenated compounds such as $CHBr_2I$ (Milkova *et al.*, 1997; Neilson, 2003; Amachi *et al.*, 2006). Punitha *et al.* (2017) gives a detailed explanation of the reactions. Trihalogenated compounds e.g. $CHBr_3$ have been widely postulated to be the result of the oxidisation of halides by haloperoxidases (e.g. bromoperoxidase) using H_2O_2 to yield hypohalide, which then either reacts with the ketones in the seaweeds or dissolved organic matters (DOM) in the environment to produce the haloform (Manley, 2002; Wever & van der Horst., 2013). Overall the mechanisms involved in the production of halocarbons by seaweeds remains unclear. The various mechanisms proposed were discussed in detail by Manley (2002).

The availability and composition of dissolved organic matter affects halocarbon emissions (Lin & Manley, 2012; Liu *et al.*, 2015). Production of hypobromous acid, HOBr from hydrogen peroxide (H_2O_2) and Br^- and H^+ by bromoperoxidase reacts with DOM to form DOM-Br. $CHBr_3$ is then formed (Wever *et al.*, 1991; Opsahl & Benner, 1997; Lin & Manley, 2012; Wever & ven der Horst, 2013; Liu *et al.*, 2015). Lin and Manley (2012) found higher halocarbon production from near-shore than offshore waters and during spring phytoplankton blooms. They attributed this to the involvement of DOM in the production of halocarbons, and the quantitative change between CH_2Br_2 and $CHBr_3$ emissions to changes in DOM composition. Specific cell metabolites including mannose, glycolic acid, alginic acid, citric acid, humic acid, and urea all increase the production of

brominated halocarbons (see Liu *et al.,* 2015 for a complete list of the effect of different DOM compounds on the production of brominated volatile compounds).

2.1.1 Main halocarbon compounds released by seaweeds with focus on $CHBr_3$

Research on biogenic short-lived brominated compounds (e.g. $CHBr_3$, CH_2Br_2) resurfaced (Ziska *et al.*, 2013; Liang *et al.*, 2014; Hossaini *et al.*, 2016) in recent years due to knowledge of the increasingly significant contribution of very short-lived brominated substances to the tropospheric and stratospheric bromine load, which can potentially alter ozone abundance and radiative impact (Stemmler *et al.*, 2015; WMO, 2018). However, the short atmospheric lifetime of some of these compounds means that they can only reach the lower stratosphere through rapid convective uplifting, particularly from the tropics (Dessens *et al.*, 2009). It was reported that short-lived halocarbons contributed around 5 ppt of the total 20 ppt of bromine in the stratosphere (WMO, 2018). This is highly significant as bromine is 40 – 60 times more efficient in depleting the ozone layer than chlorine (Daniel *et al.*, 1999). However, estimation and prediction of current and future emissions of VSLH are hindered by the uncertainties surrounding contributing sources especially those of coastal origin, and including those from anthropogenic activities (Leedham *et al.*, 2013; Ziska *et al.*, 2017).

Seaweeds release a suite of short-lived halocarbons such as $CHBr_3$, CH_2Br_2, CH_2BrI, CH_2BrCl, $CHBr_2Cl$, and $CHBrCl_2$, most probably through bromoperoxidase activity in the seaweeds, which can catalyse production of brominated and iodinated compounds. Among these compounds, seaweeds release significantly more $CHBr_3$ than other halocarbons (Wever & van der Horst, 2013). This holds true for seaweeds from temperate, polar and tropical regions. Supporting evidence include a temperate rock pool study where emission of $CHBr_3$ was highest among a suite of 13 iodinated and brominated

halocarbons emitted by a population dominated by Laminariales and Fucales (Carpenter *et al.*, 2000). A similar trend was observed for polar seaweeds. A comprehensive study by Laturnus (2001) showed that 29 out of a total of 30 polar seaweeds emitted $CHBr_3$ as the major compound, with emission rates ranging from 0.7 – 645 pmol g FW^{-1} hr^{-1}, while tropical seaweeds have also shown high $CHBr_3$ emission rates (Keng *et al.*, 2013; Leedham *et al.*, 2013; Mithoo-Singh *et al.*, 2017). CH_2Br_2, CH_2BrI and CH_2I_2 as the dominant halocarbon released were also reported from tropical seaweeds (Table 2.1; Leedham *et al.*, 2013). Available data at the time of writing are compiled in Table 2.1.

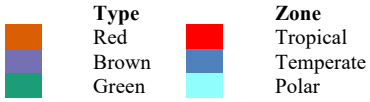
From Table 2.1 it can be seen that red (0.71 – 4960 pmol gFW^{-1} hr^{-1}) and brown seaweeds (0.1 – 1100 pmol gFW^{-1} hr^{-1}) are strong emitters of $CHBr_3$, with lower emission rates reported for green seaweeds (0.4 – 344 pmol gFW^{-1} hr^{-1}). For uncertain reasons, polar seaweeds generally showed lower emission rates than tropical and temperate seaweeds, except for a few brown species. The highest emission seen for a polar brown alga was for *Desmarestia anceps* (645 pmol gFW^{-1} hr^{-1}, Laturnus, 2001). Amongst the recorded species, the $CHBr_3$ emission rate was exceptionally high for the temperate red seaweed, *Asparagopsis armata* at 4960 pmol gFW^{-1} hr^{-1} (Carpenter *et al.*, 2000).

Table 2.1: Compilation of halocarbon emission rates reported in the literature for a range of tropical, temperate and polar seaweeds, arranged in descending rate of emission; in each case the emission rate (pmol g FW^{-1} hr^{-1} and/or pmol g DW^{-1} hr^{-1}) is provided for the dominant halocarbon emitted by the species (Published in Keng *et al.*, 2020)

Seaweeds	Type	Zone	Dominant halocarbon emitted				Rate (pmol g FW^{-1} hr^{-1} and/or pmol g DW^{-1} hr^{-1})		Reference
			$CHBr_3$	CH_2Br_2	CH_2BrI	CH_2I_2	Fresh Weight	Dry Weight	
Asparagopsis armata			✓				4960	45200	Carpenter *et al.*, 2000
Gracilaria changii			✓				3285 (1129 – 4461)		Leedham *et al.*, 2013
Gracilaria salicornia			✓				1463 (478 – 3205)		Leedham *et al.*, 2013
Kappaphycus alvarezii			✓				1122 (512 – 1731)		Leedham *et al.*, 2013
			✓				[5] 480 – 930	4800 - 9300	Mithoo-Singh *et al.*, 2017
Turbinaria conoides			✓				[5] 48.2 – 1100	279 – 6500	Keng *et al.*, 2013
			✓				[5] 272 – 918	1600 – 5400	Mithoo-Singh *et al.*, 2017
					✓		526 (491 – 562)		Leedham *et al.*, 2013
[1]Various polar species			✓				1.46 – 645		Laturnus *et al.*, 2001
Sargassum binderi			✓				0.7 – 458	4.7 – 2900	Keng *et al.*, 2013
				✓			93 (45 – 141)		Leedham *et al.*, 2013
			✓				[5] 25.6 – 104	160 – 650	Mithoo-Singh *et al.*, 2017
Enteromorpha intestinalis			✓				344	2690	Carpenter *et al.*, 2000
Gelidium elegans			✓				166 (38 – 295)		Leedham *et al.*, 2013
Laminaria digitata			✓				107 – 196	705 – 1290	Carpenter *et al.*, 2000
Macrocystis pyrifera			✓				4 – 186		Goodwin *et al.*, 1997
Ulva reticulata			✓				90 (24 – 157)		Leedham *et al.*, 2013
Laminaria saccharina			✓				125	1054	Carpenter *et al.*, 2000
Pelvetia canaliculata			✓				101	404	Carpenter *et al.*, 2000
Bryopsis sp.			✓				69		Leedham *et al.*, 2013
[2]Various polar species			✓				0.71 – 52		Laturnus *et al.*, 2001
Sargassum siliquosum			✓				36		Leedham *et al.*, 2013
			✓					1600 – 4900	Mithoo-Singh *et al.*, 2017
Padina australis			✓				5.4 – 32.4	30 – 180	Mithoo-Singh *et al.*, 2017
			✓				8		Leedham *et al.*, 2013
			✓				0.1 – 12.1	0.4 – 68.7	Keng *et al.*, 2013
[3]Various temperate species			✓					[6] 1.6 – 167	Bravo-Linares *et al.*, 2010
Fucus vesiculosus			✓				19.4	90.1	Carpenter *et al.*, 2000
Cladophora sp.				✓			9 (4 – 14)		Leedham *et al.*, 2013
[4]Various polar species			✓				1.25 – 12.88		Laturnus *et al.*, 2001
Sargassum baccularia			✓				11		Leedham *et al.*, 2013
Ascophyllum nodosum			✓				9.36	28.6	Carpenter *et al.*, 2000
Fucus serratus			✓				8.2	32.8	Carpenter *et al.*, 2000

Table 2.1 continued

Halidrys siliquosa		✓	2.61	11.6	Carpenter *et al.*, 2000
Chondrus crispus		✓	5.3	16.4	Carpenter *et al.*, 2000
Caulerpa sp.		✓	3 (0.4 – 5)		Leedham *et al.*, 2013
Caulerpa racemosa		✓	3 (3 – 4)		Leedham *et al.*, 2013



[1]*Desmarestia antarctica, Desmarestia anceps, Desmarestia menziesii, Himantothallus grandifolius, Cystosphaera jaquinotii, Fucus distichus, Dictyosiphon foeniculaceus, Laminaria saccharina, Laminaria solidungula, Chordaria flagelliformis, Alaria esculenta*; [2]*Kallymenia antarctica, Plocamium coccineum, Gymnogongrus antarcticus, Gigartina skottsbergii, Iridaea cordata, Palmaria decipiens, Myriogramme mangini, Curdiea racovitzae, Devalarea ramentacea, Plocamium cartilagineum, Pantoneura plocamioides*; [3]Brown seaweeds *Ascophyllum nodosum, Fucus vesiculosus, Fucus serratus, Laminaria digitata*, green seaweeds *Ulva lactuca, Ulva intestinalis,* and red seaweeds *Palmaria palmata, Griffithsia flosculosa*; [4]*Enteromorpha bulbosa, Enteromorpha compressa, Monostroma arcticum, Blidingia minima, Urospora penicilliformis, Acrosiphonia sonderi, Ballia callitricha, Lambia antarctica;* [5]Converted based on assumption of moisture content of ~ 90% on *K. alvarezii*, ~83% for *T. conoides,* ~84% for *S. binderi*, and ~82% for *P. australis* (our unpublished data); [6]Values represent range for all seaweed species from Bravo-Linares *et al.*, 2013, as individual readings for each species is not reported.

2.2 Factors affecting halocarbon emission by seaweeds

Seaweeds constantly interact with their environment and the other organisms in their seawater habitat. The combined effect of these interactions results in variations in physiology, growth, morphology and survival of the species (Harley *et al.*, 2012). Photosynthesis and respiratory electron transport produce reactive oxygen species (ROS) such as the superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2) (Rutherford *et al.*, 2012; Wever & van der Horst, 2013). Under normal conditions, cellular ROS scavenging mechanisms involving enzymes such as superoxide dismutase are able to catalyze the conversion of superoxide into O_2 or H_2O_2, which could later form OH^- (Birben *et al.*, 2012; Yakovleva *et al.*, 2017; Younus, 2018). Oxidative stress arises when the capacity of such a mechanism is exceeded, resulting in the buildup of ROS in the seaweeds. Common factors triggering oxidative stress in seaweeds include grazing, microbial attack, exposure to varying environmental parameters like high light, desiccation, variations in temperature, salinity changes, carbon dioxide and nutrient limitation (Figure 2.1). A checklist on the effect of each environmental parameter on the emission of halocarbon by seaweeds from various studies had been compiled for referencing purposes (Table 2.2).

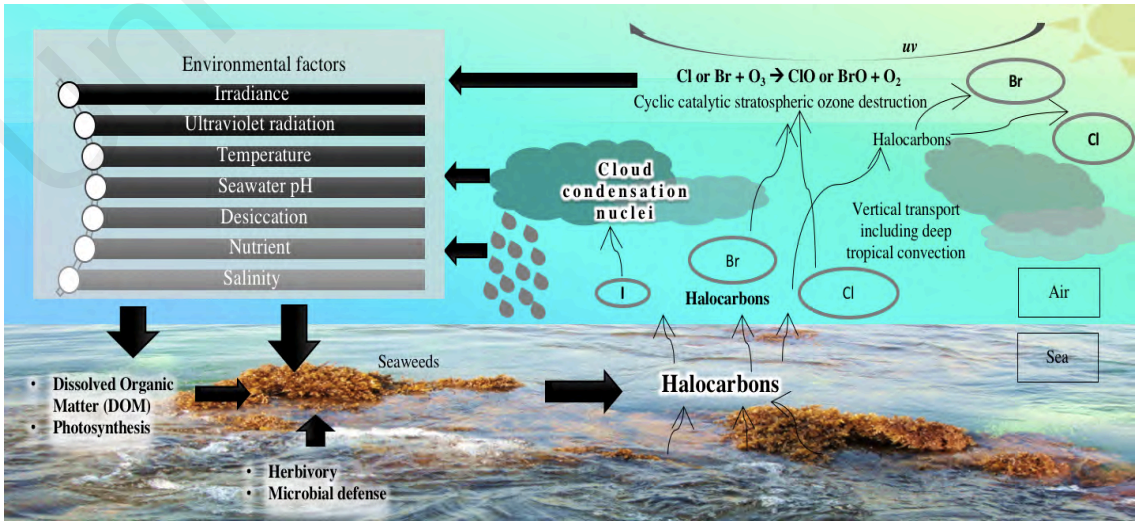


Figure 2.1: The interaction between environmental factors and the emission of halocarbon by seaweeds

2.2.1 Herbivory

Most seaweeds survive by attaching themselves to a substrate with their holdfast in order to prevent the tidal and wave action from sweeping them away. They are constantly exposed to grazing pressures from fish, sea urchins, crustaceans and molluscs and unable to evade predators and parasites, but seaweeds are thought to have developed a range of defense mechanisms to prevent grazer and pathogen attacks. They can reduce or even avoid predation through metabolic reconfiguration involving coordination in the expression of numerous defence-related genes (reviewed by Kessler & Baldwin, 2002), some of which lead to emissions of halogenated compounds that are effective against herbivores and microorganisms (Ohsawa *et al.*, 2001; Paul *et al.*, 2006; Paul & Pohnert, 2010).

Some studies investigating the effect of tissue wounding on seaweeds reported increases in the emission of halocarbon compounds (Table 2.2). When wounded by grazing-snails over 24 hours, the release rate of halocarbon e.g. $CHCl_3$ by *Ascophyllum nodosum* was ten times higher (10.5 – 10.8 pmol g DW^{-1} hr^{-1}) than the control (1.26 pmol g DW^{-1} hr^{-1}). Although no statistical significance was indicated, the release of other compounds i.e. $CHBr_3$, CH_2Br_2, $CHBrCl_2$ were also enhanced through grazing (Nightingale *et al.*, 1995, Table 2.2, Study 1). Meanwhile, Sundström *et al.* (1996) triggered tissue wounding by cutting the subtropical species, *Eucheuma denticulatum* and this also increased the emission of $CHBr_3$ (Table 2.2, Study 2). A meta-analysis of data from more prolonged grazing activity (11 – 20 days) by small crustaceans and gastropods showed induction of significant resistance in both brown and green seaweeds (Toth & Pavia, 2007). It would be interesting to know if similar halocarbon release patterns would also be observed for green and brown seaweeds after longer periods of wounding by small

gastropods, as it has been reported that the resistance response of seaweeds could increase with duration of grazing to prevent further herbivory (Toth & Pavia, 2007).

2.2.2 Microbial defense

Seaweeds are able to detect pathogen invasion through cell-level recognition of signal molecules from the invading organism or their own cell wall. Such compounds, also known as elicitors, include oligosaccharides, peptidoglycans, and lipoteichoic acid (Amsler, 2008; de Oliveira *et al.*, 2017). The brown seaweeds belonging to the families Laminariales, Desmarestiales, Ectocarpales, and Fucales, are able to rapidly detect the signals for defense elicited by simple addition of alginate oligosaccharides (Küpper *et al.,* 2002; Amsler, 2008; Chance *et al.*, 2009).

The response of seaweeds towards microbial attacks was studied in the temperate brown temperate seaweed, *Laminaria digitata*, a very well-known iodine bioaccumulator. This seaweed stores iodine in the form of inorganic iodide in the apoplast and young sporophytes are capable of accumulating up to 30,000 times more iodine than the surrounding seawater (Küpper *et al.,* 1998; Verhaeghe *et al.*, 2008). The iodide acts an inorganic antioxidant, capable of quenching aqueous oxidants through efflux into the surrounding water. Iodocarbons e.g. CH_2I_2 and CHI_3 are produced after an initial burst of reactive oxidant scavenging (Küpper *et al.,* 2008). This arises due to the fact that iodide incorporated into an organic substrate would quickly be substituted with Cl^-, Br^- or HO^- due to lower C-I bond dissociation energy (234 kJ mol^{-1}) compared to C-Br (293 kJ mol^{-1}) and C-Cl bonds (351 kJ mol^{-1}) (McMurry, 2008). Therefore, instead of an antioxidative function, the formation of iodocarbons by *L. digitata* was suggested as a defense function against microbial growth due to the harmful nature of the released iodide following nucleophilic substitutions.

The response of halocarbon emissions upon exposure to the elicitors has been investigated. Palmer *et al.* (2005) reported increased halocarbons and I_2 emissions by *L. digitata*. When subjected to oligoguluronates, the emission of iodinated compounds i.e. CH_2I_2 and CH_2ClI seemed stronger than when subjected to H_2O_2. However, when the seaweed was exposed to H_2O_2, the emissions of brominated compounds $CHBr_3$ and $CHBr_2Cl$ seemed stronger than the emission of iodinated compounds. Similar trends were also reported by Collén *et al.*, (1994) in the red seaweed *Meristiella gelidium* (Pédersen *et al.*, 1996; See Palmer *et al.*, 2005 for comparison between values). Increased amounts of halogenated compounds were also produced through the oxidative burst response to agar oligosaccharides in *Gracilaria* (Weinberger *et al.*, 2007; Table 2.2 Study 3 & 4). It is evident that microbial attack can elevate the emission of halocarbons via the scavenging of reactive oxidants: Chance *et al.* (2009) reported up to 20-fold elevated emission of iodide by *L. digitata* upon treatment with oligoguluronate elicitors. There is a study, however, that was unable to establish a direct relationship between the emissions of iodine molecules or iodocarbons, and the physiological stress of the brown kelp. This could possibly arise due to low-stress conditions (Nitschke *et al.*, 2011).

2.2.3 Oxidative stress due to the environment

Most seaweeds are benthic algae attached to hard substrates where sufficient light penetrates. Intertidal seaweeds are constantly exposed to rapidly changing environment due to the tidal cycle, which might create a stressful environment, though they have adapted to survive this. Changes in these abiotic factors e.g. irradiance, temperature, pH of the seawater, ultraviolet radiation, desiccation during tidal change, nutrient level and seawater salinity, be it temporary or as a long-long effect, affects the physiological response of the seaweeds.

In the attempt to provide a comprehensive prediction of the global halocarbon budget, considerable efforts have been made to establish and to narrow down the environmental factors responsible for the enhanced emission of halocarbons by the seaweeds (Nightingale *et al.*, 1995; Mtolera *et al.*, 1996; Carpenter *et al.*, 2000; Manley & Barbero, 2001; Abrahamsson *et al.,* 2003; Bravo-Linares *et al.,* 2010; Laturnus *et al.,* 2010; Keng *et al.*, 2013; Leedham-Elvidge *et al.*, 2015, Mithoo-Singh *et al.*, 2017). Some of these studies were carried out in a controlled environment while others were conducted *in situ*. While a controlled study might provide more straightforward findings by excluding the many possible disturbances found in an *in situ* study, *in situ* studies do embrace the complexity of the natural environment, providing a more realistic study scenario.

2.2.3.1 Irradiance

Harvesting light energy is fundamental to the survival of seaweeds. As seaweeds grow at various depths along the seabed in their natural habitat, the quality and quantity of irradiance, which is also related to the turbidity of the seawater, affects their photosynthetic responses and metabolic patterns. Irradiance is often varied in terms of duration as well as intensity. Variation arises due to seasonal changes especially in the Polar Regions where weeks of polar day or night are experienced during the polar summer and winter respectively. Variations also occur over the shorter diurnal time frame due to the angle of sun, where irradiance level starts increasing with the break of dawn, peaks at noon and decreases until sun set, or as a result of the continuous ebb and flood of tides. Transient changes in the quantity of light reaching the surface of the marine plants also arise from meteorological changes in cloud distribution, alteration in runoff and suspended sediment loads, the movement of seaweed fronds in the water column (self-shading and shading by neighbour seaweeds) and microbial blooms.

(a) ***Light vs. Dark***

In order to observe the different responses in the emission of halocarbons by seaweeds under illumination and in the dark, halocarbon emissions by seaweeds have been quantified in several experiments (summarized in Table 2.2, Studies 6–16) and the observations reveal general agreement that halocarbons were emitted in a higher concentration under illuminated conditions than in the dark (Mtolera *et al.*, 1996; Carpenter *et al.*, 2000; Manley & Barbero, 2001; Keng *et al.*, 2013).

An incubation-based study using glass vessels under natural light showed at least a two-fold increase in halocarbon emissions by *Laminaria digitata* compared to the dark (ten times higher for $CHBr_3$) (Carpenter *at al*., 2000). They found that $CHBr_3$, which is

often the most abundant biogenic brominated halocarbon released by seaweeds, increased ten-fold in the light compared to the dark. Nightingale *et al.* (1995) studied *Ascophyllum nodosum* and showed increased rates of halocarbon emissions in the light, with the exception of CH_3I, under an indoor artificial light/dark cycle setting and with an incubation period of 48 hours. The emission of $CHBr_3$ by the temperate green seaweed, *Ulva lactuca* was also elevated by up to three times under illuminated conditions (281 ± 407 pg cm^{-2} hr^{-1}), compared to the dark (64 ± 102 pg cm^{-2} hr^{-1}) (Manley & Barbero, 2001). The emission rates were lower than those reported by *L. digitata* in the Carpenter *et al.* (2000) study mentioned above. However, the emission of $CHBr_3$ decreased with prolonged incubation of *U. lactuca* in the dark for up to 14 days. This could be due to the physiological response of the seaweed, as the reduction in $CHBr_3$ was found to be linearly correlated to the log of the seaweed respiration rate (Manley & Barbero, 2001; Table 2.2 Study 22).

Other reports by Bravo-Linares *et al.* (2010) and Laturnus *et al.* (2000) have shown higher halocarbon emission rates for several temperate and polar seaweeds with exposure to increased irradiance for between 12 hours and 3 months (Table 2.2 Studies 9, 12–15). The seaweeds were either incubated under natural condition near their habitat or given an artificial light/dark treatment with the aim of making as little disturbances to the natural light regime as possible. Among these studies, the profiling of seaweed emissions from the different depths of a sampling site i.e. in a tidal range of 10m from the intertidal zone at the Menai Strait, North Wales, was also conducted (Bravo-Linares *et al.,* 2010). In addition to seaweeds showing elevated halocarbon concentrations after 12 hr of illumination as compared to a 12 hr dark treatment, it was found that while most of the brown seaweeds produced a lower amount of halocarbon after nine hours incubation, *L. digitata*, which was collected from the deepest zone displayed an increasing concentration of $CHBr_3$ emitted at the 12 hr time-point (Bravo-Linares *et al.*, 2010). The quantification

of halocarbon production after 3, 6, 9 and 12 hours of incubation in the light provided insight into the possible response of seaweeds to day length changes in the natural environment. The deeper water *L. digitata* would only be exposed to the air during extremely low tides and is possibly the seaweed that received the least light in its natural habitat among all the brown seaweeds collected from the site (Bravo-Linares *et al.*, 2010). Further studies looking at how seaweeds from different habitats or tidal depths would respond to prolonged changes in irradiance in terms of the halocarbon emissions are very much needed.

(b) ***Emissions at varying irradiance level***

Given that the presence of light triggers the emission of halocarbons by seaweeds, this suggests the process could be related to photosynthesis (see earlier section entitled *Photosynthesis*), so investigating the effect of varying irradiance levels on halocarbon emission by the seaweeds could give interesting insight into the mechanisms responsible. Since higher irradiance levels could result in more hydrogen peroxide being released by the algal cells, one could postulate that this might then be accompanied by higher emission of halocarbons by the seaweeds.

Three short term (less than 24 hours) incubation studies showed that when seaweeds were exposed to varying irradiance levels under controlled laboratory conditions, their halocarbon emission rates increased with the intensity of the irradiance (Mtolera *et al.*, 1996; Sundström *et al.*, 1996; Keng *et al.*, 2013). In a commercially important tropical seaweed collected from Tanzania, *Eucheuma denticulatum*, the production of $CHBr_3$, indicated by the brominating activity of the seaweed (r = 1.0; p<0.05), increased up to five fold at light intensity of 600 μmol photon m^{-2} s^{-1} compared to the dark and at 15 μmol photon m^{-2} s^{-1} (Sundström *et al.*, 1996; Table 2.2 Study 6). Using the same seaweed

species, Mtolera *et al.* (1996) also reported higher release rates for seven halocarbons i.e. $CHBr_3$, CH_2I_2, $CHBr_2Cl$, C_2Cl_4, CH_2ClI, *sec*-C_4H_9I and $CHCl_3$ at higher light intensities. Their observations were based on the exposure of the seaweed to either 400 and 1500 μmol photon m^{-2} s^{-1} for one hour (Mtolera *et al.*, 1996; Table 2.2 Study 6). Our own study on the tropical brown seaweeds *Sargassum binderi, Padina australis* and *Turbinaria conoides* from Port Dickson, Malaysia (Keng *et al.*, 2013; Table 2.2 Studies 17–19) showed positive correlations (Pearson Correlation Coefficient, *r,* 0.6 – 0.9, $p < 0.01$) with increasing irradiance levels (0 – 126 μmol photon m^{-2} s^{-1}) and the emissions of CH_2BrI, CH_2I_2 and the brominated compounds CH_2Br_2, $CHBr_3$ and its derivatives, CH_2BrCl, $CHBrCl_2$, and $CHBr_2Cl$. The results were obtained after a four-hour exposure to five different irradiance levels under controlled laboratory conditions, indicating the possible influence of natural environmental changes on the emission of these compounds.

In addition to the findings from the laboratory-based controlled studies, similar trends have also been observed in studies investigating the effect of diurnal light changes on halocarbon emissions in rockpools with temperate seaweed species (Table 2.2 Studies 23–24). Increased emissions of halocarbons were observed with increased irradiance level from dawn to midday, with concentrations 2-fold higher near midday compared to pre-dawn despite temperature being almost constant (Carpenter *et al.*, 2000). The concentration of $CHBr_3$ and CH_2I_2 released by the rockpool algae were above 300 and 8 pmol L^{-1} respectively near midday and below 160 and 5 pmol L^{-1} before 7am (Carpenter *et al.,* 2000). A similar pattern (Table 2.2 Study 24) was reported by Ekdahl *et al.* (1998) for a rockpool in the Canary Islands, Spain where an increase in the halocarbon concentration was reported for midday in air and seawater samples. In addition, there was another spike in the halocarbon concentration after sunset that the authors attributed to algal respiration (Ekdahl *et al.,* 1998). The first rockpool study (Nightingale *et al.*, 1995) showed rapid increase in concentrations of CH_2I_2, CH_2Br_2 and $CHBr_3$ when the rockpool

was exposed between 1400 – 2000 hours in May 1990. Seaweeds found inside the rockpool include *Fucus serratus*, *Ascophyllum nodosum*, *Dumontia contorta*, *Enteromorpha* sp., *Cladophora albida*, *Chaetomorpha* sp. and *Gigartina stellata*. Data for irradiance, however, was not provided, and the increase in halocarbon concentration could be due to increase in temperature and pH, as well as accumulation over time.

The collective evidence shows that higher irradiance levels elevate halocarbon emissions in seaweeds. Diurnal patterns drive changes in many environmental factors such as irradiance, temperature, seawater depth, as well as photosynthetic and other related metabolic activities, so these parameters are intimately related. Physical constraints such as tidal interval (Stewart *et al.*, 2013) pose constraints and challenge for *in situ* studies in the intertidal zone but nonetheless this is an important consideration in halocarbon studies where the interactive effects of multiple environmental drivers are likely to be important. Single stressor studies remain beneficial in providing useful understanding on halocarbon emissions by seaweeds, but more *in situ* studies are needed to provide a better representation of natural seaweed halocarbon emission rates in nature.

(c) ***Regional considerations***

Most of the studies discussed so far concern halocarbon emissions from temperate and tropical seaweeds. Here we briefly consider halocarbon versus light data for seaweeds from the Antarctic. The growth season is relatively short for Earth's polar regions, especially compared to the Tropics, and there are periods in the seasonal cycle with close to continuous light or dark conditions.

Laturnus *et al.* (1998) reported higher release rates of halocarbons in general (except $CHBr_3$, CH_2Br_2 and $CHBrCl_2$) at low irradiance level or in darkness by Antarctic

seaweeds, including the red alga *Gymnogongrus antarcticus* at irradiances of 0 – 80 µmol photon m^{-2} s^{-1} (Table 2.2 Studies 9 & 20). Their findings were somewhat species-dependent and they did note differences in the physical appearance of the seaweeds collected: the eulittoral seaweed, *Ulva compressa* (formerly *Enteromorpha compressa)* was able to retain its green thallus colour at higher photon fluxes of 50 – 80 µmol m^{-2} s^{-1}, while lower sublittoral seaweeds e.g. *Phycodrys quercifolia*, *Georgiella confluens,* were completely bleached at these light intensities (Laturnus *et al.*, 1998). Overall the relationship between light intensity and halocarbon release was indistinct. In another study, a longer term two-month exposure to irradiance at 15 µmol photon m^{-2} s^{-1} or darkness was found to exert no obvious influence on the halocarbon emissions by *G. antarcticus*. However, at a higher irradiance of 30 µmol photon m^{-2} s^{-1}, both short (24 hours) and long term (two months) exposures triggered the enhancement of halocarbon emissions by the seaweed (Laturnus *et al.*, 2000; Table 2.2 Study 9).

The reasons behind the differences in halocarbon emissions by seaweeds from different regions and shore zones is hard to ascertain and further research on the potential for biogeographic variation is warranted. This is particularly true for the polar regions that are undergoing highly significant environmental and climatic change and 'baseline' data is very limited. However, there are many other geographic areas where data are also sparse, limited by season, by the species examined or the incubation methods applied. A concerted international level approach is needed to uncover whether there are consistent geographical differences amongst seaweed species from temperate, tropical and polar areas, and whether the zone of collection alters the halocarbon emission responses of seaweeds towards irradiance. If differences were uncovered it would point to a need to further investigate variation in underlying physiology and mechanism of halocarbon production by seaweeds.

2.2.3.2 Photosynthesis

In irradiance-related experiments several authors have attributed increased seaweed emissions of halocarbons with exposure to increased irradiance level to the underlying influence of photosynthetic activity. The theoretical explanations have been that halocarbons are formed through the involvement of ROS produced during oxidative stress or through the efflux of iodide during an oxidative burst, which results in the formation of iodinated halocarbons (Nightingale *et al.*, 1995; Ekdahl *et al.*, 1998; Bravo-Linares *et al.*, 2010; Keng *et al.*, 2013; Küpper *et al.*, 2013) or respiration (Ekdahl *et al.*, 1998). Indeed, irradiance is indispensable for the generation of chemical potentials for the fixation and reduction of inorganic carbon during photosynthesis.

While investigating the possible constraints on the production of $CHBr_3$ in *Ulva lactuca*, Manley and Barbero (2001), as with the previously discussed works, reported increased $CHBr_3$ production by the seaweed under illumination. The production of $CHBr_3$ was decreased to 47% of the amount of $CHBr_3$ produced (376 pg cm^{-2} hr^{-1}) in the light (control) condition, when the photosynthetic inhibitor, DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] was dissolved in the incubation seawater. This observation agrees with the hypothesis that irradiance influences the emission of halocarbon by seaweeds via a photosynthesis-related mechanism (Goodwin *et al.*, 1997; Ekdahl *et al.*, 1998). Ekdahl *et al.* (1998) reported the highest halocarbon emission rates for temperate seaweeds dominated by *Cystoseria abies-marina* (See Table 2.2 Study 23 for the complete list of seaweed investigated) at midday when seaweed photosynthesis is generally maximal (Ekdahl *et al.*, 1998). Although depression of photosynthetic efficiency can occur around midday, the down-regulation of photosynthesis might not occur in high light acclimated algae (Hanelt *et al.*, 2003). In studies on the giant kelp, *Macrocystis pyrifera,* Goodwin *et al.* (1997) also used DCMU to confirm the influence of photosynthetic activity on the

emissions of $CHBr_3$ and CH_2Br_2. Emissions of $CHBr_3$ and CH_2Br_2 by the seaweeds were not detected in the presence of DCMU when incubated under illuminated conditions.

Many recent algal studies have utilized a convenient and non-invasive way of measuring the efficiency of photosystem (PS) II through the use of pulse amplitude modulated (PAM) fluorometry. Here the parameter F_v/F_m, the ratio of variable to maximum chlorophyll fluorescence, is taken as a measurement of maximal quantum efficiency of PSII which is sensitive to changes in abiotic factors including light and nutrient deficiency. In the field of halocarbon measurements, Hughes *et al.* (2006) reported the F_v/F_m values to indicate possible stress in phytoplankton cultures triggered by different irradiance levels. Although they found a pronounced decrease in F_v/F_m values of the microalgae (*Emiliania huxleyi*, *Tetraselmis* sp., *Thalassiosira pseudonana*) at high irradiance relative to low irradiance level, there was no increase in the iodocarbon emissions that might be expected under stress conditions.

Light-dependent processes in seaweeds like pseudocyclic photophosphorylation or the Mehler reaction produce superoxide radicals (O_2^-), and subsequently H_2O_2, as a result of electron transport from the ferrodoxin of PSI to the oxygen molecule during the photosynthesis process (Collén *et al.,* 1995; Manley & Barbero, 2001; Dummermuth *et al.,* 2003). The Mehler reaction is in turn influenced by environmental factors such as irradiance, temperature, nutrients (Dietz, 2016) and these affect the release of VSLH by seaweeds.

2.2.3.3 Ultraviolet radiation (UVR)

Excessive UVA (320 – 400 nm) and UVB (290 – 320 nm) can affect primary productivity in seaweeds through damaging critical cell components. Exposure to these UVR wavebands has been related to decreases in photosynthetic activity, including the loss of Photosystem II, the electron transport system, a decrease in chlorophyll content and an increase in radical formation in algae (White & Jahnke, 2002; Xue *et al.*, 2005; Figueroa *et al.*, 2009). The inhibition of photosynthesis in seaweeds exposed to UVB is species-dependent, and may be influenced by their habitat e.g. seaweeds from deep sublittoral zones are more sensitive towards UVB exposure than those from shallow-water (Bischof *et al.*, 2009).

Though multiple studies have investigated the effect of increased radiation on seaweeds, only one study to date has looked at the response of seaweeds to UVR in terms of halocarbon emissions. Laturnus *et al.* (2010) found that, with the exception of CH_3I, most of the halocarbons analysed showed no significant changes when the brown seaweed *Saccharina latissima* was exposed to UVR for 4 hours. However, with a longer irradiation period of 28 days, the presence of UVR significantly affected the sum of reactive organic halogens (chlorine and iodine) released by the seaweed (Table 2.2 Study 31). Longer exposure to UVR of 28 days increased emissions of reactive iodine, while decreasing emissions of reactive chlorine (Laturnus *et al.*, 2010). This highlights the possible contribution of seaweeds towards stratospheric halogen load as a result of increased UVR due to the loss of stratospheric ozone, thereby exacerbating the problem. However, further studies are needed to better determine the magnitude of the impact on VSLH production caused by increased levels of UVR especially UVB.

A better understanding of the effect of UVR on the halocarbon emissions of seaweeds could contribute to modelling of potential seaweed emissions from different depths,

latitudes, water turbidity conditions, intertidal position, etc. It is especially important for assessing the contribution of cultivated seaweeds towards the halocarbon budget, as seaweeds are often farmed just below the water surface, in shallow, clear, tropical waters, which are far more sensitive to UVR flux.

2.2.3.4 Temperature

Temperature affects the enzyme activities and growth of seaweeds, and the tolerance level varies between species and even intra-species, whereby factors such as geographical distribution could affect the response levels observed when seaweeds are collected from different regions (Raikar *et al.*, 2001). The latest Intergovernmental Panel on Climate Change (IPCC) reported an expected average rise in temperature of 1.5 °C in the period 2030–2052 based on the current warming rate (IPCC, 2018). Greater warming is expected in the Arctic (3–4.5 °C of regional warming), and on land (>1.5°C) rather than in water (<1.5°C). This will also manifest in short-term extreme weather events such as hurricanes and cyclones, as well as long-term seawater level rise (IPCC, 2018). Changes in temperature could induce biome transformation, species loss, extinctions, and phenology changes, as well as altered physiology in seaweeds, including photosynthetic activities. An investigation on the red seaweed *Gracilariopsis lemaneiformis* showed an increased rate of photosynthesis from ~30 μmol O_2 g^{-1} FW hr^{-1} to 70 μmol O_2 g^{-1} FW hr^{-1} when temperature was increased from 12°C to 26°C. A model had been developed that showed +2 and +4 °C in seawater temperature from the normal temperature of 14°C would lead to significant increase in photosynthesis activities of seaweeds from the Washington coastline (Colvard *et al.*, 2014).

Responses of seaweeds in terms of their halocarbon emissions after exposure to varied temperatures settings have been reported in the literature. In their study on five brackish-water algal species, Abrahamsson *et al.* (2003) did not see a general response pattern, suggesting that halocarbon emission rates by seaweeds are strongly species-dependent. Their study also showed that the emission of CH_2I_2 by *Cladophora glomerata* growing at 23°C in the field ceased (0 ± 0 pmol g ash-free dry weight^{-1} hr^{-1}) compared to those collected at a field temperature of 12°C (8.6 ± 1.7 pmol g ash-free dry weight^{-1} hr^{-1}) when the emission was determined six hours after incubation in the laboratory at 23°C (Abrahamsson *et al.,* 2003; Table 2.2 Studies 33 & 34). Under a 10 hr cross-incubation experiment, where seaweeds from the field at 12°C were incubated at 23°C in the laboratory, and *vica versa* (23°C in field then 12°C in laboratory) showed insignificant changes in the emissions of $CHBr_3$, CH_2I_2 and $CHCl_3$ from *C. glomerate* and *Ulva ahlneriana* (Abrahamsson *et al.,* 2003; Table 2.2 Studies 33 & 34). Similar studies on the Antarctic red seaweed *Gymnogongrus antarcticus*, showed around two-fold increased production of $CHBr_3$ in a short-term 24 hr incubation experiment where the temperature was increased from 0°C (standard culture condition with light intensity of 15 μmol m^{-2} s^{-1}) to 10°C (with light intensity of 30 μmol m^{-2} s^{-1}). The emission of $CHBr_3$ was lower compared to standard culture conditions when the incubation period was extended to two months under the same temperature regime (Laturnus *et al.*, 2000; Table 2.2 Study 32). Part of the two-fold increase in the short-term incubation experiment could be attributed to the increase in irradiance (from 15 to 30 μmol m^{-2} s^{-1}).

Although temperature affects enzymatic and chemical reactions, any direct effect on halocarbon emissions could be difficult to decipher as the change in temperature could affect anything from a single reaction step to an entire pathway of reactions involved in the formation of halocarbons. Even this ignores the different temperature tolerance ranges exhibited by seaweeds from different niches and habitats (Raikar *et al.*, 2001). To date

there are a limited number of studies that look at the effect of temperature on halocarbon emissions by seaweeds, with only 5 temperate and 1 polar seaweed investigated so far. These studies showed that although the responses to temperature could well be species-specific. In addition, short term temperature rise as a form of stress could elevate halocarbon production rate (Laturnus *et al.*, 2000; Abrahamsson *et al.*, 2003). The magnitude of this could be crucial for efforts to estimate the halocarbon load encompassing diurnal and seasonal variation in temperature as well as under future predicted climate scenarios.

2.2.3.5 pH

Haloperoxidase activity has a clear association with the production of halocarbons (Wever & van der Horst, 2013; Punitha *et al.*, 2017; see also Introduction). A wide range of pH values of between 4 to 8.3 has been reported as an optimum range for haloperoxidase activities, while deviation from the optimal pH range adversely affects enzyme performance (Baden & Corbett, 1980; Krenn *et al.*, 1987; Punitha *et al.*, 2017),

The effect of seawater pH on halocarbon emissions has been reported for several subtropical and tropical seaweed species (Mtolera *et al.,* 1996; Mithoo-Singh *et al.*, 2017). Both studies altered the seawater pH using the acid/base titration method. Mithoo-Singh *et al.* (2017) reported that increasing and decreasing pH from the ambient seawater pH of 7.8 triggered enhanced emission of halocarbons by the mass-cultivated seaweed, *Kappaphycus alvarezii*. While testing pH values of 7.2, 7.4, 7.6 and 8.0 against pH 7.8, the lower seawater pH values of 7.2 and 7.4 enhanced emissions of halocarbons including CH_3I by *Sargassum siliquosum* and *Padina australis,* though with varied enhancement levels between ~200% to ~1500 % (Mithoo-Singh *et al.*, 2017). Mtolera *et al.* (1996) demonstrated increased emissions of $CHBr_3$, CH_2I_2, $CHBr_2Cl$, C_2Cl_4 when pH was

increased from 8.0 to 8.8 with *Eucheuma denticulatum*, at a higher irradiance level of 1500 µmol photon m^{-2} s^{-1}. At lower irradiance of 400 µmol photon m^{-2} s^{-1}, the emissions of $CHBr_3$, C_2Cl_4 and $CHBr_2Cl$ decreased when pH was increased from 8.0 to 8.8. The authors suggested that high pH induces H_2O_2 formation which leads to the production of halocarbons by haloperoxidases in this seaweed (Mtolera *et al.* 1996; Table 2.2 Study 35).

The IPCC have predicted a decrease in seawater pH ranging from 0.14 to 0.43 under Representative Concentration Pathways RCP 2.6 and RCP 8.5 respectively due to the increasing amount of dissolved CO_2 in seawater (Hoegh-Guldberg *et al.*, 2014). The RCP pathways have been developed based on the predicted trajectory concentrations of greenhouse gases emitted and represent the scenarios of radiative forcing in the range of 2.6 to 8.5 Wm^{-2} for the year 2100 (van Vuuren *et al.*, 2011). Although seaweeds might benefit from the increase in DIC (Celis-Plá *et al.*, 2015), this is dependent upon their DIC acquisition capability (Chung *et al.,* 2017). Increasing levels of pCO_2 have recently been found to increase iodine accumulation through the alleviation of oxidative stress for several kelp and other coastal seaweeds, including cultivated *Saccharina japonica,* in China (Xu *et al.,* 2019). This study, done in the laboratory and *in situ* mesocosms, also indicated a down-regulation of genes for vanadium-dependent haloperoxidases with increasing pCO_2. The increase in accumulation of iodine in coastal seaweed species and their grazers, plus down-regulation of haloperoxidases could affect the global biogeochemical iodine cycle and iodocarbon pool in coastal ecosystems as ocean acidification advances. However, at the time of writing, there has been no direct research on how increasing pCO_2 affects halocarbon emission by seaweeds. In the case of phytoplankton, mesocosm studies have found no distinct effects of ocean acidification on the emission of halocarbons (Hopkins *et al.*, 2010; Hopkins *et al.*, 2013; Webb *et al.*, 2016).

2.2.3.6 Desiccation

Studies on how seaweed desiccation affects halocarbon emissions by seaweeds have been carried out to better understand the response of seaweeds to tidal changes (Bravo-Linares *et al.,* 2010). They are also highly relevant in determining the contribution of farmed seaweeds towards the emission of halocarbon as industrial-scale processing usually includes a drying process (Leedham Elvidge *et al.*, 2015). Seaweed production is an important source of revenue for some coastal communities. Generally, there is some agreement that the emission of halocarbons by seaweeds is influenced by desiccation perhaps due to easing the seaweed-to-air gas transfer process in the initial stages of the desiccation process by removal of the aqueous phase.

Three studies on halocarbon emission by seaweeds have attempted to simulate natural tidal change experimentally. Nightingale *et al.* (1995) found increased emissions of $CHBr_3$, CH_2Br_2, CH_3I, $CHCl_3$ and $CHBr_2Cl$ from the temperate brown seaweed *Ascophyllum nodosum* upon re-immersion in seawater after desiccation for 6 hours in the light compared to seaweed that had been immersed in seawater for the same period (Table 2.2 Study 41). In a similar study using the same approach, the total brominated halocarbons emitted by the same species decreased with 2-, 4- and 6-hours desiccation (Bravo-Linares *et al.*, 2010; Fig. 2 Study 41). *L. digitata*, however, showed increased emission of iodinated halocarbons with increasing period of desiccation (Bravo-Linares *et al.*, 2010; Table 2.2 Study 44).

A third study measured the release of halocarbons from two temperate seaweed species during exposure to air. The initial desiccation period saw a rapid increase in $CHBr_3$ and CH_2Br_2, though this flattened out or decreased within 1 – 3 hrs. This was attributed to the volatilisation of pre-formed halocarbons near the seaweed surface rather than a physiological response. To simulate rainfall in the environment, the desiccated seaweeds

were then rewetted with freshwater and again, an increase in the emissions of these halogenated compounds from both *Fucus vesiculosus* and *Ulva intestinalis* was recorded (Leedham Elvidge *et al.*, 2015; Table 2.2 Study 43 & 45).

In addition to the difference in responses of the seaweeds towards desiccation and resubmersion in terms of halocarbon emission (Nightingale *et al.,* 1995; Leedham Eldvidge *et al.,* 2015), transport of halocarbons emitted by seaweeds to the atmosphere could vary at different tidal levels, due to the presence or absence of a water phase. It is interesting to note that aside from potential physiological effects of freshwater, Ho *et al.* (2004) demonstrated that rain can enhance the air-sea gas exchange process and suggested that short and intense rainfall could accelerate gas exchange in the ocean. Küpper *et al.* (2008) observed that during low tide, iodide released by *Laminaria digitata* was able to scavenge atmospheric ozone, leading to the formation molecular iodine, which can then go on to be involved in aerosol formation. This is supported by observations of particle bursts over kelp beds during daytime low tides. The formation of the iodine molecule by iodide during low tide is five orders of magnitude higher than the contribution of the iodocarbons combined (Küpper *et al.*, 2011). A study of halocarbon flux from a seagrass meadow found that air exposure, together with tidal change (tidal ebb and flood), produced the highest emission of up to 130 nmol m^{-2} h^{-1} for CH_3Br. In their second campaign, highest fluxes of CH_3Br, CH_3Cl, CH_3I, $CHBr_3$ were also recorded during incoming tide and ebb flow. These results suggest that re-immersion due to the flood tide might increase the emission of halocarbons from seaweeds compared to continuous air exposure (Weinberg *et al.*, 2015).

Studies such as these will be useful in refining predictions of future coastal halogen loads in the event of higher evaporation and precipitation due to climate-related temperature rise, and related changes to water density such as seawater salinity (Hoegh-Guldberg *et al.*, 2018).

2.2.3.7 Nutrient levels and salinity

Projected increases in pCO_2 levels in the future ocean would increase acidity in the shallower coastal regions, affecting mineralisation processes by the coastal microbial community and thereby potentially altering nutrient availability (Satoh *et al.,* 2007; Wood *et al.*, 2009). Seaweeds need nutrients for photosynthesis and growth and nutrient availability affects uptake and assimilation by the seaweeds (Gordillo, 2012). However, nutrient uptake is also affected by several other factors including light (through photosynthesis), temperature, water motion, surface-area to volume ratio, tissue type and seaweed age (Lobban & Harrison, 1997). Nutrient-rich cold seawater from an oceanic upwelling was found to increase halocarbon emissions of a phytoplankton community in the open ocean (Quack *et al.*, 2007; Carpenter *et al.*, 2009; Raimund *et al.*, 2011; Hepach *et al.*, 2014; Hepach *et al.*, 2015), while coastal eutrophication can cause short-term blooms of both seaweeds and phytoplankton (Gordilo, 2012; Egerton *et al.*, 2014), potentially increasing the emission of halocarbons from coastal areas. However, *Laturnus et al.* (2000) found enhanced halocarbon emissions in non-enriched as opposed to Provasoli-enriched seawater, during a short term (24 hours) and a long term (2 months) tests. The emission rates of CH_2ClI, CH_2I_2 and $CHBr_3$ by the polar brown seaweed *Gymnogongrus antarcticus,* were all higher in the longer-term exposure to the non-enriched medium compared to the short-term (24 hours) exposure in the same medium.

Although increased nutrient levels could stimulate growth of seaweeds in the coastal region, cascading effects of increased nutrient level may eventually decrease seaweed biomass due to reduced light penetration and a decline in oxygen level (Rabalais *et al.,* 2009). The contrasting results between the *in situ* measurements from temperate phytoplankton community and laboratory-based single polar seaweed experiment mentioned above, highlights the need for further research to give greater insight into future nutrient level change. This is important given that further coastal eutrophication is predicted with global climate change scenarios (Hoegh-Guldberg *et al.*, 2018) and this could affect community composition, biomass and cellular level nutrient uptake and assimilation by seaweeds.

Global climate change also leads to salinity change. A global mean sea level rise of 0.26 – 0.77 m relative to 1986 – 2005 is expected with the increase of 1.5 °C in the next decade or so (IPCC, 2018). Regions at higher latitudes could experience a decrease in seawater salinity as a result of glacier and sea ice melting, while other regions could see an increase in salinity due to increased episodes of evaporation (Durack *et al.*, 2012; Hoegh-Guldberg *et al.*, 2018).

Sessile intertidal seaweeds are especially susceptible to salinity changes that lead to hyper- and hyposaline conditions during evaporation, snow, and rain events. However, when compared with sublittoral seaweeds the intertidal seaweeds are expected to have higher tolerance and survival rates with respect to salinity changes. Salinity changes affects photosynthetic activity in seaweeds, and to a lesser extent, respiration (Wong & Chang, 2000; Tropin *et al.*, 2003). Inorganic ions like K^+, Na^+, and Cl^- are important in short-term osmotic acclimation (Karsten, 2012). Furthermore, hypersaline conditions could induce the formation of reactive oxygen species, as observed in *Ulva fasciata*,

where upregulation of several antioxidants including superoxide dismutase was seen (Sung *et al.*, 2009).

Increased emission of several iodinated halocarbon compounds including CH_2I_2 (Table 2.2 Study 48) has been reported for the polar red seaweed *Gymnogongrus antarcticus* at a salinity of 27 psu compared to 34 psu. Emissions from seaweeds exposed to a 2-month period (longer term exposure) of low salinity were higher after a 24-hr incubation than those from a 24-hr exposure (shorter term exposure) (Laturnus *et al.*, 2000). Seawater salinity is far more than a simple measure of the amount of salt in a solution, as it is closely associated with seawater density, light refraction, electrical conductivity, ion concentrations (including halogens), and osmotic pressure (Kalle 1971, Lobban & Harrison, 1997).

Although a burst of halocarbon emission was reported upon rewetting seaweeds with freshwater post-desiccation (see *Desiccation* section above), there remain many unknowns concerning the effect of freshwater on the emission of halocarbons by seaweeds. In line with the increased risk of increasingly heavy precipitation in the future (IPCC, 2018), dedicated studies on the effect of salinity fluctuations on halocarbon emissions by seaweeds, especially in their natural coastal environment should be intensified. This could bridge the gap in the uncertainties related to salinity change in different regions.

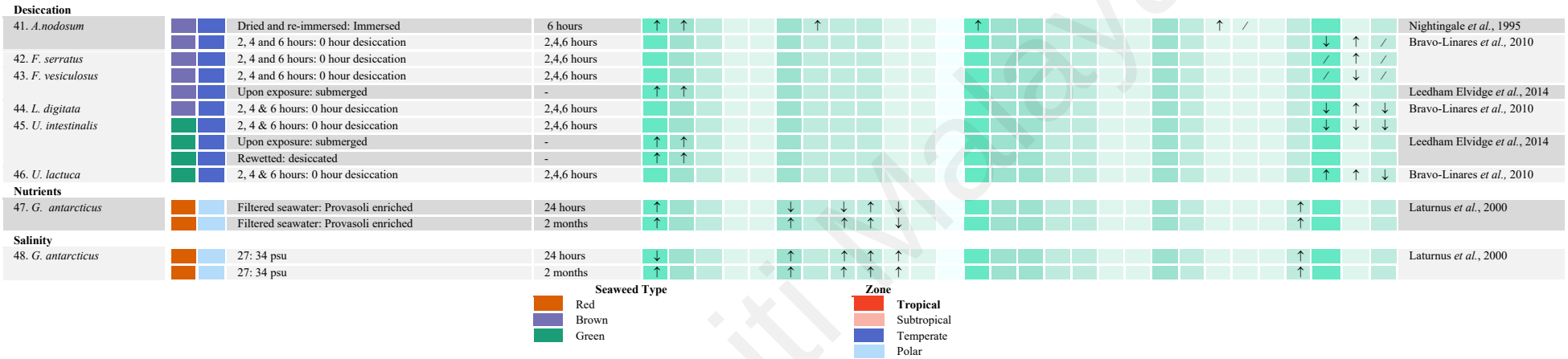
Table 2.2: A summary of studies on the emissions of halocarbons by seaweeds, arranged by experimental treatment or the stress factor applied (Table published in Keng *et al.*, 2020)

Study Number/ Seaweeds	Type	Zone	Experimental treatment/ Stress factor	Duration of Treatment	Halocarbon compounds: Brominated: $CHBr_3$	CH_2Br_2	CH_3Br	C_2H_5Br	1,2-$C_2H_4Br_2$	Iodinated: CH_2I_2	CH_3I	C_2H_5I	*n*-C_3H_7I	*sec*-C_3H_7I	C_4H_9I	*sec*-C_4H_9I	Chlorinated: $CHCl_3$	CH_2Cl_2	CH_3Cl	CCl_4	CCl_4	CH_3CCl_3	C_2HCl_3	Mixed: CH_2BrI	$CHBr_2I$	$CHBr_2Cl$	$CHBrCl_2$	CH_2BrCl	CH_2ClI	Total+: Bromine	Iodine	Chlorine	References
Herbivory																																
1. *Ascophyllum nodosum*			Snail (Herbivory)[X]	48 hours	↑	↑					≈						↑										↑						Nightingale *et al.*, 1995
			Chopped (Wounding)[X]	48 hours	↑	↑					↑						↑									↓							
2. *Eucheuma denticulatum*			Cutting (Wounding)[**]	2 hours	↑																												Sundström *et al.*, 1996
Microbial defense																																
3. *Gracilaria chilensis*			Agar oligosaccharide	30 minutes	/	/					/	↑					/							/		/	/		/				Weinberger *et al.*, 2007
4. *Gracilaria* sp.			Agar oligosaccharide	30 minutes	↑	↑					↑	↑					↑							↑		↑	↑		/				
5. *Laminaria digitata*			Oligoguluronate	< 1 hour	↑					↑	↑															↑			↑				Palmer *et al.*, 2005
Irradiance																																
6. *E. denticulatum*			Light (600 µmol photon m^{-2} s^{-1}): Dark	2 hours	↑																												Sundström *et al.*, 1996
			1500: 400 µmol photon m^{-2} s^{-1}	1 hour	↑					↑						↑	↑			↑						↑			↑				Mtolera *et al.*, 1996
7. *Georgiella confluens*			Light (80 µmol photon m^{-2} s^{-1})•: Dark	3 months	↑	↑	↑				↑								↑														Laturnus *et al.*, 1998
			Light (80 µmol photon m^{-2} s^{-1})°: Dark	3 months	↓	↓	/				/								/														
			80: 5 µmol photon m^{-2} s^{-1}	3 months				↓	/	/		/															/	↑	↓				
8. *Griffithsia flosculosa*			Light (68 µmol photon m^{-2} s^{-1}): Dark	12 hours			/			/										-						-			↑	↑	|	Bravo-Linares *et al.*, 2010
9. *Gymnogongrus antarcticus*			Light (80 µmol photon m^{-2} s^{-1})•: Dark	3 months	↑	↑	↓				/								/														Laturnus *et al.*, 1998
			Light (80 µmol photon m^{-2} s^{-1})°: Dark	3 months	↑	↓	/				/								↓														
			80: 5 µmol photon m^{-2} s^{-1}	3 months				↓	↓	↓		↓															↑	/	/				
			Light (15 µmol photon m^{-2} s^{-1}): Dark	24 hours	/					/		/	/	/	/	/													/				Laturnus *et al.*, 2000
			30: 15 µmol photon m^{-2} s^{-1} [X]	24 hours	↑					/		↑	↑	↑															↑				
			30: 15 µmol photon m^{-2} s^{-1} [X]	2 months	↑					↑		↑	↑	↑															↑				
10. *Palmaria palmata*			Light (68 µmol photon m^{-2} s^{-1}): Dark	12 hours			↑			↑										-						-				|	|	↓	Bravo-Linares *et al.*, 2010
11. *Phycodrys quercifolia*			Light (80 µmol photon m^{-2} s^{-1})•: Dark	3 months	/	↑	/				/								↑														Laturnus *et al.*, 1998
			Light (80 µmol photon m^{-2} s^{-1})°: Dark	3 months	↓	/	/				/								/														
			80: 5 µmol photon m^{-2} s^{-1}	3 months				↓	↑	↓		↓															/	↓	↑				
12. *Ascophyllum nodosum*			Light: Dark[X]	48 hours	↑	↑					↓						↑									↑							Nightingale *et al.*, 1995
			Light (68 µmol photon m^{-2} s^{-1}): Dark	12 hours			↑			↑									↑							↑			↑	|	↑	Bravo-Linares *et al.*, 2010
13. *Fucus serratus*			Light (68 µmol photon m^{-2} s^{-1}): Dark	12 hours			↑			↑									/							↑			↑	|	↑	
14. *Fucus vesiculosus*			Light (68 µmol photon m^{-2} s^{-1}): Dark	12 hours			↑			↑									↓							↑			↑	↑	|	
15. *L. digitata*			Light (68 µmol photon m^{-2} s^{-1}): Dark	12 hours			↑			↑									↓							↑			↑	↑	↓	
			[N] Light: Dark	6 hours	↑	↑	/			/	/													↑	↑	↑			↑				Carpenter *et al.*, 2000
16. *Macrocystic pyrifera*			Light (80 µmol m^{-2} s^{-1}): Dark	2 hours	↑	↑																											Goodwin *et al.*, 1997
			Light (80 µmol m^{-2} s^{-1}): Dark	2 hours			/				/								/														Manley & Dastoor, 1987

Table 2.2 continued

17. *Padina australis*	Increasing levels ^ +	4 hours	/	/				/	/													/		/	/	/					Keng *et al.*, 2013
18. *Sargassum binderi*	Increasing levels ^ +	4 hours	↑	↑				/	↑													/		↑	↑	↑					
19. *Turbinaria conoides*	Increasing levels ^ +	4 hours	↑	↑				↑	/													↑		↑	↑	↑					
20. *Ulva compressa*	Light (80 μmol photon m^{-2} s^{-1})•: Dark	3 months	/	↓	/				/								/														Laturnus *et al.*, 1998
	Light (80 μmol photon m^{-2} s^{-1})°: Dark	3 months	↑	↓	/				/								↓														
	80: 5 μmol photon m^{-2} s^{-1}	3 months				↓	↓	↓		↓															↑	/	↓				
21. *Ulva intestinalis*	Light (68 μmol photon m^{-2} s^{-1}): Dark	12 hours			↑			↑										-							↑			↑		↓	Bravo-Linares *et al.*, 2010
22. *Ulva lactuca*	Light (68 μmol photon m^{-2} s^{-1}): Dark	12 hours			↑			↑										-							↑			↑		↑	
	Light (530μmol photon m^{-2} s^{-1}): Dark	2 or 4 hours	↑																												Manley & Barbero, 2001
23. Rockpool seaweeds [//]	[N] Diurnal variation; Light: Dark 12 hour photoperiod; light period from 0600 to 1800; Temperature between 21 (dark) 29 °C, highest during midday. [X]	-	↑	↑				/					↑		↑					↑				↑							Ekdahl *et al.*, 1998
24.Rockpool seaweeds [///]	[N] Diurnal variation; Light: Dark; Temperature between 13 – 16°C. [X]	-	↑					↑																							Carpenter *et al.*, 2000
Photosynthesis																															
25. *E. denticulatum*	DCMU	2 hours	↑						↑						↑						↑										Sundström *et al.*, 1996
26. *M. pyrifera*	DCMU	2 hours	↓	↓																											Goodwin *et al.*, 1997
27. *P. australis*	Increased F_v/F_m	4 hours	↑	/				/	/													↑		↑	↑	↑					Keng *et al.*, 2013
28. *S. binderi*	Increased F_v/F_m	4 hours	/	↑				↑	/													/		/	/	/					
29. *T. conoides*	Increased F_v/F_m	4 hours	/	/				/	/													/		/	/	/					
30. *U. lactuca*	DCMU	2 or 4 hours	↓																												Manley & Barbero, 2001
Ultraviolet radiation																															
31. *Saccharina latissima*	PAR+UVR: PAR	4 hours	/	/			/	/	↑						/	/		/	/	/	/			/	/		/				Laturnus *et al.*, 2010
	PAR+UVR: PAR	28 days																										/	↑	↓	
Temperature																															
32. *G. antarcticus*	10: 0 °C	24 hours	↑					↓		↑	↑	↓															↑				Laturnus *et al.*, 2000
	10: 0 °C	2 months	↓					↓		↑	↑	↓															↑				
33. *Cladophora glomerata*	23: 12 °C in the field	6 hours	/					/							/																Abrahamsson *et al.*, 2003
	23: 12 °C cross-incubation	10 hours	/					/							/																
	12: 23 °C cross-incubation	10 hours	/					/							/																
34. *U. ahlneriana*	23: 12 °C in the field	6 hours	/					/							/																
	23: 12 °C cross-incubation	10 hours	/					/							/																
	12: 23 °C cross-incubation	10 hours	/					/							/																
pH																															
35. *E. denticulatum*	pH 8.8: 8.0; 400 μmol photon m^{-2} s^{-1}	1 hour	↓					↑				↑			/			↓						↓			↑				Mtolera *et al.*, 1996
	pH 8.8: 8.0; 1500 μmol photon m^{-2} s^{-1}	1 hour	↑					↑				↑			↑			↑						↑			↑				
36. *Kappaphycus alvarezii*	pH 8.0: 7.8	4 hours	↑	↑				-	/													/		↑	↑	/					Mithoo Singh *et al.*, 2017
	pH 7.2: 7.8	4 hours	↑	/				-	↑													/		↑	↑	/					
	pH 8.0 – 7.2	4 hours	↑					-														↓		↑	↑			↓	/	↑	
37. *P. australis*	pH 8.0: 7.8	4 hours	/	/				↑	/													/		/	/	/					
	pH 7.2: 7.8	4 hours	/	/				/	↑													/		/	/	/					
	pH 8.0 – 7.2	4 hours	/	/				/	↑													↑		/	↑	/		/	/	↑	
38. *S. binderi*	pH 8.0: 7.8	4 hours	/	/				↑	-													/		/	/	↑					
	pH 7.2: 7.8	4 hours	/	/				↑	-													↑		/	/	↑					
	pH 8.0 – 7.2	4 hours	/	/				/	-													/		/	/	/		/	/	/	
39. *Sargassum siliquosum*	pH 8.0: 7.8	4 hours	/	/				/	/													/		/	/	/					
	pH 7.2: 7.8	4 hours	/	/				/	↑													/		/	/	/					
	pH 8.0 – 7.2	4 hours	/	/				/	↑													/		/	/	/		/	↑	/	
40. *T. conoides*	pH 8.0: 7.8	4 hours	↑	/				/	/													/		/	/	/					
	pH 7.2: 7.8	4 hours	↑	/				/	/													/		↑	/	/					
	pH 8.0 – 7.2	4 hours	/	/				↓	/													↓		/	/	/		↑	↓	↑	

Table 2.2 continued



Rockpool seaweeds arranged according to decreasing abundance:
// including *Cystoseria abies-marina, Codium adherens, Grateloupia doryphore, Hypnea spinella, Sargassum* sp., *Spirida hypnoides, Padina pavonia*;
/// including *Enteromorpha prolifera*, *Cladophora rupestris* and *Ulva* sp. covering >50% of bottom surface, *Halopteris scoparia, Fucus serratus, Fucus spiralis, Halidrys siliquosa, Laminaria digitata, Himanthalia elongate, Chondrus crispus, Polysiphonia brodiaei, Corallina elongate, Hildenbrandia rubra, Palmaria palmate, Callithamnion tetragonum, Codium fragile*

All studies were conducted under controlled laboratory conditions except those denoted by ^{N} where the studies were conducted in the natural environment; $^{+}$Denotes the total of the brominated, iodinated and chlorinated halocarbons (Bravo-Linares *et al.*, 2010) or the reactive organic halogen which was the molar sum derived from the halocarbon compounds investigated in the respective studies (Laturnus *et al.*, 2010; Mithoo Singh *et al.*, 2017).

↑ Increased emission of compounds; ↓ Decreased emission of compounds; / Insignificant; ≈ Uncertain effect; ^{X} Statistical significance not stated; | lacking replicates; - Not detected; ** CHB_3 production assumed through the formation of tetrabromophenol by brominating activity of the seaweed - the production of $CHBr_3$ was reported as a linear function of brominating activity (production of tetrabromopheol); $^{\wedge}$ $CHBr_2Cl$, $CHBrCl_2$ and CH_2BrCl were represented as derivatives of $CHBr_3$; Treatment of 1500/40 µmol photon m^{-2} s^{-1}= Trend observed based on the irradiance at 1500 µmol photon m^{-2}

s^{-1} relative to 40 µmol photon m^{-2} s^{-1}; + Increasing irradiance of 0, 47, 58, 82, 126 µmol photon m^{-2} s^{-1}; • Seaweeds acclimatized at photoperiod of 6.45 hour; ° Seaweeds acclimatized at photoperiod of 17.45 hour

DCMU = 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PAR = Photosynthetically Active Radiation; cross-incubation indicates a temperature change in the laboratory from the field.

CHAPTER 3: METHODOLOGY

3.1 Investigating the effect of temperature variation on the halocarbon emissions of selected tropical seaweeds under controlled laboratory conditions

3.1.1 Sample collection

Gracilaria manilaensis Yamamoto & Trono (red) and *Ulva reticulata* Forsskål (green), were collected from Tanjong Kupang, Johor, West Peninsular Malaysia, at a sandy/muddy beach area in the vicinity of a land reclamation project (Figure 3.1). The area was dominated by *U. reticulata* and seagrass meadows with the presence of seahorses. By sight, both *U. reticulata* and *G. manilaensis* were dominant seaweeds present at the sampling site during the time of sampling. *Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva, a commercially important red seaweed, was purchased from a seaweed farm off Semporna, Sabah, East Malaysia, while the fourth species, *Turbinaria conoides* (J.Agardh) Kützing, was collected from a fringing coral reef at Port Dickson, West Peninsular Malaysia, and had been reported to be one of the dominant seaweed species present at the site (Keng *et al.,* 2013). For collection dates and coordinates of the seaweed sites, see Table 3.1. All seaweeds were transported back to the University of Malaya hatchery and maintained under a flowing seawater system at an average temperature of 27.4 (25 – 29.7) °C for no longer than eight weeks.

Figure 3.1: Seaweeds used in the temperature study; from left to right: *Gracilaria manilaensis* Yamamoto & Trono, *Ulva reticulata* Forsskål, *Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva and *Turbinaria conoides* (J.Agardh) Kützing

Table 3.1: Location and date of collection of seaweed samples used in the temperature study

Seaweeds	Coordinates	Location	Date
Gracilaria manilaensis Yamamoto & Trono (Red)	1°20'26" N 103°36'16" E	Tanjong Kupang, Johor	22.5.2017
Ulva reticulata Forsskål (Green)			22.5.2017
Kappaphycus alvarezii (Doty) Doty ex P. C. Silva (Red)	4°30'6" N 118°37'40" E	Semporna, Sabah	17.7.2017
Turbinaria conoides (J. Agardh) Kützing (Brown)	2°24'56" N 101°51'20" E	Cape Rachado, Port Dickson	9.8.2017

3.1.2 Experimental setup

Prior to exposure to the temperature treatments, seaweeds from the hatchery were transported back to the laboratory, cleansed of visible epiphytes and acclimatised to the laboratory conditions in a shaking incubator (HiPoint 600SR) for between 16 and 20 hours, at an irradiance level of 81 ± 7 μmol photons m^{-2} s^{-1} (LICOR, Inc LI-250A light meter with LI-190SA quantum sensor) and temperature of 25 ± 2 °C in pre-filtered seawater with a constant air supply. The incubator was set to shake at approximately 30 rpm.

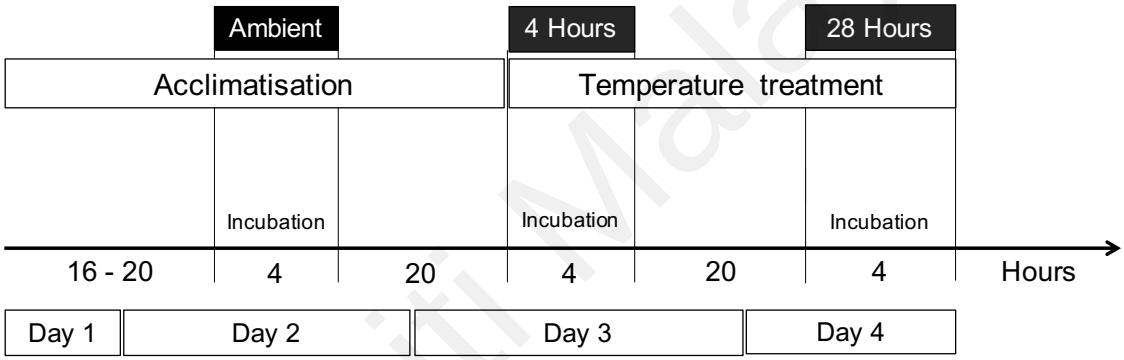


Figure 3.2: Treatment of seaweeds for the temperature study. Seaweeds were acclimatised at laboratory condition i.e. 25 °C, prior to the exposure to varying temperature levels of 40, 35, 30, 25 and 20 °C for up to 28 hours. Halocarbon emissions were measured four hours and 28 hours post-exposure to the temperature treatments. The ambient sample was taken after 16 – 20 hours of acclimatisation.

The treatment consisted of a set of five different temperatures, i.e. 40, 35, 30, 25 and 20 °C, starting with an 'ambient' treatment where seaweeds were incubated in custom-made stoppered flasks (with a Luer port at the bottom) for 4 hours at laboratory temperature, i.e. 25 ± 2 °C, to determine the halocarbon emissions prior to temperature treatment (Figure 3.2). The 'ambient' treatment was carried out to enable comparison of halocarbon emissions between the starting and the treatments. This temperature was close to the average seawater temperature of 27.4 (25–29.7) °C in the hatchery, where the seaweeds were maintained (HOBO logger). This temperature value is close to previously

reported sea surface temperature of 25.7–33.9 °C (Tan *et al.* 2002) and was within the range of 20.9–33.5°C reported at Port Dickson (Hamzah *et al*., 2011). As this could be the first dedicated temperature-based experiment on tropical seaweeds, extreme temperatures of 20 and 40 °C were included to investigate the response in terms of halocarbon emissions of the selected tropical seaweeds toward these temperatures. Intertidal seaweeds during tidal ebb at Morib could be exposed to temperatures as high as 38.2 ± 1.1°C.

The incubation flasks were filled with seawater with no headspace. Likewise, control flasks with only seawater were filled in a similar manner for the determination later of seaweed halocarbon emissions. The seawater from the control flasks and incubation flasks was immediately and individually extracted using a 100 mL gas-tight glass syringe.

Upon completion of the ambient treatment, seaweeds were returned to their previous vessels and acclimatised to the laboratory conditions as mentioned earlier until the next day, when the seaweeds were subjected to one of the temperature regimes, e.g. 40 °C. For this, seaweeds were incubated at the respective temperature for 4 hours, again in the custom-made stoppered flask filled to the top with pre-filtered seawater without headspace. Control flasks were also prepared and subjected to the same treatment. Seawater from the flasks was then extracted using a glass syringe. Upon completion of this 4-hour exposure treatment, the seaweeds were returned to their acclimation vessels and maintained at the same conditions, except this time at the respective treatment temperatures for a further 20 hours.

The same batches of seaweeds were once again placed into the incubation flasks, 24 hours after their first exposure to the temperature treatment. Seawater was extracted from the flasks immediately after another 4-h incubation for halocarbon analyses. These steps were repeated at all treatment temperatures, i.e. 40, 35, 30, 25 and 20 ± 2 °C (Figure 3.2).

Halocarbons emitted by the seaweeds were derived from the net difference in seawater halocarbon concentrations between seawater-filled flasks containing seaweeds and seawater-filled flasks without seaweeds (control). The seawater used in this experiment was sourced naturally from Port Dickson and was filtered (0.7 μm GF/F, Whatman) prior to experimental use.

Seaweed biomasses used for each flask were between 10g and 15g and were weighed prior to the start of each temperature treatment (Day 1). To determine the moisture content (Table 3.2) of the seaweeds, the dry weights of seaweeds were determined after 72 hours of drying in the oven at 60 °C.

Table 3.2: Moisture content (mean ± standard deviation, %) and the dry weight (DW) to fresh weight(FW) ratio of the selected seaweed species

Seaweed	Moisture content (%)	DW: FW
G. manilaensis	85.50 ± 1.08	0.1450
U. reticulata	81.91 ± 1.17	0.1809
K. alvarezii	90.45 ± 0.26	0.0955
T. conoides	84.97 ± 4.96	0.1503

n= 7, 8, 3, 3 for *G. manilaensis*, *U. reticulata, K. alvarezii, T. conoides*

In addition to the halocarbon emission rates, seawater nutrient contents (phosphate, nitrate, nitrite, and ammoniacal nitrogen), the maximal quantum yield, F_v/F_m, and the pigment contents (Chl-*a* and carotenoids) of the seaweeds were determined.

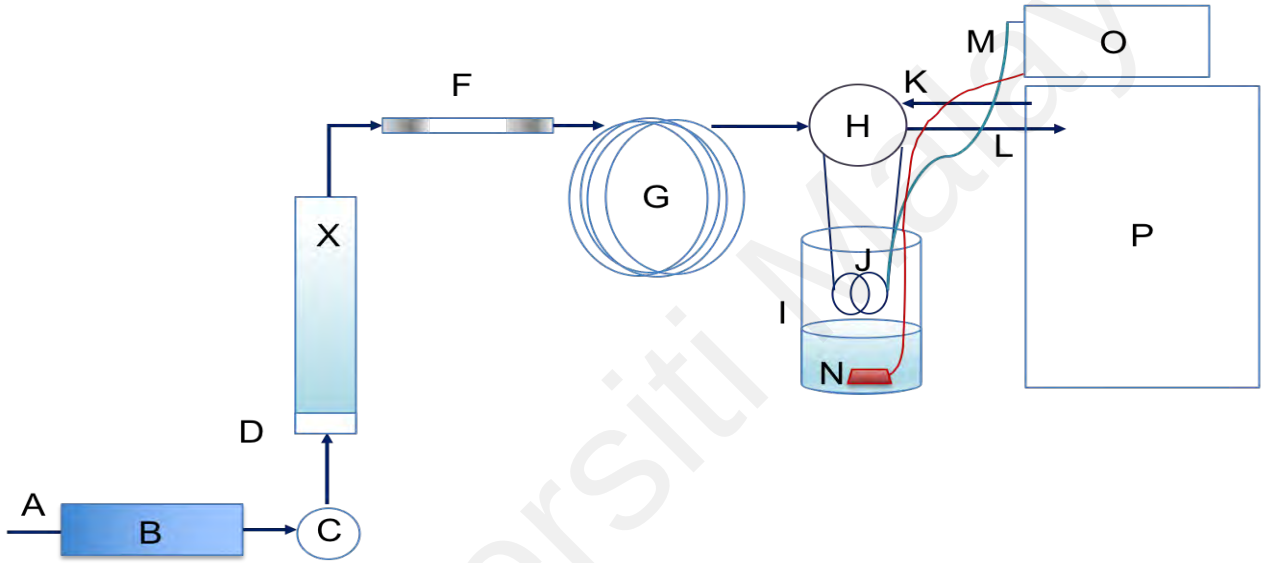
3.1.3 Halocarbon analysis

Seawater samples extracted from the incubation flasks were subsequently injected into a self-assembled Purge-and-Trap (P&T) system (Figure 3.3; Keng *et al.*, 2013; Leedham *et al.*, 2013; Mithoo-Singh *et al.*, 2017). The P&T system was constructed based on a similar loaned unit from the University of East Anglia, United Kingdom. The seawater samples were injected into the sampling vessel and purged with oxygen-free nitrogen at a rate of 40 mL min^{-1} for 15 minutes. The purged gas was channelled through a glass tube fitted with glass wool followed by channelling through a Nafion dryer (Perma Pure) at a counterflow rate of 100 mL min^{-1} with oxygen-free nitrogen to remove aerosol particles and water vapour. The analytes were than trapped and concentrated in a sampling loop attached to a six-port two-way valve (VICI) using liquid nitrogen maintained at −150 °C through a thermostatic liquid nitrogen boiler (University of East Anglia).

At about 15 minutes after purging, the position of the six-port valve was changed from 'trap' to 'inject'. With a quick switch between liquid nitrogen and boiling water at the sampling loop, the trapped analytes were desorbed by high purity helium (Linde Malaysia) at 1 mL min^{-1} into a gas chromatography (GC) system (Agilent Technologies, 7890B), through a heated transfer line maintained at 91 ± 2 °C. The GC system was fitted with a 60 m capillary column (J&W DB-VRX, film thickness 1.40 µm; internal diameter 0.25 mm). The GC oven was programmed to hold the temperature at 40 °C for 4 minutes and ramp up to 200 °C at a rate of 20 °C min^{-1} and held for 2 minutes, followed by a ramp up of 40 °C min^{-1} until 240 °C and held for 5 minutes. The detection and quantification of analytes were done by the mass spectrometry system (Agilent Technologies, 5977B MSD) coupled to the GC.

A total of six compounds were monitored in this study, through the Single Ion Monitoring mode. These include the brominated compounds, i.e. bromoform ($CHBr_3$) and dibromomethane (CH_2Br_2), the iodinated compounds diiodomethane (CH_2I_2) and methyl iodide (CH_3I), and the mixed compounds dibromochloromethane ($CHBr_2Cl$) and dichlorobromomethane ($CHBrCl_2$).

Concentrations of target compounds were determined through five-point calibration curves of compound standards (Sigma-Aldrich, Merck) at a temperature of 25 ± 1 °C. The commercially available liquid standards were gravimetrically prepared and diluted in methanol (Fisher Scientific, HPLC grade) for this purpose. Surrogate analytes, i.e. deuterated methyl iodide (CD_3I) and deuterated diiodomethane (CD_2I_2), were added to each of the samples prior to P&T injection to monitor for system drift. Peak areas were corrected according to the purging efficiencies at temperatures of 40, 35, 30 and 20 °C relative to 25 °C determined through our system. The detection limits of the system for each compound were determined from the standard deviation (SD) of the blanks (three times SD) (Abrahamsson & Pedersén, 2000). The detection limit for each halocarbon compound was 10 pmol L^{-1}.



A	In-flow of OFN	G	Nafion™ driers (PermaPure)
B	Hydrocarbon trap (Agilent Technologies)	H	Six-port valve (VICI Valco)
C	Metering valve (Swagelok Malaysia)	I	Liquid nitrogen filled dewar
D	Purge vessel with fritted sparger	J	Sample loop for trapping
X	Sample injection inlet	K	High Purity Helium (HPH) flow from GCMS
F	Glass wool trap	L	Heated transfer line to GCMS
		M	Thermocoupler
		N	Liquid nitrogen heater (RS Components)
		O	Thermostatted heating device (UEA)
		P	GCMS (Agilent Technologies)

Figure 3.3: Schematic diagram of the self-assembled P&T system

3.1.4 F_v/F_m measurements

Photosynthetic performance of the seaweeds can be determined using Pulse Amplitude Modulated (PAM) Chlorophyll Fluorescence (Keng *et al.,* 2013; Mithoo Singh *et al.,* 2017). PAM parameters including maximal quantum yield (F_v/F_m) are useful for indicating the photosynthetic performance of the seaweeds. F_v/F_m was used in this experiment as a measure of the physiological health of the seaweeds. F_v/F_m of the seaweeds pre- and post-incubation (Figure 3.1) was determined using a Walz Inc., DIVING-PAM. To obtain the F_v/F_m values, seaweeds were dark adapted for at least 15 minutes using the dark leaf clips prior to measurement. This was done to create an 'open' state in the reaction centres of the photosynthetic pigments. A weak modulating light beam (0.15 μmol photons m^{-2} s^{-1}) was then applied for the determination of the ground fluorescence (F_0), followed by a saturation pulse of 800 μmol photons m^{-2} s^{-1} for 0.6 s^{-1} to determine the maximal fluorescence (F_m). F_v/F_m was then determined through the formula $F_v/F_m = (F_m - F_0)/F_m$. The value of F_v/F_m was represented as average (n= 12), or as a change (%) of values between pre- and post-exposure relative to the pre-exposure F_v/F_m values of the seaweeds to the various treatments.

3.1.5 Nutrient analysis

The nutrient content i.e. phosphate (PO_4^{3-}), ammonia (NH_3-N), nitrate (NO_3^--N) and nitrite (NO_2^--N) of the seawater medium used throughout the experiment was tested. The nutrient analyses were carried out based on the protocols laid out in the manual for the Hach Odyssey DR/2500 Spectrophotometer (Hach, 2001) spectrophotometer using powder pillows. Protocols used were based on the following methods:

Table 3.3: Test methods used for nutrient analyses using HACH Spectrophotometer (Hach, 2001)

Method	Test
Salicylate method (Reardon *et al.*, 1966)	Ammonia (NH_3-N)
Diazotization method (USEPA, 1979)	Nitrite (NO_2^--N)
Cadmium reduction method (APHA, 1998)	Nitrate (NO_3^--N)
Ascorbic acid method (APHA, 1998)	Phosphate (PO_4^{3-})

Salicylate Method (Reardon *et al.*, 1966): Briefly, 10 mL of seawater was poured into a round sample cell, while another round sample cell was filled with deionised water as a blank reading. The contents of one Ammonia Salicylate Powder Pillow was added to each cell. The cells were then stoppered and shaken to dissolve the powder. After 3 minutes, the contents of one Ammonia Cyanurate Reagent Powder Pillow was added to each cell. After 15 minutes, the blank was placed in the spectrophotometer and the reading was set to zero. This was followed by taking the reading for the second cell. The presence of ammoniacal nitrogen was indicated by a greenish colour.

Federal Register's Diazotization Method (USEPA, 1979): A round sample cell was filled with 10 mL of seawater and the contents of one NitriVer 3 Nitrite Reagent Powder Pillow was added. This was then capped and shaken to dissolve to powdered reagent. The presence of nitrite was determined through the formation of a pinkish solution. This sample cell was then read through with the spectrophotometer after 20 minutes. The NO_2^--N content was measured by comparison to a blank sample cell filled with 10 mL seawater.

Cadmium Reduction Method (APHA, 1998): Approximately 10 mL seawater was poured into a round sample cell. The contents of one NitraVer 5 Nitrate Reagent Powder Pillow was added into the same sample cell and capped. The sample cell was then shaken vigorously for one minute. It was then left for 5 minutes for reaction. The presence of

nitrate was indicated by the formation of an amber-coloured solution. Meanwhile, a blank was prepared by pouring 10 mL of seawater into a second round sample cell. The blank cell was placed in the spectrophotometer and the reading was set to zero. This was followed by taking the reading for the sample cell.

Ascorbic Acid Method (APHA, 1998): A round sample cell was filled with 10 mL of seawater and the contents of one PhosVer 3 phosphate Reagent Powder Pillow was added. It was then immediately swirled to mix and left to settle for 2 minutes. A blank was subsequently prepared by filling a second, round sample cell with 10 mL of seawater. After 2 minutes, the blank was placed into the cell holder of the spectrophotometer and the reading was set to zero. The blank was then taken out and replaced with the other sample cell for the reading of the results.

The seawater used throughout the experiment was source from Port Dickson, and were of the same batch. The seawater nutrients of ammonia (NH_3-N), nitrite (NO_2^--N), nitrate (NO_3^--N) and phosphate (PO_4^{3-}) were found in the ranges of 0.04–0.11 mg/L, 0.001–0.013 mg/L, 0.6–1.2 mg/L and 0.01–0.09 mg/L, respectively.

3.1.6 Determination of pigment content in seaweeds

The pigment contents of seaweeds were determined upon the completion of each temperature treatment to determine the effect of temperature on the pigment contents of the seaweeds, and to determine the correlation between the pigment contents of seaweeds with the halocarbon emission rates.

Around 0.3–3g of seaweeds were ground with a mortar and pestle in a chilled and dark condition, with the occasional addition of acetone. The extracts were then drained into a centrifuge tube and topped up with acetone to 20 mL. The extracts were kept in the dark and chilled (4 °C) overnight. The extracts were then centrifuged at 3000 rpm for 10 minutes. Around 4 mL of supernatant was pipetted out into a quartz cuvette and all pigment extracts were read at wavelengths of 665, 645, 630 and 452 nm with a spectrophotometer (Shimadzu UV-1800 UV spectrophotometer). The chlorophyll and carotenoid contents of the seaweeds were determined through Equation 1 and Equation 2 (Strickland and Parsons, 1968).

Equation 1

$$\text{Chlorophyll a} = \frac{\text{Ca} \times \text{Volume of Acetone (mL)}}{\text{Seaweed fresh weight (g)}}$$

Where $\text{Ca} = 11.6\,(\text{OD } 665\text{nm}) - 1.31\,(\text{OD } 645\text{nm}) - 0.14\,(\text{OD } 630\text{nm})$; Chlorophyll a is given in μg g^{-1}

Equation 2

$$\text{Carotenoid} = \frac{\text{OD } 452\text{ nm} \times 3.86 \times \text{Volume of Acetone (mL)}}{\text{Seaweed fresh weight (g)}}$$

Where Carotenoid is given in μg g^{-1}

3.1.7 Statistical analysis

All statistical analyses were carried out using the SPSS Statistics software (IBM, Version 22). One-way ANOVA was conducted to test the difference between halocarbon emission rates at different exposure duration, the difference in F_v/F_m values of the seaweeds at different temperatures and for different treatment durations, and to observe the difference across exposure duration in the F_v/F_m values. One-way ANOVA was also conducted to test the effect of temperature changes on the pigment content of the seaweeds (Table 4.2). Meanwhile, the relationships between temperature change and halocarbon emissions (Table 4.1) and between halocarbon emissions and pigment content were determined using Pearson's Product-Moment Correlation.

3.2 Investigating the combined effect of temperature and irradiance on halocarbon emissions by *Kappaphycus alvarezii* under controlled laboratory conditions

The red seaweed *Kappaphycus alvarezii* (Figure 3.4) collected from Semporna, Sabah, (4°30'6" N, 118°37'40" E) on 5th October 2019 was kept at the University of Malaya outdoor hatchery under aerated flowing seawater system for at least a week before the start of the experiment. The salinity of the seawater was maintained at 32 ± 1 ppt by the addition of freshwater or high-salinity seawater prepared through natural evaporation process.

Figure 3.4: *Kappaphycus alvarezii* maintained at the outdoor hatchery at University of Malaya for use in the temperature and irradiance study

Prior to the start of the experiment, the seaweeds were brought back to the laboratory, cleansed of visible epiphytes and acclimatised to the experimental treatment conditions for 16–20 hours in a shaking incubator (HiPoint 600SR) set at 30 rpm. To test the combined effects of temperature and irradiance on the halocarbon emissions of the commercially important seaweed, four temperature levels, i.e. 25 ± 1 (T1), 28 ± 1 (T2), 31 ± 1 (T3) and 34 ± 1 (T4) °C, were crossed with four irradiance levels, i.e. 0 ± 0 (L1), 62 ± 8 (L2), 117 ± 13 (L3) and 177 ± 26 (L4) μmol photons^{-2} s^{-1} (LICOR, Inc LI-250A light meter with LI-190SA quantum sensor). The fresh seaweed was blotted dry before being weighed to determine the fresh weight. This was followed by measuring the F_v/F_m values (See Section 3.1.4) of the seaweed, prior to the start of the incubation.

For each exposure treatment, a 500 mL custom-made stoppered flask with a Luer port at the bottom was filled with pre-filtered (GF/F, Whatman) seawater at a salinity of 32 ± 1 ppt, adjusted using distilled water and high-salinity seawater prepared through natural evaporation process. Nutrient analysis of pre-filtered seawater stock was carried out on the HACH DR3900 spectrophotometer using the methods described in Section 3.1.5. The ranges of nutrients in the pre-filtered seawater were 0.00–0.02 mg/L ammonia (NH_3-N), 4.9–5.3 mg/L nitrite (NO_2^--N), 0.005–0.006 mg/L nitrate (NO_3^--N) and 0.001–0.002 mg/L phosphate (PO_4^{3-}).

Seaweeds were then added to the flask filled with the pre-filtered seawater for 4 hours to determine the halocarbon emission rates. As with the previous experiment, control flasks with only pre-filtered seawater were used to enable the determination of halocarbon emissions by the seaweeds alone.

After the 4-hour incubation, 40 mL of seawater was extracted from each flask for injection into the P&T and GCMS system using a 100 mL gas-tight glass syringe. The F_v/F_m values of the seaweeds were once again measured. The seaweeds used in the incubation were then left to dry in the oven at 60 °C for 72 hours to determine the dry weight.

The determination of halocarbon content in the seawater was done as described in Section 3.1.3. A total of four compounds were monitored in this study: $CHBr_3$, CH_2Br_2, CH_2I_2 and CH_3I. The two mixed halocarbon compounds from the previous experiment, i.e. $CHBr_2Cl$ and $CHBrCl_2$, were omitted due to the low emission rates released by *K. alvarezii*. Calibration curves at each of the exposure temperatures were established for the determination of halocarbon concentrations.

3.2.1 Statistical analysis

One-way ANOVA was conducted to determine the effect of irradiance and temperature (Figure 4.4 and Table 4.6) on halocarbon emission rates. It was also used to determine the effect of irradiance (Figures 4.5 and 4.7, Appendix G) and temperature (Appendix F) on the F_v/F_m values of the seaweed samples. Normality and homogeneity of variance were also assessed.

Two-way ANOVA was conducted to examine the effects of temperature and irradiance level on halocarbon emissions by the seaweeds. Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were assessed by inspection of a boxplot; normality was assessed using Shapiro-Wilk's normality test for each cell of the design and the homogeneity of variances was assessed by Levene's test.

The Pearson Product-Moment Correlation was run to assess the relationships of halocarbon emission rates with irradiance and with temperature, the emission rates of each halocarbon, and the percentage changes in F_v/F_m of halocarbon emissions with variations in temperature and irradiance. All statistical analyses were done using SPSS Statistics Version 22 (IBM).

3.3 *In situ* measurement of halocarbon emissions during cultivation

3.3.1 Onshore tank cultivation system

This study was conducted to profile the halocarbon emission by *G. manilaensis*, *K. alvarezii* and *U. reticulata* (Figure 3.5) cultivated in onshore tank cultivation system, during daylight and dark.

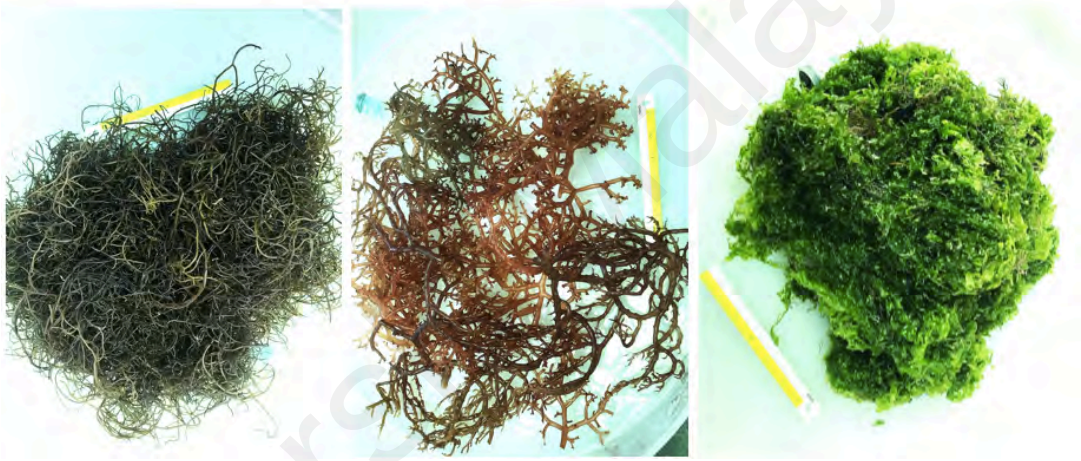


Figure 3.5: The cultivated seaweed species; from left to right: *G. manilaensis, K. alvarezii* and *U. reticulata*

The overflow cultivation system was set up during the Grand Challenge project at the Bachok Marine Research Station (BMRS), Institute of Ocean and Earth Sciences (IOES), Kelantan. Each of the overflow system consist of three inter-connected plastic tanks each measuring 1.3 x 0.9 x 0.5 m attached to a reservoir (Figure 3.6). Seawater collected 300 m from shore was pumped in and filtered twice using sand filter followed by filtration net prior to filling the tanks. Approximately 1000 L of seawater was filled between the reservoir and the three tanks, and continuous flow of the seawater was maintained by

underwater pumps. The designated tanks were each filled with 1kg of *G. manilaensis*, 500 g of *K. alvarezii* and 200 g of *U. reticulata. U. reticulata* was only cultivated during the first sampling trip. Seaweeds sampled during sampling trips 2 and 3 were of the same batch (Table 3.4).

For measurement of environmental parameters, data loggers were attached to the system to record the irradiance and temperature, and seawater salinity was determined during sampling and seawater samples were collected and transported back in a chilled condition for analysis of nutrient contents.

Air samples collected after sunrise and before sunset were categorized as 'daylight' samples, while air samples collected after sunset were categorized as 'dark'. Both daylight (n = 5) and dark (n = 4) air samples without seaweeds (Control) were also collected throughout the sampling trips for comparison to the seaweed air samples.

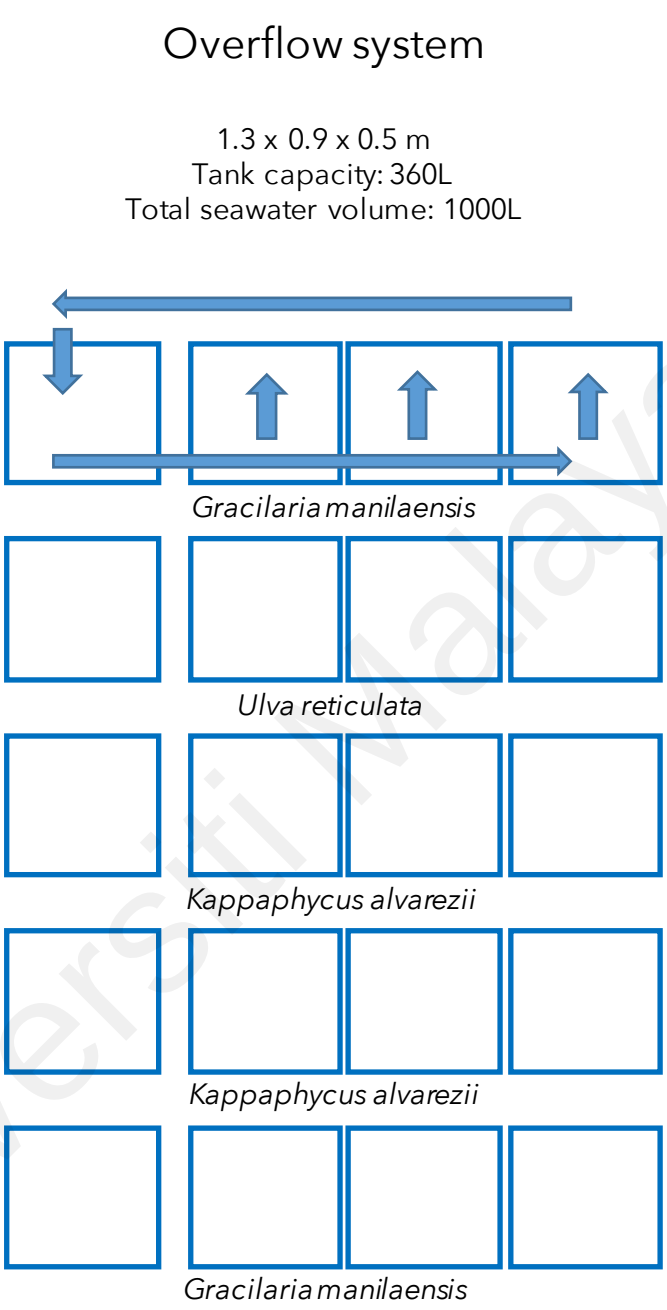


Figure 3.6: The layout of the overflow system used in the onshore tank cultivation at BMRS, Bachok. Arrows indicate seawater flow direction. Blue squares indicate individual tanks; reservoir tank (left) of each system was separated from the three inter-connected tanks containing seaweeds as labelled.

Table 3.4: Date of sampling trips, the culture age of seaweeds from which sample samples (through the use of flux chamber and air canisters) were collected, the sampling condition i.e. daylight and dark, and the number of air samples taken from the onshore tank cultivation system

Sampling Trip		Seaweed Culture Age	Sampling Condition / Number of samples collected							
No.	Date		Daylight				Dark			
			C	GM	KA	UR	C	GM	KA	UR
1	4 – 5 Apr 2018	1 week	1	2	1	1	2	1	1	1
2	8 – 10 Oct 2018	<3 days	1	3	3	-	1	3	-	-
3	30 – 31 Oct 2018	4 weeks	1	2	2	-	1	3	2	-

C = Control (without seaweeds); GM = *G. manilaensis*; KA = *K. alvarezii*; UR = *U. reticulata*;

3.3.2 Offshore platform

To profile the emission of halocarbon by *G. manilaensis* and *K. alvarezii* during farming cycle, an offshore platform measuring 5 x 5 m made of wooden plank and blue tongs was constructed. The platform was anchored at 600–650 m from the coast and was deployed for a duration of four weeks for the cultivation of *G. manilaensis* and *K. alvarezii*.

Seaweeds of the same batch as the onshore tank cultivation system in October 2018 were used for this cultivation system. Baskets of 0.3 x 0.3 x 0.1 m made of fishing net wound around PVC tubings, were tied to a rope line and kept afloat with plastic bot. Each basket contains 500 g of *G. manilaensis*, and three baskets were evenly hanged along a 50 m nylon rope line (Figure 3.7). The monoline culture method was used for the cultivation of *K. alvarezii* at the offshore platform. Bunches of *K. alvarezii* each weighing 150 g were tied along a rope at an interval of 20 cm, with a 15 cm gap between the seaweed bunches and the rope. All lines were 0.5 m apart from each other. Data loggers to collect irradiance and temperature data were tied to the rope lines. There was a total of three rows of *G. manilaensis* in the hanging baskets and five lines of *K. alvarezii* bunches cultivated at the platform between 4 – 31 October 2018.

Air samples of the seaweeds were collected during the 1st (day 1), 2nd (day 11th) and 4th (day 23rd) week of cultivation at the offshore platform. To achieve this, one random rope line containing the hanging baskets of *G. manilaensis* and one with the *K. alvarezii* bunches were collected by boat and sent back to the hatchery, the same place where air samples of seaweeds cultivated at the onshore tank system was taken. Randomly chosen seaweeds were then detached from the line for air sampling (See Table 3.5). All air sampling of seaweeds from the offshore platform were carried out during the day and seawater samples were collected for determination of nutrient contents. As with the

onshore tank sampling, Control air samples were collected during daylight for comparison with the air samples from the seaweeds. As the sampling dates of this cultivation system coincides with the sampling dates of the onshore tank culture (Trips 2 and 4; Table 3.4), the daylight Control measurements were used to compared between the onshore tank and the offshore platform samples (n = 3, one from each sampling trip).

Table 3.5: Sampling details of seaweeds cultivated at the offshore platform for the collection of air samples using flux chamber attached to canister

Sampling Date	Time into cultivation	Culture Age	Number of samples collected		
			C	GM	KA
8 – 10 Oct 2018	Week 1	1 day	1	3	2
18 Oct 2018	Week 2	11 days	1	3	3
30 – 31 Oct 2018	Week 4	23 days	1	3	2

C = Control; GM = *G. manilaensis*; KA = *K. alvarezii*

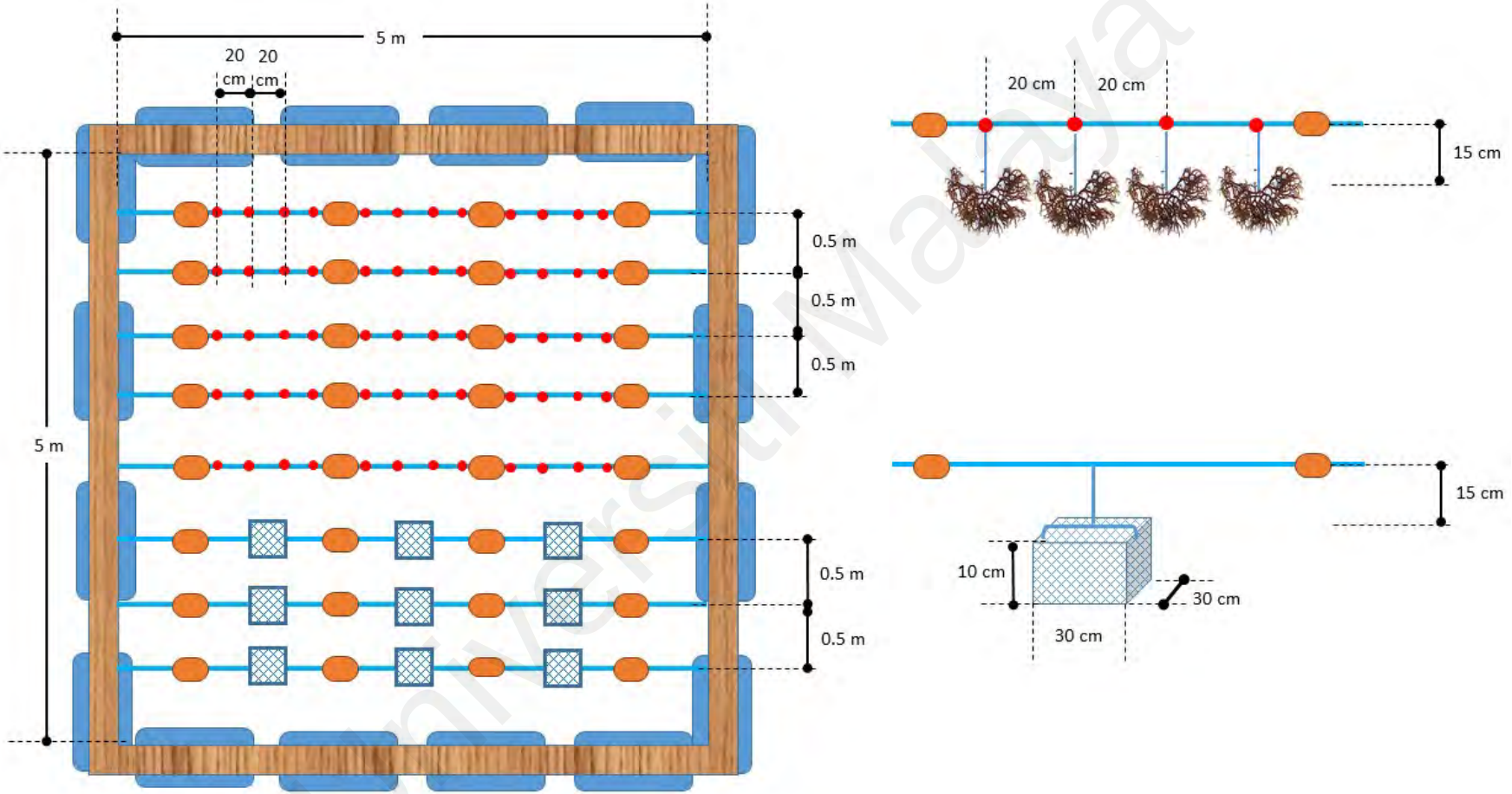


Figure 3.7: Layout of the offshore platform built for the cultivation of *G. manilaensis* and *K. alvarezii*

3.3.3 Cage culture

To measure the emission of halocarbon released by seaweed cultivated in cages, a cage culture system with three compartments as replicates was constructed out of the same material as the hanging basket used in the offshore platform cultivation system. The three compartments were each filled with 500 g of *G. manilaensis.* The cage culture system measuring 1.5 x 1.0 x 0.5 m (Figure 3.8 (D)) was then deployed at the river mouth of Sungai Melawi (6º1'23.24" N, 102º25'3.47" E), close to the BMRS.

Air sampling was conducted two weeks into the cultivation on 18th October 2018, two air samples from *G. manilaensis* and a Control air sample (without seaweeds) was collected at the riverside at around 3 pm. Seawater samples were collected for determination of nutrient content (see Section 3.1.5) and salinity during air sampling. Data loggers attached to the cultures provided temperature and irradiance readings.

Figure 3.8: The three culture systems from which seaweeds were cultivated; A: Onshore tanks, B: Offshore platform, C: Sungai Melawi, D: Cage culture

3.3.4 Air sample collection

Air samples containing halocarbons from the sampling sites were collected using SilcoCan canisters (Entech Instruments) made for air monitoring. The Silonite-coated stainless-steel canister has been reported to be inert and is able to contain volatile organic compounds for up to five months after sampling (Brinckmann *et al.*, 2012). Canisters were cleaned through repeated baking (at 90 °C) and flushing with high purity nitrogen gas (Linde Malaysia) for six cycles before bringing down the pressure to −25 psi (Canister Cleaning System, Entech).

A custom-made flux chamber (Figure 3.9) was deployed for the collection of air. The 40 L flux chamber was designed and modified according to the EPA-recommended flux chamber (Eklund, 1992) and as described by Sartin *et al.* (2002). A small battery-operated (12V) axial fan unit was built-in to provide better air circulation in the chamber. Temperature fluctuations during incubation were observed via a thermocouple inserted through an inlet on the flux chamber. The average observed temperature increase in the flux chamber during the 30 minutes of incubation was 0.65 °C and ranged between +0.06 (dark samples) to +2.01 °C. A temperature change of up to +15 °C has been reported elsewhere (Dimmer *et al.*, 2001). The lower fluctuation in temperature in this study could be due to the built-in axial fan and the onshore location where incubations were conducted.

Whole seaweeds weighing around 500 g were placed in the flux chamber for an incubation time of 30 minutes (Sartin *et al.*, 2002) prior to collection of the air samples. In this study, all halocarbon mixing ratios (ppbv) measured from the air samples of cultivated seaweeds represent the emissions from 500 g of seaweed biomass. In cases where seaweed samples were more or less than 500 g, the mixing ratios of halocarbons emitted were calculated from the emissions per gram of seaweed and standardized to the 500 g level.

The seaweeds were then blotted dry for weighing, then moistened by sprinkling seawater or river water for incubation purposes. Control air samples for comparison with seaweed air samples were collected by incubating the flux chamber without seaweeds and collecting the air 30 minutes after incubation. Data logger was placed within the flux chamber during each incubation to collect irradiance data.

The air samples were collected into the canisters by the use of PFA transfer tubing connected to the canister valve. A motor pump (Air Dimensions Inc.) connected to a rechargeable battery (12V) was used to enable filling of the canister to a pressure that was higher than the atmospheric level (Figure 3.10). The pre-evacuated canisters were flushed once with sampling air by filling the canisters with the sample air up to approximately 10 psi before the content was vented. The sample was then collected by filling the cannisters to 10 psi. Samples were then transported back to the laboratory for analysis within three weeks.

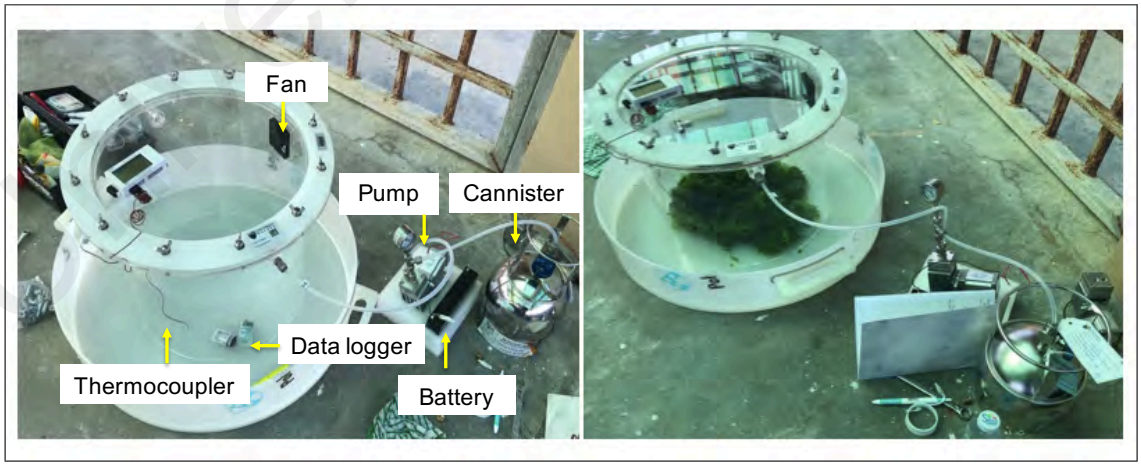


Figure 3.9: Flux chamber deployed at Bachok Marine Station, for *in situ* study of halocarbon emissions by cultivated seaweed species; left: Control, right: measurement of emissions from *Ulva reticulata*

3.3.5 Sample analysis

Filled canisters were attached to the inlet tubing of an Entech pre-concentrator (7200 Sample Preconcentrator, Entech Instruments) coupled to an Agilent GCMS (7890B/5977B, Agilent Technologies). The pre-concentrator utilises a three-stage trapping known as 'Extended Cold Trap Dehydration' to improve the sensitivity of the analyses. This includes passing the air samples through an empty trap (M1) treated with the inert Silonite-D pre-cooled to −40 °C, eliminating the water in the samples. The analytes were then transferred to a second stage Tenax trap (M2) at −50 °C for trapping. This was followed by a back-desorption of the Tenax trap to the third and final focusing trap (M3) prior to injection into the GCMS system (Markle *et al.*, 2017). Further details on the pre-concentration parameters are summarised in Table 3.6.

A sample volume of 100 cc was filled into the pre-concentrator each time during analysis. A series of pre-concentrations ensued with the use of liquid nitrogen and Tenax before injection into the GCMS. The GC system was fitted with a 60 m capillary column (J&W DB-VRX; film thickness 1.40 μm, internal diameter 0.25 mm). The GC oven was programmed to hold the temperature at 35 °C for 10 minutes and ramp up to 220 °C at a rate of 8 °C min^{-1} and hold for 3 minutes (Markle *et al.*, 2017). The analyte concentrations were then determined by comparing the peak areas against the peak areas of the standard gas. The standard gas containing TO14 and TO15 compounds was blended down to a concentration of 10 ppbv (courtesy of Entech Instruments; see Appendix B for compound list), with a calibration volume of 10 cc. Of these, only four abundant compounds, CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$ and $CHBr_3$, were chosen for monitoring. A total of three technical replicates were analysed from each canister containing air samples.

Table 3.6: Trapping conditions for air samples containing halocarbons in the pre-concentrator prior to GCMS analysis

	Trap	Sweep	M1-M2	M2-M3	Bakeout
M1 Empty Trap (°C)	−40	−40	10	10	150
M2 Tenax Trap (°C)	−50	−50	−50	230	220
M3 Focuser	N/A	−175	N/A	−150	N/A
Volume (cc)	100	75	50	20	N/A
Flow rate (cc/min)	60	60	10	6	N/A

The precision of the analytical system in this study was determined by the variability of the replicates of the standard and is represented as percentage standard deviation (%1σ, Table 3.7). The detection limit was derived from the standard deviation (σ) and the blank mean ($\bar{x}$), as shown in Equation 3 (Kaiser, 1970).

Equation 3: Detection limit = $\bar{x} + 3\sigma$

Table 3.7: System precision and detection limits for air analysis

Compound	Precision (%1σ)	Detection Limit (ppbv)
CH_2Br_2	4.48	<1
$CHBrCl_2$	3.21	<1
$CHBr_2Cl$	9.11	<1
$CHBr_3$	14.9	<1

3.3.6 Statistical analysis

Mann-Whitney U test was conducted to compare the difference between daylight and dark emissions at the onshore tank cultivation system (Table 4.12). One-way ANOVA was conducted to determine the effect of different cultivation systems on halocarbon emission rates of *G. manilaensis* (Table 4.16) and to compare the means between seawater nutrient and salinity levels among the different cultivation systems (Table 4.19). Meanwhile, a Student's t-test was used to test the difference between onshore and offshore cultivation systems on the emissions of halocarbons by *K. alvarezii* (Table 4.18).

Pearson's bivariate and Pearson's partial correlation was run to assess the relationship between temperature and halocarbon emissions by *G. manilaensis* (Table 4.21) and *K. alvarezii* (Table 4.23) after adjusting for irradiance. The linear relationships between these factors were assessed by scatterplots and partial regression plots. The normality of transformed data assessed by Shapiro-Wilk's test ($p > 0.05$), and there were no univariate or multivariate outliers, as assessed by boxplots and Mahalanobis Distance respectively.

The Pearson Product-Moment Correlation was run to assess the relationship between the halocarbon mixing ratios in the air samples of *G. manilaensis* (Table 4.22) and *K. alvarezii* (Table 4.24), with seawater nutrient and salinity levels measured throughout the sampling period. All statistical analyses were done using SPSS Statistics Version 22 (IBM).

CHAPTER 4: RESULTS

4.1 Effect of temperature on halocarbon emissions by selected tropical seaweeds under controlled laboratory conditions

4.1.1 Emissions of halocarbons by the selected seaweeds

The emission rates of $CHBr_3$ by all four seaweeds were found be the highest among the six halocarbon compounds investigated, followed by CH_2Br_2 and CH_2I_2 (Figure 4.1). The averaged emission rates of $CHBr_3$ by both *K. alvarezii* and *T. conoides* under the ambient conditions, 4 hours post-exposure and 28 hours post-exposure were generally higher compared to *G. manilaensis* and *U. reticulata* except for the latter at 30 °C, 4 hours post-exposure at 528 ± 190 pmol gFW^{-1} Hr^{-1} (Appendix C). The highest averaged emission rate observed throughout the experiment was of $CHBr_3$ by *K. alvarezii* at 561 ± 46 pmol gFW^{-1} Hr^{-1}. The average emission rates of $CHBr_3$ under ambient conditions from *K. alvarezii* and *T. conoides* ranged from 330 ± 138 pmol gFW^{-1} Hr^{-1} to 561 ± 46 pmol gFW^{-1} Hr^{-1} and from 332 ± 13 pmol gFW^{-1} Hr^{-1} to 454 ± 66 pmol gFW^{-1} Hr^{-1} respectively. Emissions of $CHBr_3$ from *G. manilaensis* and *U. reticulata* under the same conditions ranged from 16 ± 1.7 pmol g FW^{-1} Hr^{-1} to 34 ± 11 pmol gFW^{-1} Hr^{-1} and from 4.6 ± 1.7 pmol gFW^{-1} Hr^{-1} to 140 ± 59 pmol gFW^{-1} Hr^{-1} respectively. Based on the averaged emission rates at the ambient condition (Appendix C), *T. conoides* was the highest emitter of CH_2Br_2, CH_2I_2 and CH_3I at most of the exposure durations compared to the other three seaweeds. *K. alvarezii* was the second highest emitter of CH_2Br_2 and CH_2I_2 after *T. conoides*. *K. alvarezii* is also the highest emitter of $CHBrCl_2$ at 2.0 ± 0.8 pmol gFW^{-1} Hr^{-1} under ambient conditions. *U. reticulata* emitted the highest amount of $CHBr_2Cl$ at 26 ± 16 pmol gFW^{-1} Hr^{-1}.

4.1.2 Effect of exposure duration and varying temperature treatments on halocarbon emissions by the selected seaweeds under controlled laboratory conditions

The effect of temperature on the halocarbon emission rates of the four seaweeds is summarised in Figure 4.1. Exposure at 40 °C for 4 hours did not significantly ($p< 0.05$) alter the emission rates of $CHBr_3$ and CH_2Br_2 by *G. manilaensis* and *U. reticulata* from the ambient condition. On the contrary, *K. alvarezii* and *T. conoides* showed significant drops in the emission rates of the two compounds, compared to the ambient conditions (Figure 4.1). Upon extended exposure duration from 4 to 28 hours at 40 °C, the emission rates of $CHBr_3$ and CH_2Br_2 for all four seaweeds decreased significantly ($p<0.05$), except for the emission of $CHBr_3$ by *T. conoides* where the decrease in emissions was insignificant. A huge decrease in emissions is particularly evident in *G. manilaensis.*

At the same temperature, a shorter exposure period (4 hours) did not significantly change the emission rates of CH_2I_2 by *G. manilaensis* and *U. reticulata*, while a decrease in the emission rates of CH_2I_2 by *K. alvarezii* and *T. conoides* was observed. Prolonged exposure saw overall decreased emission rates of CH_2I_2 from all four seaweeds. The emission rates of CH_3I at 4 hours post-40 °C treatment by both *G. manilaensis* and *U. reticulata* increased from ambient conditions and increased further upon an extended exposure duration. *K. alvarezii* and *T. conoides*, however, showed contrasting results.

At 40 °C, emission rates of $CHBr_2Cl$ by all seaweeds start decreasing at 4 hours post-exposure, and significantly ($p<0.05$) lowered emission rates of $CHBr_2Cl$ were observed at 28 hours post-exposure to 40 °C treatment by all four seaweeds, compared to ambient conditions, except *U. reticulata*. $CHBrCl_2$ emissions were somewhat similar between *G. manilaensis* and *U. reticulata,* and between *K. alvarezii* and *T. conoides* at this temperature.

Similarities were also observed between the emissions of $CHBr_3$, CH_2Br_2, CH_2I_2, and $CHBr_2Cl$ by the four seaweeds when the conditions change from ambient to 4-hours and 28-hours exposure at 40 °C.

At 35 °C, the changes in the emissions of $CHBr_3$ by *G. manilaensis* and *K. alvarezii* remained insignificant across the different exposure periods, while *U. reticulata* and *T. conoides* showed increased emissions at 4 hours post-exposure followed by a decrease at 28 hours (Figure 4.1). Emission rates of CH_2Br_2 by *G. manilaensis* decreased from ambient conditions when first exposed to the temperature treatment, followed by an increase when the treatment time was prolonged (Figure 4.1). As with $CHBr_3$, the emission rates of CH_2I_2 and CH_3I at 4 hours and 28 hours post-exposure by the same species did not differ significantly (p<0.05), while $CHBrCl_2$ increased 28 hours after exposure to 35 °C. *U. reticulata* showed a uniform trend of increased emission rates for all the halocarbon compounds at 4 hours post-exposure to 35 °C, followed by a decrease after 28 hours at the same temperature. At the same temperature, however, albeit the greater standard deviations observed, *K. alvarezii* showed insignificant decrease (p<0.05) in the average emission rates of all the halocarbon compounds across the exposure durations from the ambient conditions to 4 hours and 28 hours post-exposure to 35 °C treatment. The emissions of $CHBr_3$, $CHBr_2Cl$ and $CHBrCl_2$ by *T. conoides* showed that 4-hour exposure to 35 °C triggered a surge in emissions of the compounds.

The emission trends across the exposure durations showed by *U. reticulata* and *K. alvarezii* at 30 °C were similar to those at 35 °C, except for the emissions of CH_2I_2 by *U. reticulata* and $CHBrCl_2$ by both seaweeds. At this temperature, however, the standard deviations were not as large as those observed at 35 °C by *K. alvarezii*. Insignificant differences in the emission rates of CHB_3, CH_2Br_2, CH_2I_2 and CH_3I were observed at 30 °C by *G. manilaensis*, similar to 35 °C. Difference in the emission rates of $CHBr_3$, CH_2Br_2

and $CHBr_2Cl$ by *T. conoides* across the different exposure periods were rather similar at a temperature of 30 °C.

At the lowest temperature of 20 °C, *G. manilaensis* showed a similar trend of decreasing emission rates for all compounds except $CHBrCl_2$ from ambient conditions to 4-hours and 28-hours treatment, rather similar to that observed at 25 °C. *U. reticulata* showed an increased rate of emission followed by decrease for all compounds when the temperature fell from ambient to 20 °C for the first 4 hours, followed by prolonged exposure at 20 °C for 28 hours. *K. alvarezii* showed decreased emission rates for $CHBr_3$, CH_2Br_2 and CH_2I_2 at 4 hours after exposure at 20 °C, while no significant differences ($p<0.05$) were observed for emissions of all other compounds. At the same temperature, the emission rates of all compounds except for CH_3I by *T. conoides* were rather similar throughout the exposure periods from ambient to 28 hours.

The trend of halocarbon emissions at higher temperatures of 40, 35 and 30 °C were comparably different from the trend observed at 25 °C (ambient).

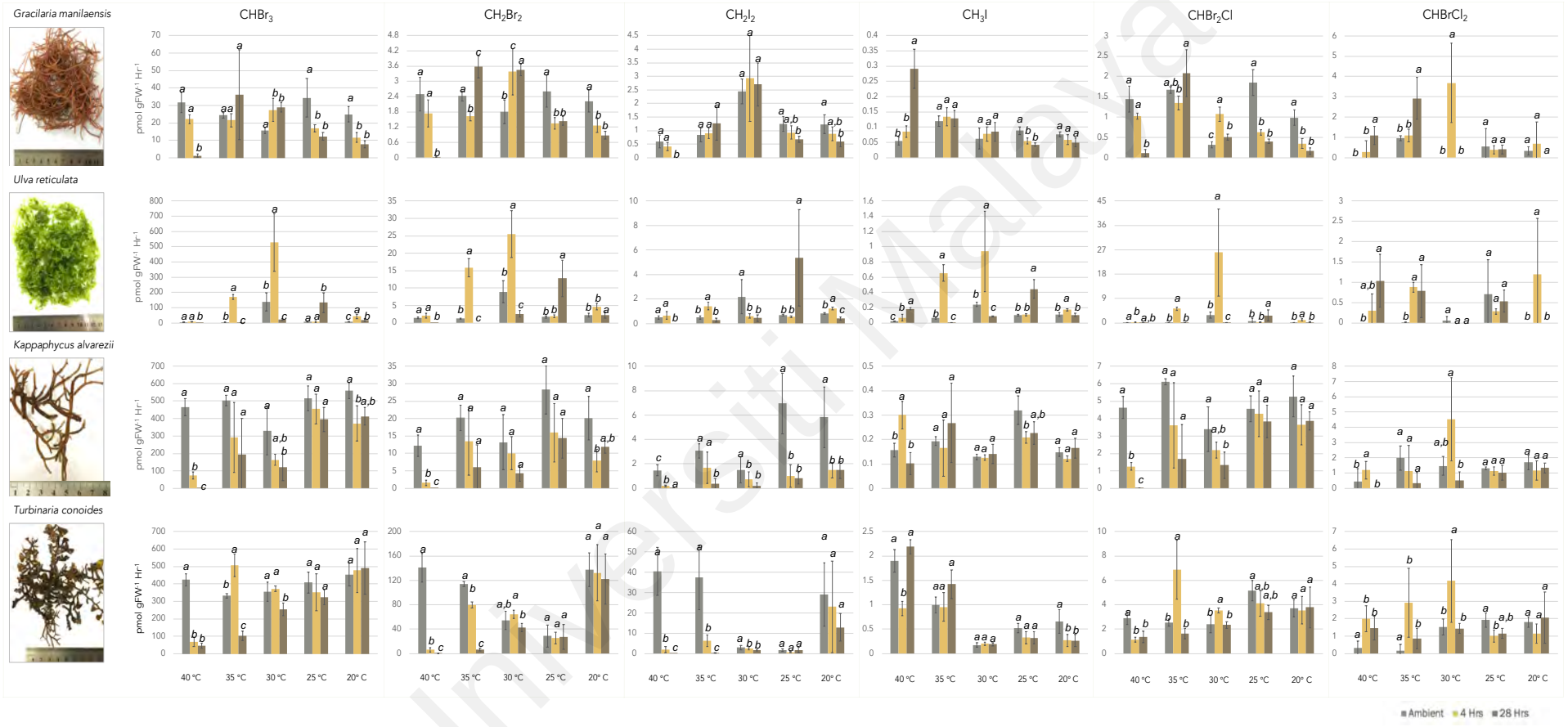


Figure 4.1: Average emission rates of halocarbons ± standard deviation (pmol gFW^{-1} hr^{-1}; n = 4) by the four seaweeds (*G. manilaensis, U. reticulata, K. alvarezii* and *T. conoides*) at the ambient conditions, 4 hours and 28 hours post-exposure to the varying temperature levels, i.e. 40, 35, 30, 25 and 20°C, tested using one-way ANOVA; ^{a,b,c} indicate homogenous groups across the incubation period based on Tukey's post-hoc test (p<0.05)

4.1.3 Correlation between halocarbon emissions and temperature

Significant correlations were observed between the emission of certain halocarbon compounds and temperature (Table 4.1). Significant positive correlations (p<0.05) occurred between the emissions by *G. manilaensis* and temperature during the first 4 hours of temperature treatment. Emission rates of halocarbons were mainly negatively (p<0.05) correlated to temperature change.

At the shorter (4 hours) duration of temperature exposure, the emission rates for $CHBr_3$, CH_3I and $CHBr_2Cl$ by *G. manilaensis* showed a significant positive correlation ($0.59 \leq r \leq 0.81$; p<0.01) with temperature change (Table 4.1). Compounds with a strong positive correlation to temperature change include $CHBr_3$ ($r = 0.64$; p<0.01) and $CHBr_2Cl$ ($r = 0.81$; p<0.01). The emissions of all halocarbons by *U. reticulata*, however, did not correspond well with temperature change in the first 4 hours of exposure. At the same exposure duration, the emissions of CH_3I by *K. alvarezii* and *T. conoides* showed strong ($r = 0.72$ and 0.75; p<0.01) positive correlation with temperature change, while emissions of $CHBr_3$ by the two seaweeds were negatively correlated to temperature ($r = -0.69$ and -0.63; p<0.01).

Stronger correlations were observed at 28 hours post-exposure to temperature treatment. Positive correlations were observed in the emissions of CH_3I by *G. manilaensis* and *T. conoides*, and $CHBrCl_2$ by *G. manilaensis* and *U. reticulata*. *U. reticulata,* having showed no correlation between the halocarbons emitted with temperature at the exposure duration of 4 hours, showed strong negative correlations ($r = -0.50$ to -0.74; p<0.01) in the emissions of $CHBr_3$, CH_2Br_2, CH_2I_2 and $CHBr_2Cl$ at 28 hours post-exposure to temperature treatment. Five out of six of the halocarbon compounds released by *K. alvarezii* and *T. conoides* showed a significant negative correlation with temperature at 28 hours post-exposure ($-0.95 \leq r \leq -0.80$; p<0.01). *U. reticulata, K. alvarezii* and *T.*

conoides showed strong negative correlations ($-0.95 \leq r \leq -0.69$; $p<0.01$) in the emissions of $CHBr_3$, CH_2Br_2 and CH_2I_2 with temperature 28 hours after exposure.

Table 4.1: Pearson Product-Moment Correlation Coefficient, *r*, of the halocarbon compounds to changes in temperature between 40 °C and 20 °C

	4 hours				28 hours			
	G. manilaensis	*U. reticulata*	*K. alvarezii*	*T. conoides*	*G. manilaensis*	*U. reticulata*	*K. alvarezii*	*T. conoides*
$CHBr_3$	0.641**	0.021 NS	−0.694**	−0.630 **	−0.223NS	−0.728**	−0.828**	−0.951**
CH_2Br_2	0.269 NS	−0.091 NS	−0.434 NS	−0.654**	−0.337 NS	−0.742**	−0.814**	−0.889**
CH_2I_2	−0.271 NS	−0.129 NS	−0.428 NS	−0.445 NS	−0.516 *	−0.689**	−0.801**	−0.927**
CH_3I	0.591**	0.029 NS	0.718**	0.745**	0.892**	−0.277 NS	−0.263 NS	0.822**
$CHBr_2Cl$	0.809**	0.039 NS	−0.543 *	−0.334 NS	0.344 NS	−0.495*	−0.827**	−0.821**
$CHBrCl_2$	−0.078 NS	−0.217NS	−0.151 NS	0.393 NS	0.671**	0.615**	−0.845**	−0.253 NS

** Correlation is significant at the 0.01 level ($p<0.01$; 2-tailed); * Correlation is significant at the 0.05 level ($p<0.05$; 2-tailed); NS Non-significant ($p>0.05$; 2-tailed); $n = 20$.

4.1.4 Temperature effect on F_v/F_m of seaweeds

Most of the maximum quantum yield (F_v/F_m) of the seaweeds did not differ significantly ($p<0.05$) at the start of the experiment (ambient conditions) (Appendix D). At 4 hours post-exposure to temperature treatments of 40 °C and 35 °C, both *K. alvarezii* and *T. conoides* showed significant drops of up to 93% and 95% (Figure 4.2, Appendix D) respectively from F_v/F_m values prior to treatment. All four seaweeds showed the biggest drops in F_v/F_m values at 40 °C, 4 hours after exposure (Figure 4.2). At the same exposure duration, changes in the F_v/F_m values of *G. manilaensis* and *U. reticulata* were not significant between temperature treatments of 35 °C to 25 °C. *T. conoides* showed insignificant differences in the F_v/F_m values at temperatures of 30 °C to 20 °C.

At 28 hours post-incubation, the F_v/F_m values of all seaweeds were lowest at 40 °C, at near to zero. *G. manilaensis* showed higher F_v/F_m values at temperatures of 35–20°C, which did not significantly vary with the different temperature treatments. *U. reticulata* showed higher F_v/F_m values at 30 °C and 20 °C, followed by 35 °C and 25 °C. The F_v/F_m values of *K. alvarezii* and *T. conoides* were lowest at 40 °C and 35 °C, and the values were not significantly affected by temperature treatment between 30 °C and 20 °C.

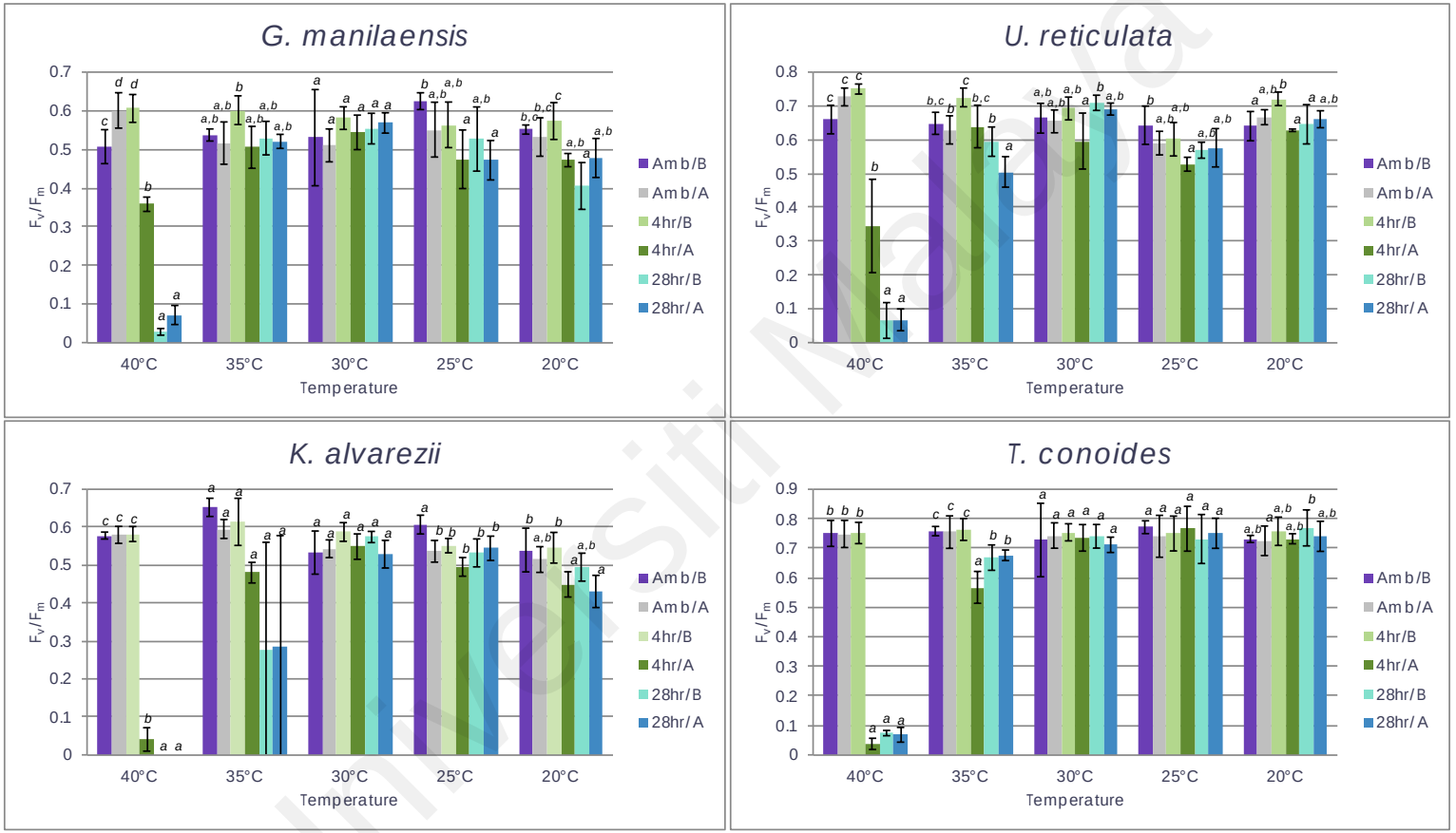


Figure 4.2: Average F_v/F_m values (with standard deviation; *n* = 4) of seaweeds measured before (/B) and after (/A) incubation, under ambient (Amb) conditions, 4 hours post-exposure (4hr) and 28 hours post-exposure (28hr) to temperature treatments of 40, 35, 30, 25 and 20 °C tested using one-way ANOVA; ^{a,b,c} indicate homogenous groups based on Tukey's post-hoc test ($p<0.05$)

4.1.5 Temperature effect on pigment contents of seaweeds

Pigment contents of the seaweeds showed different responses towards the temperature treatments 28 hours after exposure (Table 4.2). In *G. manilaensis*, a lower chlorophyll-a (Chl-*a*) concentration (9.17 ± 3.52 μg g^{-1}) was observed at 40 °C, while higher Chl-*a* concentrations (66.96–73.69 μg g^{-1}) were observed at lower temperatures of 25 °C and 20 °C (Table 4.2). Carotenoid content was highest at 40 °C, followed by the two lowest temperatures. Lowest Chl-*a* to carotenoid ratio (Chl-*a*: Car) was observed at 40 °C, followed by 25 and 20 °C, then 30 and 35 °C. In *U. reticulata*, a higher Chl-*a* content was observed at 35 °C (275.23 ± 28.45 μg g^{-1}) instead of 40 °C, while higher concentrations of carotenoids occur at the two temperature extremes of 40 °C and 20 °C, and also at 35 °C (120–158 μg g^{-1}). Lower Chl-*a*: Car was observed at 40, 25 and 20 °C compared to 35 and 30°C. *K. alvarezii* showed a significant decrease in Chl-*a* and carotenoid concentration as well as Chl-*a*: Car at 40 °C (1.43 ± 0.11 μg g^{-1} and 0.63 ± 0.05 μg g^{-1} respectively), while insignificant changes were observed at temperatures of 35–20 °C. The same response was observed in *T. conoides* except that no bleaching was visible. Among the four seaweeds, *K. alvarezii* contains the lowest amount of Chl-*a* and carotenoids, while *U. reticulata* contains the highest amounts at 28 hours after the temperature treatment.

When halocarbon emission rates from all temperature levels at 28 hours post-exposure were plotted against the pigment content of seaweeds, positive correlations were found between the Chl-*a* content of *G. manilaensis* and *K. alvarezii* with the emission rates of $CHBr_3$, CH_2Br_2 and CH_2I_2 (r = 0.48–0.90; p<0.05). Negative correlations were observed between carotenoid content and the emission rates of $CHBr_3$, CH_2Br_2 and CH_2I_2 by *G. manilaensis* and with the emission rates of $CHBr_3$ and CH_2Br_2 by *U. reticulata* (r = −0.49 and −0.59); p<0.05). However, both the carotenoid and Chl-*a* contents were found to be

positively correlated to all halocarbons in *K. alvarezii* (r = 0.62–0.90; p<0.01). Pigment content in *T. conoides* was not strongly correlated to the emission rates of all halocarbon (Table 4.3). Negative correlations (r = −0.96–(−0.49); p<0.05) between the Chl-*a* to carotenoid ratio (Chl-*a*: Car) and the emission rate of most halocarbon compounds (except CH_2Br_2) were observed in *G. manilaensis. K. alvarezii* showed positive correlations (r = 0.54–0.66; p<0.05) between Chl-*a*: Car and the emission rate of all halocarbon compounds except CH_2I_2 and CH_3I. *U. reticulata* showed negative correlation between the emission rate of CH_3I (r = −0.60; p<0.01) with Chl-*a*: Car, while no strong correlations were established by *T. conoides* (Table 4.3).

Table 4.2: Chl-*a* (µg g^{-1}), carotenoid (µg g^{-1}) contents and the chlorophyll-a to carotenoid ratio (Chl-*a*: Car) of the four seaweeds measured upon completion of temperature exposure at 40, 35, 30, 25 and 20 °C; data was statistically analysed using one-way ANOVA to determine the effect of temperature on the respective pigment contents and Chl-a: Car ratios

Temperature	Chl-*a*	Carotenoids	Chl-*a*: Car
G. manilaensis			
40 °C	9.17 ± 3.52^{a}	73.69 ± 10.99^{c}	0.1 ± 0.1^{a}
35 °C	45.48 ± 3.42^{b}	10.47 ± 1.42^{a}	4.4 ± 0.5^{c}
30 °C	54.05 ± 6.03^{b}	13.04 ± 2.03^{a}	4.1 ± 0.3^{c}
25 °C	66.96 ± 6.52^{c}	21.17 ± 3.00^{b}	3.2 ± 0.2^{b}
20 °C	73.69 ± 10.99^{c}	22.52 ± 2.93^{b}	3.3 ± 0.1^{b}
U. reticulata			
40 °C	$182.41 \pm 76.47^{a,b}$	$120.43 \pm 46.03^{b,c}$	1.5 ± 0.1^{a}
35 °C	275.23 ± 28.45^{c}	157.95 ± 14.33^{c}	1.7 ± 0.0^{b}
30 °C	$160.28 \pm 29.93^{a,b}$	$90.22 \pm 16.93^{a,b}$	1.8 ± 0.1^{b}
25 °C	122.56 ± 25.10^{a}	81.82 ± 15.74^{a}	1.5 ± 0.1^{a}
20 °C	195.30 ± 63.82^{b}	131.80 ± 41.97^{c}	1.5 ± 0.1^{a}
K. alvarezii			
40 °C	1.43 ± 0.11^{b}	0.63 ± 0.05^{a}	2.3 ± 0.0^{a}
35 °C	5.55 ± 2.07^{a}	1.81 ± 0.83^{b}	3.2 ± 0.4^{b}
30 °C	5.62 ± 0.39^{a}	1.65 ± 0.22^{b}	3.4 ± 0.4^{b}
25 °C	8.19 ± 1.42^{c}	2.50 ± 0.89^{b}	3.5 ± 0.6^{b}
20 °C	6.03 ± 1.57^{a}	1.78 ± 0.53^{b}	3.4 ± 0.3^{b}
T. conoides			
40 °C	44.41 ± 18.11^{a}	17.65 ± 9.13^{a}	2.3 ± 0.2^{a}
35 °C	$50.34 \pm 11.14^{a,b}$	26.67 ± 5.12^{b}	$1.9 \pm 0.1^{a,b}$
30 °C	64.93 ± 4.94^{b}	26.97 ± 2.04^{b}	2.4 ± 0.4^{b}
25 °C	$48.48 \pm 1.80^{a,b}$	$24.69 \pm 0.75^{a,b}$	$2.0 \pm 0.1^{a,b}$
20 °C	$48.68 \pm 6.13^{a,b}$	$24.00 \pm 2.43^{a,b}$	$2.0 \pm 0.1^{a,b}$

^{a,b,c} indicate homogenous groups across temperature for each pigments and the Chl-*a*: Car ratio based on Tukey's post-hoc test ($p<0.05$); $n = 12$.

Table 4.3: Pearson Product-Moment Correlation coefficient, *r*, between the halocarbon emission rates at 28 hours post-exposure and the chlorophyll-a (Chl-*a*), carotenoid (Car), and chlorophyll-*a* to carotenoid ratio (Chl-*a*: Car) of the seaweeds

Temperature	Chl-*a*	Carotenoids	Chl-*a*: Car
G. manilaensis			
$CHBr_3$	0.481^{*}	-0.809^{**}	-0.840^{**}
CH_2Br_2	0.628^{**}	-0.924^{**}	0.962^{**}
CH_2I_2	0.729^{**}	-0.946^{**}	-0.957^{**}
CH_3I	-0.951^{**}	0.686^{**}	-0.639^{**}
$CHBr_2Cl$	-0.006^{NS}	-0.566^{*}	-0.624^{**}
$CHBrCl_2$	-0.508^{*}	-0.518^{*}	-0.485^{*}
U. reticulata			
$CHBr_3$	-0.504^{*}	-0.488^{*}	-0.149^{NS}
CH_2Br_2	-0.630^{**}	-0.586^{**}	-0.242^{NS}
CH_2I_2	-0.299^{NS}	-0.334^{NS}	-0.056^{NS}
CH_3I	-0.658^{**}	-0.528^{*}	-0.601^{**}
$CHBr_2Cl$	-0.411^{NS}	-0.327^{NS}	-0.414^{NS}
$CHBrCl_2$	0.178^{NS}	0.213^{NS}	-0.239^{NS}
K. alvarezii			
$CHBr_3$	0.899^{**}	0.800^{**}	0.589^{**}
CH_2Br_2	0.891^{**}	0.768^{**}	0.661^{**}
CH_2I_2	0.730^{**}	0.640^{**}	0.424^{NS}
CH_3I	0.631^{**}	0.618^{**}	0.308^{NS}
$CHBr_2Cl$	0.881^{**}	0.775^{**}	0.586^{**}
$CHBrCl_2$	0.756^{**}	0.660^{**}	0.544^{*}
T. conoides			
$CHBr_3$	0.186^{NS}	0.344^{NS}	-0.205^{NS}
CH_2Br_2	0.284^{NS}	0.384^{NS}	-0.119^{NS}
CH_2I_2	0.185^{NS}	0.314^{NS}	-0.181^{NS}
CH_3I	-0.344^{NS}	-0.371^{NS}	-0.009^{NS}
$CHBr_2Cl$	0.072^{NS}	0.267^{NS}	-0.242^{NS}
$CHBrCl_2$	0.052^{NS}	-0.057^{NS}	0.281^{NS}

** Correlation is significant at the 0.01 level ($p<0.01$; 2-tailed); * Correlation is significant at the 0.05 level ($p<0.05$; 2-tailed); ^{NS} Non-significant ($p>0.05$; 2-tailed); $n = 20$

4.2 Combined effect of temperature and irradiance on the halocarbon emissions of *Kappaphycus alvarezii*

4.2.1 The halocarbon emission rates and trends at varying temperature and irradiance

K. alvarezii emitted $CHBr_3$ at the highest rate among the four compounds investigated (Figure 4.3). The highest average emission rate was 609 ± 75 pmol gFW^{-1} hr^{-1}, observed at L3 at 28 °C (Appendix E). The lowest emission rates were observed for CH_3I across all temperatures. The highest emission rate of CH_3I was 0.15 ± 0.03 pmol gFW^{-1} hr^{-1} at L3, at the lowest temperature of 25 °C (Appendix E). The emission rates in decreasing order from *K. alvarezii* based on the highest recorded rate of each compound at each temperature was as follows: $CHBr_3 > CH_2Br_2 > CH_2I_2 > CH_3I$. *K. alvarezii* emitted higher levels of brominated than iodinated halocarbons, up to approximately 600 times higher, based on a comparison of the highest emission rate of $CHBr_3$ (609 ± 75 pmol gFW^{-1} hr^{-1}) and lowest emission rate of CH_3I and CH_2I_2 (0 ± 0 pmol gFW^{-1} hr^{-1}).

As observed from the results, increasing irradiance levels led to an increase in halocarbon emissions from dark samples (L1) – up to 117 ± 13 pmol gFW^{-1} hr^{-1} (L3) or 177 ± 26 pmol gFW^{-1} hr^{-1} (L4) – at most temperatures (Figure 4.3). There was an upward trend in the emission of CH_3I when irradiance was raised from L1 to L3, peaking at L3 before decreasing, when the exposure temperature was set at 25, 28 and 31 °C. At the highest temperature of 34 °C, however, the CH_3I emission rate showed an upward trend from L1 to L4, with emissions at L4 significantly higher (0.14 ± 0.04 pmol gFW^{-1} hr^{-1}) than in the dark (L1, 0.07 ± 0.01 pmol gFW^{-1} hr^{-1}).

The emission rates of CH_2I_2, CH_2Br_2 and $CHBr_3$ were somewhat similar (Figure 4.3). Lower emission rates were observed during dark treatment at all temperatures except at 31 °C. Lower emission rates were observed at higher temperatures, i.e. 31 and 34 °C, especially for CH_2I_2 and $CHBr_3$ (Figures 4.3 and 4.4). The trend in emission rates at 31 °C was not evident at L1, L2 and L3, but peaked at L4, indicating higher irradiance, i.e. 177 ± 26 μmol photons m^{-2} s^{-1}, which could trigger an increase in the emission rates of the three compounds.

The emissions of CH_2I_2 were generally highest at 28 °C and lowest at 31 °C. At 28 °C, the emission rate increases with increasing irradiance, while at 25 and 34 °C, emissions increase with increasing irradiance but drop when irradiance increases beyond L3 (Figure 4.3). This could mean that irradiance influences the emission of halocarbons at these two temperature extremes, i.e. 25 and 34 °C.

The same trend was observed in the emission of CH_2B_{r2} by *K. alvarezii* at 25, 28 and 34 °C. The emission rate of this compound increased up to L3 and dropped at L4. Insignificant differences in the emission rate of CH_2Br_2 were observed at irradiance levels of L1, L2 and L3 at 31 °C (Figure 4.3).

The emission of $CHBr_3$ increased with increasing irradiance at the lowest temperature of 25 °C. At 28 and 34 °C, the emission rate increases with irradiance from dark to L3 and falls at L4. The same trend as for the emissions of CH_2I_2 and CH_2Br_2 was observed for $CHBr_3$ at 31 °C (Figure 4.3).

When observed across temperature levels (Figure 4.4), the emission trends for three of the four halocarbon compounds were hard to establish, with the exception being $CHBr_3$. The average emission rate of CH_2I_2 was highest at 28 °C with irradiance, i.e. L2, L3, L4. The emission rates of CH_2Br_2 were similar at all temperature treatments in the dark.

The statistical analysis showed that emissions of CH_3I, CH_2I_2, CH_2Br_2 and $CHBr_3$ by *K. alvarezii* were significantly (p<0.01) affected by irradiance, temperature and the interaction between the two factors (Table 4.4). Pooled data on the emission rates of CH_3I were generally highest at L3 (Table 4.5). Lower emissions for both CH_3I and CH_2Br_2 were observed at a temperature of 31 °C (Table 4.6). Higher emission rates of $CHBr_3$ and CH_2Br_2 were observed at L2, L3 and L4 (Table 4.5), and at lower temperatures of 25 and 28 °C (Table 4.6). Higher emission rates of CH_2I_2 were generally found at L3 and L4 (Table 4.5) at the temperature of 28 °C (Table 4.6), while lower rates were observed at 31 °C in the dark (Tables 4.7 and 4.8).

Meanwhile, significant correlations (p<0.01; n = 64) were observed between irradiance and the emissions of all four of the halocarbon compounds (Table 4.7). This corresponds well with the ANOVA test results showing significantly lower emissions being observed in the dark, which was discussed at the start of this section (Figure 4.3). Correlations were not particularly high although significant (p<0.01; n = 64), ranging between 0.421 (CH_3I) and 0.554 (CH_2Br_2). A stronger correlation (−0.633; p<0.01; n = 64; Table 4.8) was observed between the emission of $CHBr_3$ and temperature. This is the only halocarbon compound that has a significant correlation with temperature, although all four halocarbon compounds showed an inverse relationship with temperature (Table 4.8).

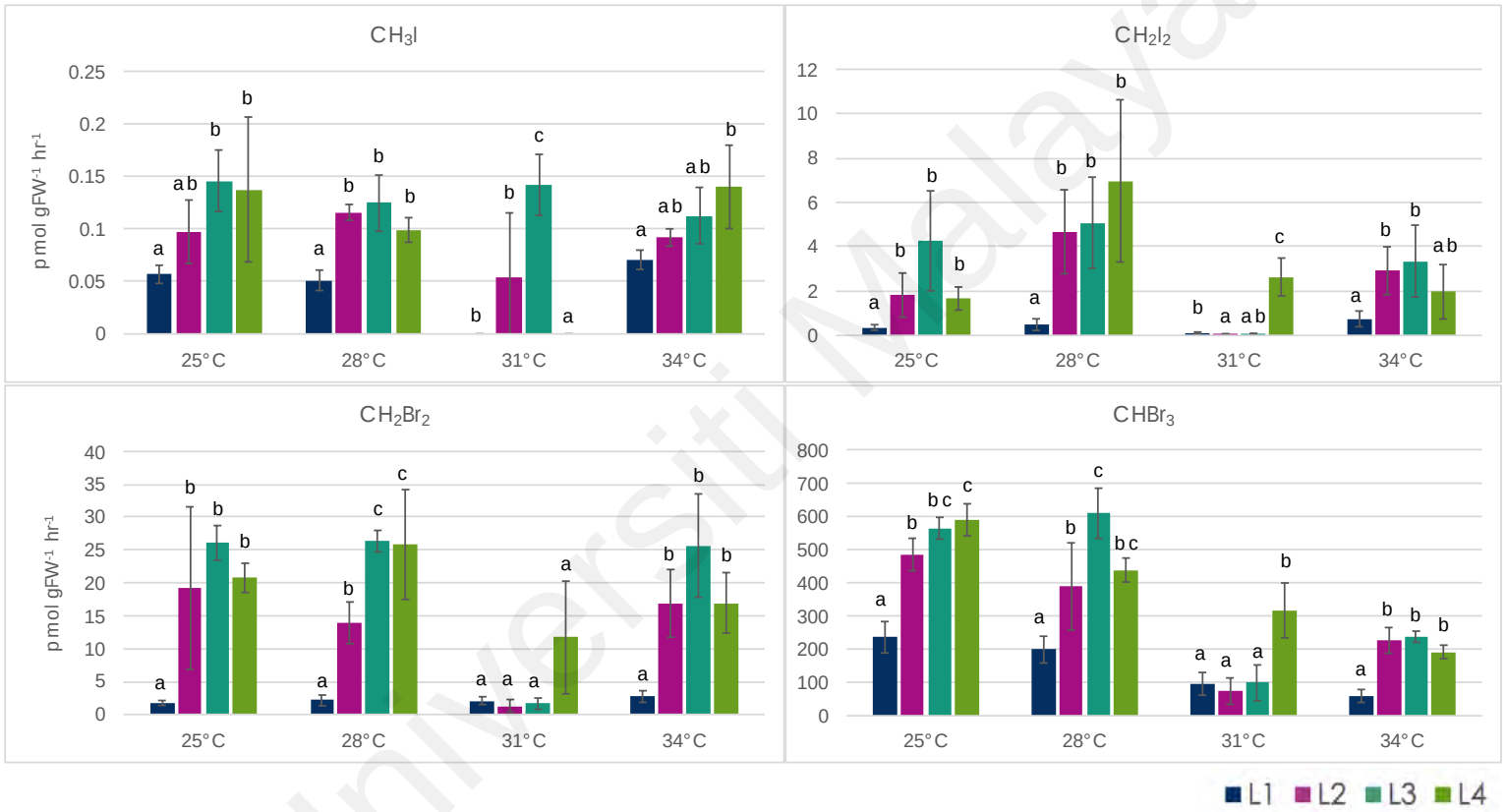


Figure 4.3: Emission rates (average ± standard deviation, pmol gFW^{-1} hr^{-1}, $n = 4$) of CH_3I, CH_2I_2, CH_2Br_2 and $CHBr_3$ by *Kappaphycus alvarezii* at temperatures of 25, 28, 31 and 34 °C, crossed with irradiance levels of 0 ± 0 (L1), 62 ± 8 (L2), 117 ± 13 (L3) and 177 ± 26 (L4) µmol photons m^{-2} s^{-1} tested using one-way ANOVA; ^{a,b,c} denotes homogeneous groups based on Tukey's post-hoc test ($p<0.05$)

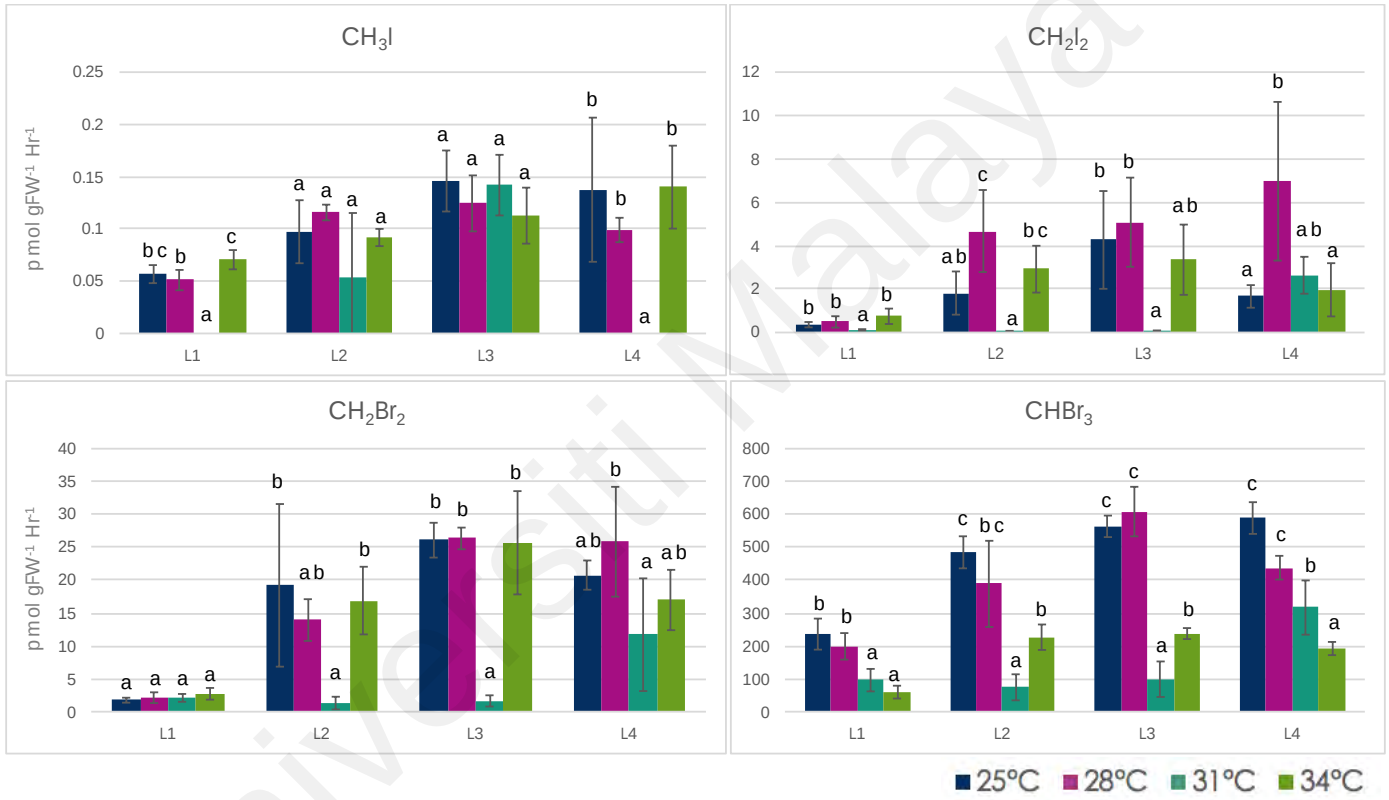


Figure 4.4: Emission rates (average ± standard deviation, pmol gFW^{-1} hr^{-1}, $n = 4$) of CH_3I, CH_2I_2, CH_2Br_2 and $CHBr_3$ by *Kappaphycus alvarezii* at irradiance levels of 0 ± 0 (L1), 62 ± 8 (L2), 117 ± 13 (L3) and 177 ± 26 (L4) µmol photons m^{-2} s^{-1}, crossed with temperatures of 25, 28, 31 and 34 °C tested using one-way ANOVA; [a,b,c] denotes homogeneous groups based on Tukey's post-hoc test ($p<0.05$)

Table 4.4: Two-way ANOVA tests on the emissions of CH_3I, CH_2I_2, CH_2Br_2 and $CHBr_3$ by *K. alvarezii* at different temperatures and irradiances

Compound	Parameter	*df*	*F*	*p*
CH_3I	Temperature	3	11.147	0.000
	Irradiance	3	21.694	0.000
	Interaction	9	4.142	0.001
CH_2I_2	Temperature	3	63.447	0.000
	Irradiance	3	42.815	0.000
	Interaction	9	12.398	0.000
CH_2Br_2	Temperature	3	28.661	0.000
	Irradiance	3	34.214	0.000
	Interaction	9	4.285	0.000
$CHBr_3$	Temperature	3	118.071	0.000
	Irradiance	3	55.545	0.000
	Interaction	9	9.744	0.000

Table 4.5: Emission rates (average ± standard deviation, pmol gFW^{-1} hr^{-1}, *n* = 16) of CH_3I, CH_2I_2, CH_2Br_2 and $CHBr_3$ by *Kappaphycus alvarezii*, at irradiance levels of 0 ± 0 (L1), 62 ± 8 (L2), 117 ± 13 (L3) and 177 ± 26 (L4) µmol photons m^{-2} s^{-1}, pooled from the entire study tested using one-way ANOVA; [a,b,c] denotes homogeneous irradiance groups based on Tukey's post-hoc test ($p<0.05$)

Irradiance	CH_3I	CH_2I_2	CH_2Br_2	$CHBr_3$
L1	0.05 ± 0.02^{a}	2.18 ± 0.72^{a}	147.58 ± 81.51^{a}	0.43 ± 0.31^{a}
L2	0.09 ± 0.04^{b}	12.84 ± 9.44^{b}	293.57 ± 175.29^{b}	2.36 ± 2.04^{b}
L3	0.13 ± 0.03^{c}	19.94 ± 11.56^{b}	377.24 ± 226.78^{c}	3.19 ± 2.50^{bc}
L4	0.10 ± 0.07^{b}	18.83 ± 7.90^{b}	383.99 ± 158.69^{c}	3.31 ± 2.84^{c}

Table 4.6: Emission rates (average ± standard deviation, pmol gFW^{-1} hr^{-1}, *n* = 16) of CH_3I, CH_2I_2, CH_2Br_2 and $CHBr_3$ by *Kappaphycus alvarezii*, at temperatures of 25, 28, 31 and 34 °C, pooled from the entire study tested using one-way ANOVA; [a,b,c] denotes homogeneous temperature groups based on Tukey's post-hoc test ($p<0.05$)

Temperature	CH_3I	CH_2I_2	CH_2Br_2	$CHBr_3$
25 °C	0.11 ± 0.05^{b}	16.96 ± 11.05^{b}	468.40 ± 149.61^{c}	2.03 ± 1.84^{b}
28 °C	0.10 ± 0.03^{b}	17.07 ± 11.06^{b}	408.58 ± 167.07^{c}	4.30 ± 3.20^{c}
31 °C	0.06 ± 0.06^{a}	4.19 ± 5.94^{a}	146.42 ± 113.60^{a}	0.72 ± 1.21^{a}
34 °C	0.10 ± 0.03^{b}	15.57 ± 9.71^{b}	178.96 ± 76.71^{b}	2.25 ± 1.47^{b}

Table 4.7: Pearson Product-Moment Correlation Coefficients, *r*, between the emission rates of CH_3I, CH_2I_2, CH_2Br_2 and $CHBr_3$ by *Kappaphycus alvarezii* and irradiance

Irradiance	CH_3I	CH_2I_2	CH_2Br_2	$CHBr_3$
Correlation	0.421**	0.494**	0.554**	0.480**
Significance	0.001	0.000	0.000	0.000

**Correlation is significant at the 0.01 level (2-tailed); $n = 64$; emission rates were pooled from the entire study

Table 4.8: Pearson Product-Moment Correlation Coefficients, *r*, between the emission rates of CH_3I, CH_2I_2, CH_2Br_2 and $CHBr_3$ by *Kappaphycus alvarezii* and temperature

Temperature	CH_3I	CH_2I_2	CH_2Br_2	$CHBr_3$
Correlation	−0.132	−0.135	−0.152	−0.633**
Significance	0.298	0.287	0.232	0.000

**Correlation is significant at the 0.01 level (2-tailed); $n = 64$; emission rates were pooled from the entire study

4.2.2 Correlations among the halocarbon compounds

When the halocarbon emission rates of the four compounds were pooled from all irradiance and temperature treatments, and the relationships between the emissions were determined, strong correlations (Table 4.9) were reported between the compounds. These include the emissions of CH_2I_2 and CH_2Br_2 (r = 0.798; p<0.01), CH_2I_2 and $CHBr_3$ (r = 0.724; p<0.01), and CH_2Br_2 and $CHBr_3$ (r = 0.717; p<0.01). The strongest correlation was observed between CH_2Br_2 and CH_2I_2 (r = 0.798; p<0.01; n = 64), while emissions of CH_3I seem to correlate weakly with CH_2I_2 (r = 0.369; p<0.01) and $CHBr_3$ (r = 0.385; p< 0.01).

Table 4.9: Pearson Product-Moment Correlation Coefficients, *r,* between the emission rates of CH_3I, CH_2I_2, CH_2Br_2 and $CHBr_3$ by *Kappaphycus alvarezii*

		CH_3I	**CH_2I_2**	**CH_2Br_2**
CH_2I_2	Correlation	0.369**		
	Significance	0.003		
CH_2Br_2	Correlation	0.525**	0.798**	
	Significance	0.000	0.000	
$CHBr_3$	Correlation	0.385**	0.724**	0.717**
	Significance	0.002	0.000	0.000

**Correlation is significant at the 0.01 level (2-tailed); n = 64

4.2.3 The F_v/F_m values of *K. alvarezii* under varying temperature and irradiance levels

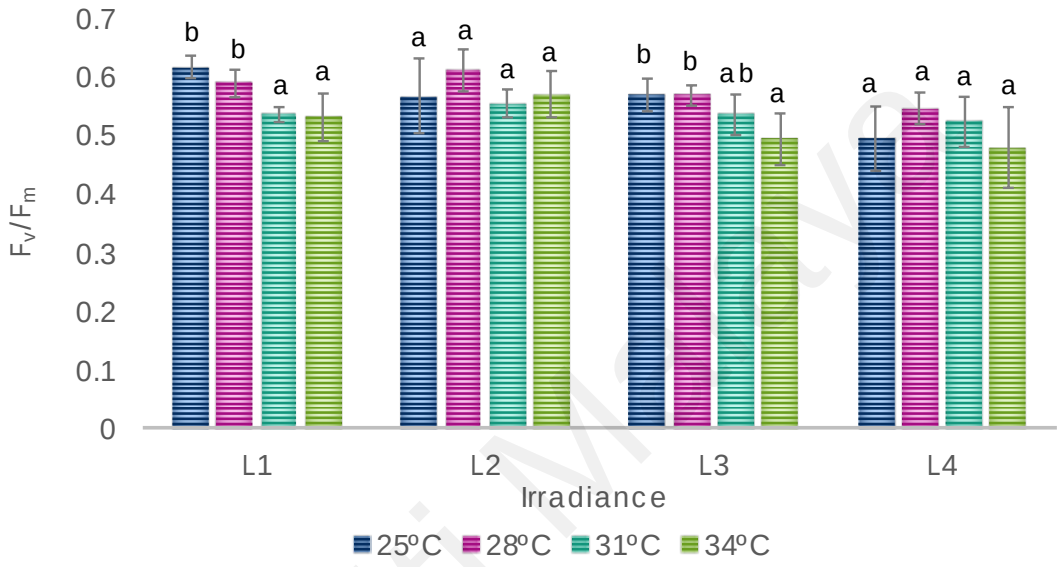


Figure 4.5: The effect of temperature on the F_v/F_m values (average ± standard deviation, *n* = 8) of *Kappaphycus alvarezii* at irradiance levels of 0 ± 0 (L1), 62 ± 8 (L2), 117 ± 13 (L3) and 177 ± 26 (L4) µmol photons m^{-2} s^{-1} tested using one-way ANOVA; ^{a,b,c} denotes homogeneous temperature groups based on Tukey's post-hoc test ($p<0.05$)

F_v/F_m values (n = 8), taken prior to and post-incubation of the seaweeds at a particular irradiance and temperature level, were pooled and represented as an average value. When plotted against irradiance levels at each temperature (Figure 4.5), most of the F_v/F_m values of the seaweed were found to be affected by temperature treatments at 25, 28, 31 and 34 °C. The highest average F_v/F_m value of 0.619 ± 0.019 was recorded at L1 at 25 °C. Most F_v/F_m values were above 0.5 (Figures 4.5 and 4.6). F_v/F_m values below 0.5 were observed at the highest temperature of 34 °C at L3 and L4, and at 25°C at L4. In the dark (L1), higher F_v/F_m values were observed at lower temperatures of 25 and 28 °C, while significantly ($p<0.05$) lower values were associated with a higher temperature. At L2, there were no significant differences among the F_v/F_m values across the various temperatures. At L3, the average F_v/F_m value was lowest at the highest temperature of 34 °C (0.495 ± 0.044; Appendix G), while at the highest irradiance level of L4, no significant difference was observed among the F_v/F_m values at the tested temperatures.

The highest irradiance level, L4, shows the lowest F_v/F_m values (Appendix F), although the lower values are only significant in the dark (L1). The effect of irradiance on the F_v/F_m values of *K. alvarezii* was significant (Appendix F), except at the temperature of 31 °C where changes in irradiance levels generally seemed to not significantly affect F_v/F_m values.

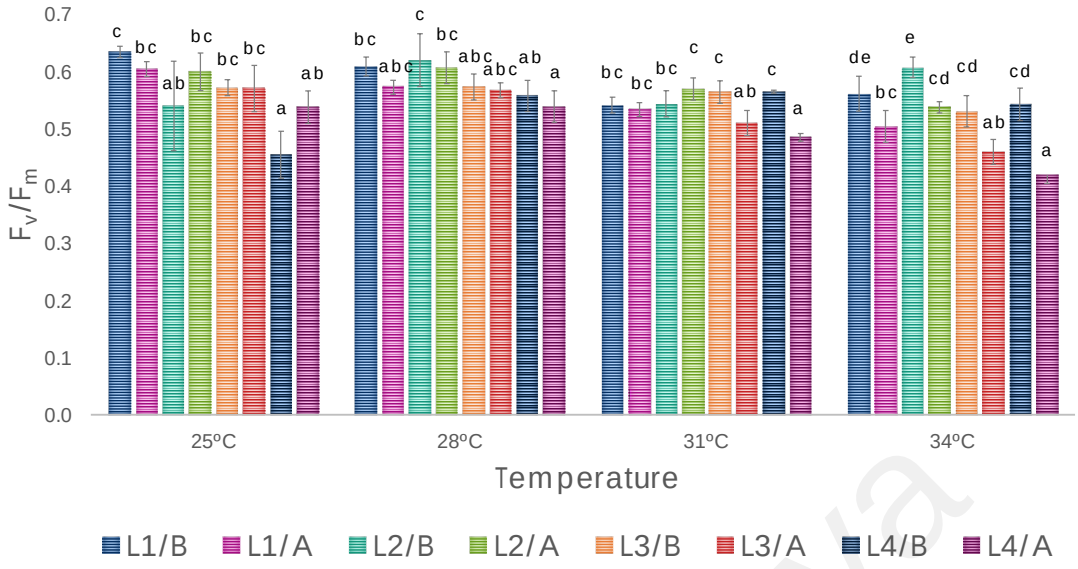


Figure 4.6: F_v/F_m values (average ± standard deviation, *n* = 4) of *Kappaphycus alvarezii,* taken prior to and post-incubation of seaweeds at temperatures of 25, 28, 31 and 34 °C; /A indicates measurements taken prior to the incubation, while /B indicates measurements taken post-incubation; L1, L2, L3 and L4 are irradiance levels of 0 ± 0, 62 ± 8, 117 ± 13 and 177 ± 26 µmol photons m^{-2} s^{-1} tested using one-way ANOVA; ^{a,b,c,d,e} denotes homogeneous irradiance groups based on Tukey's post-hoc test ($p<0.05$)

When the F_v/F_m values were categorised into two groups, i.e. 'before' and 'after', with values measured prior to and post-incubation individually plotted out (Figure 4.6), no significant differences were observed at 25 and 28 °C between values taken prior to the incubation and values taken post-incubation. However, at higher temperatures and higher irradiance levels, the F_v/F_m values taken prior to and post-incubation differed significantly ($p< 0.05$; Figure 4.6). Therefore, the percentage change in F_v/F_m, derived from the difference in F_v/F_m prior to and post-incubation relative to the values prior to incubation, will be used in determining the correlations between the F_v/F_m values and other parameters studied (Appendix G).

The percentage changes in F_v/F_m showed a rather strong correlation with temperature change ($r = -0.616$; $p<0.01$), compared to irradiance ($r = -0.053$; $p>0.05$; Table 4.10). It did not correlate well with the emission rates of the halocarbons studied. A rather weak ($r = 0.343$; $p<0.01$) correlation between percentage changes in F_v/F_m with $CHBr_3$ was noted

Table 4.10: Pearson Product-Moment Correlation Coefficients between the percentage of F_v/F_m change and temperature, irradiance and the emission rates of CH_3I, CH_2I_2, CH_2Br_2 and $CHBr_3$ by *Kappaphycus alvarezii*

F_v/F_m change	Temperature	Irradiance	CH_3I	CH_2I_2	CH_2Br_2	$CHBr_3$
Correlation	−0.616**	−0.053	0.151	−0.065	0.090	0.343
Significance	0.000	0.679	0.235	0.609	0.479	0.005

**Correlation is significant at the 0.01 level (2-tailed); $n = 64$; emission rates were pooled from the entire study

4.3 *In situ* measurements of halocarbon emissions by cultivated seaweeds

4.3.1 Onshore tank cultivation system

The mixing ratios (ppbv) of halocarbon released by the seaweeds cultivated at the onshore tank cultivation system were in the sequence of $CHBr_3$> CH_2Br_2> $CHBr_2Cl$> $CHBrCl_2$ except for the emission of *U. reticulata* where CH_2Br_2> $CHBr_3$ (Table 4.11). Higher emissions were recorded from *G. manilaensis* as compared to *K. alvarezii* and *U. reticulata* during first sampling. Higher amount of CH_2Br_2 (5.12 ppbv) compared to $CHBr_3$ (0.94 ppbv) was released by *U. reticulata* while *G. manilaensis* and *K. alvarezii* releases higher amount of $CHBr_3$ compared to CH_2Br_2 (Sampling Trip 1, Table 4.11).

Seaweeds sampled during the second and fourth trip were of the same batch (Table 3.4). This enables comparison between the mixing ratios of halocarbon by seaweeds from the two trips. The emission of all four halocarbons were generally higher in the fourth sampling compared to the second sampling in all daylight samples. The mixing ratios of CH_2Br_2 and $CHBr_3$ emitted by *G. manilaensis* during the second trip was between 2.96 – 8.98 and 6.26 – 31.66 ppbv and was higher at 8.98 – 21.99 and 82.26 – 96.61 ppbv during the fourth trip. Meanwhile, although lower mixing ratios were observed for all halocarbons emitted by *K. alvarezii* as compared to *G. manilaensis* during Sampling Trip 4, the values showed similar trend observed in *G. manilaensis* – that the mixing ratios of all compounds except $CHBrCl_2$ in the fourth sampling trip were higher than the second trip (Table 4.11). The mixing ratios of halocarbons released by *G. manilaensis* in the dark did not differ much between second and fourth sampling trip, and was low compared to the daylight emission. The mixing ratios of halocarbons released by *K. alvarezii*, especially those of the brominated compounds, was higher than *G. manilaensis* during the night of the fourth sampling.

A Mann-Whitney U test was run to determine if there were differences in the daylight and dark halocarbon emissions by the Control (without seaweed), *G. manilaensis* and *K. alvarezi*. The results showed no significant difference (p = 1.000; n = 7) between the Control during daylight and in the dark. *G. manilaensis* showed significantly ($p < 0.05$) higher emission of all halocarbon compounds during daylight than dark except $CHBrCl_2$. Meanwhile, all four halocarbon compounds were emitted more ($p< 0.05$) during daylight than dark by *K. alvarezii* (Table 4.5).

Table 4.11: Mixing ratios (ppbv) of seaweeds cultivated at the onshore tank cultivation system throughout the sampling trips.

	Daylight (ppbv)				Dark (ppbv)			
	CH_2Br_2	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$	CH_2Br_2	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
Sampling Trip 1								
Control	0.43	0.01	0.03	0.70	0.17 ± 0.15 (0.06 – 0.27)	0.01 ± 0.01 (0.00 – 0.01)	0.02 ± 0.02 (0.00 – 0.03)	0.47 ± 0.28 (0.27 – 0.67)
G. manilaensis	20.03 ± 25.27 (2.16 – 37.90)	0.12 ± 0.13 (0.03 – 0.22)	3.01 ± 3.26 (0.71 – 5.32)	122.79 ± 118.64 (38.90 – 206.68)	4.37	0.09	0.67	17.37
K. alvarezii	30.08	0.17	3.00	61.77	4.83	0.02	0.46	17.31
U. reticulata	5.12	0.03	0.13	0.94	2.61	0.01	0.06	0.88
Sampling Trip 2								
Control	0.00	0.00	0.00	0.24	0.00	0.00	0.00	0.20
G. manilaensis	6.15 ± 3.03 (2.96 – 8.98)	0.06 ± 0.05 (0.00 – 0.09)	0.45 ± 0.28 (0.16 – 0.72)	19.02 ± 12.70 (6.26 – 31.66)	1.07 ± 0.34 (0.85 – 1.45)	0.04 ± 0.01 (0.03 – 0.04)	0.17 ± 0.03 (0.14 – 0.19)	3.98 ± 0.78 (3.39 – 4.86)
K. alvarezii	27.31 ± 1.39 (26.33 – 28.29)	0.37 ± 0.11 (0.29 – 0.45)	1.47 ± 0.36 (1.21 – 1.72)	91.10 ± 16.00 (79.78 – 102.41)				
Sampling Trip 4								
Control	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.10
G. manilaensis	15.48 ± 9.20 (8.98 – 21.99)	0.86 ± 0.84 (0.27 – 1.46)	7.94 ± 5.88 (3.78 – 12.10)	89.44 ± 10.15 (82.26 – 96.61)	0.53 ± 0.10 (0.41 – 0.60)	0.02 ± 0.00 (0.01 – 0.02)	0.17 ± 0.05 (0.12 – 0.21)	7.34 ± 2.47 (4.50 – 9.00)
K. alvarezii	69.83 ± 52.43 (32.76 – 106.91)	0.13 ± 0.11 (0.05 – 0.21)	1.91 ± 1.25 (1.02 – 2.79)	71.39 ± 48.67 (36.97 – 105.81)	7.23 ± 6.38 (7.23 – 11.74)	0.04 ± 0.04 (0.04 – 0.07)	0.50 ± 0.04 (0.50 – 0.78)	25.92 ± 19.82 (25.92 – 39.94)

Table 4.12: The central tendency (median, ppbv) of the halocarbon mixing ratios of the Control, *G. manilaensis* and *K. alvarezii* samples and the Mann-Whitney U test results

Seaweed	Median (ppbv)		*n*	*U*	*z*	*p*
	Daylight	Dark				
Control						
CH_2Br_2	0.00	0.03	7	6.00	0.000	1.000
$CHBrCl_2$	0.00	0.00		7.00	0.390	1.000
$CHBr_2Cl$	0.00	0.00		6.00	0.000	1.000
$CHBr_3$	0.24	0.23		6.00	0.000	1.000
G. manilaensis						
CH_2Br_2	8.98	0.85	14	2.00	-2.875	0.002
$CHBrCl_2$	0.09	0.03		12.00	-1.597	0.128
$CHBr_2Cl$	0.72	0.18		6.00	-2.364	0.017
$CHBr_3$	38.9	4.86		3.00	-2.747	0.004
K. alvarezii						
CH_2Br_2	29.19	4.83	9	0.00	-2.324	0.024
$CHBrCl_2$	0.25	0.02		1.00	-2.066	0.048
$CHBr_2Cl$	1.53	0.46		0.00	-2.324	0.024
$CHBr_3$	80.48	17.31		1.00	-2.066	0.048

n = number of replicates, *U* = Mann-Whitney's U, *z* = standardized test statistics, *p* = significance

4.3.2 Offshore platform

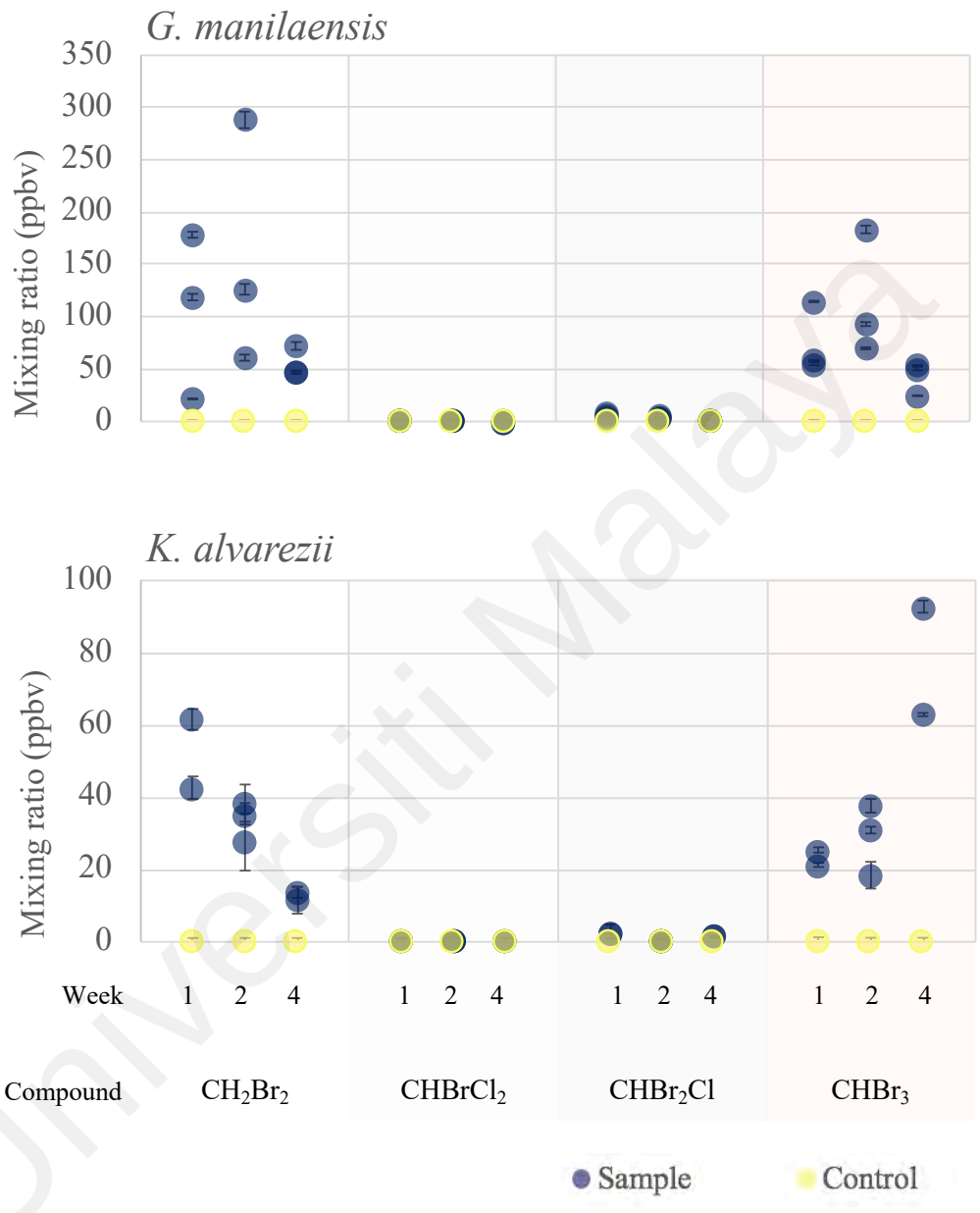


Figure 4.7: The mixing ratios (ppbv, with standard deviation; *n* = 3) of CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$ and $CHBr_3$ released by *G. manilaensis* and *K. alvarezii* throughout the cultivation period; *n* = 3, 3, 3 for *G. manilaensis* and *n* = 2, 3, 2 for *K. alvarezii* at Week 1, 2 and 4 respectively

Two of the seaweeds, *G. manilaensis* and *K. alvarezii*, cultivated at the offshore platform showed higher emissions of CH_2Br_2 and $CHBr_3$ relative to the emissions of $CHBrCl_2$ and $CHBr_2Cl$ (Figure 4.7).

Throughout the 4 weeks of farming, higher mixing ratios of CH_2Br_2 (21.4–178.4 ppbv) were observed for *G. manilaensis* compared to $CHBr_3$ (55–114.6 ppbv). A higher mixing ratio of CH_2Br_2 (60.8–288.2 ppbv) was observed during the second week of farming, compared to the first, and falls to between 46.9–73.0 ppbv during the fourth week of farming (Figure 4.7). The same trend was observed in the emissions of $CHBr_3$ by the same seaweed. The emissions of the mixed halocarbons, i.e. $CHBrCl_2$ and $CHBr_2Cl$, remained rather low and constant (Table 4.13).

The emissions of CH_2Br_2 (52.1 ± 13.4, 33.8 ± 5.6, 12.5 ± 1.5 ppbv) by *K. alvarezii* showed a decreasing trend, while emissions of $CHBr_3$ (23.3 ± 2.9, 29.0 ± 9.8, 77.8 ± 21.1 ppbv) increased from Week 1 to Week 4 (Figure 4.7). Emissions of mixed compounds remained rather constant. The changes in emission trends of CH_2Br_2 and $CHBr_3$ by *K. alvarezii* can be observed when the mixing ratios of CH_2Br_2:$CHBr_3$ were determined (Table 4.13). A gradual decrease in the ratio of CH_2Br_2:$CHBr_3$ was observed for *K. alvarezii* from Week 1 to Week 4, while the ratio for *G. manilaensis* remained rather constant.

Table 4.13: Mixing ratios (average ± standard deviation and range, ppbv) of halocarbons emitted by *G. manilaensis* and *K. alvarezii* cultivated at the offshore platform throughout the cultivation period

Farming	Halocarbon	Control (ppbv)	*G. manilaensis* (ppbv)	*K. alvarezii* (ppbv)
Week 1	CH_2Br_2	0.00	106.2 ± 79.2	52.1 ± 13.4
			(21.4 – 178.4)	(42.6 – 61.5)
	$CHBrCl_2$	0.00	0.9 ± 0.5	0.3 ± 0.2
			(0.4 – 1.4)	(0.2 – 0.5)
	$CHBr_2Cl$	0.00	5.3 ± 1.7	2.5 ± 0.0
			(4.0 – 7.3)	(2.4 – 2.5)
	$CHBr_3$	0.21	75.9 ± 33.6	23.3 ± 2.9
			(55.0 – 114.6)	(21.2 – 25.4)
Week 2	CH_2Br_2	0.00	158.4 ± 117.1	33.8 ± 5.6
			(60.8 – 288.2)	(27.6 – 38.4)
	$CHBrCl_2$	0.00	0.8 ± 0.3	0.1 ± 0.0
			(0.5 – 1.0)	(0.1 – 0.1)
	$CHBr_2Cl$	0.00	4.8 ± 1.7	0.6 ± 0.0
			(3.8 – 6.8)	(0.6 – 0.7)
	$CHBr_3$	0.07	115.4 ± 60.1	29.0 ± 9.8
			(18.4 – 37.6)	(18.4 – 37.6)
Week 4	CH_2Br_2	0.00	55.3 ± 14.5	12.5 ± 1.5
			(46.9 – 72.0)	(11.5 – 13.6)
	$CHBrCl_2$	0.00	0.1 ± 0.0	0.1 ± 0.0
			(0.1 – 0.2)	(0.1 – 0.1)
	$CHBr_2Cl$	0.00	1.5 ± 0.6	1.5 ± 0.0
			(0.9 – 2.0)	(1.5 – 1.5)
	$CHBr_3$	0.06	42.8 ± 16.0	77.8 ± 21.1
			(24.4 – 53.5)	(62.9 – 92.8)

Samples collected during the day; $n = 1$ for Control; all the rest of measurements $n = 3$ except for * where $n = 2$ *for K. alvarezii*

Table 4.14: Mixing ratios of CH_2Br_2:$CHBr_3$ emitted by *G. manilaensis* and *K. alvarezii* during cultivation at the offshore platform

	G. manilaensis	*K. alvarezii*
Week 1	1.3 ± 0.9	2.2 ± 0.3*
Week 2	1.3 ± 0.6	1.2 ± 0.3
Week 4	1.4 ± 0.5	0.2 ± 0.1*

$n = 3$ except for * where $n = 2$ *for K. alvarezii*

4.3.3 Cage culture

G. manilaensis from the cage culture at the river mouth emitted high amount of CH_2Br_2 and $CHBr_3$ compared to the mixed compounds $CHBr_2Cl$ (5 ± 0.6 ppbv) and $CHBrCl_2$ (0.9 ± 0.2 ppbv), two weeks into their cultivation. The emission of CH_2Br_2 was particularly high at 1165 ± 243 ppbv while the mixing ratios of $CHBr_3$ in the flux chamber containing the seaweed was 278 ± 11 ppbv (n = 2; Figure 4.8).

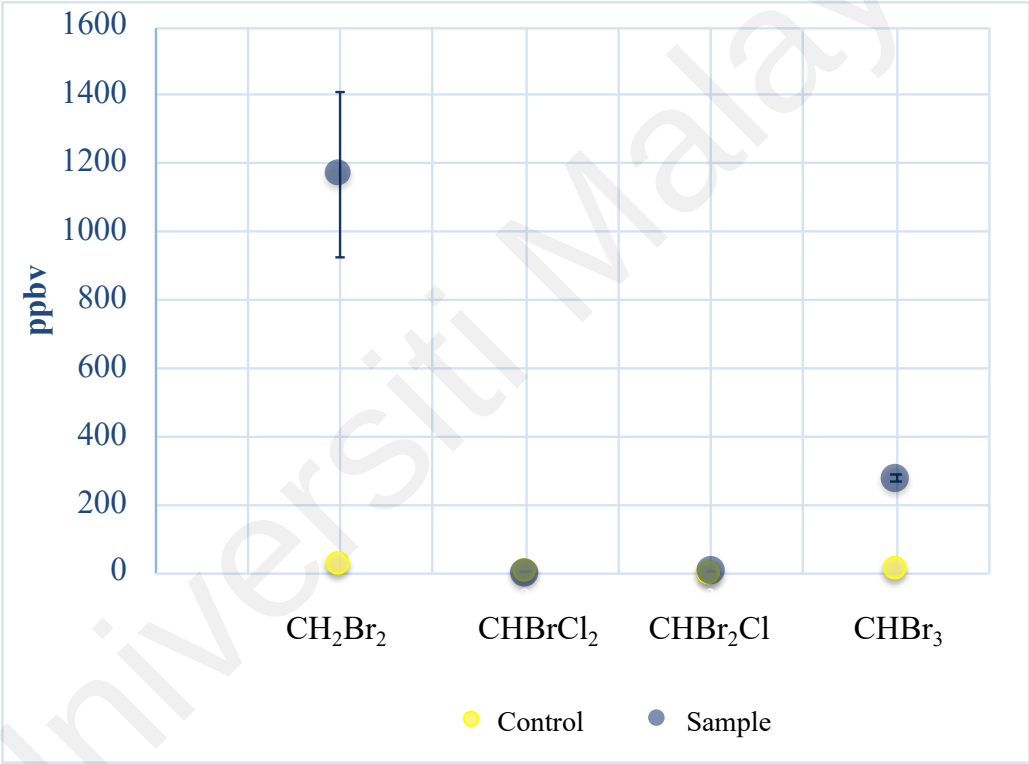


Figure 4.8: Halocarbon mixing ratios (ppbv) in the air samples of *G. manilaensis* in cage culture deployed at Sungai Melawi, Kelantan

4.3.4 Halocarbon mixing ratios released by seaweeds from all three cultivation systems

The emission profile of *G. manilaensis, K. alvarezii,* and *U. reticulata* from all three cultivation systems were compiled (Figure 4.9; Table 4.15). Emissions during daylight recorded higher mixing ratios of CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$ and $CHBr_3$ by all three seaweeds, relative to their emissions in the dark. Higher mixing ratios of the halocarbons were recorded by *G. manilaensis* during daylight compared to the dark. This was followed by *K. alvarezii* and, lastly, by *U. reticulata* (Figure 4.7).

The compounds with the highest mixing ratio recorded during the day were all released by *G. manilaensis* i.e. CH_2Br_2 (1337.46 ppbv), followed by $CHBr_3$ (286.34 ppbv), $CHBr_2Cl$ (12.10 ppbv) and $CHBrCl_2$ (1.46 ppbv). In the dark, the highest mixing ratios of all halocarbon compounds except $CHBrCl_2$ were comparably lower than those released by *K. alvarezii.* The emissions were in the order of $CHBr_3$ (17.37 ppbv) > CH_2Br_2 (4.37 ppbv) > $CHBr_2Cl$ (0.67 ppbv) > $CHBrCl_2$ (0.09 ppbv) (Table 4.15). The two highest data points (Table 4.8) in the mixing ratio graphs of CH_2Br_2 and $CHBr_3$ and the highest Control point of $CHBrCl_2$ were measurements conducted at the river mouth where *G. manilaensis* was cultivated.

The highest mixing ratio of halocarbons emitted by *K. alvarezii* was recorded for CH_2Br_2 at 106.91 ppbv in daylight, closely followed by $CHBr_3$ (105.81 ppbv), $CHBr_2Cl$ (3 ppbv) and $CHBrCl_2$ (0.49 ppbv) during the day. Although the highest single recorded emission was of CH_2Br_2, the highest average mixing ratio was of $CHBr_3$ at 46.76 ± 30.89 ppbv (Table 4.15). The mixing ratios of all compounds in the dark were within, if not lower than, the range of observed mixing ratios during the day (Figure 4.7).

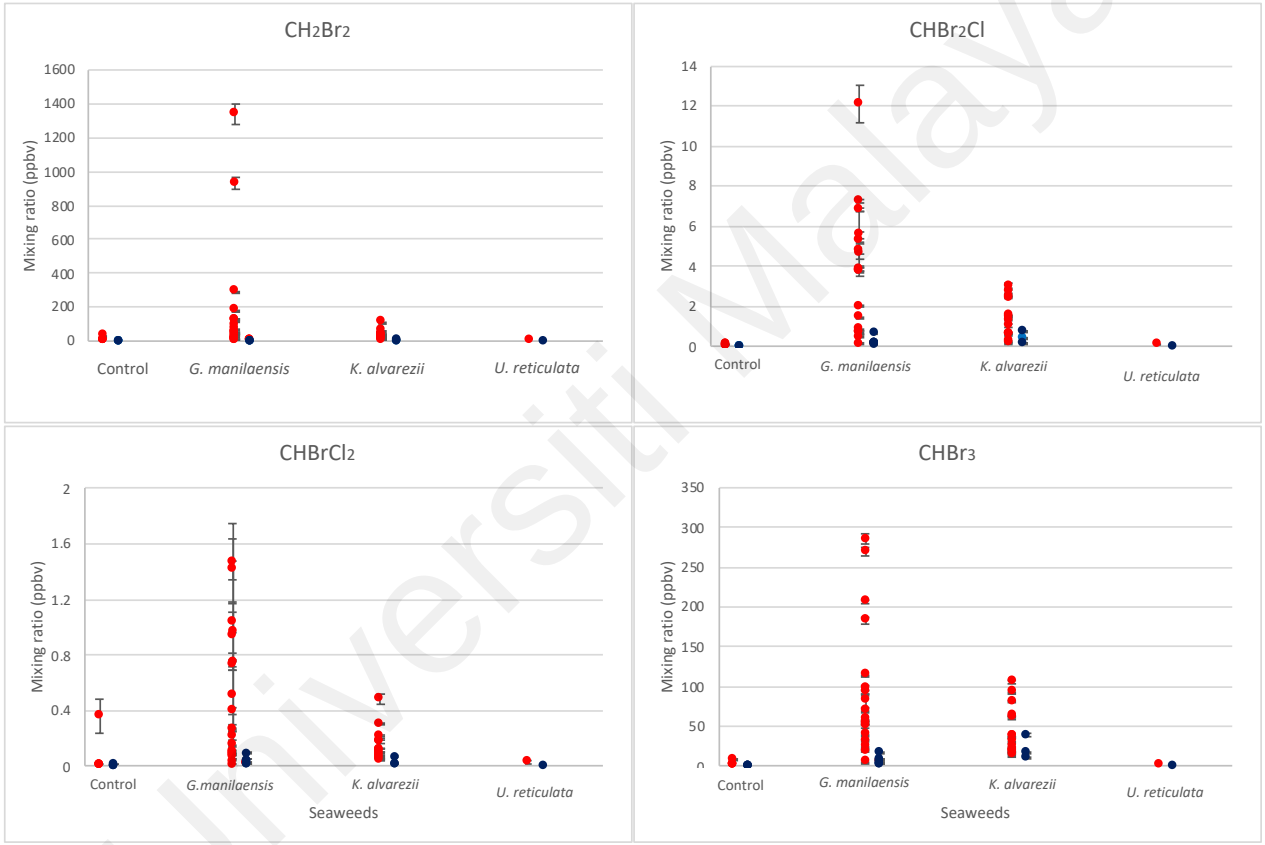


Figure 4.9: Mixing ratios (ppbv) of CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$ and $CHBr_3$ released by 500 g of *G. manilaensis, K. alvarezii,* and *U. reticulata* during daylight (red circle) and in the dark (navy circle) for 30 minutes; *n* = 5, 16, 13, 1 and *n* = 4, 7, 3, 1 for daylight and dark measurements of Control, *G. manilaensis, K. alvarezii,* and *U. reticulata* respectively. Each data point is an average value of three technical replicates analysed (error bars indicate standard deviations of the technical replicates), therefore represents a single separate measurement.

Table 4.15: Average mixing ratios ± standard deviation and the range (ppbv) of CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$, and $CHBr_3$ released by 500 g of *G. manilaensis, K. alvarezii,* and *U. reticulata* from all three cultivation systems during daylight and in the dark for 30 minutes; *n* = 5, 13, 18, 1 and *n* = 4, 3, 7, 1 for daylight and dark measurements of Control, *G. manilaensis*, *K. alvarezii* and *U. reticulata* respectively

		CH_2Br_2	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
Control	Daylight	5.3 ± 11.62 (0.00–26.08)	0.07 ± 0.16 (0.00–0.36)	0.05 ± 0.06 (0.00–0.13)	1.89 ± 3.65 (0.06–8.41)
	Dark	0.08 ± 0.13 (0.00–0.27)	0.00 ± 0.01 (0.00–0.01)	0.01 ± 0.02 (0.00–0.03)	0.31 ± 0.25 (0.10–0.67)
G. manilaensis	Daylight	186.53 ± 359.64 (2.16–1337.46)	0.51 ± 0.48 (0.00–1.46)	3.80 ± 3.15 (0.16–12.10)	96.68 ± 84.46 (6.26–286.34)
	Dark	1.31 ± 1.39 (0.41–4.37)	0.04 ± 0.03 (0.01–0.09)	0.24 ± 3.53 (0.12–0.67)	7.33 ± 4.96 (3.39–17.37)
K. alvarezii	Daylight	32.83 ± 27.30 (5.44–106.91)	0.16 ± 0.12 (0.04–0.49)	1.43 ± 0.97 (0.23–3.00)	46.76 ± 30.89 (14.49–105.81)
	Dark	6.43 ± 4.72 (2.72–11.74)	0.04 ± 0.12 (0.02–0.07)	0.49 ± 0.28 (0.22–0.78)	23.05 ± 14.87 (11.91–39.94)
U. reticulata	Daylight	5.12 (-)	0.03 (-)	0.13 (-)	0.94 (-)
	Dark	2.61 (-)	0 (-)	0.06 (-)	0.88 (-)

4.3.5 Comparison of halocarbon emissions between the different cultivation systems

G. manilaensis was cultivated in all three of the cultivation systems, i.e. onshore tank, offshore platform and the cage culture. *K. alvarezii*, meanwhile, was cultivated in the onshore tanks and at the offshore platform.

A comparison of means of the daylight data through one-way ANOVA showed that the three different cultivation systems significantly (p<0.05) affect the emissions of brominated halocarbons by *G. manilaensis* (Table 4.16). The mixing ratio of CH_2Br_2 emitted by *G. manilaensis* was significantly higher when cultivated at the river at 1165 ± 243 ppbv, followed by offshore cultivation (108 ± 83 ppbv) and onshore tank (13 ± 13 ppbv). Emissions of $CHBr_3$ by *G. manilaensis* cultivated at the river mouth cages were also highest among the systems (278 ± 11 ppbv), while the emissions of $CHBr_3$ by *G. manilaensis* between the onshore tanks and offshore platform did not differ much (Table 4.16). The emissions of mixed halocarbon, i.e. $CHBrCl_2$ and $CHBr_2Cl$, by *G. manilaensis*, did not differ significantly (p<0.05) among the various systems (Table 4.16).

Table 4.16: Halocarbon mixing ratios (ppbv ± standard deviation) by *G. manilaensis* released at the various cultivation systems during daylight

Cultivation System	CH_2Br_2	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
Onshore Tanks	13 ± 13^{a}	0.3 ± 0.5^{a}	3.3 ± 4.3^{a}	69 ± 69^{a}
Offshore Platform	108 ± 83^{b}	0.6 ± 0.5^{a}	3.9 ± 2.2^{a}	78 ± 47^{a}
Cage culture	1165 ± 243^{c}	0.9 ± 0.2^{a}	5.2 ± 0.6^{a}	278 ± 11^{b}

n = 7, 9, 2 for onshore, offshore and river cultivation (daylight) respectively; ^{a,b,c} indicate homogenous groups based on Tukey's post-hoc test (p< 0.05).

Meanwhile, *K. alvarezii* released a higher amount of $CHBr_3$ compared to CH_2Br_2 both onshore and offshore, followed by $CHBr_2Cl$ and $CHBrCl_2$ (Table 4.17). The average mixing ratios of all four halocarbons except CH_2Br_2 ($p< 0.01$) emitted by *K. alvarezii* at both cultivation sites did not differ significantly ($p>0.10$), as shown by a student t-test (Table 4.18).

Table 4.17: Mixing ratios (average ± standard deviation, ppbv) of halocarbons emitted by *K. alvarezii* in onshore tanks and at the offshore platform during daylight

Cultivation System	CH_2Br_2	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
Onshore Tanks	41 ± 33	0.3 ± 0.1	1.8 ± 0.8	78 ± 26
Offshore Platform	33 ± 17	0.2 ± 0.1	1.4 ± 0.8	41 ± 27

n = 6, 7 for daylight onshore and offshore samples, respectively.

Table 4.18: Student's t-test on the effect of different system (onshore tank and offshore cultivation) on the halocarbon mixing ratios for *K. alvarezii*

Compound	t	df	Sig. (2-tailed)
CH_2Br_2	−5.252	14	0.000
$CHBrCl_2$	−1.705		0.120
$CHBr_2Cl$	−1.098		0.302
$CHBr_3$	−0.810		0.439

Halocarbon data was fourth root transformed; homogeneity was assessed by Levene's test.

The means of seawater nutrient content and salinity at the three cultivation systems were compared (Table 4.19). Results showed that the ammoniacal nitrogen, nitrate and nitrite contents did not show significant differences between the cultivation systems ($p< 0.05$). Readings from onshore tanks and offshore platform did not differ significantly. The only significant differences were the seawater phosphorus content and salinity of the cage culture system, located in the river mouth. Phosphorus content was highest 0.39 ± 0.01 while salinity was lowest (4 ± 0 ppt) at the river mouth where the seaweeds were cultivated (Table 4.19).

Table 4.19:Nutrient content and salinity readings (average ± standard deviation) at the various seaweed cultivation systems at Bachok, Kelantan, testing using one-way ANOVA

Nutrient	Onshore tanks	Offshore platform	Cage
Phosphorus (mg/L)	0.05 ± 0.07^{a}	0.10 ± 0.07^{a}	0.39 ± 0.01^{b}
Nitrogen, Ammonia (mg/L)	0.05 ± 0.09^{a}	0.10 ± 0.12^{a}	0.20 ± 0.02^{a}
Nitrate (mg/L)	1.0 ± 0.8^{a}	1.9 ± 0.7^{a}	1.4 ± 0.1^{a}
Nitrite (mg/L)	0.057 ± 0.079^{a}	0.103 ± 0.104^{a}	0.007 ± 0.000^{a}
Salinity (ppt)	25 ± 7^{b}	29 ± 2^{b}	4 ± 0^{a}

a,b denotes homogeneous group of cultivation systems based on Tukey's post-hoc test ($p< 0.05$); n = 15, 16, 2 and n = 24， 16， 2 for the readings of nutrients and salinity levels at the onshore, offshore and cage cultivation systems.

Readings from data loggers attached to the cultivation systems showed highest temperature of 43.24 °C experienced by seaweeds cultivated at the river mouth in the cages, where they experienced a greater temperature change (19.08 °C) throughout the cultivation period, while seaweeds cultivated in the onshore tanks experienced the least change in temperature (11.01 °C) and irradiance (1019 μmol photons m^{-2} s^{-1}; Table 4.20).

Table 4.20:Temperature and irradiance data collected from various culture systems

Culture systems	Average ± Standard Deviation	Range	Difference in range
Onshore Tanks			
Temperature, °C	29.21 ± 1.81	24.64–35.65	11.01
Irradiance, μmol photons m^{-2} s^{-1}		0–1019	1019
Offshore Platform			
Temperature, °C	30.73 ± 2.38	24.73–40.30	15.56
Irradiance, μmol photons m^{-2} s^{-1}		0–3466	3466
Cage culture			
Temperature, °C	29.48 ± 2.60	24.16–43.24	19.08
Irradiance, μmol photons m^{-2} s^{-1}		0–1478	1478

The recorded temperature and irradiance, and seawater nutrient content and salinity, from the three cultivation systems were extracted and tested against the mixing ratios of halocarbons emitted by *G. manilaensis* (Table 4.22) and *K. alvarezii* (Table 4.23).

A bivariate Pearson's correlation established that there were strong positive correlations (r = 0.668–0.805; p<0.001) between the mixing ratios of the four halocarbons for *G. manilaensis* and the air temperature in the flux chamber. Positive correlation (r = 0.565–0.724; p<0.001) were also observed between irradiance level with the mixing ratios of the four halocarbons. However, Pearson's partial correlation showed that the strength of this linear relationship became less ($r_{partial}$ = 0.318 – 0.518), but still statistically significant (p< 0.05) for all compounds except $CHBrCl_2$ when irradiance level was accounted for. Meanwhile, emissions of CH_2Br_2 and $CHBr_3$ by *G. manilaensis* were strongly affected (r = 0.734, 0546; p<0.005) by the phosphate levels in the seawater (Table 4.22).

Table 4.21: Bivariate Pearson's correlations (r) and Pearson's Partial ($r_{partial}$) when irradiance was controlled for between halocarbon emissions by *G. manilaensis* pooled from all three habitats with temperature and irradiance at time of sampling.

Control Variables			CH_2Br_2	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
-none-[a]	Temperature	r	0.668	0.742	0.805	0.761
		Significance (2-tailed)	0.001	0.000	0.000	0.000
		df	20	20	20	20
	Irradiance	r	0.565	0.724	0.723	0.653
		Significance (2-tailed)	0.006	0.000	0.000	0.001
		df	20	20	20	20
Irradiance	Temperature	$r_{partial}$	0.436	0.318	0.513	0.518
		Significance (2-tailed)	0.048	0.160	0.017	0.016
		df	19	19	19	19

[a] Cells contain zero-order (Pearson) correlations; data for temperature and irradiance were pooled from all samples from the three cultivation systems; df = degree of freedom

Table 4.22: Pearson Correlation Coefficient, *r*, between the mixing ratios of halocarbons emitted by *G. manilaensis* during daylight and the seawater nutrients and salinity

Parameters		CH_2Br_2	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
Phosphate	Pearson Correlation	0.734**	0.116	0.108	0.546*
	Sig. (2-tailed)	0.002	0.680	0.700	0.035
Ammonia	Pearson Correlation	0.328	−0.153	−0.157	0.131
	Sig. (2-tailed)	0.232	0.586	0.577	0.643
Nitrate	Pearson Correlation	0.209	0.064	0.059	0.081
	Sig. (2-tailed)	0.455	0.821	0.835	0.775
Nitrite	Pearson Correlation	−0.181	−0.305	−0.281	−0.298
	Sig. (2-tailed)	0.518	0.268	0.310	0.280
Salinity	Pearson Correlation	−0.436	−0.080	−0.073	−0.298
	Sig. (2-tailed)	0.080	0.760	0.781	0.246
	n	17			

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); *n* = 15 unless otherwise stated; only daylight emission data was considered for the correlation with seawater nutrients and salinity

Weak non-significant positive correlations (r = 0.243 to 0.513; p> 0.05) were observed between the mixing ratios of all halocarbon compounds emitted by *K. alvarezii*, with the air temperature in the flux chamber (Table 4.23). Non-significant correlations were observed between irradiance and the emission of all halocarbons by *K. alvarezii*. The emission of CH_2Br_2 by *K. alvarezii* was negatively correlated (r = −0.578 to −0.694; p<0.05) to the ammoniacal nitrogen, nitrate and nitrite contents of the seawater while positively correlated to seawater salinity (r = 0.601, p< 0.05; Table 4.24).

Table 4.23: Bivariate Pearson's correlations (r) and Pearson's Partial Correlation Coefficient ($r_{partial}$) when irradiance was controlled for between halocarbon emissions by *K. alvarezii* pooled from all three habitats with temperature and irradiance at time of sampling.

Control Variables			CH_2Br_2	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
-none-[a]	Temperature	r	0.452	0.435	0.243	0.513
		Significance (2-tailed)	0.104	0.120	0.403	0.061
		df	12	12	12	12
	Irradiance	r	0.337	0.485	−0.073	−0.127
		Significance (2-tailed)	0.238	0.079	0.805	0.666
		df	12	12	12	12
Irradiance	Temperature	$r_{partial}$	0.338	0.226	0.343	0.710
		Significance (2-tailed)	0.259	0.457	0.251	0.007
		df	11	11	11	11

[a] Cells contain zero-order (Pearson) correlations; data for temperature and irradiance were pooled from all samples from the three cultivation systems; df = degree of freedom

Table 4.24: Pearson Correlation Coefficient, *r*, between the mixing ratios of halocarbons by *K. alvarezii* during daylight and the seawater nutrients and salinity

Parameters		CH_2Br_2	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
Phosphate	Pearson Correlation	−0.519	−0.572	−0.051	0.032
	Sig. (2-tailed)	0.084	0.052	0.874	0.921
Ammonia	Pearson Correlation	−0.694*	−0.335	0.096	0.422
	Sig. (2-tailed)	0.012	0.287	0.767	0.171
Nitrate	Pearson Correlation	−0.578*	−0.422	−0.041	−0.043
	Sig. (2-tailed)	0.049	0.172	0.900	0.894
Nitrite	Pearson Correlation	−0.600*	−0.230	0.284	0.303
	Sig. (2-tailed)	0.039	0.471	0.371	0.338
Salinity	Pearson Correlation	0.601*	−0.508	0.006	−0.365
	Sig. (2-tailed)	0.030	0.076	0.985	0.220
	n	13			

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); n = 12 unless otherwise stated; only daylight emission data was considered for the correlation with seawater nutrients and salinity

CHAPTER 5: DISCUSSION

5.1 Effect of temperature on halocarbon emissions by tropical seaweeds, under controlled laboratory conditions

This study was designed to measure the effect of a range of temperatures, under controlled conditions, on the halocarbon emissions from the selected seaweeds (see Section 3.1 and 4.1). Temperature is an important factor that generally influences physiological processes including photosynthesis and growth. Temperatures above the tolerated threshold will cause physiological stress which may result in increased halocarbon emission due to formation of reactive oxygen species followed by haloperoxidase activities. This was a short-term study (4 and 28 hours of exposure) that can contribute to understanding how seaweeds respond to temperature increase. However, it must be noted that such fast change in temperature are relatively uncommon in the natural environment.

5.1.1 Trends in halocarbon emission under temperature variation

Of the six halocarbon compounds analysed, $CHBr_3$ and CH_2Br_2 were the most dominant compounds released by all the seaweeds. Of the four seaweeds studied, *K. alvarezii* (red seaweed) and *T. conoides* (brown seaweed), were found to be strong emitters of $CHBr_3$ with *K. alvarezii* showing a slightly higher average emission rate of 561 pmol g FW^{-1} hr^{-1}. This corresponds well with the findings of Leedham *et al.* (2013), who reported that red seaweeds including *K. alvarezii* produce higher amounts of $CHBr_3$, followed by the brown seaweed *T. conoides*. The range of emission rates observed for *K. alvarezii* and *T. conoides* were comparable to values previously reported by Leedham *et al.* (2013) and Mithoo-Singh *et al.* (2017; Table 5.1), although *K. alvarezii* is at the lower end of the values. The averaged emission rates from the work of Leedham *et al.* (2013)

were determined at 24 hours post-incubation at 35 °C and at a light range of 120–130 μmol photons m^{-2} s^{-1}, a slightly higher light intensity than the 81 ± 7 μmol photons m^{-2} s^{-1} used in our experiment, while emission rates from the work of Mithoo-Singh *et al.* (2017) were collected from seaweeds exposed to varying pH levels at a temperature of 30 °C with irradiance levels similar to this work. Due to the differences in objectives and the exposure conditions used in these studies, direct comparisons between the values in Table 5.1 therefore should be executed with caution.

Table 5.1: Comparison between the emission rates of $CHBr_3$ (pmol g FW^{-1} hr^{-1}) by *Kappaphycus alvarezii* and *Turbinaria conoides*

Studies	*K. alvarezii*	*T. conoides*
Leedham *et al.*, 2013	512–1731	260–554
Mithoo-Singh *et al.*, 2017	* 480–930	* 272–918
Keng *et al.*, 2013	N/A	48.2–1100
This study	0.5–561	46–505

* Converted based on assumption of moisture content of ~ 90% for *K. alvarezii* and ~83% for *T. conoides* (our unpublished data); emission rates are mean emission rates derived from replicates

The emission trends of $CHBr_3$, CH_2Br_2 and CH_2I_2 across the exposure periods from the ambient (control, at a temperature of 25 °C) to 4 and 28 hours at higher temperatures of 30–40 °C were noticeably different, especially for *K. alvarezii* and *T. conoides,* compared to lower temperatures of 20 and 25°C (Figure 4.1), indicating the possible influence of higher temperatures on the halocarbon emissions of these seaweeds. It was observed that upon exposure to 40 °C, the two seaweeds above with high $CHBr_3$ emissions, had a bigger reduction in halocarbon emission rate (Figure 4.1) compared to *G. manilaensis* and *U. reticulata*. This reduction, together with a large decrease between F_v/F_m values before and after 4 hours treatment at the higher temperatures, could indicate that *K. alvarezii* and *T. conoides* are less heat tolerant compared to *G. manilaensis* and *U. reticulata* (Figure 4.2). It is generally accepted that the threshold value for a healthy state

of a seaweed is F_v/F_m = 0.5, although lower values have been reported by seaweeds in the natural environment (Li *et al.*, 2016; Rabiei *et al*., 2016; Wang *et al.*, 2016). This could possibly indicate that *G. manilaensis* and *U. reticulata* are more tolerant to rapid temperature change, i.e. 4 hours, compared to *K. alvarezii* and *T. conoides.* The large standard deviations in the emissions of $CHBr_3$ and CH_2Br_2 from *K. alvarezii* observed at 35 °C (Figure 4.1) compared to lower temperatures, could be attributed to the low resilience of this seaweed to temperatures higher than 30 °C. At the high temperatures, stress of the seaweeds was indicated by the large decrease in the F_v/F_m values (Figure 4.2). A longer duration of exposure to 40 °C, however, saw the emission rates of $CHBr_3$, CH_2Br_2 and CH_2I_2 by all four seaweeds diminishing (Figure 4.1) compared to the shorter exposure, during which the F_v/F_m values for all four seaweeds fell below 0.1 (Figure 4.2). It should be noted that inherent biological variability exists among seaweeds of the same species (Leedham *et al*., 2013; Keng *et al*., 2020), and therefore larger deviation bars are inevitable and could be observed even at the ambient temperature i.e. 25 °C.

Positive correlations (r = 0.59–0.81; p<0.01) occurred between the halocarbon emission rates for *G. manilaensis* and temperature during the first 4 hours of temperature treatment. This indicates that a short-term increase in temperature could possibly enhance halocarbon emissions, although this effect could depend on seaweed species. This could happen during the daily diurnal change. Carpenter *et al.* (2000) reported higher halocarbon emission rates from temperate rockpool seaweeds during midday. However, the direct effect of temperature on the increased emissions could not be ascertained as factors such as irradiance and photosynthesis that could influence halocarbon emissions co-exist (Keng *et al.*, 2013). Under prolonged exposure, there were strong negative correlations (r = (−0.69)–(−0.95); p<0.01) between the decreasing rates of halocarbon emissions and increasing temperature (Table 4.1), indicating that the halocarbon

emissions by tropical seaweeds such as *U. reticulata, K. alvarezii* and *T. conoides* alter further with prolonged temperature changes. The decrease in halocarbon emissions with increasing temperature could possibly be caused by the destruction of photosynthetic apparatus in the seaweeds, indicated by the low F_v/F_m values. At a temperature of 35 and 40 °C, *K. alvarezii* showed signs of bleaching, which was even evident at 40 °C, and at 28 hours post-exposure, the seaweeds were completely bleached. This might be the result of the effects of reactive oxygen species (ROS) leading to cell death under the extreme temperatures. As the negative correlations could be attributed to cell mortality in the seaweeds at temperatures of 35 and 40 °C, the correlations between temperature and halocarbon emissions could be different should the temperature range be narrowed down to a tolerable level by the seaweeds. Temperature treatments at 20–30 °C, on the other hand, did not affect the F_v/F_m values of the seaweeds much. This coincides with the findings on another tropical brown seaweed, *Sargassum polycystum,* where changes of F_v/F_m values were insignificant between temperatures of 15–30 °C (Zou *et al.*, 2018).

The production of $CHBr_3$, CH_2Br_2 and CH_2I_2 had been commonly associated with bromoperoxidase activity in the seaweeds, with these compounds proposed to arise from the same production pathway (Lin & Manley, 2012; Wever & van der Horst, 2013). Photosynthesis and respiratory electron transport produce ROS, such as the superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2) (Rutherford *et al.*, 2012; Wever & van der Horst, 2013). Under stressful conditions, such as high temperature, oxidative stress arises when the capacity to regulate the ROS in the seaweeds is exceeded, resulting in the damage of the seaweeds. The presence of H_2O_2 and other ROS could activate the oxidation of halides, forming hypohalous acid through the activity of the haloperoxidases. The unstable hypohalous acid then decays through the haloform reaction and produces $CHBr_3$ (dominant compound) and CH_2Br_2 in the presence of dissolved organic matter.

Through nucleophilic substitutions, a variety of halocarbon compounds such as $CHBr_2Cl$ are then formed (Wever & van der Horst, 2013; Lin & Manley, 2012). While the bromoperoxidases involved in halocarbon production could remain active at temperatures as high as 50 °C (Kongkiattikajorn & Ruenwongsa, 2006), the decrease in the production of $CHBr_2$, CH_2Br_2, CH_2I_2 and $CHBr_2Cl$ seemed to be affected by the photosynthetic yield of the seaweeds as shown in this study, especially at 40 °C (Figure 4.1, Appendix D). Exposure to 40 °C causes stress and affects the health of the seaweeds, as indicated by the percentage reduction of F_v/F_m values at 4 hours post-exposure compared to pre-exposure values at the ambient temperature of 25 °C, and the near-zero values at 28 hours after exposure (Appendix D). The near-zero values could suggest that the seaweeds were dead from prolonged exposure to the high temperature.

The results from this study also showed that responses in terms of $CHBr_3$, CH_2Br_2, CH_2I_2 and $CHBr_2Cl$ emissions during short and long durations of exposure to higher temperatures were similar for the four compounds. For example, *G. manilaensis* responded to the higher temperatures by a slight decrease in emissions of the compounds during the first 4 hours, followed by a significant decrease after 28 hours, while *U. reticulata* responded by showing an increase in emissions after 4 hours, followed by a decrease after 28 hours. The emission trends, however, were unique to each of the seaweeds. The different responses in the halocarbon emissions of polar seaweeds to different exposure periods to temperature were previously published. The initial doubling of $CHBr_3$ emissions from *Gymnogongrus antarcticus* was recorded when the temperature was increased from 0 to 10 °C, but the emission rates decreased to values lower than those of normal culture conditions when the exposure period was extended to two months (Laturnus *et al.,* 2000).

Although exposure duration and temperature change could affect the halocarbon emissions by seaweeds, the response towards these factors is difficult to ascertain. A similar short-term study with six- and ten-hours incubation time has been conducted on temperate brackish water seaweeds. No general response pattern was observed (Abrahamsson *et al.*, 2003). The ten-hour cross-incubation experiment conducted showed insignificant changes in the emissions of $CHBr_3$, CH_2I_2 and $CHCl_3$ from *Cladophora glomerate* and *Ulva ahlneriana*. The cross-incubation involved the incubation of seaweeds at 23 °C in the laboratory after collection from the field at 12 °C, and vice versa (23 °C in field and then 12 °C in the laboratory) (Abrahamsson *et al.*, 2003).

Tropical seaweeds and corals are currently at their lethal limit (Bartsch *et al.*, 2012). The temperature threshold for coral bleaching was reported to be between 27.5 – 32 °C, and as dead corals could exert a negative effect on seaweed growth (Liu *et al*., 2009), a small rise in temperature in the face of climate change could affect the abundance and distribution of tropical seaweeds, which may then affect the regional halocarbon pool.

5.1.2 The relationship between F_v/F_m, pigment contents and the morphology of seaweeds on halocarbon emission

The maximal quantum yield, F_v/F_m, during photosynthesis, was shown to be a reliable indicator of the state of health of the seaweeds and could be correlated to the halocarbon emission rate. During photosynthesis, the scavenging of H_2O_2 from over-production in stressful condition is achievable via both enzymatic and non-enzymatic pathways (de Silva *et al.*, 2017). Enzymatic pathways include those involving antioxidant enzymes such as catalase, peroxidase and ascorbate peroxidase, while non-enzymatic pathways involve antioxidants such as carotenoids (de Silva *et al.*, 2017; Sharma *et al*., 2012).

Studies have shown that the concentration of carotenoids can increase during temperature stress, while chlorophyll content decreases with increased temperature, with differential responses of individual seaweeds observed when subjected to moderate and high temperatures (Ismail & Osman, 2016; de Silva *et al.*, 2017). Significantly lowered ratios of Chl-*a*: Car were observed in *G. manilaensis, U. reticulata* and *K. alvarezii* at 40 °C. Results of significantly low chlorophyll-a content in *G. manilaensis* and *K. alvarezii* at 40 °C (Table 4.2) and significantly high carotenoid content in *G. manilaensis* (40 °C), *U. reticulata* (40, 35 and 20 °C) and *T. conoides* (35 and 30°C) relate well to these previous findings and possibly indicate a stressful condition at these temperatures for the seaweeds. The low carotenoid content in *K. alvarezii* at 40 °C could be due to the bleaching of the seaweed.

Emissions of $CHBr_3$ and CH_2Br_2 were negatively correlated with carotenoid content of *G. manilaensis* and *U. reticulata*, but they were positively correlated with carotenoid content of *K. alvarezii.* Meanwhile, the halocarbon emissions of $CHBr_3$, CH_2Br_2 and CH_2I_2 were positively correlated to Chl-*a* content in *G. manilaensis* and *K. alvarezii* while negatively correlated to Chl-*a* content in *U. reticulata* (Table 4.3). Correlations between

Chl-*a*: Car with the emission rates of most halocarbon were strongly negative in *G. manilaensis* but were positive in *K. alvarezii.* This suggest that any direct correlation of pigment content on the emissions of these compounds could be hard to determine, although species-specific responses of pigment content towards temperature changes has been recorded (de Silva *et al.*, 2017). This study could nevertheless serve as a starting point for future research into the relationships between pigments, temperature and halocarbon emissions in seaweeds.

The morphology and structure of the seaweed thallus may influence its response to temperature. The multi-layered seaweeds with main axis and branches (Hay, 1986), such as *K. alvarezii* and *T. conoides*, which also have higher moisture content (90 and 85 %, Table 3.2) were found to emit higher amount of the $CHBr_3$, CH_2Br_2 and CH_2I_2, compared to *U. reticulata,* which has thin, translucent blades (Hay, 1986). *T. conoides* belongs to the brown seaweeds which are known to contain iodine in the vesicles. The physode-like vesicles of temperate brown seaweeds such as *Laminaria digitata* are able to store iodine (Küpper *et al*., 2008). The high concentration of iodine in *L. digitata* resulted in the increased production of iodinated halocarbons (Küpper *et al.* 2008). This could be an additional factor that could contribute for the higher emission of halogenated compounds especially CH_2I_2 by *T. conoides*.

The effect of temperature on halocarbon emission is species-specific. In general, with the multi-layered branched seaweeds, *K. alvarezii and T. conoides*, there were stronger negative correlations (r = (−0.95)–(−0.80); p<0.01) between the emission rates of $CHBr_3$, CH_2Br_2 and CH_2I_2 at 28 hours post-exposure with temperature compared to *U. reticulata* (r = (−0.74)–(−0.69); p<0.01). *G. manilaensis* however, did not show any strong correlations between the emission rates of $CHBr_3$, CH_2Br_2 and CH_2I_2 at 28 hours post-exposure with temperature (Table 4.1). A bigger decrease in the F_v/F_m values from these

two multi-layered, branched seaweeds was observed 4 hours post-exposure to 40 °C although all four seaweeds species showed significant decrease in the F_v/F_m at higher temperatures of 40 and 35 °C both 4- and 28-hours post-exposure (Appendix D). The F_v/F_m value decreased 93% and 95% in both *K. alvarezii* and *T. conoides,* 4 hours post exposure at 40 °C while in *G. manilaensis* and *U. reticulata*, the values were at 40 and 54% (Appendix D). At 35 °C with the same exposure duration, both *K. alvarezii* and *T. conoides* again showed more than 20% decrease in F_v/F_m compared to *G. manilaensis* and *U. reticulata* at 12 – 16%.

The responses of seaweeds towards temperature change could involve changes in both enzymatic and chemical reactions leading to halocarbon production or changes to other aspects of physiology that subsequently affect the halocarbon production in the seaweeds. Factors such as the resilience capability of the individual seaweed could contribute to the variability of the acquired results. It is therefore crucial for more studies to explore this area, especially the formation pathway of halocarbons. Meanwhile, in view of future climate scenarios, a broader approach incorporating multiple environmental factors (Boyd *et al.*, 2018, Hopkins *et al*., 2020) at the mesocosm level and at a longer exposure duration could help to reduce the uncertainties in prediction arising from scarcity of data in this area.

5.2 Combined effect of temperature and irradiance on the halocarbon emissions by *Kappaphycus alvarezii* under controlled laboratory conditions

K. alvarezii is an economically important seaweed and one of the most commonly species in Malaysia. Production of farmed seaweeds in the country increased from 60,000 tonnes in 2006 to 202,966 tonnes in 2017 (FAO, 2018 and 2020). In this study, four halocarbon compounds, i.e. $CHBr_3$, CH_2Br_2, CH_2I_2 and CH_3I, were chosen for investigation due to: the high emission rates by *K. alvarezii* of $CHBr_3$, CH_2Br_2 and CH_2I_2 (see Section 4.1); the significant roles of $CHBr_3$ and CH_2Br_2 towards the loss of stratospheric ozone (WMO, 2018); and the ability of CH_2I_2 and CH_3I to contribute towards cloud nuclei formation (Saiz-Lopez *et al.*, 2011). The average water temperature logged at the hatchery was 27.32 ± 0.85 °C (n = 3166), with the range of 25.32–30.05 °C, while irradiance had a range of 0–2141 µmol photons m^{-2} s^{-1} (Figure 5.1). The temperature and irradiance levels selected in this experiment were either within (irradiance) or covered the entire (temperature) range observed in the hatchery.

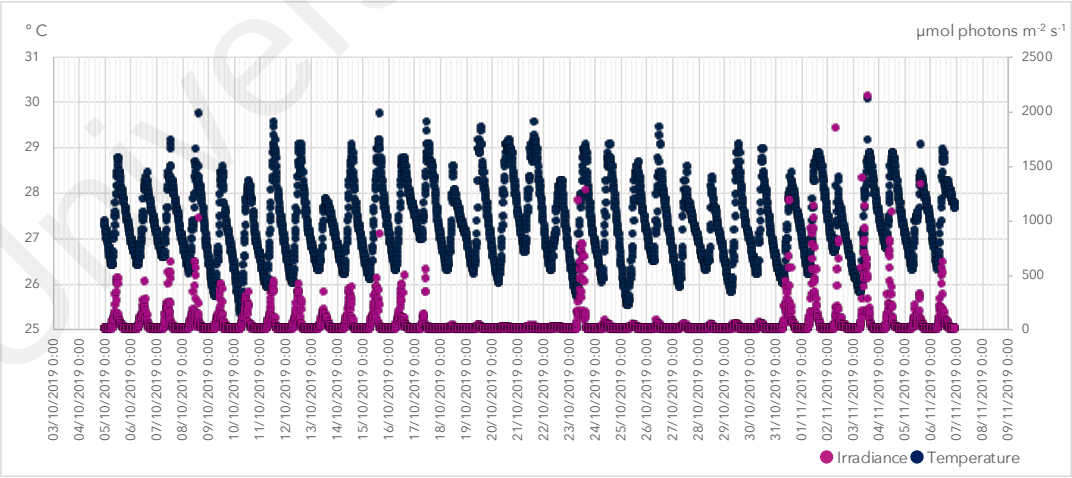


Figure 5.1: Irradiance and temperature data logged at the University of Malaya hatchery during the entire cultivation and experimentation period

Most previous published reports concerning the effect of irradiance on halocarbons showed that seaweeds released higher concentrations of halocarbons during the presence of light compared to the dark (Mtolera *et al.*, 1996; Carpenter *et al.*, 2000; Manley & Barbero, 2001; Keng *et al.*, 2013; Keng *et al.*, 2020). The short-term six hours incubation of a brown temperate seaweed, *Laminaria digitata*, under natural light showed at least a two-fold increase in the emissions of compounds such as CH_2Br_2, CH_2BrI and $CHBr_2I$ compared to the dark. Releases of the dominant compound, $CHBr_3$, were 10 times higher in the light compared to the dark (Carpenter *et al.*, 2000). Nightingale *et al.* (1995) showed an increase in $CHBr_3$, CH_2Br_2, $CHCl_3$ and $CHBr_2Cl$ after 48-hours incubation under irradiated conditions compared to the dark, while the emissions of CH_3I decreased (Table 2.2). Similarly, emission rates of $CHBr_3$, CH_2Br_2, CH_2I_2 and CH_2BrI from Malaysian specimens of *Turbinaria conoides* and *Sargassum binderi* were higher under irradiated conditions compared to the dark (Keng *et al.*, 2013). Results from this study were consistent with these previous findings, with lower emission rates observed in the dark treatment at most of the temperatures. In addition, the upward trend of CH_3I observed across the irradiance levels, and the significantly higher emission rate at L4 and at 34 °C, indicate that at high temperature, CH_3I emissions could increase with irradiance, and could be suggestive of photochemical production. Oxidative stress conditions could increase CH_3I production by seaweeds, and the production of CH_3I could involve an *S*-adenosyl-methionine-dependent methyl transferase, which is independent of the type of ROS present. During stress, the enzyme reacts with *S*-adenosyl-L-methionine and halide to yield *S*-adenosyl-L-homocysteine and methyl halide (Küpper *et al*., 2018).

The effect of irradiance was also significant at 31 °C where emission rates of CH_2I_2, CH_2Br_2 and $CHBr_3$ were higher at L4 compared to L1, L2 and L3 (Figure 4.3). A previous study on Malaysian *T. conoides* and *S. binderi* showed a positive correlation between irradiance levels and halocarbon emission rates, while an insignificant correlation was observed in *Padina australis* (Keng *et al.*, 2013). The present study showed all four halocarbon compounds to be positively correlated to irradiance ($0.421 \leq r \leq 0.554$; $p<0.001$; $n = 64$). The drop in emission rates at higher temperature, i.e. 34 °C, could have affected the strength of this correlation.

Changes in temperature affect halocarbon emissions. Higher temperatures at 31 and 34 °C decrease emission rates of $CHBr_3$ (Figure 4.4.). However, responses were rather specific to each compound under different temperature settings. While the emission of CH_2Br_2 was not affected by increased temperature when incubated in the dark, the emission of $CHBr_3$ decreases with increasing temperature at L4 and in the dark (Figure 4.4). Therefore, a general response did not apply for all of the halocarbons investigated. There was only a significant negative correlation observed between temperature change and the emission of $CHBr_3$ ($r = -0.633$; $p<0.01$; $n = 64$) from this study. Strong negative correlation ($r = -0.83$; $p<0.01$; $n = 20$) were observed at temperature changes of 20 to 40 °C with *K. alvarezii* at 28 hours after temperature shift (see Section 4.1), while exposure to the same temperature range for 4 hours showed a correlation of $r = -0.69$ ($p<0.01$; $n = 20$). The lesser extent of correlation between the halocarbon emission rates and temperature found in this study compared to the previous laboratory study at 28 hours exposure duration could be due to the influence of irradiance. The finding from the previous experiment on polar seaweeds corresponds well to these data. Investigation of polar *Gymnogongrus antarcticus* showed increased emissions of $CHBr_3$ but decreased emissions of CH_2I_2 when the red seaweed was exposed to a temperature increase of 10

°C from 0 °C for 24 hours. Prolonged exposure to this temperature saw decreases in $CHBr_3$ emissions (Laturnus *et al.*, 2000). Studies on temperate seaweeds *Cladophora glomerata* and *Ulva ahlneriana*, involving 6 and 10 hours of temperature switches from 12 to 23 °C and vice versa under both laboratory and field conditions, did not show a general trend in the effect of temperature on halocarbon emissions (Abrahamsson *et al.*, 2003). It is notable that only a handful of studies in this area are currently available.

A two-way ANOVA test (Table 4.4) confirmed that the combined effect of irradiance and temperature affects the emission rates of all four halocarbons emitted by *K. alvarezii*. Limited data are available on the combined effect of irradiance and temperature on the halocarbon emissions of seaweeds at the current time. In-depth study on the effect of these two factors on halocarbon emissions by seaweeds is lacking. There are, however, *in situ* observations from rockpool studies that noted higher halocarbon concentrations at midday when both irradiance and temperature could be high. One of the studies mentioned that temperature was rather constant at midday at the time of sampling (Carpenter *et al.* 2000; Table 2.2 Study Number 24), pointing to the possibility of dominance in the effect of irradiance over temperature, in the two-fold increase in emissions. A second study by Nightingale *et al*. (1995) showed a rise in the emissions of several halocarbon compounds, including CH_2Br_2, $CHBr_3$ and CH_3I, by temperate seaweeds in a rockpool, which corresponds to an increase in temperature of around 5 °C between 14:00 and 20:00 hours. The irradiance level was not reported. Seaweeds found in the rockpool included *Fucus serratus, Ascophyllum nodosum, Dumontia contorta, Enteromorpha* sp., *Cladophora albida, Chaetomorpha* sp. and *Gigartina stellata.* A third rockpool study dominated by *Cystoseria abies-marina* in Spain showed an increase in halocarbon emissions at midday when the temperature increased to around 28 °C from 21 °C (Ekdahl *et al.*, 1998). This included a four-fold increase in the $CHBr_3$ and CH_2Br_2

concentrations. In contrast, the concentration of CH_3I fluctuated throughout the day. The effect of irradiance was not studied. The authors suggested that the hike in the emission rate of the halocarbons could be due to the increase in photosynthesis activities at midday.

Photosynthetic activity could produce reactive oxygen species, which act as a substrate to bromoperoxidases present in the seaweed to produce halocarbons. Increased photosynthetic activities could potentially increase halocarbon emissions by seaweeds, as does oxidative stress from abiotic environmental change (Keng *et al.*, 2020). Investigations into the effects of irradiance and temperature on the photosynthesis and growth of *K. alvarezii* showed that the photosynthetic rate increases with irradiance, provided that the irradiance level does not exceed the estimated saturation irradiance of 154 μmol photons m^{-2} s^{-1} (Terada *et al.*, 2016). At all temperature levels, the F_v/F_m values observed in our experiment decreased at L4 (177 ± 26 μmol photons m^{-2} s^{-1}), if not at L3 (117 ± 13 μmol photons m^{-2} s^{-1}) except at 31 °C, where changes across the irradiance level did not significantly affect the F_v/F_m values (Appendix F). A high growth rate of *K. alvarezii* has been reported at temperature levels of 28 and 32 °C (Terada *et al.*, 2016), while a decline in F_v/F_m has been observed at temperatures above 30 °C (Borlongan *et al.*, 2016). In this study, the changes in F_v/F_m pre- and post-incubation were correlated with temperature change (r = −0.616; $p<0.0005$; n = 20), but a relationship between changes in F_v/F_m values (pooled data; Table 4.10) with irradiance could not be established.

The production of $CHBr_3$, CH_2Br_2, and CH_2I_2 has been proposed to arise from haloperoxidase activity. Bromination of H_2O_2 by bromoperoxidases produces hypohalous acid, which gets released into the seawater. The hypohalous acid will then react with dissolved organic matter (DOM) in the seawater to form DOM-halide, resulting in the production of poly-brominated and poly-iodinated compounds (Wever & van der Horst,

2013). Emission rates of $CHBr_3$, CH_2Br_2 and CH_2I_2 were strongly correlated with each other (r = 0.7 – 0.8; p<0.01; n = 64; Table 4.9). Correlations of CH_3I with other compounds were comparatively weaker. As mentioned, the production of CH_3I has been suggested to involve the *S*-adenosylmethionine (SAM): halide ion methyl transferase reactions (La Barre *et al.*, 2010), and could be contributed by other factors via photochemical production (Ref).

Halocarbon emission rates were rather low at 31 °C compared to other temperatures (Figure 4.3). The average F_v/F_m values at the same temperature did not indicate any significant differences from those at temperatures of 25, 28 and 34 °C (Figure 4.5). Similar experimental procedures were taken throughout the study, with the same batch of seaweed samples used and the same cultivation conditions maintained throughout. Therefore, the low emissions observed at 31 °C could be due, though not limited, to differential physiological responses of the seaweeds (n = 16) at this temperature. The physiological responses not measured in this experiment, such as respiration, and bromoperoxidase and catalase activities (Ekdahl *et al.*, 1998) could be affecting the availability of H_2O_2 for the reaction of haloperoxidases and therefore causing the low rate of emissions.

The average emission rate for the most abundant compound, $CHBr_3$, by *K. alvarezii* in the dark was 199–237 pmol gFW^{-1} hr^{-1} at 25 and 28 °C. Taking into consideration the 12-hour light–dark cycle in Malaysia, the dark phase itself could have contributed around 2388–2844 pmol $CHBr_3$ gFW^{-1} day^{-1}. If extrapolated to the production scale of seaweeds in Malaysia (FAO, 2020), this rate could be approximately six magnitudes higher. On the other hand, emission rates during daylight at 25 and 28 °C showed emissions two to three times higher than in the dark. This should be taken into account in the estimation of emission rates over diurnal fluctuations. Also, since both temperature and irradiance

affect the emissions of $CHBr_3$, CH_2Br_2, CH_2I_2 and CH_3I by *K. alvarezii*, the combination effect of these two parameters at different levels should be assessed carefully. A comprehensive approach encompassing the considerations on environmental change, physiological responses of the seaweeds and community interactions should be included for better estimation of the contribution by *K. alvarezii* to the regional halocarbon pool. Estimation and prediction should involve mesocosm studies of seaweeds under various environmental settings, while laboratory-based studies could provide a better understanding of the physiological background to the emissions of halocarbons by seaweeds.

A 1.5 °C increase in global temperatures is expected in the next decade (IPCC, 2018). This might create more favourable farming conditions in subtropical waters (Largo *et al.*, 2017), increasing the capacity for the farming of *K. alvarezii*. As *K. alvarezii* has been reported to be photosynthetically tolerant to high levels of irradiance, i.e. no photoinhibition was observed at 1000 μmol photons m^{-2} s^{-1} (Borlongan *et al.*, 2016), an increase in the production of this seaweed could be accompanied by increased halocarbon load in the atmosphere. This increased load could contribute further to stratospheric ozone depletion and potentially affect local climate. However, the net outcome is still hard to predict as other factors have to considered as well. For example, increased temperature and the practise of monoclonal seaweed farming could in fact weaken the resistance of the seaweed (Largo *et al.*, 2017) to disease and other infestations. These factors could counteract the positive effect of temperature increase on seaweed farming in the sub-tropical regions. In addition, temperature also affect ocean acidification and salinity fluctuations in the ocean, and these can also impact on the halocarbon emissions (Punitha *et al*. 2017). Therefore, more detailed studies looking at the interactions of all these

factors could give a clearer understanding of what will happen to halocarbon emission by seaweed farms in future climate conditions.

5.3 *In situ* measurements of halocarbon emissions by cultivated seaweeds

This study was designed to characterise the halocarbons released by seaweeds cultivated using three different systems: onshore tanks, an offshore platform and cage culture at the river mouth. These three cultivation systems were located at three different yet nearby locations with varying levels of irradiance, temperature, seawater nutrient and salinity levels.

5.3.1 Halocarbon emission trends at the three different cultivation systems

Both $CHBr_3$ and CH_2Br_2 were released in higher amounts by all seaweeds i.e. *G. manilaensis*, *K. alvarezii* and *U. reticulata* cultivated at the various cultivation systems, followed by $CHBr_2Cl$ and $CHBrCl_2$ (Table 4.11, 4.16; Figure 4.8). This observation agrees well with the two previous laboratory-based experiments, as well as previously published data (Table 2.1).

At the onshore cultivation system, higher halocarbon emissions were observed during the fourth sampling trip (4-week-old culture) as compared to the second sampling trip (<3 days culture) by both *G. manilaensis* and *K. alvarezii* during daylight (Table 4.11). This might be due to more stressful conditions and less healthy seaweeds at the onshore cultivation system, 4 weeks into cultivation, as compared to the < 3 days culture measured during the second sampling trip. As the cultivation condition at the Bachok Marine Research Station (BMRS) is different from the original habitat of the seaweeds prior to cultivation, a change in environment could trigger oxidative stress responses in the seaweeds, causing the build-up of ROS (Dietz, 2016), eventually leading to a rise in halocarbon production.

The emissions measured in the dark did not differ much between the sampling trips and remained comparably low against daylight emissions by seaweeds cultivated at the onshore tank system. Significantly higher amounts of halocarbons were released by both *G. manilaensis* and *K. alvarezii* (except $CHBrCl_2$ by *G. manilaensis*, Table 4.12) during daylight compared to dark. This indicates a strong link between photosynthesis and the production of halocarbons in seaweeds. Photosynthesis and respiratory electron transport produce ROS i.e. superoxide anion radical (O^{2-}), the hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) (Rutherford *et al*., 2012; Wever & van der Horst, 2013). While respiration happens throughout the day, processes such as pseudocyclic photophosphorylation or the Mehler reaction which produce ROS as a result of electron transport from the ferrodoxin of PSI to the oxygen molecule are limited to daylight (Collén *et al.*, 1995; Manley & Barbero, 2001; Dummermuth *et al*., 2003). Higher amount of ROS present increases halocarbon production (Wever & van der Horst, 2013; Abrahamsson *et al*., 2018).

The emission of $CHBrCl_2$ by *G. manilaensis* did not differ significantly ($p>0.1$) between daylight and dark. This could indicate that the production of this halocarbon by the seaweed might not be directly affected by changes in environment, although significant difference was noted in the emission by *K. alvarezii* (Table 4.12). In the production of halocarbon by haloperoxidase, $CHBr_3$ is produced as a result of halide oxidation by H_2O_2, which increases in stressful conditions. This is followed by the reaction with ketones or dissolved organic matter (DOM) in the environment (Wever *et al.*, 1991; Opsahl & Benner, 1997; Lin & Manley, 2012; Wever & van der Horst, 2013; Liu *et al*., 2015). Other brominated compounds such as CH_2Br_2, $CHBr_2Cl$ and $CHBrCl_2$ could be formed either through the same enzymatic pathway or through subsequent nucleophilic substitution of $CHBr_3$ (Abrahamsson *et al.*, 2018). The higher $CHBrCl_2$ emission during daylight by *K. alvarezii* could be due to higher amount of $CHBr_3$

produced by the seaweed (Table 4.11), which in turn, increases the amount of $CHBrCl_2$ in the sample, creating the significant difference in the emission of $CHBrCl_2$ between the daylight and dark sample.

The Mann-Whitney U test (Table 4.12) showed that the mixing ratios of halocarbons in all the Control samples were not affected by irradiance, as no significant difference was noted between the daylight and dark readings. The Control samples were background air incubated in the flux chamber without the presence of seaweeds. Therefore, the background halocarbon mixing ratios could not have contributed to the significant differences in the daylight and dark samples released by the seaweeds.

Different trends in the emissions of CH_2Br_2 and $CHBr_3$ were evident in the offshore seaweed samples throughout the cultivation period, particularly by *K. alvarezii. G. manilaensis* releases a greater amount of CH_2Br_2 than $CHBr_3$ and the average ratio of CH_2Br_2 to $CHBr_3$ (CH_2Br_2: $CHBr_3$) remained rather constant (1.3 – 1.4) throughout the cultivation cycle while CH_2Br_2: $CHBr_3$ of *K. alvarezii* decreased (Table 4.14). As these two seaweeds were cultivated at the same offshore platform within a space of 5 x 5 m, the difference in the ratio change in *K. alvarezii* could not be due to differences in DOM distribution (discussed in the next paragraph) but rather, an unknown factor such as different tolerance level of the seaweeds that causes the difference in emission behaviour between *G. manilaensis* and *K. alvarezii*.

The CH_2Br_2: $CHBr_3$ emissions ratio of *G. manilaensis* cultivated at the offshore system (1.4 ± 0.6) was higher than the onshore system (0.3 ± 0.2), while CH_2Br_2: $CHBr_3$ was highest in the river culture (4.2 ± 1.0; Table 5.2). The same was observed in *K. alvarezii* cultivated onshore (0.5 ± 0.3) and offshore (1.2 ± 0.9). A search through previously published literature on halocarbon emissions by *Gracilariales* species, including *G.*

changii and *G. salicornia*, showed $CHBr_3$ to be the dominant halocarbon released, rather than CH_2Br_2 (see Section 2.1.1; Table 2.1). This corresponds to the findings from the onshore emissions, but not the offshore and river emissions where emission of CH_2Br_2 became dominant. The difference between the CH_2Br_2: $CHBr_3$ ratios at these two systems could reflect the different locations where the seaweeds were cultivated. The seawater for the onshore cultivation system was pumped in from nearshore, approximately <5 m from the shoreline, while the offshore platform was around 600 – 650 m away from the shore. The difference in the location could possibly indicate a different distribution of DOM in the seawater. Changes in the composition of DOM have been related to the quantitative change between CH_2Br_2 and $CHBr_3$ emissions (Lin & Manley, 2012; Liu *et al*., 2015), causing a difference to the CH_2Br_2: $CHBr_3$ between samples collected from the coastal regions and the open ocean (Carpenter *et al*., 2009). The different chemical compositions of DOM may react differently to HOBr, an intermediate compound formed through the bromination of H_2O_2 by the reaction of haloperoxidases in seaweeds (Wever & van der Horst, 2013).

Table 5.2: Comparison of CH_2Br_2: $CHBr_3$ between seaweeds at the different cultivation systems

Cultivation systems	*G. manilaensis*	*K. alvarezii*
Onshore tank	0.3 ± 0.2 (0.1 – 0.4)	0.5 ± 0.3 (0.2 – 1.0)
Offshore platform	1.4 ± 0.6 (0.4 – 2.0)	1.2 ± 0.9 (0.1 – 2.4)
Cage culture	4.2 ± 1.0 (3.5 – 5.0)	-

5.3.2 Halocarbon emissions and the environmental parameters

The comparison of halocarbon mixing ratios by *G. manilaensis* between the three cultivation systems showed that cage culture at the river at Sungai Melawi enhanced the emission of the brominated compounds, CH_2Br_2 and $CHBr_3$ by the seaweed. CH_2Br_2 seemed to be the compound most affected by the type of cultivation system used, as the emission of this compound by *G. manilaensis* significantly varies with the different cultivation systems (Table 4.16). The emissions of CH_2Br_2 were significantly ($p< 0.05$) lower at the onshore tank system, followed by the offshore platform, then the river cage culture.

As the seaweeds cultivated at the three systems originated from the same source, the difference in emissions is attributed to the environmental factors. The comparison of seawater nutrient contents and salinity readings between the three cultivation systems showed a significantly high level of phosphorus (0.39 ± 0.01 mg/L, $p< 0.05$) and an extremely low salinity level (4 ± 0 ppt, $p< 0.05$) at the river where the seaweeds were cultivated in cages (Table 4.19). These results correspond to the significantly high ($p< 0.05$) emissions of CH_2Br_2 and $CHBr_3$ at the river (Table 4.16). Meanwhile, temperature ranged from 24.2 – 43.2 °C at the river throughout the cultivation period. This was the highest range recorded among the three cultivation systems, followed by the offshore system (24.7 – 40.3 °C) and onshore tank system (24.6 – 35.7 °C) (Table 4.20). This too, corresponds to the increasing CH_2Br_2 emissions from onshore, offshore and cage cultivated *G. manilaensis* (Table 4.16).

To further determine the relationship between environmental factors with halocarbon emissions by *G. manilaensis,* the emissions data was pooled from all three cultivation systems and plotted against the irradiance and temperature readings at the time of measurements (in the flux chamber), and all daylight emission data against the seawater

nutrients and salinity (Table 4.22, 4.26). Positive correlations were recorded between temperature and the emissions (r = 0.668 – 0.805; p< 0.005), and between irradiance and the emissions of all halocarbons (r = 0.565 – 0.724; p< 0.01). This was in agreement with the findings from Section 4.1 of the effect of temperature on *G. manilaensis* in the laboratory (Table 4.1), as well as the effect of both temperature and irradiance on *K. alvarezii* (Table 4.2). The correlation between temperature and emission became weak ($r_{partial}$ = 0.436 – 0.518; p< 0.05; Table 4.22) when irradiance was controlled. The emission of CH_2Br_2 and $CHBr_3$ was also positively correlated to seawater phosphate levels (r = 0.734, 0.546; p< 0.05; Table 4.23). However, a direct relationship between seawater salinity and the halocarbon emission could not be established. This could indicate that higher phosphate level at the river mouth could be exerting a dominant effect in driving higher emissions of CH_2Br_2 and $CHBr_3$ instead of the salinity change, since *G. manilaensis* is tolerant to a wide range of salinity e.g. able to grow in fish ponds and has been reported to be able to grow well at a salinity level of 15 ppt (Mohamad Hidayat *et al.*, 2015). However, an under-representation of river samples (Appendix D) in the pooled data, with majority of the input coming from onshore-tank and offshore cultures, it is necessary that this work be expanded to include cultivation at brackish water and the mangrove areas for a better representation of salinity effect.

Meanwhile, *K. alvarezii,* which was only cultivated at the onshore and offshore platform, showed significantly (p< 0.0005) higher emission of CH_2Br_2 in the onshore cultivation system compared to the offshore site. The emission of other halocarbon compounds was not affected by the different cultivation systems (Table 4.17, 4.21). Although seawater nutrient contents and salinity did not fluctuate much between the onshore and offshore cultivation system (Table 4.19), further testing on the relationship between these factors and the halocarbon emission by *K. alvarezii* found negative correlations between the ammoniacal nitrogen, nitrate and nitrite with the emission of

CH_2Br_2 (Table 4.24). Positive correlation (r = 0.601, p< 0.601; Table 4.24) was found between the emission of CH_2Br_2 with seawater salinity within the range 21 – 38 ppt. This suggested that halocarbon emission by cultivated *K. alvarezii* was more sensitive to changes in seawater salinity than *G. manilaensis*.

Nitrogen and phosphorus are the two most important nutrients for seaweed growth (Roleda & Hurd, 2019). Nitrogen is indispensable for making amino acids, the basic building blocks for proteins/ enzymes involved in respiration and photosynthesis, while phosphorus is critical for DNA and RNA. Nutrient requirement in the seaweeds depends on supply and demand, whereby growth is limited by the nutrient in most limited supply (Hurd *et al*., 2014; Roleda & Hurd, 2019). This may probably relate to the observation that the emission of CH_2Br_2 and $CHBr_3$ by *G. manilaensis* was affected by phosphate levels but was not affected by the seawater nitrogen contents, and the emission of CH_2Br_2 by *K. alvarezii* negatively correlates with the nitrogen content of the seawater but did not respond to changes in phosphate levels. The distribution of organisms such as zooplankton at the various cultivation systems could contribute to increased levels of phosphate, and at the same time excretes DOM, which could affect halocarbon production by seaweeds (Condon *et al.,* 2010; Lin & Manley, 2012; Findlay & Parr, 2017).

5.3.2.1 Emission changes between daylight and dark by the cultivated seaweeds

In general, daylight halocarbon emissions were higher than dark emissions. This finding is in line with what has been observed and discussed in Section 5.2 and the previous literatures (Table 2.2), although halocarbon measurements were carried out at different temperature (25 – 28 °C, 24 – 40 °C) and irradiance levels (0 – 177, 0 – 352 μmol photons m^{-2} s^{-1}) between the laboratory-based study and the cultivation study. For a clearer picture of how halocarbon emissions from tropical seaweeds could vary between daylight and dark, the ratios of daylight to dark (L:D) emissions by all seaweeds from this and a previous study was calculated and the lower bound (LB) and upper bound (UB) to the ratios were determined (Table 5.3).

The averaged L:D ratios showed that halocarbon emissions by *G. manilaensis* in the day could be around 15 to 105 times higher than dark emissions. The daylight emission of CH_2Br_2 by *G. manilaensis* was 105 times higher compared to the dark, followed by 17 times in the $CHBr_2Cl$ emission, 15 times in $CHBr_3$ and $CHBrCl_2$ emission. Meanwhile, the LB ($\leq$1) determined from the present study showed that while it is possible that emissions during the dark could be close to or exceeds the daylight emissions, this might be due to the data being compiled irrespective of the different cultivation systems and the age of the seaweed culture. A 3000-fold increase in CH_2Br_2 emission by *G. manilaensis* during daylight compared to the dark was recorded. The exceptionally high upper bound value for CH_2Br_2 emission in daylight to dark was due to the high data input of emission from the cage culture at the river (1165 ± 143 ppbv), compared to the average low value of 1.3 ± 1.4 ppbv of dark emissions collected from the onshore and offshore seaweeds. Without it, the emission of CH_2Br_2 by *G. manilaensis* was approximately 15 times higher in daylight compared to the dark.

Carpenter *et al.* (2000) reported a 10-fold increase in $CHBr_3$ during daylight compared to dark emissions by the temperate brown seaweed *Laminaria digitata*. However, this was the largest increase of all halocarbons investigated, with most of the other compounds, including CH_2Br_2 and $CHBr_2Cl$, reporting at least a two-fold increase during the day. The quantification was derived from seawater measurements of the compounds (Carpenter *et al.*, 2000).

K. alvarezii reported an average increase of two to five times of emissions of the investigated halocarbons during daylight relative to the dark. This value agrees with the previous laboratory-based experiment where *K. alvarezii* showed a two- to three-fold higher emission of halocarbons by *K. alvarezii* under illuminated conditions. Although calculations from the previous experiment were based on seawater contents of halocarbons, the values were still in the same order of magnitude as observed in this study. These ratios could provide details on the changes in emissions between dark and illuminated conditions, and perhaps reduce the uncertainty about the diurnal emissions of farmed tropical seaweeds.

Table 5.3: Lower bound (LB) and upper bound (UB), and the average (bracketed) light:dark ratios of halocarbons emitted by tropical seaweeds during illuminated or daylight and dark incubation

Seaweed	CH_2Br_2		$CHBr_2Cl$		$CHBr_3$		$CHBrCl_2$	
	LB	UB	LB	UB	LB	UB	LB	UB
This study								
G. manilaensis	1	3241	0	103	0	85	0	100
	(105) (15*)		(17) (13*)		(15) (52*)		(15) (10*)	
K. alvarezii	1	39	0	14	0	9	1	25
	(5)		(3)		(2)		(4)	
U. reticulata	(2)		(2)		(1)		-	
Keng *et al.*, 2013								
S. binderi	6	49	101	485	161	654	-	-
T. conoides	15	32	5	11	9	23	1	3
P. australis	0	27	0	37	1	121	-	-

*average ratios by *G. manilaensis* cultivated at the onshore tank and offshore platform

The L:D ratios determined from the three wild seaweeds (Keng *et al.,* 2013) showed that daylight emission could reach as high as 650-fold higher than in the dark by the brown seaweed *S. binderi*. Factors were higher in the brown seaweeds from the wild than the *in situ* measurements on red seaweeds in this study. This could also be due to the derivation of the ratio from published average emission data. Also, emissions data were determined from seawater measurements, while ratios from this study were determined from *in situ* studies involving air samples. It is as yet unclear how or why a different analytical matrix could alter the apparent formation by seaweeds under illuminated and dark conditions. Although L:D ratios determined from air samples of *K. alvarezii* in this study were in agreement with the laboratory-based result determined from seawater samples i.e. less than a magnitude of 10 in both case (Figure 4.3), differences could possibly occur considering the halocarbon production and loss mechanism, and the differences in sampling conditions and techniques of sampling and analysis. This is a

potential field for further exploration to minimise the uncertainties for the comparison of halocarbon emissions derived from seawater or air sample data. Halocarbon production could escalate through the production of H_2O_2 during possible desiccation stress (Leedham *et al*., 2015). Meanwhile, as DOM in seawater enhanced halocarbon production (Lin & Manley, 2012), the amount of seawater and hence the availability of DOM during air sampling could affect the halocarbon production by the seaweeds. Although the effect of desiccation might not be prevalent as the seaweeds were moistened prior to air sampling and a short incubation time of 30 minutes was used, the introduction of a standardised approach could make comparisons easier. A standardised sampling and analytical approach, including the use of the same analytical technique for air and seawater analyses (Leedham, 2013), could minimise the unknown.

5.3.3 Estimation of halocarbon emission from the farming of *K. alvarezii* in Malaysia

While data on *G. manilaensis* production is limited, a recent statistic from FAO (2020) showed a total production of 202,966 tons (FW) of *K. alvarezii* was produced in Malaysia in 2017. Simple extrapolation based on this biomass data could give an estimate of the potential contribution of *K. alvarezii* farming in Malaysia in terms of its halocarbon emissions. As the air samples from this study were collected from fresh and moistened *K. alvarezii*, this estimate could reflect the potential increase in halocarbon load into the atmosphere during harvesting of the seaweeds at the *K. alvarezii* farms in Malaysia.

The estimated range of bromine contribution based on the release of the dominant compound, $CHBr_3$, during harvesting of *K. alvarezii* alone is between 72 and 360 mol Br hr^{-1} (Table 5.4). This was based on the mixing ratios of $CHBr_3$ emitted from *K. alvarezii* collected from the offshore platform (Table 4.13). This could be the first estimate of halocarbon emissions determined through *in situ* measurements of farmed seaweeds in the tropics, which is an important region for the vertical transport of reactive bromines into the upper troposphere/lower stratosphere.

Table 5.4: Estimation of halocarbon emissions by farmed *K. alvarezii* in Malaysia based on total production in year 2017

	Mixing ratios (ppbv)	Estimated Range* (ppbv kg FW^{-1} hr^{-1})	Estimated Range (mol Br hr^{-1})
CH_2Br_2	11.6–61.5	46–246	30–160
$CHBrCl_2$	0.1–0.5	0.4–2.0	0.1–0.6
$CHBr_2Cl$	0.6–2.5	2.4–10	1.6–6.5
$CHBr_3$	18.4–92.8	74–371	72–360

* Scaled-up from the mixing ratios measured when 500 g of *K. alvarezii* was incubated in the 40L flux chamber for 30 minutes; conversion to mol Br were calculated based on an average air temperature of 32.35 °C.

A bottom-up estimation could be derived based on the FAO (2020) *K. alvarezii* biomass data and the flux rate previously determined by Leedham *et al.* (2013) for comparison with estimated value of 72 – 360 mol Br hr^{-1} determined from this study. The $CHBr_3$ flux rate of 17–73 nmol $CHBr_3$ m^{-2} hr^{-1} was derived from the seawater incubation studies of Malaysian seaweeds (Leedham *et al*., 2013). An acre (4046.86 m^2) in the sea could produce up to a total of 1500 kg DW (38–50% moisture content; Phang *et al.*, 2019) of *K. alvarezii* (Sade *et al.*, 2006). If all this information (assuming an average of 44% moisture content), as well as the biomass figure from FAO (2020), is incorporated into the flux rate (Leedham *et al*., 2013), the expected contribution of farmed *K. alvarezii* in 2017 would be around 12.3–52.8 mol Br hr^{-1}. This is based on the assumption that the total biomass, according to FAO (2020), was produced only once from a total sea surface area of 2.4 x 10^8 m^2 (see Table 5.5 for calculations). This bottom-up estimation derived from the flux rate by Leedham *et al.* (2013) is somewhat lower but close to the emission rate of 72–360 mol Br hr^{-1} in this study, which was determined through direct measurement from the biomass of farmed *K. alvarezii* (Figure 5.2).

As with all comparison studies, differences in the estimations could arise due to the various assumptions used. While the emission rate in this study was measured directly from the source, the flux rate from Leedham *et al*. (2013) was determined from laboratory incubations of a myriad of red, brown and green seaweeds. Red seaweeds are generally greater emitters of halocarbons compared to brown and green seaweeds (Leedham *et al.*, 2013). Table 2.1 showed that production rate of brown and green seaweeds reported by Leedham *et al.* (2013) could be 2 – 20 times lower than the emission rate of *K. alvarezii* derived here. Also, the flux rate was determined based on the seaweed distribution at Cape Rachado, Port Dickson, which was dominated by brown species instead of red (Keng, 2013). The density of red seaweed in the wild at that time was 0.1 kg FW m^{-2}. This is eight times lower than the density of farmed *K. alvarezii* at 0.84 kg FW m^{-2},

assuming maximum farming capacity was achieved (Sade *et al.*, 2006). Meanwhile, diurnal variations were taken into consideration by Leedham *et al*. (2013) by reducing the initial emission rates by 60 %. These could explain the differences between the lower value of 12.3–52.8 mol Br hr^{-1} estimated through flux rate by Leedham *et al.* (2013) and the extrapolated value of 72–360 mol Br hr^{-1}. Despite the inherent difference between these two estimations, the values fit into the overall estimation by Leedham *et al*. (2013) which showed that the Malaysian coastline could contribute between 0.3 and 12 Mmol Br $year^{-1}$, equivalent to 34–1370 mol Br hr^{-1}.

The bottom-up estimation using flux rate determined by Leedham *et al*. (2013) and the estimation extrapolated from the emissions by the cultivated *K. alvarezii* in this study are the only data available for the estimation of contribution by farmed *K. alvarezii* in Malaysia. These estimations are only gross estimations and should be interpreted with caution. More laboratory-based and *in situ* studies need to be carried out before the estimation could be refined.

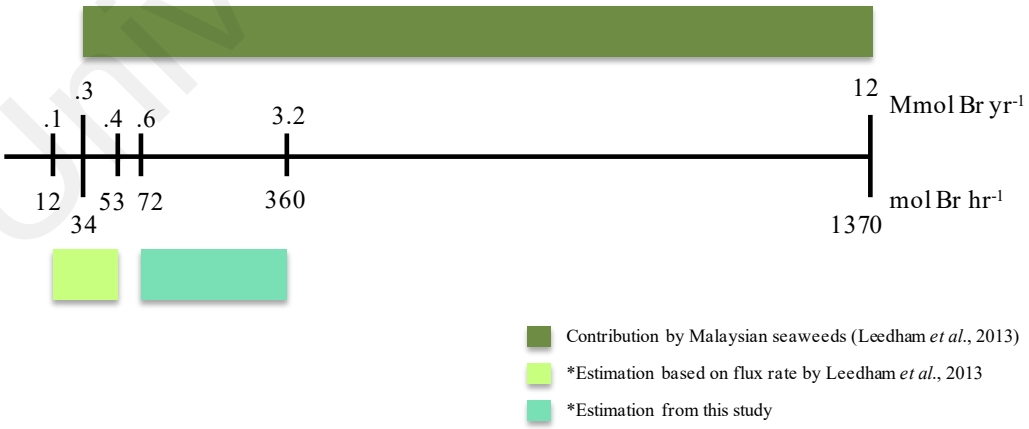


Figure 5.2: The estimations of Br released by Malaysian seaweeds (values derived based on the emission of $CHBr_3$)

*Estimations were fitted to the 2017 biomass production of *K. alvarezii* (FAO, 2020)

Table 5.5: Contribution of Br by *K. alvarezii* in 2017 through bottom-up estimation from the flux rate reported by Leedham *et al.* (2013)

A	*K. alvarezii* production in 2017
	202,966,000 kg FW (FAO, 2020)
	Production capacity
	1 acre = 1500 kg DW (Sade *et al.*, 2006)
	Moisture content of *K. alvarezii* = 44 % (38–50 %, Phang *et al.*, 2019)
	1 acre = 3409 kg FW
	4046.86 m^2 = 3409 kg FW
B	Total area for farming in 2017
	240,942,870.8 m^2 = 202,966,000 kg FW
C	Estimated flux rate
	17–73 nmol $CHBr_3$ m^{-2} hr^{-1} (Leedham *et al.*, 2013, Supplementary Data)
	51–219 nmol Br m^{-2} hr^{-1}
D	Estimated contribution of Br by *K. alvarezii* in 2017
	12.3–52.8 mol Br hr^{-1}

5.4 Future research

5.4.1 In relation to this study

Although laboratory-based studies provide a better understanding of how halocarbon production by seaweeds is affected by various environmental changes, establishing mesocosm studies would allow for better estimation and prediction of emissions from tropical seaweeds and their contributions towards regional halocarbon emissions. A more complete approach would include the effects of various factors such as environmental change, physiological responses and community interactions in the predictions. Incorporating multiple factors into laboratory-based studies is also important. As with all multi-factorial experiments such studies would be challenging and would require a multidisciplinary research team.

In view of the likely future expansion of seaweed farming, the profiling of halocarbon emissions during farming would be beneficial in providing a detailed account of the trend of emissions and the contribution of this 'anthropogenic' activity towards regional and global halocarbon loads. On the other hand, the standardisation of sampling and techniques used in analyses could avoid irregularities arising from the differences in these aspects, enabling easier comparison of results. A database to deposit relevant results from sources of halocarbons, such as seaweeds, should also be established to enable easy access and data availability. Also, more laboratory-based and *in situ* studies should be conducted for a refined estimation on the halocarbon emissions by Malaysian seaweeds.

5.4.2 In relation to the future climate

There is no doubt that seaweeds are important emitters of volatile halocarbons. As shown through the studies conducted, changes in the environment – for example, irradiance and temperature – will affect the emissions of halocarbons by seaweeds. The responses of seaweeds towards these changes are very likely species- and compound-specific. While the halocarbon emission responses of seaweeds towards the changes in environmental parameters have been documented, there is still no clear picture of what the future holds. It has been predicted that global climate change and ocean acidification will affect the distribution, abundance and diversity of seaweeds in the future through changes in the marine environments and this could also influence where seaweeds can be farmed. The possible increase of 1.5 °C in temperature, increased episodes of coastal eutrophication and stratification, which could affect light penetration into the water, and elevated levels of pCO_2 in seawater that bring about changes in seawater chemistry can all potentially affect seaweed physiology and growth responses. This creates many unknowns concerning how these changes could affect the halocarbon emissions by seaweeds. Brodie *et al*. (2014) predicted a shift in seaweed community composition in the event of warming, including kelp forests being wiped out in the north-east Atlantic and the increase in growth of some of the non-calcifying seaweeds including the red seaweeds *Porphyra* and *Gracilaria* (Gao *et al*., 1991; Gao *et al*., 1993) as a result of increased CO_2. On the other hand, increased CO_2 could result in increased productivity of the seaweeds. Such major changes in seaweed distribution could affect the regional and even the global contribution that seaweeds make to the halocarbon pool. In addition, environmental changes affecting plankton blooms could contribute to significant compositional and molecular changes in dissolved organic matter (Thornton, 2014; Zark *et al.,* 2015). This could in turn affect the production of halocarbons through the proposed (Lin & Manley, 2012; Liu *et al.*, 2015) haloperoxidase mechanisms.

Future climate change might increase the stratospheric load of VSLH compounds (Dessens *et al.*, 2009; Hossaini *et al.*, 2012). It has been predicted that the emissions of halocarbons, particularly $CHBr_3$, CH_2Br_2 and CH_3I, could increase by as much as 29.4 %, 23.3 % and 5.5 % respectively under the RCP8.5 future climate scenario, and by 9 %, 6.4 % and 1.5 % under RCP2.6 due to an increase in sea surface temperature and the sea-air flux (Ziska *et al.*, 2017). The continuing rapid expansion of seaweed cultivation industries could also contribute to further stratospheric loading of halogens in a future warmer climate (Hossaini *et al.*, 2012). However, many uncertainties exist regarding future environmental changes and the potential for alterations in the abundance and distribution of marine sources of halocarbons (Ziska *et al.*, 2017). Therefore, there is a need for further 'baseline' studies on how seaweeds from a wide variety of different natural habitats, and farmed species, from different areas would respond to sustained changes in the environment, and how these changes could alter seaweed halocarbon emissions.

On the basis of the current published evidence and the findings from this work, the following gaps could be targeted for further research:

- Treatment exposure time: Will the selected approach be sufficient to provide insights into biological adaptations, including phenotypic and genotypic modification?
- The lack of data on standing biomass (crop) of seaweeds from different geographical locations and identification of the key/dominant seaweed species in specific habitats. This area could benefit from the integration of remote-sensing techniques.

These points are important as data for oceanic sources could allow for better estimation of the halocarbon load through bottom-up modelling approaches (Ziska *et al.*, 2013). Figure 5.3 also highlights the lack of datasets for tropical and subtropical regions. This is a critical gap as the tropics and the subtropics have been identified as potentially important regions for halocarbon emissions and vertical transport to the upper troposphere/lower stratosphere (Quack & Wallace, 2003; Hossaini *et al.*, 2012; Tegtmeier *et al.,* 2012; Ziska *et al.*, 2013).

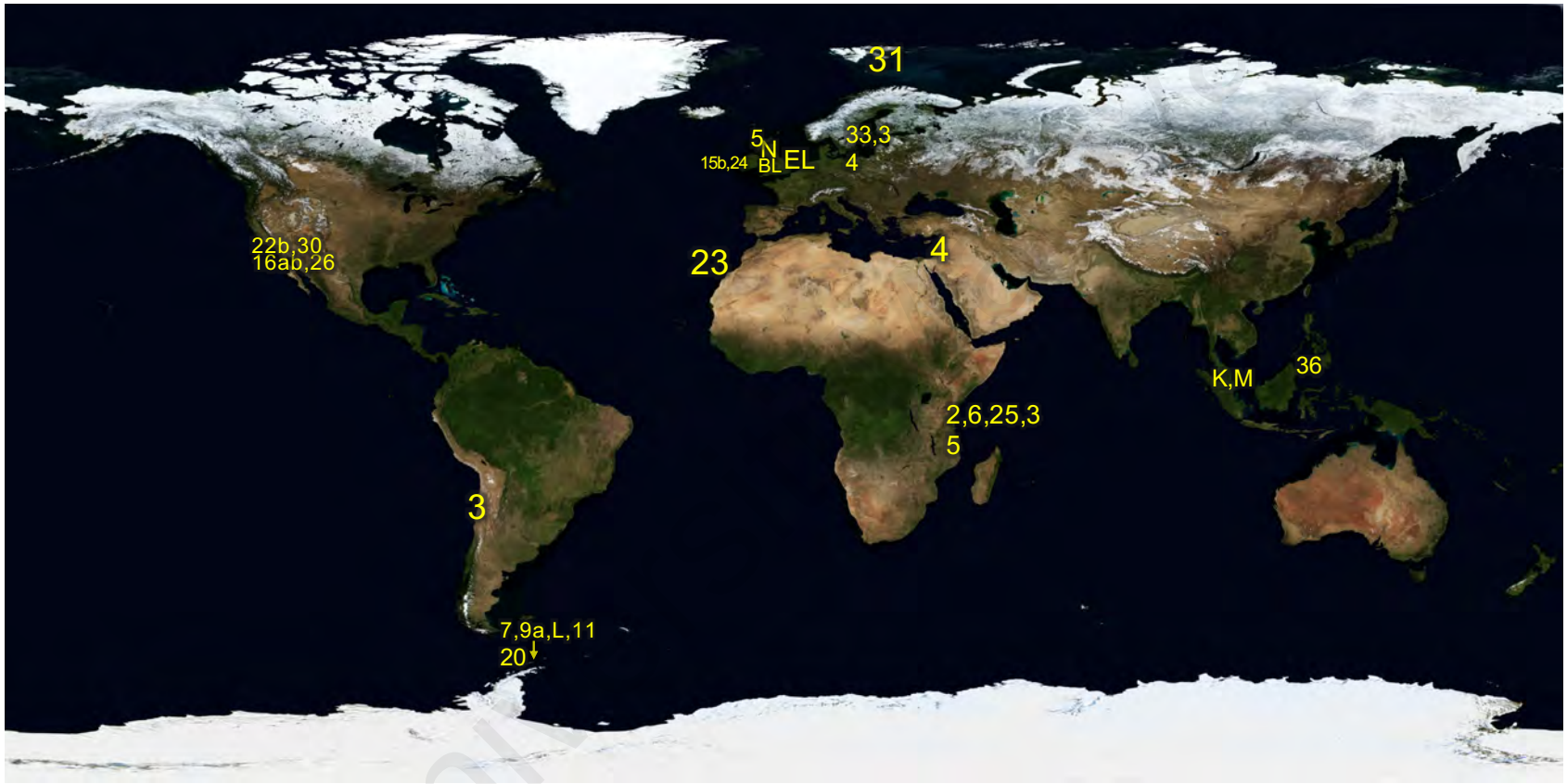


Figure 5.3: Geographical overview of the various locations from which seaweeds were collected for studies relating the effect of environmental change on the emissions of halocarbon by seaweeds

Numbers are based according to the Study Number mentioned in Table 2.2. BL = Study Numbers 8, 10, 12b, 13–15a, 21, 22a, 41b–43a, 44, 45a, 46; EL = Study Numbers 43b, 45b; K = Study Numbers 17–19, 27–29; L = Study Numbers 9b, 32, 47, 48; M = Study Numbers 37–40; N = Study Number 1, 12a, 41a. Photo credit: NASA Earth Observatory.

CHAPTER 6: CONCLUSION

The emission rates of $CHBr_3$ by all four seaweeds, namely *G. manilaensis, U. reticulata, K. alvarezii* and *T. conoides*, were found to be the highest among the six halocarbon compounds investigated, with the highest averaged emission rate observed in *K. alvarezii*. Changes in temperature and exposure duration affected the emissions of halocarbons by all four seaweeds. Differences in emission trends at higher temperatures, i.e. 40–30°C, were observed compared to lower temperatures, especially by *K. alvarezii* and *T. conoides.* Strong negative correlations ($r = (-0.69)–(-0.95)$; $p<0.01$) were reported after 28 hours exposure to varying temperatures in the emissions of $CHBr_3$, CH_2Br_2 and CH_2I_2 by *U. reticulata, K. alvarezii* and *T. conoides*, indicating decreasing emissions of the compounds with increasing temperature at longer exposures. Should the same trend be observed under longer term exposure in a future climate mesocosm setting, the contribution of seaweeds towards the total coastal halocarbon emissions could be reduced. The highest temperature of 40 °C bleached the commercially important seaweed *K. alvarezii* and, at the same time, the lowest halocarbon emission rates ($CHBr_3$, CH_2Br_2, CH_2I_2, $CHBr_2Cl$, $CHBrCl_2$), F_v/F_m values (<0.1) and Chl-*a* and carotenoid contents (1.43 ± 0.11 μg g^{-1}, 0.63 ± 0.05 μg g^{-1}) were observed. The changes in pigment content to temperature changes were unique to the species, with varying degrees of correlation between halocarbon emission rates and pigment content.

The combined effect of irradiance and temperature affects the emissions of CH_3I, CH_2I_2, CH_2Br_2 and $CHBr_3$ by *K. alvarezii.* Individual effects of irradiance and temperature were also observed in the study. Lower emission rates were observed during dark treatments at most of the temperatures tested, while higher irradiance levels increased the emissions of all four compounds by *K. alvarezii.* Correlations between halocarbon emission rates and irradiance levels, *r*, were between 0.421 and 0.554

(p<0.001, n = 64). Higher temperatures of 31 and 34 °C decreased the emission rates of CH_2I_2 and $CHBr_3$ by *K. alvarezii*. The emission rate of $CHBr_3$ was negatively correlated with temperature (r = −0.633; p<0.01; n= 64). Changes in F_v/F_m values corresponded to changes in temperature (r = −0.616; p<*0.*0005; n = 20), but a direct relationship with the emission rates of the halocarbon compounds could not be established under the influence of both factors. The emission rates of CH_2I_2, CH_2Br_2 and $CHBr_3$ were strongly correlated (r = 0.7–0.8; p<0.01; n = 64), supporting the suggestion that these three compounds are produced via a similar mechanism. Based on the active emissions of halocarbons observed in this study, the expansion of global *K. alvarezii* cultivation could increase the regional load of reactive bromine. This contribution could be magnified if future global warming creates a suitable farming condition, i.e. temperatures of less than 31 °C with sufficient irradiance for the mass cultivation of *K. alvarezii* in the subtropical waters or at higher latitudes.

In situ measurements of cultivated seaweeds showed higher emission of CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$ and $CHBr_3$ during daylight than in the dark, which is in general agreement with previous studies involving both temperate and tropical seaweed species. Daylight emissions by *G. manilaensis* from both onshore and offshore cultivation systems were found to be 10 to 52 times higher than dark emissions, while emissions by *K. alvarezii* were between two and five times higher during the day. Measurements carried out during the farming cycle showed that *G. manilaensis* emits higher concentrations of all four halocarbons compared to *K. alvarezii* and *U. reticulata*. The ratio of CH_2Br_2 to $CHBr_3$ produced by *G. manilaensis* remained fairly constant throughout the 4-week cultivation, while the ratio decreased for *K. alvarezii. G. manilaensis* was found to be an active emitter of CH_2Br_2 in the field, contrary to the findings in the laboratory. Meanwhile, cultivation of *G. manilaensis* using different systems affected the emissions of CH_2Br_2 and $CHBr_3$, while *K. alvarezii* showed no significant difference in the

emissions of halocarbons when cultivated at the onshore tanks or offshore platforms. The emission of halocarbons by *G. manilaensis* in the different cultivation systems was affected by changes in temperature, irradiance and seawater phosphate levels while seawater nutrient levels such as ammoniacal nitrogen, nitrate, nitrite and salinity appeared to affect the release of halocarbons by *K. alvarezii.* The harvesting activity of *K. alvarezii* in Malaysia could release 72–360 mol Br hr^{-1}, a value which is slightly higher than the value estimated based on a predicted flux rate estimated from simple incubations of wild Malaysian seaweed species.

Although the effect of a few environmental factors on the halocarbon emissions of Malaysian seaweeds has been investigated, there remain many unexplored areas in halocarbon-seaweed research, especially in the tropics. Inputs from all aspects of the research are needed in order to build a more comprehensive understanding of what is happening now and to better evaluate the effect of climate change e.g. increased primary productivity due to increased CO_2 and temperature on halocarbon emissions by seaweeds in the future.

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