EMISSION OF SELECTED HALOCARBONS BY SEAWEEDS INHABITING A CORAL REEF

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ABSTRACT

Since the discovery of the Antarctic stratospheric ozone hole in 1985 there has been increasing scientific interest in the halocarbon species that can cause ozone destruction. Although an important region for halocarbons in terms of atmospheric circulation, the tropics are underrepresented in terms of halocarbon measurements, especially those biogenic short-lived halocarbon compounds. A fringing coral reef flat at Cape Rachado, west coast Peninsular Malaysia was selected for a study on the emissions of halocarbons by seaweeds. A portable, automated gas chromatograph with electron capture detector was used to measure a suite of halocarbon species trimonthly over a 15-month period at the study site. The measurements of the halocarbon atmospheric mixing ratios were then correlated to the seaweed standing biomass to investigate its influence on the halocarbon mixing ratios at the survey site. Although it was found that the atmospheric mixing ratio for the biogenic halocarbon compounds were poorly correlated ($\rho < 0.5$) to some of the important seaweed species at the sampling site, there was no significant correlation between the total seaweed standing biomass with the atmospheric concentration of biogenic halocarbon compounds. This may be due to many contributing factors such as localized emissions, wind direction and speed that might influence the halocarbon contents in the atmosphere.

To better understand the halocarbon emissions by the seaweeds, a laboratory-based incubation study was conducted to observe if the halocarbon emissions by the seaweeds varied with irradiance. Three selected seaweed species, *Sargassum binderi* Sonder ex J.

Agardh, *Padina australis* Hauck, and *Turbinaria conoides* (J. Agardh) Kützing were collected from the sampling site and exposed to a range of irradiance in the laboratory. The halocarbon contents in the seawater were then analyzed using a purge-and-trap system attached to a gas chromatograph with mass selective detector. Release of halocarbons especially dibromochloromethane, $CHBr_2Cl$ (r= 0.79; p< 0.01) was found to be influenced by irradiance. Correlations were also observed between emission of certain halocarbons with photosynthetic activity, especially bromoiodomethane, CH_2BrI (r = 0.85; p< 0.01) and bromoform, $CHBr_3$ (r = 0.79; p< 0.01) suggesting that environmental factors such as light can affect the release of these volatile halogenated compounds by the seaweeds into the atmosphere. From this study, it was also found that upon comparison with temperate and polar brown seaweeds, tropical species, such as *Turbinaria conoides*, may emit higher levels of bromoform, $CHBr_3$ and other halocarbons. It is therefore important to investigate the contribution of tropical seaweeds towards the local atmospheric composition of halocarbons.

ABSTRAK

Sejak penemuan lubang ozon stratosfera Antartika pada tahun 1985, minat saintifik terhadap spesies halokarbon yang terlibat dalam pemusnahan ozon telah kian meningkat. Walaupun kawasan tropika merupakan suatu rantau yang penting bagi halokarbon dalam aspek peredaran atmosfera, namun kawasan ini masih kurang dikaji dalam aspek ukuran halokarbon, terumanya halokarbon biogenik yang berhayat singkat. Suatu pinggir terumbu karang di Cape Rachado, pantai barat Semenanjung Malaysia, telah dipilih untuk kajian pembebasan halokarbon oleh rumpai laut. Satu kromatograf gas mudah alih dan automatik berserta pengesan tangkapan elektron telah digunakan untuk mengukur pelbagai spesies halokarbon di tapak kajian sekali setiap tiga bulan untuk tempoh 15 bulan. Ukuran nisbah pencampuran halokarbon atmosfera kemudiannya dikorelasi dengan biojisim rumpai laut untuk mengkaji pengaruh biojisim rumpai laut terhadap nisbah pencampuran halokarbon biogenik di tapak kajian. Walaupun terdapat korelasi yang lemah ($\rho < 0.5$) antara nisbah pencampuran sebatian halokarbon biogenik di atmosfera dengan biojisim beberapa spesies rumpai laut yang penting di tapak pensampelan, namun jumlah keseluruhan biojisim rumpai laut didapati tidak berkorelasi dengan kandungan halokarbon biogenik di atmosfera. Ini berkemungkinan disebabkan oleh banyak faktor penyumbang seperti pembebasan setempat, hala dan kelajuan angin yang mungkin mempengaruhi kandungan halokarbon biogenik dalam atmosfera.

Untuk lebih memahami pembebasan halokarbon oleh rumpai laut, eksperimen inkubasi di dalam makmal telah dijalankan untuk mengaitkan pengaruh sinaran cahaya terhadap

pembebasan halokarbon oleh rumpai laut. Tiga spesies rumpai laut yang terpilih iaitu *Sargassum binderi* Sonder ex J. Agardh, *Padina australis* Hauck, dan *Turbinaria conoides* (J. Agardh) Kützing telah dikumpul dari tapak pensampelan dan didedah kepada sejulat kekuatan sinaran cahaya di dalam makmal. Kandungan halokarbon dalam air laut kemudiannya dianalisis dengan menggunakan satu sistem singkir dan perangkap yang disambungkan kepada kromatograf gas yang dipasang dengan pengesan pemilihan jisim. Pembebasan sebatian halokarbon terutamanya dibromoklorometana, $CHBr_2Cl$ (r = 0.79; p <0.01) didapati dipengaruhi oleh sinaran cahaya. Korelasi juga diperhatikan antara pembebasan sebatian halokarbon tertentu dengan aktiviti fotosintesis, terutamanya sebatian bromoiodometana, CH_2BrI (r = 0.85, p <0.01) dan bromoform, $CHBr_3$ (r = 0.79, p <0.01). Ini menunjukkan bahawa faktor-faktor persekitaran seperti sinaran cahaya boleh menjejaskan pembebasan sebatian berhalogen oleh rumpai laut ke atmosfera. Kajian ini juga mendapati bahawa berbanding rumpai laut berwarna perang dari rantau beriklim sederhana dan berkutub, spesies tropika seperti *Turbinaria conoides* berpotensi untuk membebaskan lebih banyak bromoform, $CHBr_3$ dan sebatian halokarbon lain. Oleh itu, adalah penting untuk kita mengkaji sumbangan pembebasan sebatian halokarbon oleh rumpai laut di rantau tropika terhadap komposisi atmosfera tempatan.

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LIST OF ABBREVIATIONS

CFC	Chlorofluorocarbon
Chl-a	Chlorophyll-a
DCMU	[3 - (3,4-dichlorophenyl)-1,1-dimethylurea]
ECD	Electron Capture Detector
EDTA	Ethylenediaminetetraacetic acid
FAO	Food & Agriculture Organization
Fig.	Figure
F_m	Maximal fluorescence
F_o	Minimal fluorescence
F_v	Variable fluorescence
F_v/F_m	Maximum quantum yield
GC	Gas chromatography
GCMS	Gas Chromatography - Mass Spectrometry
GWP	Global Warming Potential
ID	Identifying ion
IVI	Importance Value Index
L1	0 µmol photon $m^{-2}s^{-1}$
L2	47 µmol photon $m^{-2}s^{-1}$
L3	58 µmol photon $m^{-2}s^{-1}$
L4	81 µmol photon $m^{-2}s^{-1}$
L5	126 µmol photon $m^{-2}s^{-1}$
MS	Mass spectrometer

MSD	Mass Selective Detector
n.m	Not measured
N.S	Not significant
NH_3^- -N	Ammonia
NO_2^- -N	Nitrite
NO_3^- -N	Nitrate
NOAA/ESRL	National Oceanic & Atmospheric Administration / Earth System Research Laboratory
O_3	Ozone
ODP	Ozone Depletion Potential
OFN	Oxygen Free Nitrogen
PAM	Pulse Amplitude Modulator
PCA	Principle Component Analysis
PO_4^{3-}	Orthophosphate
PPFD	Photosynthetic photon flux density
PSII	Photosystem II
RT	Retention Time
S.D.	Standard Deviation
S.E	Standard Error
SIM	Single Ion Mode
UV	Ultraviolet
UV-B	Ultraviolet-B

LIST OF SYMBOLS & UNITS

%	Percentage
<	Less than
>	More than
℃ min^{-1}	Degree Celsius per minute
µg L^{-1}	Microgram per liter
µm	Micrometer
µmol photon m^{-2} s^{-1}	Micromole photon per meter square per second
g $CHBr_3$ yr^{-1}	Gram $CHBr_3$ per year
g L^{-1}	Gram per liter
Gg Br yr^{-1}	Gigagram bromine per year
gm^{-2}	Gram per meter square
H_1	Alternative hypothesis
ha	Hectare
H_o	Null hypothesis
m	Meter
m/z	Mass to charge ratio
m^2	Meter square
mg L^{-1}	Milligram per liter
min	Minute
mL	Milliliter
mL min^{-1}	Milliliter per minute
n	Number of replicates / samples

pmol g DW^{-1} hr^{-1}	Picomole per gram dry weight per hour
pmol L^{-1}	Picomole per liter
ppt	Parts per trillion
pptscc	Parts per trillion x volume (mL)
r	Pearson Product-Moment Correlation coefficient
r^2	Regression
ρ	Spearman Rank Order Correlation coefficient

CHAPTER 1

INTRODUCTION

Halocarbons are chemical compounds consisting of the carbon atom bonded to one or more halogen atoms, with or without the presence of hydrogen. Halocarbons that contain bromine, chlorine and limited amount of iodinated halocarbons are found to cause ozone layer depletion. The Ozone Depletion Potential (ODP) of 1.0 over a scale of 0-1 has been reported by few chlorofluorocarbons (CFC) e.g. CFC-11 (WMO, 2007). Iodine atoms may be involved in the cyclic catalytic destruction of ozone, yet the number of iodine atoms that reach the stratosphere is greatly limited due to rapid tropospheric loss (WMO, 2007). The contribution of fluorine atoms towards ozone destruction is insignificant as the atoms are rapidly transformed into a stable repository in the form of hydrogen fluoride (HF). Besides causing ozone loss, some of the halocarbon compounds are greenhouse gases that exert very high Global Warming Potential (GWP) e.g. CFC-11 with GWP of 6730 compared to carbon dioxide with GWP of 1 (WMO, 2007). These compounds are involved in the absorption of infrared radiation from the Earth's surface, and contribute to the radiative forcing of the climate system, thereby implicating the global environment (WMO, 2007).

Since the discovery of the stratospheric ozone hole above Antarctica in 1985 (Farman *et al*., 1985), concerns have been raised over the increasing emission of the ozone-depleting compounds. The destruction of ozone was caused by the presence of reactive radical species in the stratosphere. These reactive radical species are made available from ozone-depleting compounds such as halocarbon through catalytic photolysis. Subsequent research

found CFC compounds responsible for the drastic loss of stratospheric ozone (Molina & Rowland, 1974; Farman *et al.,* 1985). In an effort to prevent further stratospheric ozone loss, the production of the man-made, ozone-depleting halogenated compound, CFCs, and some other anthropogenic ozone-depleting substances, were controlled through the ratification of the Montreal Protocol 1987 and its subsequent amendments by all of the world's nations. Apart from the contribution of anthropogenic activities, scientists are now looking further into the natural activities that might result in the release of volatile organic compounds, in order to minimize the uncertainties in the estimation of global halocarbon budget. Through their studies, it was found that in places where anthropogenic sources become insignificant, biogenic oceanic sources play a fundamental role in the emission of the volatile halogenated compounds. This is especially apparent in the coastal region. As a result, a number of short-lived halocarbon compounds released from oceanic sources such as the seaweeds and microalgae (e.g., Hughes *et al*., 2006; Tokarczyk and Moore 1994) had been reported following the discovery of increased levels of CH_3I over kelp beds (Lovelock, 1975). Recent evidence suggested the possibility of these biogenic short-lived halocarbons contributing to the stratospheric halogen load (Laube *et al*., 2008).

Halocarbon emissions from temperate and polar seaweeds had been recently addressed. These include few common seaweed species such as *Laminaria digitata, Laminaria saccharina, Fucus serratus* and *Ulva lactuca* (Baker *et al.*, 2001; Carpenter *et al*., 2000; Laturnus, 2001; Nightingale *et al*., 1995). The dominant halocarbon compound released by the seaweeds was reported to be bromoform ($CHBr_3$) (Carperter *et al.,* 2000; Giese *et al*., 1999; Laturnus, 2001; Nightingale *et al*., 1995) while the dominant iodinated halocarbon compound was found to be released in the form of diiodomethane (CH_2I_2) (Carperter *et al.,*

2000; Giese *et al*., 1999). In-depth studies on the mechanism behind such productions suggested the possible involvement of a certain haloperoxidase widely found in red, green, and mainly brown seaweeds (Butler & Walker, 1993) to overcome stressful conditions, although the actual mechanism that takes place is yet unclear. Effects of environmental conditions that enhanced stress level in seaweeds including temperature, pH, irradiance and desiccation had been found to influence the emission of halocarbons by the seaweeds (Carpenter *et al.*, 2000; Giese *et al*., 1999; Mtolera *et al.*, 1996; Nightingale *et al*., 1995). Also, grazing activity (Nightingale *et al*., 1995) and the photosynthesis of the seaweeds (Ekdahl *et al.*, 1998; Goodwin *et al.*, 1997; Laturnus *et al.*, 2000) have been reported to influence halocarbon emissions.

Though literature on halocarbon emissions by the temperate and polar seaweeds is available, reports from the tropics are scarce. The tropics are an important region for the mass cultivation of economically important seaweed species such as *Kappaphycus* and *Eucheuma*. 1,500 ha of seaweed farms in Malaysia alone are able to produce over 100 tonne DW per month (Phang, 2006). These seaweeds are made into semi-refined carrageenan chips, with production of dried carrageenan in year 2010 recorded as 15,000 tonne (Tan *et al*., 2013). Atmospheric measurements of halocarbons showed that these farmed species could contribute to high concentrations of $CHBr_3$ at coastal location (Pyle *et al.*, 2011). The transport of these halocarbon from the Earth's surface into the stratosphere is likely to be aided by the deep tropical convection (Aschmann *et al.*, 2009), an important driver in the global atmospheric circulation. The fundamental roles of the deep convection in the tropics include vapor transfer, heat transfer (Riehld & Malkus 1958), and vertical exchange between the troposphere and the stratosphere (Holton *et al.*, 1995; Sherwood &

Dessler, 2000). Therefore, it is vital that some work in the tropical region be done to better understand the local atmospheric chemistry. In view of the conceivable deleterious consequences the halocarbon gases might bring to the atmosphere if released in quantities that greatly exceed those from natural sources, it is crucial that the sensitivity and mechanisms of their release be assessed, as the mariculture of seaweeds are rapidly expanding.

The overall aim of this project was to study the emission of halocarbons by seaweeds. This would be achieved firstly, through testing the hypothesis that concentrations of halocarbons in the atmosphere at a selected study site are influenced by seaweed biomass in the natural environment. Secondly, to test the effect of irradiance on the halocarbon emission by selected seaweed species through a small-scale laboratory study in an effort to find a possible trigger that enhances the natural emission of halocarbons by seaweeds.

Hypothesis I:

H_o: Atmospheric concentrations of halocarbons are not affected by seaweed biomass.

H_1: Atmospheric concentrations of halocarbons are affected by seaweed biomass.

Hypothesis II:

H_o: Irradiance does not affect halocarbon emission by seaweeds.

H_1: Irradiance affects halocarbon emission by seaweeds.

The objectives of this study are:

1. To correlate seaweed biomass and atmospheric halocarbon concentration at Cape Rachado, Port Dickson.
2. To study the effect of irradiance on the emission of halocarbons by selected seaweed species in the laboratory.
3. To correlate the maximal quantum yield (indicator of photosynthesis performance) of selected seaweed species with their halocarbon emissions.

CHAPTER 2

LITERATURE REVIEW

2.1 Ozone

2.1.1 Importance of ozone

The relatively unstable ozone (O_3) molecule in the atmosphere is essential to life on earth. Although only present in small amount, the ozone layer in the upper part of the atmosphere shields the harmful ultraviolet (UV) radiation from the Earth's surface, making humans less susceptible to related medical conditions (e.g. skin cancer, cataracts and impaired immune system) arising from the excessive exposure to UV-B, while the harmful tropospheric ozone pollutes and causes damage to lung tissues and plants (Fahey & Hegglin, 2011). An upset in the balance of these ozone types will have serious implications on life.

2.1.2 Ozone production and loss

Production of ozone in the stratosphere involves the breaking up of molecular oxygen by UV radiation to make atomic oxygen, which later combines with molecular oxygen to produce ozone. Destruction of ozone in the stratosphere happens via the Chapman Mechanism and the cyclic chemical reaction (Fahey & Hegglin, 2011; WMO, 2011). The Chapman Mechanism involves the reaction of ozone with oxygen atoms while the cyclic chemical reaction involves naturally occurring species like halogen radicals, nitrogen oxide radicals and hydrogen radicals. Small changes in radical concentrations will cause serious implications on the ozone as they get regenerated through the ozone-destructing catalytic cycles (Fahey & Hegglin, 2011).

Ozone is most obvious in the lower and upper stratosphere. Temporal variation causes the total ozone amount to vary daily, seasonally and multiannually (WMO, 2011). The variation includes meteorological variability, changes of stratospheric temperature and wind, changes of solar input, variation in source gas emission and interannual variability in stratospheric wind (Fahey & Hegglin, 2011; WMO, 2011).

In the 1970s, anthropogenic emissions of nitrogen oxide from supersonic aircraft and the stable chlorofluorocarbons (CFCs) were found to deplete the layer of protective ozone in the stratosphere. In the spring of year 1985, a massive ozone hole was observed above Antarctica. Human-produced CFCs and other brominated and chlorinated ozone depleting substances were implicated in the loss of this stratospheric ozone. Since then, ozone is found to be destroyed every year over the Antarctic stratosphere in the late August to early October period, while large Arctic depletion of the ozone can be found during some springtime (Fahey & Hegglin, 2011).

2.2 Halocarbons

2.2.1 Halocarbons and the environment

Halocarbon is a compound that consists of a carbon atom bonded to one or more halogens, with or without the presence of hydrogen. It is a major sub-class of volatile organic compounds which creates photochemical smog episodes, promoting secondary air pollutant including ozone (Rivett *et al*., 2003).

Halocarbons that contain bromine and chlorine are found to cause ozone layer depletion which indirectly affects temperature (Forster & Joshi, 2005). These halocarbons are source gases that contribute to the halogen radical loading to the stratosphere. It is produced by

both natural (e.g., biogenic pathways at the Earth's surface) and anthropogenic processes (e.g., industrial processes) (WMO, 2011). There is a big possibility of these halocarbon gases released from the Earth's surface reaching the stratosphere through vertical transport i.e. deep convection (Aschmann *et al.*, 2009). In the stratosphere, the halogen radical produced from halogenated source gases reacts with the ozone molecules in the presence of UV radiation. Both bromine- and chlorine- containing halocarbons are found to cause ozone layer depletion. A single chlorine atom in the stratosphere can destroy many ozone molecules through a catalytic cycle when ultraviolet radiation is present. Bromine is even more efficient, that is, up to 40-100 times in destroying the ozone molecules than chlorine in the stratosphere (Penkett *et al*., 1995). Besides causing ozone loss, halocarbons are involved in the absorption of infrared radiation from the Earth's surface, implicating the global environment (WMO, 2007). Halocarbon like CFC-11 exerts very high value of Global Warming Potential (GWP), some 6000 times higher than other greenhouse gases like carbon dioxide, making it much more efficient in absorbing the radiation from the Earth's surface (WMO, 2007). As good absorbers of radiation, these gasses are good emitters of radiation, emitting the radiation back to the Earth's surface, upsetting the Earth's energy balance, thereby increasing the temperature at the Earth's surface and the lower atmosphere (IPCC, 2007).

Compared to the brominated and chlorinated halocarbons, iodinated halocarbons are less likely to be contributing to the ozone depletion. Iodine atoms may be involved in the cyclic catalytic destruction of ozone, yet the number of iodine atoms that reach the stratosphere is greatly limited due to rapid tropospheric loss (WMO, 2007). Although iodine-containing halocarbons are not directly involved in the depletion of stratospheric ozone, they do, however, release iodine through photolysis in the atmosphere. These iodine released forms

iodine oxides (IO/OIO) rapidly with ozone, which will influence the tropospheric oxidizing capacity (McFiggans *et al*., 2000) and greenhouse gas processing. Iodine compounds released from seaweed sources e.g. CH_2I_2 (Carpenter *et al.,* 2000) at coastal region during low tide was found to correlate well with new particle formation (O'Dowd *et al.*, 1998). Iodine oxides promote formation of potential cloud condensation nuclei in the atmosphere (O'Dowd & Hoffman, 2005). Iodine compounds give rise to particle burst at the coastal region through nucleation process (O'Dowd *et al.*, 2002). Having a photodissociation lifetime of only a few minutes during midday (Mössinger *et al.*, 1998), compounds like CH_2I_2 released at the coastal area through ocean-atmosphere exchange, are able to photodissociate rapidly within the marine boundary layer, forming iodine atoms, which react with ozone to form IO radicals. These IO radicals then self-act to form OIO. Formation of stable iodine oxide clusters occurs through the multiple addition of OIO (Hoffman *et al*., 2001). The involvement of atmospheric aerosols in cloud condensation could change the cloud characteristics (Lohmann & Feichter, 2005). The compound CH_2I_2 had been reported to be the most fundamental iodine precursor among a suite of organoiodines measured (Carpenter *et al*., 1999).

2.2.2 Long-lived compounds

Halocarbon compounds with lifetimes longer than six months are considered long-lived (WMO, 2007). Because they exist longer than the period of time needed for their transport to the stratosphere, they remain undamaged throughout their transportation to the stratosphere (WMO, 2007). Systematic measurements of long-lived halocarbon compounds including the chlorofluorocarbons (CFCs) has been established since the late-1970s, by means of both flask and on site measurements of these gases, carried out by networks such as the Advanced Global Atmospheric Gases Experiment (AGAGE), System for

Observation of Halogenated Greenhouse Gases in Europe (SOGE) and National Oceanic and Atmospheric Administration/Earth System Research Laboratory (NOAA/ESRL). Universities e.g. University of East Anglia (UEA) and University of California at Irvine (UCI) are also actively involved in the constant monitoring of such gases (WMO, 2007). Examples of long-lived halocarbons include the CFCs, hydrofluorocarbons (HCFCs), halons, carbon tetrachloride (CCl_4), methyl chloroform (CH_3CCl_3) and methyl chloride (CH_3Cl). These gases are predominantly anthropogenic (WMO, 2007). Although natural processes such as volcanic eruption could release some of these long-lived compounds e.g. CCl_4, CH_3Cl (Frische *et al.,* 2006), the contribution of CFCs and few other long-lived compounds through natural processes are negligible (Martinerie *et al.*, 2009).

CFCs including CFC-11 (CCl_3F), CFC-12 (CCl_2F_2) and CFC-113 (CCl_2FCClF_2) are compounds having a very long lifetime – lasting between 45 years (CFC-11) to 100 years (CFC-12) (WMO, 2011). The CFCs were widely used since being developed in the 1930s. They were commonly used as propellants, refrigerants and solvents (McCulloch, 1999) while CCl_4 act as feedstock for CFCs production (Simmonds *et al.*, 1988). The chlorine atoms released from these source gases were found responsible for the major stratospheric ozone loss (Molina & Rowland, 1974; Farman *et al*., 1985) over Antarctica in 1985. The potential of such gases as both ozone depleting substances and greenhouses gasses brought about the breakthrough of atmospheric chemistry research. Although it was suggested that the emission of CFCs was mainly released in the Northern Hemisphere (UNEP, 2010), the long lifetimes of these gases enables mixing throughout the atmosphere, resulting in the similarity in their concentrations in the Northern and Southern Hemisphere (Martinerie *et al*., 2009). In an attempt to minimize and prevent further loss of the stratospheric ozone, the Montreal Protocol on Substances that Deplete the Ozone Layer (1987) and its subsequent

amendments saw the complete phase-out of CFCs, halons, and carbon tetrachloride. As a result of such treaties, there was significant decline in the surface concentrations of the CFCs and carbon tetrachloride globally (WMO, 2011).

The long lifetime of the long-lived halocarbon compounds creates a time lag between the decline in the source gas emissions and the actual concentration of such gases existing in the atmosphere (Martinerie *et al*., 2009). This would mean that the 'complete loss' of such compounds in the atmosphere would take up years and even centuries depending on the lifetime of gases. The loss of halocarbon in the atmosphere occurs through several processes including oceanic uptakes, terrestrial uptake, photolysis and hydroxyl radical oxidation. All these processes influence the lifetime of a compound (WMO, 2011).

2.2.3 Short-lived compounds

Although the long-lived halocarbons contribute a greater part to the stratospheric halogen load, it was reported that the short-lived halocarbons with lifetimes of 6 months or less play a part in the contribution of free halogen radicals to the stratosphere (Laube *et al*., 2008). Chlorinated short-lived compounds e.g. dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), tetrachloroethene (CCl_2CCl_2) have been measured from both anthropogenic and natural sources. CH_2Cl_2 is mainly used for paint removal, $CHCl_3$ in the production of fluoropolymers and HCFC-22, as by-products of water chlorination, paper and pulp bleaching, while C_2Cl_4 is important in the texture industry (WMO, 2011). Natural sources of the short-lived chlorinated compound, $CHCl_3$, by seaweeds had been reported by Nightingale *et al*., (1995) both in the rockpool and laboratory. Apart from the marine sources, terrestrial sources including vegetation and soil production also contributes the emission of chlorinated short-lived compounds (Laturnus *et al.,* 2002).

Release of brominated short-lived compounds is mainly contributed by natural sources. Anthropogenic release of brominated short-lived compounds is very minute compared to the huge amount released by natural sources (WMO, 2011). Seaweeds, microalgae, and terrestrial vegetation e.g. rice are natural sources that were found to release short-lived brominated compounds including bromoform ($CHBr_3$) and dibromomethane (CH_2Br_2).

Although there are huge uncertainties involved in the estimation of emission rates due to a vast variety of unaccounted coastal sources (Butler *et al*., 2007), the oceanic emission of $CHBr_3$ and CH_2Br_2 had been estimated to be around 430-1400 Gg Br yr^{-1} and 57-280 Gg Br yr^{-1} (WMO, 2007). There are many reports available concerning the release of $CHBr_3$ by seaweeds from the coastal region, suggesting the possible involvement of bromoperoxidase activities in the seaweeds (Manley & Barbero, 2001; Ohsawa *et al*., 2001). $CHBr_3$ had been reported to be the dominant brominated short-lived compound released by seaweeds (Carpenter *et al.,* 2000). Seaweed was found to be responsible for 70% of the world's $CHBr_3$ emission, with a reported production rate of 2.2×10^{11} g $CHBr_3$ yr^{-1} (Carpenter & Liss, 2000).

Majority of iodinated short-lived compounds are released through natural processes especially from the ocean (WMO, 2011) but anthropogenic release of several iodinated compounds including CH_3I through biomass burning (Mead *et al.,* 2008) and fumigation-use (UNEP/TEAP, 2010) has been reported. Enhanced emissions of CH_3I could be caused by increased sea surface temperature (Yokouchi *et al.,* 2008) and abiotic chemical mechanism stimulated by dust deposition (Williams *et al*., 2007). Oceanic sources may include phytoplankton (Hughes *et al*., 2006) and seaweeds (Carpenter *et al*., 2000).

Carpenter *et al*. (2000) reported CH_2I_2 as the dominant iodocarbon released by all the temperate brown species i.e. *Laminaria digitata*, *Laminaria saccharina*, *Ascophyllum nodosum*, *Fucus vesiculosus* and *Fucus serratus* observed in their study.

2.3 Seaweed as a natural source of halocarbon emission

2.3.1 Introduction to seaweeds

Seaweeds are macroscopic, autotrophic marine plants found in the marine or brackish water environment. They are adapted to grow in coastal regions between the top of the intertidal zone and the maximum depth where sufficient light to support growth can penetrate. Rocky shores are abundantly covered by seaweeds, while muddy and sandy areas have fewer seaweeds as most species cannot anchor there (Lobban & Harrison, 1997). Many environmental parameters such as irradiance, temperature, depth, exposure, and tides are thought to attribute to seaweed distribution.

Seaweeds are classified based on their pigmentation, with three major classes: Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae). They reproduce both sexually and asexually through fragmentation and the latter, formation of flagellate and non-flagellate spores. The growth and reproduction of seaweeds are affected by irradiance, temperature, nutrients and photoperiod.

Seaweeds are ecologically and economically important. Ecologically, they are the primary producers in the marine environment, providing food for other marine consumers. Also, they are important in the structuring of the marine ecosystems, forming algae turfs and meadows, kelp beds and forest as well as coralline algae reefs. Seaweeds, through their photosynthetic capability, have the potential to sequester carbon dioxide. The reported sea

carbon removal rate of commercial seaweeds accounted to 0.7 million tonnes C yr^{-1} (Turan & Neori, 2010).

Economically, seaweeds with high protein content like Nori (*Porphyra*) are served as food. Phycocolloids from the seaweeds e.g. agar, carrageenan and alginate are highly required as they are used in the cosmetic and pharmaceutical industries. Seaweed-based industries like the semi-refined carrageenan production from *Kappaphycus* are growing. According to the Food and Agriculture Organization (FAO), the world production from algae cultivation has been increasing regularly from 1980 to 2005. The seaweed production was 14.8 million tonnes in 2005 while 1.3 million tonnes were from seaweed gathering (FAO, 2010). The production for brown, red and green seaweeds was 7.8 million tons, 4.8 million tons and 13,000 tons wet weight respectively. Many seaweed farms are located in Asia. The driving force behind the growth of seaweed culture is likely to be related to their new found usage – as a biofuel source and carbon sequestration, besides serving as a source of food and industrial material.

In Malaysia, mass cultivation of seaweeds can be found in Sabah. It was shown that the aquaculture sector in Sabah contributed an estimated export value of RM114 million (1994-1997) of fisheries products, with 30% of the export value contributed by seaweed production. At least six varieties are cultivated from the two seaweed species namely *Kappaphycus* Doty and *Eucheuma* J. Agardh, (Phang *et al.*, 2010; Sade *et al*., 2006). The reported production of seaweeds from Sabah was 6,000 million tonne in 2009 (Phang *et al*., 2010).

Besides the cultivated seaweed species, a total of 386 taxa comprising the Chlorophytes, Rhodophytes, and Phaeophytes had been recorded by Phang *et al*. (2007). The seaweed community in Cape Rachado, Port Dickson, Negeri Sembilan had been extensively studied. The total number of seaweed species reported at the three sampling sites at Cape Rachado (Fig. 2.1) had been increased from 69 in the first survey carried out in 1987/88 to 81 in the 1998 survey. The dominant species reported in the 1987/88 survey for the three sites in Cape Rachado was *Sargassum oligocystum* while the 1998 survey reported dominance by *Gracilaria salicornia* at site A, *Sargassum baccularia* for site B and C (Phang, 1995; Ooi, 2001). A decrease in the total number of seaweed species at site B was observed from the 1987/88 to the 1998 survey (Table 2.1). A detailed table on the number of seaweed species found at Cape Rachado, Port Dickson is described in Table 2.1.

Figure 2.1: Map of Cape Rachado, Port Dickson at West Coast of Peninsular Malaysia. (Source: Google Maps)

Table 2.1: Number of seaweed species found at Cape Rachado, Port Dickson in the 1987/88 and 1998 survey

Site	Seaweed	1987/88	1998
A	Phaeophyta	12	9
	Chlorophyta	15	17
	Rhodophyta	22	23
	Total species	51	54
B	Phaeophyta	14	8
	Chlorophyta	10	8
	Rhodophyta	26	17
	Total species	50	35
C	Phaeophyta	18	11
	Chlorophyta	8	23
	Rhodophyta	19	31
	Total species	45	69

2.3.2 Discovery of halocarbon emissions by seaweeds

With seaweed farming and processing industry being a potential investment option, and the prospect of seaweed as a possible CO_2 sink and biofuel, it will certainly encourage more cultivation of seaweeds. However, just like how most man-made activities would influence natural ecosystems, there's bound to be some drawbacks with the increase in seaweed cultivation. Though seaweeds are beneficial, they do release halocarbons.

Halocarbon emission by seaweeds in the seawater was first discovered by Lovelock in 1975. They found concentration of methyl iodide (CH_3I) to be 1000 times higher in seawater above the Irish *Laminaria digitata* kelp bed compared to pelagic North Atlantic seawater. This discovery later paved the way for more detailed studies on halocarbon emission by seaweeds (Baker *et al.* 2000; Ekdahl *et al.*, 1998; Klick, 1992; Nightingale *et al.*, 1995).

Since then, many laboratory studies on different seaweed species was able to provide a glimpse into the wide variety of halocarbon compounds emitted by the seaweeds, from brominated-, chlorinated- and iodinated-compounds to mixed compounds. Among them are the common brominated compounds such as bromoform ($CHBr_3$) and dibromomethane (CH_2Br_2), the chlorinated compounds e.g. chloroform ($CHCl_3$) and methyl chloride (CH_3Cl), the iodinated compounds diiodomethane (CH_2I_2) and methyl iodine (CH_3I) as well as the mixed compounds e.g. dichlorobromomethane ($CHBrCl_2$) and dibromochloromethane ($CHBr_2Cl$). Bromoform ($CHBr_3$) was found to be the halocarbon compound emitted most abundantly by algae (Carpenter & Liss, 2000; Quack & Wallace, 2003; Weinberger *et al*., 2007). It was believed that 70% of the world's bromoform are sourced from the seaweeds (Carpenter & Liss, 2000; Quack & Wallace, 2003). These halocarbon compounds are found to be emitted with varying rates from different species, greatly hinting the probability of seaweed halocarbon emissions being species-dependent.

A more detailed compilation of the halocarbon compounds emitted from seaweeds by different group of researchers can be found in Table 2.2. It is evident that most studies relating halocarbon emission by seaweeds was done on temperate and some polar seaweed species with just a few on tropical species. This shows the importance and need for more similar research on tropical seaweed species in order to minimize the uncertainties where estimation of the global halocarbon budget estimation is concerned.

Table 2.2: List of seaweed halocarbon studies

Seaweed class	Seaweed species	Species type	Halocarbon compounds observed	Condition studied	Reference
Phaeophyta	*Fucus distichus* L., *Dictyosiphon foeniculaceus* (Huds.) Grev, *Pilayella littoralis* (Lyngb.) Kjellm., *Laminaria digitata* (Huds.) Lamour, *Chordaria flagelliformis* (O.F. Müll.) C. Ag., *Desmarestia aculeate* (L.) Lamour., *Desmarestia viridis* (Müll.) Lamour, *Chorda filum* (L.) Stackh., *Chorda tomentosa* Lyngb.	Polar (Arctic)	CH_2Br_2, $CHBrCl_2$, CH_2ClI, $CHBr_2Cl$, 1,2-C_2H_4Br, CH_2I_2, $CHBr_3$	Laboratory storage	Laturnus, 1996
Phaeophyta	*Laminaria saccharina* (L.) Lamour, *Laminaria solidungula* J.Ag., *Alaria* sp.	Polar (Arctic)	CH_2Br_2, $CHBrCl_2$, CH_2ClI, $CHBr_2Cl$, 1,2-C_2H_4Br, CH_2I_2, $CHBr_3$	Laboratory storage; thallus part	Laturnus, 1996
Phaeophyta	*Desmarestia Antarctica* Moe et Silva, *Phaeurus antarcticus* Skottsberg,, *Desmarestia menziesii* J. Agardh, *Halopteris obovata* (Hooker et Harvery) Sauvageau, *Adenocystis utricularis* (Bory) Skottsberg	Polar (Antarctic)	CH_2Br_2, $CHBrCl_2$, CH_2ClI, $CHBr_2Cl$, 1,2-C_2H_4Br, CH_2I_2, $CHBr_3$	Laboratory storage	Laturnus *et al.*, 1995
Phaeophyta	*Desmarestia anceps* Montagne, *Cystosphaera jacquinotii* (Montagne) Skottsberg, *Ascoseira mirabilis* Skottsberg, *Himantothallus grandifolius* (A. et E.S.Gepp) Zinova	Polar (Antarctic)	CH_2Br_2, $CHBrCl_2$, CH_2ClI, $CHBr_2Cl$, 1,2-C_2H_4Br, CH_2I_2, $CHBr_3$	Laboratory storage; thallus part	Laturnus *et al.*, 1995
Chlorophyta	*Enteromorpha compressa*	Polar (Antarctic)	$CHBr_3$, CH_2Br_2, CH_3Cl, CH_3Br, CH_3I, CH_2I,CH_2BrCl_2, C_2H_5Br, 1,2-$C_2H_4Br_2$, C_2H_5I, CH_2ClI	Light	Laturnus *et al.*, 1998

Chlorophyta	*Urospora penicilliformis* (Roth) Areschoug, *Blidingia minima* (Näg. Ex Kütz.) Kylin, *Acrosiphonia sonderi* (Kütz.) Kronm, *Monostroma arcticum* Wittr., *Enteromorpha compressa* (L.) Grev., *Chaetomorpha melagonium* (Web. ex Mohr) Kütz	Polar (Arctic)	CH_2Br_2, $CHBrCl_2$, CH_2ClI, $CHBr_2Cl$, 1,2-C_2H_4Br, CH_2I_2, $CHBr_3$	Laboratory storage	Laturnus, 1996
Chlorophyta	*Monostroma hariotii* Gain, *Enteromorpha bulbosa* (Suhr) Montagne	Polar	CH_2Br_2, $CHBrCl_2$, CH_2ClI, $CHBr_2Cl$, 1,2-C_2H_4Br, CH_2I_2, $CHBr_3$	Laboratory storage	Laturnus *et al.*, 1995
Rhodophyta	*Gymnogongrus antarcticus*	Polar	C_3H_7I, C_4H_9I, CH_2I_2, CH_2ClI, $CHBr_3$	Temperature, light, salinity, nutrient	Laturnus *et al.*, 2000
Rhodophyta	*Georgiella confluens*	Polar (Antarctic)	$CHBr_3$, CH_2Br_2, CH_3Cl, CH_3Br, CH_3I, CH_2I,CH_2BrCl_2, C_2H_5Br, 1,2-$C_2H_4Br_2$, C_2H_5I, CH_2ClI	Light	Laturnus *et al.*, 1998
Rhodophyta	*Gymnogongrus antarcticus*	Polar (Antarctic)	$CHBr_3$, CH_2Br_2, CH_3Cl, CH_3Br, CH_3I, CH_2I,CH_2BrCl_2, C_2H_5Br, 1,2-$C_2H_4Br_2$, C_2H_5I, CH_2ClI	Light	Laturnus *et al.*, 1998
Rhodophyta	*Phycodrys quercifolia*	Polar (Antarctic)	$CHBr_3$, CH_2Br_2, CH_3Cl, CH_3Br, CH_3I, CH_2I,CH_2BrCl_2, C_2H_5Br, 1,2-$C_2H_4Br_2$, C_2H_5I, CH_2ClI	Light	Laturnus *et al.*, 1998
Rhodophyta	*Rhodomela lycopodioides* (L.) Ag., *Palmaria palmate* (L.) Kuntze, *Devalaerea ramentacaea* (L.) Guiry, *Polysiphonia arctica* J. Ag.	Polar (Arctic)	CH_2Br_2, $CHBrCl_2$, CH_2ClI, $CHBr_2Cl$, 1,2-C_2H_4Br, CH_2I_2, $CHBr_3$	Laboratory storage	Laturnus, 1996
Rhodophyta	*Porphyra endiviifolium*, *Kallymenia Antarctica* Hariot, *Plocamium coccineum* (Hudson) Lyngbye, *Gymnogongrus antarcticus* Skottsberg, *Iridaea cordata* Kützing, *Georgiella lancifolia* (J.D. Hooker) J. Agardh, *Myriogramme mangini* (Gain) Skottsberg, *Pantoneura plocamioides* Kylin, *Picconiella plumose* (Kylin) DeToni, *Hymenocladiopsis crustigena* Moe, *Phyllophora ahnfeltioides* Skottsberg	Polar	CH_2Br_2, $CHBrCl_2$, CH_2ClI, $CHBr_2Cl$, 1,2-C_2H_4Br, CH_2I_2, $CHBr_3$	Laboratory storage	Laturnus *et al.*, 1995

Rhodophyta	*Gigartina papillosa* (Bory) Setchell et Gardner, Palmaria decipiens (Reinsch) Ricker	Polar	CH_2Br_2, $CHBrCl_2$, CH_2ClI, $CHBr_2Cl$, 1,2-C_2H_4Br, CH_2I_2, $CHBr_3$	Laboratory storage; thallus part	Laturnus *et al.*, 1995
Phaeophyta	*Fucus vesiculosus*	Temperate	$CHCl_3$, $CHBr_3$, CH_2I_2	Temperature	Abrahamsson *et al.*, 2003
Phaeophyta	*Fucus serratus*	Temperate	CH_2Cl_2, CH_2ClI	Laboratory storage	Baker *et al.*, 2001
Phaeophyta	*Fucus vesiculosus*	Temperate	CH_3Br, CH_3Cl, CH_2Cl_2, $CHCl_3$, CH_3I, CH_3CH_2I, CH_2ClI	Laboratory storage	Baker *et al.*, 2001
Phaeophyta	*Ascophyllum nodosum*	Temperate	CH_3I, C_2H_5I, CH_2ICl, CH_2IBr, CH_2I_2, $CHIBr_2$, CHI_2Cl, CHI_2Br, CH_3Br, CH_2Br_2, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Carpenter *et al.*, 2000
Phaeophyta	*Fucus serratus*	Temperate	CH_3I, C_2H_5I, CH_2ICl, CH_2IBr, CH_2I_2, $CHIBr_2$, C_2H_5Br, CH_2Br_2, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Carpenter *et al.*, 2000
Phaeophyta	*Fucus vesiculosus*	Temperate	CH_3I, C_2H_5I, CH_2ICl, CH_2IBr, CH_2I_2, $CHIBr_2$, C_2H_5Br, CH_2Br_2, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Carpenter *et al.*, 2000
Phaeophyta	*Halidrys siliquosa*	Temperate	CH_3I, C_2H_5I, CH_2ICl, CH_2IBr, CH_2I_2, $CHIBr_2$, CH_3Br, C_2H_5Br, CH_2Br_2, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Carpenter *et al.*, 2000
Phaeophyta	*Laminaria digitata*	Temperate	CH_3I, C_2H_5I, CH_2ICl, CH_2IBr, CH_2I_2, $CHIBr_2$, CHI_2Cl, CHI_2Br, CH_3Br, C_2H_5Br, CH_2Br_2, $CHBr_2Cl$, $CHBr_3$	Laboratory storage; light	Carpenter *et al.*, 2000
Phaeophyta	*Laminaria saccharina*	Temperate	CH_3I, C_2H_5I, CH_2ICl, CH_2IBr, CH_2I_2, $CHIBr_2$, CHI_2Cl, CHI_2Br, CH_3Br, C_2H_5Br, CH_2Br_2, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Carpenter *et al.*, 2000
Phaeophyta	*Pelvetia canaliculata*	Temperate	CH_3I, C_2H_5I, CH_2ICl, CH_2IBr, CH_2I_2, $CHIBr_2$, CHI_2Cl, CHI_2Br, CH_3Br, C_2H_5Br, CH_2Br_2, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Carpenter *et al.*, 2000
Phaeophyta	*Macrocystis pyrifera*	Temperate	$CHBr_3$, CH_2Br_2	Laboratory storage	Goodwin *et al.*, 1997
Phaeophyta	*Fucus serratus, Laminaria saccarina, Laminaria digitata, Desmaresstia aculeata, Chorda filum*	Temperate	C_2HCl_3	Laboratory storage	Abrahamsson *et al.*, 1995
Phaeophyta	*Ascophyllum nodosum*	Temperate	CH_3I, $CHCl_3$, CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$, $CHBr_3$	Laboratory storage; desiccation; light; grazing; tissue wounding	Nightingale *et al.*, 1995
Phaeophyta	*Fucus serratus*	Temperate	CH_3I, $CHCl_3$, CH_2Br_2, $CHBrCl_2$, $CHBr_3$	Laboratory storage	Nightingale *et al.*, 1995
Phaeophyta	*Laminaria digitata*	Temperate	CH_3I, $CHCl_3$, CH_2Br_2, $CHBr_2Cl$, $CHBr_3$	Laboratory storage; tissue age	Nightingale *et al.*, 1995
Phaeophyta	*Laminaria saccharina*	Temperate	CH_3I, $CHCl_3$, CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Nightingale *et al.*, 1995

Phaeophyta	*Pelvetia canaliculata*	Temperate	CH_3I, $CHCl_3$, CH_2Br_2, $CHBrCl_2$, $CHBr_3$	Laboratory storage	Nightingale *et al*., 1995
Chlorophyta	*Cladophora glomerata,Enteromorpha ahlneriana, Enteromorpha flexuosa, Enteromorpha intestinalis*	Temperate	$CHCl_3$, $CHBr_3$, CH_2I_2	Temperature	Abrahamsson *et al*., 2003
Chlorophyta	*Cladophora pellucida*	Temperate	CH_3Br, CH_2Cl_2, $CHCl_3$	Laboratory storage	Baker *et al.*, 2001
Chlorophyta	*Enteromorpha compressa*	Temperate	CH_3Br, CH_3Cl, CH_2Cl_2, $CHCl_3$, CH_3I, CH_3CH_2I, CH_2ClI	Laboratory storage	Baker *et al.*, 2001
Chlorophyta	*Ulva lactuca*	Temperate	CH_3Br, CH_3Cl, CH_2Cl_2, $CHCl_3$, CH_3I, CH_3CH_2I, CH_2ClI	Laboratory storage	Baker *et al.*, 2001
Chlorophyta	*Ulva lactuca*	Temperate	$CHBr_3$	Light; photosynthesis and respiratory inhibition	Manley & Barbero, 2001
Chlorophyta	*Enteromorpha intestinalis*	Temperate	CH_3I, C_2H_5I, CH_2ICl, CH_2IBr, CH_2I_2, $CHIBr_2$, CHI_2Cl, CHI_2Br, CH_3Br, C_2H_5Br, CH_2Br_2, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Carpenter *et al*., 2000
Chlorophyta	*Cladophota albida*	Temperate	CH_3I, $CHCl_3$, CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Nightingale *et al.*, 1995
Chlorophyta	*Enteromorpha* sp.	Temperate	CH_3I, $CHCl_3$, CH_2Br_2, $CHBrCl_2$, $CHBr_3$	Laboratory storage	Nightingale *et al.*, 1995
Chlorophyta	*Ulva lactuca*	Temperate	CH_3I, $CHCl_3$, CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Nightingale *et al.*, 1995
Rhodophyta	*Corallina officinalis*	Temperate	CH_3Br, CH_3Cl, CH_2Cl_2, $CHCl_3$, CH_3I, CH_2ClI	Laboratory storage	Baker *et al*., 2001
Rhodophyta	*Asparagopsis armata*	Temperate	CH_3I, C_2H_5I, CH_2ICl, CH_2IBr, CH_2I_2, $CHIBr_2$, CHI_2Cl, CHI_2Br, CH_3Br, C_2H_5Br, CH_2Br_2, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Carpenter *et al*., 2000
Rhodophyta	*Chondrus crispus*	Temperate	CH_3I, C_2H_5I, CH_2ICl, CH_2IBr, CH_2I_2, $CHIBr_2$, CH_3Br, C_2H_5Br, CH_2Br_2, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Carpenter *et al*., 2000
Rhodophyta	*Falkenbergia* stages of *Asparagopsis armata and Asparagopsis taxoformis*	Temperate	$CHBr_3$, CH_2Br_2, $C_2H_2Br_2$, $CHBr_2Cl$,C_2HBr_3	Laboratory storage; light	Marshall *et al*., 1999

Rhodophyta	*Chondrus crispus, Phyllophora pseudoceranoides, Porphyra umbilicalis, Polysiphonia nigrescens, Furcellaria lumbricalis, Ceramium rubrum, Ahnfeltia plicata*	Temperate	C_2HCl_3	Laboratory storage	Abrahamsson *et al*., 1995
Rhodophyta	*Corallina officinalis*	Temperate	CH_3I, $CHCl_3$, CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Nightingale *et al, 1995*
Rhodophyta	*Gigartina stellata*	Temperate	CH_3I, $CHCl_3$, CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Nightingale *et al, 1995*
Rhodophyta	*Polysiphonia lanosa*	Temperate	CH_3I, $CHCl_3$, CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$, $CHBr_3$	Laboratory storage	Nightingale *et al, 1995*
Mixed	*Cystoseria abies-marina, Codium adherens, Grateloupia doryphora, Hypnea spinella, Sargasum* sp., *Spirida hypnoides, Padina pa ́nia,*	Temperate	CH_3I, CHC_{l3}, CCl_4, CH_3CCl_3, $CHBrCl_2$, CH_2Br_2, $CHCl_5CCl_2$, Ch_3CHICH_3, CH_2ClI, CH_3CH_2 ICH_2CH_3, $Cl_2C_5CCl_2$, $CHBr_2Cl$, $CH_3CH_2CH_2CH_2$ I, $CHBr_3$, CH_2I_2	Rockpool; Diurnal variation	Ekdahl *et al*., 1998
Chlorophyta	*Caulerpa* sp., *Ulva rigida*	Sub-tropical	C_2HCl_3	Laboratory storage	Abrahamsson *et al*, 1995
Rhodophyta	*Eucheuma denticulatum*	Sub-tropical	$CHBr_3$, CH_2I_2, $CHBr_2Cl$, C_2Cl_4,CH_2ClI, $CHCl_3$, *sec*-C_4H_9I, CH_3I, CH_3-CCl_3, CCl_4, Cl_2C=$CHCl$, C_4H_9I, CH_2Br_2, CH_3I, $CHCl_2Br$, C_3H_7I	Light; pH	Mtolera *et al*., 1996
Rhodophyta	*Eucheuma denticulatum*	Sub-tropical	$CHBr_3$	Tissue wounding; light; photosynthesis	Sundstr öm *et al*., 1996
Rhodophyta	*Hypnea musciformis, Asparagopsis taxiformis, Gelidium canariensis, Falkenbergia hillebrandii, Laurencia obtusa, Corallina officinalis, Gracilariopsis lemaneiformis*	Sub-tropical	C_2HCl_3	Laboratory storage	Abrahamsson *et al., 1995*
Rhodophyta	*Gracilaria cornea, Meristiella gelidium*	Tropical	C_2HCl_3	Laboratory storage	Abrahamsson *et al*., 1995

**Laboratory storage = laboratory experiment under standardized condition for determination of halocarbon emission rates; no particular factor studied.*

2.3.3 Mechanism behind the production of halocarbons by seaweeds

The mechanism(s) behind the production of halocarbon from seaweed is(are) yet to be fully understood. However, literature suggests distinctly different mechanisms in the formation of monohalogenated and polyhalogenated compounds.

The formation of monohalogenated compounds i.e. methyl chloride, methyl bromide and methyl iodide are reported to be synthesized by S-adenosylmethionine (SMA): halide ion methyl transferase reactions, which have been reported earlier by Wuosmaa and Hager (1990) in the seaweeds. This enzyme is not dependent upon the availability of hydrogen peroxide, H_2O_2. Due to the different nucleophilicity of the halide ions, the halide methylation reactivity is in the order of I > Br > Cl.

On the other hand, the formation of polyhalogenated compounds involves the haloperoxidases enzymes found in seaweeds. There has been two types of haloperoxidases found in seaweeds so far, one containing a heme prosthetic group (Manthey & Hager, 1989), another the vanadium-containing bromoperoxidases. Halogenating peroxidases produce not only volatile halogenated organic compounds, but also halogenated terpenes and phenols (Butler & Carter-Franklin, 2004). Heme-containing peroxidase was found in seaweeds like *Cystoclonium purpureum* (Pedersén, 1976), *Chondrus crispus* (Collén *et al*., 2006) and *Rhodomela larix* (Ahern *et al.*, 1980).

Vanadium-containing bromoperoxidase was first found in the brown seaweed *Ascophyllum nodosum* (de Boer *et al.*, 1986). Later, there were reports of vanadium-containing bromoperoxidases found in *Fucus distichus* (Soedjak & Butler, 1991), *Corallina pilulifera* (Krenn *et al.*, 1989), *Corallina vancouveriensis* (Everett *et al.*, 1990), *Laminaria saccharina*, *Laminaria digitata*, *Fucus vesiculosis*, *Pelvetia*

canaliculata, *Ascophyllum nodosum*, *Chondrus crispus*, and *Plocamiun hamatum* (Wever *et al*., 1991). Gribble (2004) reported that 71 out of 94 species of red algae exhibit bromoperoxidase activity.

Vanadium pentoxide-enhanced vanadium-dependent bromoperoxidatic activities of six *Gracilaria* species were found to be more resistant to azide, cyanide and hydrogen peroxide than those containing heme (Suthiphongchai *et al*., 2008). This vanadium-containing peroxidase can be inactivated by dialyzing against EDTA at low pH and reactivated by vanadium (Vilter, 1984), while PO_4^{3-} was able to inhibit the haloperoxidases (Soedjak *et al*., 1991). The vanadium-haloperoxidases had been found in rhodophytes, phaeophytes and chlorophytes. The haloperoxidases are named according to the oxidative ability. The chloroperoxidases oxidize all halides but F^-, bromoperoxidases oxidize I^- and Br^- while the iodoperoxidases oxidize only I^-. The bromoperoxidases are primarily found in marine algae (Manley, 2002; Neidleman & Geigert 1986).

Two mechanisms had been proposed in which the haloperoxidases are involved. First is the direct halogenation of β-keto acids and cyclic β-diketones (Theiler *et al*., 1978). Polyhalogenated compounds like $CHBr_3$ and CH_2Br_2 occur through halogenation of ketones in the seaweeds and the decay process via the pH dependent haloform reaction (Laturnus *et al*., 2000). Mixed halocarbon compounds e.g. $CHBr_2Cl$ were suggested to be formed through nucleophilic substitution of other polyhalogenated compounds like $CHBr_3$ or CH_2I_2 with the Cl^- ions available in seawater (Class & Ballschmiter, 1988). Lately, there had been suggestions that intracellular and extracellular bromoperoxidases exist to protect the seaweeds from H_2O_2 (Manley & Barbero, 2001). The second mechanism involving the haloperoxidases is the oxidation catalysis of halide ions into

hypohalous acid, which then diffuses into the seawater and reacts with organic matter to produce volatile halogenated compounds (Wever *et al*., 1991). However, this mechanism was not widely supported as the brominating activity of the bromoperoxidase was found to be not consistent with the catalytic release of hypohalous acid in the laboratory experiments involving the seaweeds (Tschirret-Guth & Butler, 1994). Nevertheless, the mechanism behind the compounds is yet unknown.

2.3.4 Factors affecting halocarbon emissions by seaweeds

Most seaweed survives by attaching themselves to a substrate with their holdfast in order to prevent the tidal waves from sweeping them away. To compensate for their inability to evade predator and parasites, the seaweeds are thought to have developed a defense mechanism by emitting halogenated compounds against herbivores and microorganisms (Paul *et al*., 2006; Ohsawa *et al*., 2001). In the tropical reef habitats, diversified and high frequencies of chemical defenses were exhibited among the seaweeds (Hay & Fennical, 1988). In the *Gracilaria*, halogenated compounds are produced through their oxidative burst response to agar oligosaccharides, which is thought to be part of a control against bacteria (Weinberger *et al*., 2007). Also, excess H_2O_2 in seaweeds' habitat might trigger the release of halocarbon as a by-product from the production of other halogenated defense agents such as hypohalous acid (Brochardt *et al*., 2001; Gaut *et al.*, 2001) or haloterpenes (Sakata *et al.*, 1991) for the removal of toxic reactive oxygen species that may also formed during normal metabolism.

Further efforts to narrow down the factors responsible for the enhanced emission of halocarbons by the seaweeds were taken. This includes subjecting the seaweeds to different environmental parameters in the laboratory such as irradiance (Carpenter *et al.*, 2000; Manley & Barbero, 2001; Mtolera *et al*., 1996, Nightingale *et al.*, 1995),

temperature (Abrahamsson *et al*., 2003), pH (Mtolera *et al*., 1996) and even ultraviolet radiation levels (Laturnus *et al*., 2010).

Several studies found increased halocarbon emissions by the seaweeds during higher irradiances (Carpenter *et al.,* 2000; Manley & Barbero, 2001) which, together with the increased H_2O_2 level could indicate the role of halocarbon in oxidative stress protection. The production of $CHBr_3$ by the green seaweed *Ulva lactuca* was found to be three times higher in the illuminated compared to dark condition (Manley & Barbero, 2001). An irradiance study carried out on the sub-tropical red seaweed *Eucheuma denticulatum* (Mtolera *et al.*, 1996) reported elevated halocarbon emissions by the seaweed under high irradiance level, with the emissions of $CHBr_3$ and CH_2I_2 dominating over all other compounds studied. Similarly, Carpenter *et al.* (2000) found increased emission of halocarbons in the presence of incident light through their incubation-based and diurnal rockpool measurements. In their incubation-based study, it was reported that almost all of the halocarbon compounds measured were two times higher in light condition than dark, except for $CHBr_3$, which was ten times higher. Their in situ rockpool measurement results agreed well with the incubation-based study. In the natural habitat, when irradiance level got high, the halocarbon concentrations increased. The concentration of halocarbons was twice higher in mid-day compared to pre-dawn. The seaweed species dominating the rockpool includes *Enteromorpha prolifera, Cladophora rupestris, Ulva* sp., *Halopteris scoperia, Fucus serratus, F. spiralis* and *Halidrys siliquosa*. In another study, Ekdahl *et al.* (1998) concluded that there is a diurnal variation in halocarbon release by temperate seaweeds through their experiment at a rockpool in Canary Island, Spain. They observed an increase in the halocarbon concentration both in the air and seawater samples during mid-day, followed by a second concentration peak after sunset that was most probably caused by algal

respiration. The in situ and laboratory-based measurements were reported to relate well to each other.

However, some other studies do not agree that irradiance level causes higher halocarbon emissions by seaweeds. This is especially true when the Antarctic seaweed species are concerned. Klöser *et al*. (1993) and Laturnus *et al*. (1998) reported high release of halocarbons in low irradiance level or darkness. Laturnus *et al.* (1998) associated this observation with the strong ability of these Antarctic seaweeds to adapt to the considerable seasonal variation during summer and winter in Antarctic. In a study that followed, Laturnus *et al.* (2000) found no changes in the halocarbon emission rate between light and dark period by the Antarctic red species, *Gymnogongrus antarcticus* during long-term exposure, unlike short-term exposure which could trigger a stress response in the seaweeds. This finding somehow contradicted the conclusion made by Ekdahl *et al.* (1998) and Carpenter *et al.* (2000) that there is a diurnal variation in halocarbon release by seaweeds. Whether this could be caused simply by the different regions of seaweed origins, or the complexity of seaweed physiology that brings about the differences, it will certainly be intriguing if such contradiction could be explained.

Besides irradiance, the effect of temperature on halocarbon emissions by seaweeds was also observed over the years. There had not been any general agreement where temperature is concerned. Each study observed different or no trends between the increased temperature and the halocarbon compound emissions by the selected seaweed species. In a study involving brackish-water algae, Abrahamsson *et al*. (2003) did not see a general response pattern that is applicable to all the seaweed species studied, while suggesting that halocarbon production rates by the seaweeds are strongly species-dependent. On the other hand, Laturnus *et al*. (2000), in their study of short-term and

long-term effect of increase temperature from 0 ℃ observed in the natural habitat to 10 ℃ exerted on the Antarctic red seaweed, *Gymnogongrus antarcticus* Skottsberg, showed increased production of CH_2I_2 and $CHBr_3$ by the seaweed in their short-term incubation but no significant increase in the long-term change. Only CH_2ClI showed increased production in the long-term change but not short-term change. The increased halocarbon production during short term change could be due to stress. However, no explanation was provided concerning the increased of CH_2ClI production in the long-term change as the seaweed has been well adapted to the changes.

The production of reactive oxygen species that forms hydrogen peroxide occurs under stressed conditions. Therefore, apart from irradiance and temperature, exposure to altered pH (Mtolera *et al.*, 1996) was also found to influence the halocarbon emissions by seaweeds. The only literature reported on effect of pH on halocarbon emissions by seaweeds was reported by Mtolera *et al*. (1996). The sub-tropical seaweed species from Tanzania, *Eucheuma denticulatum*, which is also a species mass cultivated worldwide, was selected for the study. At the irradiance level of 1500 photosynthetic photon flux density (PPFD), the release of $CHBr_3$, CH_2I_2, $CHBr_2Cl$, C_2Cl_4, CH_2ClI, *sec*-C_4H9_I and $CHCl_3$ showed higher emission rates while at the irradiance level of 400 PPFD, $CHBr_3$, $CHBr_2Cl$ and C_2Cl_4 showed lower halocarbon emission rates while no trend was observed for $CHCl_3$ emissions at the same irradiance level. All other compounds exhibited increased emission rates at 400 PPFD. They suggested the promotion of halocarbon emission as a result of carbon dioxide deficiency caused by high pH level which induces the production by hydrogen peroxide (Mtolera *et al.*, 1996).

In addition to irradiance and pH, two other studies reported the effect of desiccation on seaweed halocarbon release (Bravo-Linares *et al.*, 2010; Nightingale *et al.*, 1995).

These studies were carried out to better understand the response of intertidal seaweeds in their halocarbon emission towards tidal changes. Generally, both studies seemed to agree that the emission of halocarbons by seaweeds were somehow influenced by desiccation. The emission of mixed and iodinated halocarbon compounds were found higher after the re-immersion of several common temperate intertidal seaweeds in seawater was reported by Bravo-Linares *et al.* (2010), but they reported a decrease in the chlorinated and brominated halocarbon emissions. However, another study reported increased production in both chlorinated and iodinated compounds especially CH_3I and CH_3Cl by the temperate brown seaweed *Ascophyllum nodosum* (Nightingale *et al*., 1995). It was also found that the increase in the halocarbon emissions is greater in seaweeds exposed to shorter desiccation period compared to those that are desiccated for over 24 hours before re-immersion (Nightingale *et al*., 1995).

Other factors reported to influence halocarbon emission by seaweeds include tissue wounding and tissue age (Nightingale *et al.*, 1995; Sundström *et al*., 1996). The release of CH_3I, $CHCl_3$ and $CHBr_3$ were found to increase with tissue wounding in *Ascophyllum nodosum* caused by the grazing of snails (Nightingale *et al*., 1995). Tissue wounding in the form of cutting was also carried out by Sundström *et al*. (1996) on the sub-tropical species, *Eucheuma denticulatum*. The emission of $CHBr_3$ was increased by the mechanical stress exerted upon the seaweed. On the other hand, although the emission of CH_3I was once found not to be influenced by the age of the seaweeds (Manley & Dastoor, 1988), a later study on *Laminaria digitata* showed up to 17-fold increase in the emission of another halogenated compound, $CHBr_3$, is possible between the meristem and the end tissue. Compounds including CH_3I, $CHCl_3$, CH_2Br_2 and $CHBr_2Cl$ also showed a gradated increase in emissions from the tissue near the stipe of the seaweed to the distal end of the frond (Nightingale *et al.*, 1995).

A recent literature regarding the emission of reactive organic halogens by seaweeds upon the exposure to ultraviolet radiation has been published (Laturnus *et al*., 2010). The emission of the reactive organic halogens by *Saccharina latissima* was significantly influenced by the presence of the ultraviolet radiation. The emission of reactive organic bromine and chlorine was decreased as a result, while the emission of reactive organic iodine was enhanced. This suggested the possibility of a greater contribution by seaweeds towards the stratospheric halogen loading caused by increased ultraviolet radiation reaching the Earth's surface due to more stratospheric ozone loss (Laturnus *et al*., 2000).

A comparison study of halocarbon emissions between seaweeds incubated in Provasoli enriched seawater and short term/ long term exposure of seaweeds to non-enriched seawater was carried out by Laturnus *et al*. (2000) who showed enhanced seaweed halocarbon emissions in non-enriched seawater instead of Provasoli enriched seawater. The emission rates of the three compounds studied i.e. CH_2ClI, CH_2I_2 and $CHBr_3$ were all higher in long term (two months) exposure to non-enriched medium compared to the short-term (24 hours) exposure. A similar trend was observed when another abiotic factor – salinity was tested. Increased halocarbon emissions for the three compounds were reported from lower salinity (27%) compared to higher salinity (34%), with the emission from seaweeds exposed to longer period of low salinity higher than those from the short-term exposure (Laturnus *et al*., 2000). Such study brings about the possible effect of global warming and ocean eutrophication on terms of seaweed halocarbon emissions in the natural environment.

2.3.4.1 Halocarbon emissions and photosynthesis

While investigating the possible constraints on *Ulva lactuca* in the production of $CHBr_3$, Manley and Barbero (2001) also reported that although the production of $CHBr_3$ increased in lighted condition compared to the dark, the production of $CHBr_3$ was decreased when DCMU [3-(3.4-dichlorophenyl)-1,1-dimethylurea], a photosynthetic inhibitor, was dissolved in the seawater where the green seaweed was incubated. This observation agrees to the postulation that the influence of irradiance on emission of halocarbon by the seaweeds is a photosynthesis-related mechanism (Ekdahl *et al*., 1998; Goodwin *et al*., 1997). Ekdahl *et al*. (1998) reported highest halocarbon emission rates by the seaweeds during mid-day, where the seaweed photosynthesis is assumed to be highest. While acknowledging the effect of irradiance on the emissions of $CHBr_3$ and CH_2Br_2 by the giant kelp, *Macrocystic pyrifera*, Goodwin *et al.* (1997) also confirmed that photosynthesis activity influenced the emission of the mentioned compounds through their experiment with the use of DCMU. Light-dependent process in the seaweeds like the pseudocyclic photophosphorylation or the Mehler reaction produces superoxide radical (O_2^-) as a result of electron transport from ferrodoxin of PSI to the oxygen molecule in the plant's photosynthesis process (Collén *et al.,* 1995; Dummermuth *et al.,* 2003; Manley & Barbero, 2001). The activity of superoxide dismutase produces hydrogen peroxide (H_2O_2) through the dismutation of O_2^- (Halliwell, 1994). The accumulation of H_2O_2 in turn activates the bromoperoxidase in the seaweeds to produce polybromomethanes e.g. $CHBr_3$, CH_2Br_2, where bromination of β-keto acids and cyclic β-diketones are involved (Theiler *et al*., 1978). Other sources of H_2O_2 include mitochondrial respiration, enzyme catalysis and illuminated seawater (Manley & Barbero, 2001). The findings that irradiance and photosynthesis influenced halocarbon emissions by seaweeds suggests that light-related environmental factors such as time of day, degree of cloud cover and tidal height can affect the release of

these volatile halogenated compounds by the seaweeds in their natural habitat at the coastal region into the atmosphere.

In an attempt to assess the antioxidative properties of seaweed towards H_2O_2 stress, the maximum quantum yield (F_v/F_m) of the seaweed was used as a stress indicator. Seaweeds with high ratio of F_v/F_m were treated as healthy and not susceptible to the oxidative stress (Dummermuth *et al*., 2003). The photosynthesis efficiency of the seaweeds was determined by using a pulse amplitude modulated (PAM) fluorometer. The use of PAM in the determination of the plant's photosynthesis activity has been widely reported as this technique does not stress or damage the plant itself while providing an almost-instantly sensitive measurement compared with other conventional methods e.g. radiocarbon method, dissolved oxygen measurement etc. F_v/F_m is commonly used in physiological and stress researches in plants (Dummermuth *et al*., 2003). The light energy that is absorbed into the leaf is either converted into photochemical energy for photosynthesis, or as heat dissipation or re-emitted as light in the form of chlorophyll fluorescence. An increase in either one of the converted energy form will limit the amount of the other two, hence through the quantification of chlorophyll fluorescence, efficiency of photosynthesis and heat dissipation can be assessed (Maxwell & Johnson, 2000). The Photosystem II (PSII) in the chlorophyll is able to emit fluorescence. These fluorescence acts as an indicator for energy conversion in plants capable of photosynthesis, reflecting the efficiency of the reaction centers in PSII in converting the sun's light into photochemical energy (Kraus & Weiss, 1991). In a study to determine the effect of irradiance on the release of halogenated compounds by few microalgae, Hughes *et al*. (2006) uses the F_v/F_m values to indicate possible seaweed stress triggered by the different irradiance level. Although they found pronounced decrease in F_v/F_m values of the microalgae in low irradiance level, there

wasn't increase in the iodocarbon emissions that was expected from stress condition. Although photosynthesis had long been postulated to influence halocarbon emissions in seaweeds, yet there are no other literatures available that studies the photosynthesis parameters in seaweeds that correlate to their halocarbon release.

2.4 The use of micro-Dirac gas chromatograph for atmospheric halocarbon measurements

The use of μDirac gas chromatograph in field deployment for measurements of selected halocarbon compounds had been described by Gostlow *et al*. (2009). The μDirac was designed and made by the authors. It is a light-weight, portable, flexible, power efficient and autonomous device that, compared to conventional gas chromatography device, made field deployment much easier without the need to operate the device during every sample injection. The use of such device has been reported in balloon, aircraft, ship, and also ground-based station in the measurement of selected atmospheric long-lived e.g. CFCs and short-lived halocarbons e.g. $CHCl_3$, CH_2Br_2, C_2Cl_4, $CHBr_2Cl$, $CHBr_3$ and CH_3I.

The μDirac consists of an inlet manifold that contains three solenoid valves for the selection of helium carrier gas, calibration gas, or sample gas. The pre-determined volume of each gas type is controlled by the flowmeter. The carrier gas (helium) was made to flow through stainless steel tubing fitted with molecular sieve and charcoal for moisture and contaminants removal. Gas samples taken by the sampling pump through the sampling inlet were made to flow through the adsorption / desorption system. The adsorption system consists of carbon molecular sieve adsorbent. Compounds with high boiling point are adsorbed by Carboxen ™ 1016 while compounds with lower boiling point are adsorbed by Carboxen ™ 1001 in the temperature range of 15-25℃. The

desorption of compounds were done by purging the tube with the helium gas to remove oxygen and the Valco Vici valve turned to the 'inject' position. This is followed by a flash heating at a pre-determined temperature and the target compounds will then be carried away by the carrier gas into the capillary column for separation. The capillary column used was described as a 10m (Internal Diameter: 0.18mm) MXT 502.2 (Restek®, model 71891) coated with Crossbond® diphenyl/dimethyl polysiloxane phase (film thickness: 1 μm). As the targeted compounds flow through the capillary column, they are subjected to a set of temperature programme at a certain flow rate for better separation. The targeted compounds will then pass through the electron capture detector (ECD), which sends signals to an attached computer. All compounds were then compared to the National Oceanic & Atmospheric Administration – Earth System Research Laboratory (NOAA-ESRL) calibration standards. The calibration gas is a pressurized air sample from a remote site with continental air at Niwot Ridge, CO, USA. The sample was spiked with halocarbons like $CHBr_3$ and $CHBr_2$ and compared to the NOAA-ESRL working standards and analyzed with gas chromatography-electron capture detector (GC-ECD) and gas chromatography-mass spectrometer (GC-MS).

The accuracy of the device was reported to be < 2% for long-lived halocarbons like the CFCs and <10% for short-lived halocarbons. However, the overall precision is determined through the repeated measurement of the calibration gas a few times for the calculation of Relative Standard Deviation of each compound (Gostlow *et al*., 2009).

CHAPTER 3

METHODOLOGY

3.1 General methods

3.1.1 Main study site

Cape Rachado (2° 31'N, 101° 47' E), Port Dickson has a fringing coral reef flat area extending out to about 50 m, which slopes towards the reef edge (Phang, 1995). It faces the Straits of Malacca and Site B, the study site, is bordered by a small patch of mangroves on the left and a rocky shore on the right. It is a popular picnic area. Tidal level of less than 0.3m from the chart datum will see the reef flats completely exposed.

The community structure of the fringing coral reef was extensively studied (Goh & Sasekumar, 1980). Seaweed biomass studies were also carried out during years 1987/88, 1995/96, 1998 and 2005/06 (Phang, 1995; Wong, 1997; Ooi, 2001; Andriana, 2010).

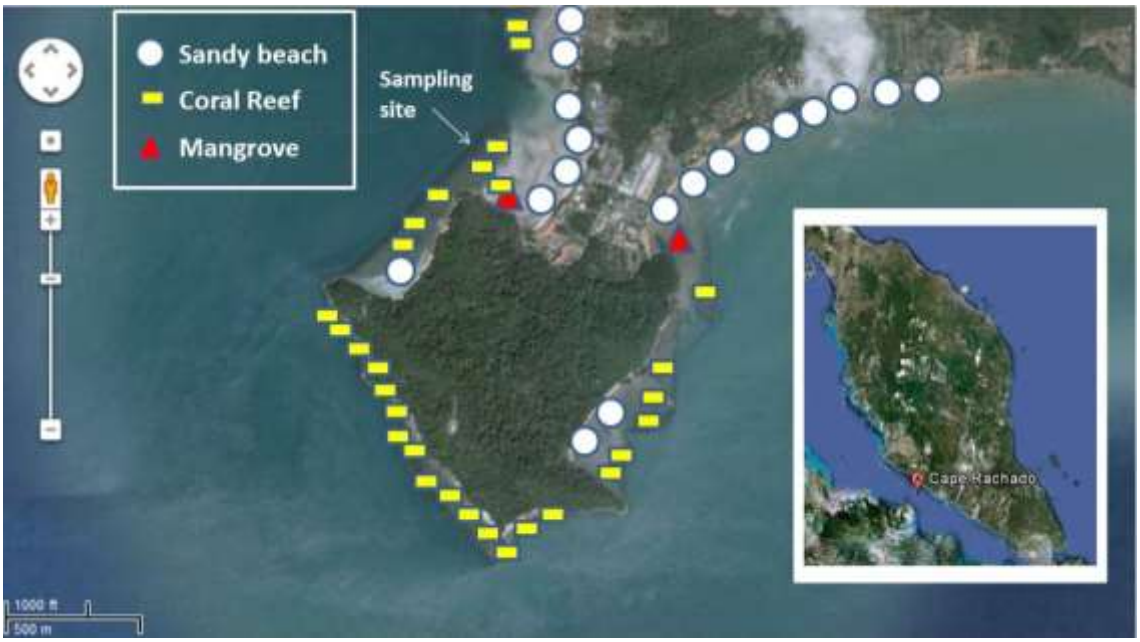


Figure 3.1: Map of the main study site at Cape Rachado, Port Dickson (Source: Google Maps)

3.1.2 Collection of seaweeds

Collections of seaweeds sample were done at the main study site at Cape Rachado. All selected seaweeds samples were collected during low tidal level with a maximum level of 0.8m. Whole plants were collected and placed in zip-lock bag with seawater before being transported back into the laboratory in ice-cooled ice-chest. All live samples were kept in bags partially-filled with seawater during transportation in the ice-chest to minimize desiccation stress upon the plants. All seaweeds samples were kept alive in the hatchery until use in the experiments.

3.1.3 Determination of seaweed photosynthetic parameters

The Pulse Amplitude Modulator (PAM) fluorometry is a sensitive non-invasive method to determine a plant's photosynthetic parameters. To access the maximal quantum yield of the seaweeds (F_v/F_m, where F_v is the variable fluorescence measured as the difference between maximal (F_m) and minimal (F_o) fluorescence in dark-adapted leaves), the diving PAM (Walz, Germany) was used.

The diving PAM measures the minimum fluorescence yield (F_o) of the plant by using a weak measuring light (0.15 µmol photons m^{-2} s^{-1}) and the maximal fluorescence yield (F_m) with a saturating pulse (>3000 µmol photons m^{-2} s^{-1}). In order to determine the F_v/F_m, the seaweeds were dark-adapted for 15 minutes prior to measurement. This period is sufficient to provide a true measurement of F_o (McMinn & Hegseth 2004; Colsalvey *et al*., 2005). During this time, the electron transport stops, allowing the full reduction of the primary electron acceptor. F_v/F_m is then determined by the following equation:

$$F_v/F_m = (F_m - F_o) / F_m$$ (Ralph and Gademann, 2005)

For the measurement of seaweeds F_v/F_m values in the laboratory incubation, photosynthesis activity of the seaweed was assessed before and after the seaweed incubation during the experiment and the average values recorded.

3.2 Field study

Field survey was carried out once in every three months between March 2010 – June 2011 at the selected site at Cape Rachado, Port Dickson. A total of six surveys had been carried out in March 2010, June 2010, September 2010, December 2010, March 2011 and June 2011. Each sampling trip lasted three days. During each sampling trip, the following measurements were made:

1. Atmospheric halocarbon measurements
2. Seaweed standing biomass
3. Seawater quality

3.2.1 Atmospheric halocarbon measurements

In-situ monitoring of atmospheric halocarbon measurements was carried out using μDirac IV, a portable, autonomous gas chromatograph (GC) with electron capture detector (ECD) built by Gostlow *et al* (Gostlow *et al*., 2009). This GC was designed to meet the challenging requirements for deployment in the field. The structure and design of the μDirac was described in Gostlow *et al*., 2009.

The μDirac was set-up and placed in an air-conditioned room at PNB Ilham Resort, Port Dickson, each time during deployment. A room at a high-floor overlooking the sampling site was selected to minimize disturbance for human activities. A leak-check on the regulators for both helium and nitrogen gas was carried out every time after

setting up the μDirac. One end of the sample inlet tube (High purity Tygon tubing) was connected to the GC while the other was tied up to the window opening of the room. A fixed volume of 20 cm^3 air was collected for every sample run. The μDirac was set to run at a pre-defined set of temperature programme. The μDirac was usually left on without running the pre-set programme for few hours depending on the baseline level as peak integration at high baseline level is impossible. High baseline upon start-up of the device was usually caused by the assemble and dissemble of the machine that introduce contaminants into the system. When the baseline went low, the pre-set programme was set on. The starting temperature of the μDirac was 25 ℃, which was then increased to 175 ℃ at a rate of 30 ℃ min^{-1}. Halocarbons were removed from the air with an adsorbent-containing micro-trap, and desorbed into the capillary column, where it was separated. The sampling time was approximately 37 minutes. The μDirac was set to run in a cycle of blank, calibration gas, blank, calibration gas, sample, and sample. The whole cycle takes around four hours to complete.

3.2.2 Survey of seaweed standing biomass

In order to achieve the first hypothesis testing the influence of seaweed standing biomass on atmospheric halocarbon contents, the seaweed standing biomass at Cape Rachado, Port Dickson was surveyed every three months. Line-transect and systematic quadrat-sampling method was selected primarily because this was used in the 1987/88, and 1998 diversity study (Phang, 1995; Ooi, 2001).

As the working time was constrained by tidal movement, sampling was carried out during low tide, when the level was approximately 0.8 meter above chart datum or less. Baseline was marked out from the fringing reef structure, running parallel to the coastline. Three transects were laid out along this baseline, running at a perpendicular

angle to it at a 20 meter interval. Quadrats measuring 0.3 meter x 0.3 meter were placed systematically along the transects with a 10 meter distance between each quadrat. All algal materials with holdfasts lying within the 0.09 m^2 (0.3 x 0.3 m) quadrats were collected, placed in plastic bags and sent back to the laboratory for identification and processing.

In the laboratory, seaweeds were quickly washed in diluted seawater to remove sand, silt and epiphytes. This was followed by identification and separation of species. Taxonomic identification was based on several references (Abbott 1988, 1992, 1994, 1995, 1997, 1999; Abbot & Norris, 1985; Ismail, 1995; Taylor 1967, 1969; Trono, 1997; Womersley 1984, 1987, 1994, 1996). All cleaned seaweed samples were then loosely wrapped in pre-weighed aluminum foil and oven dried at 60℃ for 72 hours to determine the dry weight (gm^{-2}) for the seaweed biomass in each quadrat.

The frequency and dominance of seaweed were calculated from the equations below:

$$Frequency\ (\%) = \frac{Number\ of\ quadrats\ in\ which\ a\ species\ occurs}{Total\ number\ of\ quadrats\ sampled} \times 100\%$$

$$Dominance\ (\%) = \frac{Total\ dry\ biomass\ values\ for\ a\ species\ (N)}{Total\ dry\ biomass\ values\ for\ all\ species} \times 100\%$$

The Importance Value Index (IVI), which takes into account of both relative frequency and relative dominance, provides a comprehensive estimate of the importance of a certain species (Greig-Smith, 1983). The calculations were based on the following equations (Cox, 1996):

$$Frequency\ value\ (f) = \frac{Number\ of\ quadrats\ in\ which\ a\ species\ occurs}{Total\ number\ of\ quadrats\ sampled}$$

$$Relative\ frequency\ (Rf) = \frac{Frequency\ value\ for\ a\ species\ (f)}{Total\ frequency\ values\ for\ all\ species} \times 100\%$$

$$Dominance\ value\ (d) = \frac{Total\ wet\ biomass\ values\ for\ a\ species}{Area\ sampled}$$

$$Relative\ dominance\ (Rd) = \frac{Dominance\ value\ for\ a\ species\ (d)}{Total\ dominance\ values\ for\ all\ species} \times 100\%$$

$$Important\ value\ index\ (IVI) = \frac{Relative\ frequency\ (Rf) + Relative\ dominance\ (Rd)}{2}$$

3.2.3 Seawater quality tests

In order to determine the correlation between seaweed standing biomass at the sampling site with the physicochemical, nutrients, total suspended solid, and chlorophyll-a concentration of the seawater, several seawater quality tests had been carried out. Five replicates were measured each trip. The physicochemical parameters were measured in situ between 12pm – 2pm during each sampling. As for the nutrient, total suspended solid, and chlorophyll-a concentration measurements, five replicates of seawater samples were collected in separate containers and sent back to the laboratory/ hotel for immediate analysis.

a. Physicochemical parameters

Physicochemical parameters of the seawater including temperature, conductivity, total dissolved solids, salinity, dissolved oxygen, pH, and oxidizing reducing potential were measured using YSI Water Quality Logger Model 577 during the sampling period. Five seawater samples (except the March 2010 sampling which has a total of 10 replicates) were collected at random spots within the three transect areas during each trip between 12pm – 2pm, and the average value for the five samples were recorded.

b. Nutrient analysis

Five seawater samples (except the March 2010 sampling which has a total of 10 replicates) were collected at random spots within the three transect areas during each trip between 12pm – 2pm, and the average value for the contents of ammonia (NH_3-N), Nitrite (NO_2^--N), Nitrate (NO_3^--N) and Phosphate (PO_4^{3-}) levels were collected. Water samples collected were analyzed in the laboratory on the same day of collection. All nutrient analyses were carried out based on the protocols laid out in the manual for HACH ODYSSEY DR 2500 spectrophotometer using powder pillows (HACH). Protocols used were based on the following methods:

Method	Test
Salicylate Method	Ammonia (NH_3-N)
Diazotization Method	Nitrite (NO_2^--N)
Cadmium Reduction Method (APHA, 1998)	Nitrate (NO_3^--N)
Ascorbic Acid Method (APHA, 1998)	Orthophosphate (PO_4^{3-})

The protocol (Method 8155; Hach Program: 385 N, Ammonia, Salic.) for the determination of NH_3-N was adapted from the Salicylate Method (Reardon *et al*., 1966). Briefly, 10 mL of seawater collected from the sampling site was filled into a round sample cell while another round sample cell was filled with deionized water as a blank reading. The content of one Ammonia Salicylate Powder Pillow was added to each cell.

The cells were then stoppered and shook to dissolve the powder. After three minutes, the content 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 the reading for the second cell. The presence of ammonia nitrogen was indicated by a greenish color.

The determination of No_2^--N in Method 8507, (Hach Program: 371 N, Nitrite LR) was adapted from the Federal Register's Diazotization Method. As stated in the protocol, a round sample cell was filled with 10 mL of seawater collected from the sampling site and the contents of one NitriVer 3 Nitrite Reagent Powder Pillow was added to the sample cell. It was then capped and shook to dissolve to powdered reagent. The presence of nitrite is determined through the formation of pinkish solution. This sample cell was then read through with the spectrophotometer after 20 minutes. No_2^--N content was measured by comparison to a blank sample cell filled with 10 mL seawater.

The protocol (Method 8171; Hach Program 353N, Nitrate MR) for measurement of NO_3^--N was based on the Cadmium Reduction Method (APHA, 1998). 10 mL of the collected seawater from sampling site was filled into a round sample cell. Content 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 untouched for five minutes for reaction. The presence of nitrate was indicated by formation of amber color solution. A blank was prepared in the meanwhile by filling in 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 the reading for the sample cell.

The determination of PO_4^{3-} in Method 8048, (Hach Program: 490 P, React. PV) was based on the Ascorbic Acid Method (APHA, 1998). As stated in the protocol, a round sample cell was filled with 10 mL of seawater collected from the sampling site and the contents of one PhosVer 3 phosphate Reagent Powder Pillow was added to the sample cell. It was then immediately swirled to mix and let to settle for two minutes. In the meanwhile, a blank was prepared by filling a second round sample cell with 10 mL of seawater. After the two 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.

c. Total suspended solids (TSS)

This procedure was modified from APHA (1998); to determine the total suspended solids (TSS) level of the seawater samples, pre-weighed glass microfibre filters (GF/C, Whatman) were washed with 20 mL distilled water thrice through a vacuum filtration apparatus. Seawater collected were then filtered and dried at 100 ℃ for 72 hours and left in a desiccator for 24 hours. The constant weight of the GF/F was recorded. Increase in the weight of the filter indicates the total suspended solids content per 100 mL seawater as in the calculation below:

$$\text{TSS} = \frac{(\text{Filter weight} + \text{residue, g}) - (\text{Filter weight, g})}{\text{Sample volume, L}}$$

d. Chlorophyll-a content of seawater

Five composite replicates (except the March 2010 sampling which has a total of 10 replicates) of seawater sample were collected from within the three transect area and sent back to the laboratory / hotel for extraction within the same day of seawater collection. The seawater was filtered using a glass microfibre filters (GF/C, Whatman)

placed in a vacuum filtration apparatus. The filtrate on the filter paper, together with the filter paper was collected and placed in a 15 mL Falcon centrifuge tube. 10 mL of acetone was added into the tube. The filter paper was then mashed with a glass rod. Following that, the centrifuge tube was capped and wrapped in aluminium foil and placed in a 4 ℃ refrigerator for 24 hours for extraction (Strickland & Parsons, 1968).

The extract was centrifuged at 3000 rpm (Kubato 2100) for 10 minutes the following day. The supernatant was pipetted out and filled in a quartz cuvette. Readings at wavelengths of 665, 645, and 630 nm against an acetone blank, were taken using a 160 UV Shimadzu Spectrophotometer. Chlorophyll-a concentration was then calculated from the formula below:

Chl-a ($\mu g\ L^{-1}$)

$$= \frac{[(11.6\ \times OD\ 665) - (1.31\ \times OD\ 645) - (0.14\ \times OD\ 630)]\ \times Acetone\ vol\ (mL)}{Sample\ volume\ (L)}$$

3.2.4 Meteorological data

Meteorological data including total rainfall, wind speed and wind direction was obtained from the Malaysian Meteorological Department. The total rainfall data was taken from the Tanjung Tuan Lighthouse (02 24'N; 101 51'E), which is just next to our sampling sport; while the wind data was recorded from Atherton Estate (02 33'N; 101 55'E), the next nearest weather station from the study site. The total rainfalls for every three months were summed up to correlate with the seaweed biomass measurements on the third month. Other data e.g. irradiance, water quality data was not available from any stations near the sampling site.

3.3 Laboratory study

3.3.1 Seaweed incubation

Three brown seaweed species, *Sargassum binderi* Sonder ex J. Agardh, *Padina australis* Hauck, and *Turbinaria conoides* (J. Agardh) Kützing from Cape Rachado, Port Dickson were collected and transported back to the laboratory to study effect of irradiance on halocarbon emissions. They were kept alive at the hatchery at a maximum of five days until experimental needs under ambient conditions.

For each level of irradiance studied, the seaweeds were left to acclimatize prior to experiment between 15-18 hours by placing the seaweeds in a large glass bottle with aeration, irradiated at the irradiance level the seaweeds are to be studied at a constant temperature of 32 ± 2 ℃. Visible epiphytes on the seaweeds were removed. This was followed by selecting five whole and healthy plantlets and placing them into five separate 500 mL borosilicate flasks (Schott, Duran) filled with GF/F (0.7 µm, Whatman) pre-filtered seawater without any headspace. Five flasks filled with pre-filtered seawater without seaweed were used as control. The seawater used for the five control flasks and the five seaweed incubation flasks were of the same source collected from the hatchery at the same time in order to minimize inconsistency in the measurement that may arise.

These flasks were later incubated in the laboratory at a constant temperature of 32 ± 2 ℃. During experiment, the flasks were subjected to different level of irradiances (fluorescent tubes, Philips) of 0 (L1), 47 (L2), 58 (L3), 81 (L4), and 126 (L5) µmol photons m^{-2} s^{-1} measured using LI-250A light meter with LI-190SA quantum sensor (LICOR, Inc.). The presence of ultraviolet radiation was also determined (UVX Digital Radiometer, UVP). Incubation time was set for four hours to allow sufficient time for detectable amount of the volatile halocarbons to be emitted (Laturnus *et al.*, 2010) while

avoiding additional stresses that might occur due to prolonged incubation period. The photosynthetic activity of the seaweed samples were assessed by using the diving Pulse Amplitude Modulator (Walz, Germany) prior and post-incubation, and was indicated by the F_v/F_m values.

Upon reaching the incubation time of four hours, the flasks were gently swirled to make sure the seawater was well-mixed before collecting 40 mL of seawater from the flasks using a 100 mL glass syringe fitted with a stopcock. In order to monitor and correct system sensitivity drift, deuterated surrogate analytes, CD_3I (ARMAR chemicals) and CD_2I_2 (Sigma-Aldrich) were added into every seawater sample before analysis. The seawater will then be injected into the analytical system. The concentration of halocarbons emitted by the seaweeds was determined from the differences of the halocarbon concentrations between the control flasks and the flasks with seaweeds.

Lastly, in order to determine the seaweed dry weight, the seaweed biomass of each replicate used for all five of the irradiance level study were dried in an oven at 60 ℃ for 72 hours followed by placing the seaweeds in a desiccator for 24 hours. The dry weight for each seaweed was recorded to determine the concentration of halocarbon emitted by the seaweeds in an hour per gram dry weight of seaweeds.

3.3.2 Analysis for halocarbon

All laboratory incubation analyses were carried out with the analytical system consisting of a purge-and-trap system (Figure 3.2) developed by the University of East Anglia (UEA), United Kingdom connected to an Agilent Technologies 7890A gas chromatograph and 5975C mass selective detector (GC-MSD) (Figure 3.3).

The gas chromatograph system was fitted with a J&W 60-m DB-VRX capillary column (film thickness 1.40 µm).The injected seawater sample was injected into the purge-and-trap system and purged for 15 minutes using oxygen-free nitrogen (OFN) at a flow rate of 20 mL min^{-1}. Aerosol particles were removed from the system by passing through the purged gas through a glass tube stuffed with glass wool, while water vapor was removed by passing the purged gas through a molecular sieve and a counter-flow Nafion dryer (Perma-Pure) using OFN at 100 mL min^{-1}. The target compounds were then trapped and concentrated in a stainless-steel tubing at a temperature of -150 ℃ by immersing the stainless-steel trap portion in liquid nitrogen, helped by a thermostatic heating device.

The samples were then desorbed by swapping the liquid nitrogen with boiling water, and samples introduced into the GC with the help of helium as a carrier gas at a flow rate of 1 mL min^{-1}. As the run starts, the oven was set to hold for 5 minutes at 36 ℃, which was followed by a heating of 20 ℃ min^{-1} to 200 ℃, and finally heated up to 240 ℃ at the rate of 40 ℃ min^{-1} (Hughes *et al.*, 2006). The MSD detector was set on between 4 and 18 minutes to collect data in the Single Ion Mode.

A total of eight common halocarbon compounds emitted by seaweeds were selected. These included iodomethane (CH_3I), diiodomethane (CH_2I_2), bromochloromethane (CH_2BrCl), bromoiodomethane (CH_2BrI), dibromomethane (CH_2Br_2), tribromomethane ($CHBr_3$), bromodichloromethane ($CHBrCl_2$) and dibromochloromethane ($CHBr_2Cl$).

The peak areas for the quantifying ions for each halocarbon compounds were recorded and fitted in to a linear equation determined from pre-calibrated halocarbon standards to determine their concentrations.

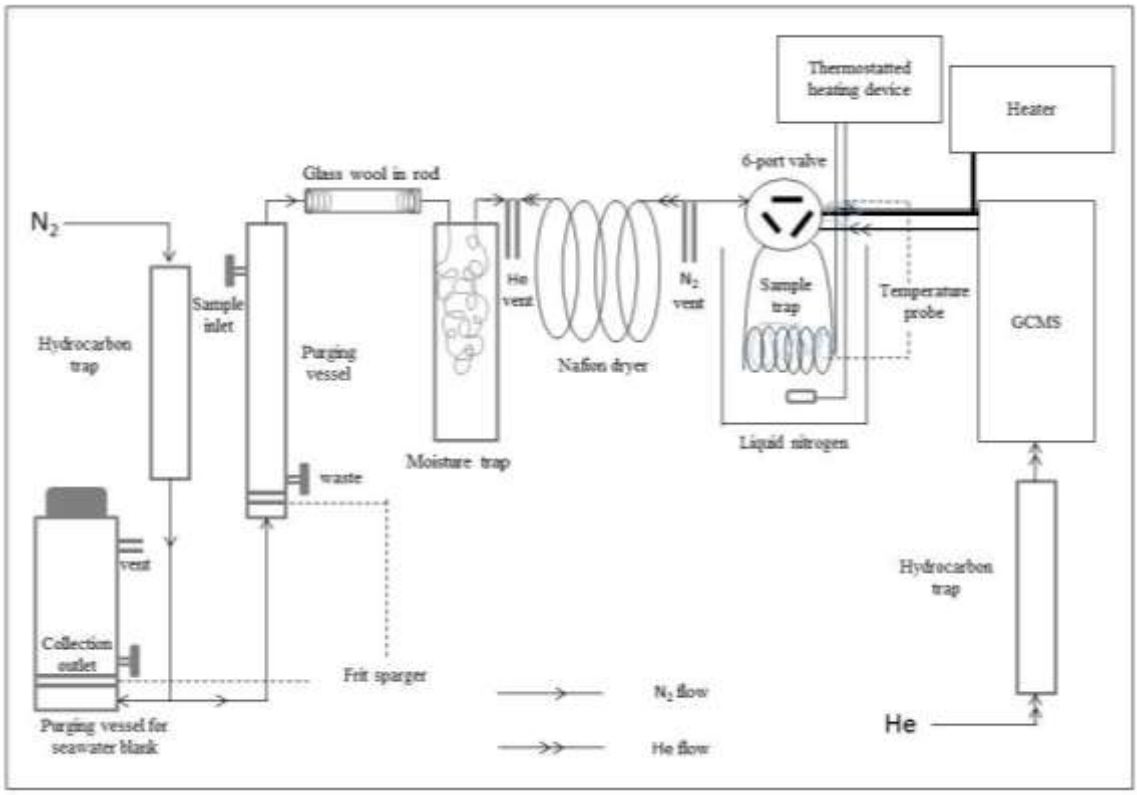


Figure 3.2: Purge-and-trap system developed by the University of East Anglia

Figure 3.3: The analytical system with the purge-and-trap system attached to the GCMS

3.3.3 Calibration of halocarbon standards

Gravimetrically prepared liquid standards (Sigma-Aldrich) in high-performance liquid chromatography-grade methanol (Fischer Scientific) were used for all calibrations. Serial dilutions were carried out to prepare five different concentrations of a single halocarbon standard over a range of pre-determined concentration.

Each of the five different concentrations of a halocarbon standard was then added to 40mL of pre-purged seawater blank contained in a 100 mL glass syringe. The pre-purged seawater blank was prepared by purging the seawater in a flask by using oxygen-free nitrogen overnight. Prior to injection into the analytical system, the two internal standards were added into the glass syringe.

The known concentration of halocarbon was plotted against their peak areas determined at the end of each system run to a linear function. All regression (r^2) for the linear calibration curve was above 0.95 except for CH_2I_2 ion 141 which was 0.94. All halocarbon compounds were identified with their individual identifying ions (Table 3.1) under the single ion mode (SIM) selected under the GCMS software programme run.

3.3.4 Determining the repeatability and detection limit of the system

The repeatability of the system was determined by the five times injection of a seawater sample into the analytical system. The percentage of standard deviation over the average value from the same sample was then subtracted. The repeatability for six of the eight halocarbon compounds studied was above 90 %, while the repeatability for $CHBr_3$ and CH_2I_2 was 89.89 % and 77.00 % due to the increased background noise with longer retention time compounds (Table 3.1).

The order of detection limits, calculated as three times the standard deviation of the blanks (Abrahamsson & Pedersén, 2000) for each of the halocarbon compounds determined are listed in Table 3.1. It was determined by examining the signal-to-noise ratio with pre-calibrated liquid standards.

Table 3.1: The halocarbon compounds studied in this experiment with their retention time, identifying ions, quantifying ions, repeatability and order of detection limit

Compounds	RT (min)	ID (m/z)	Repeatability	Detection limit
CH_3I	8.98	142*, 141	98.87%	10 pmol L^{-1}
CH_2BrCl	10.84	130*, 128	99.37%	10 pmol L^{-1}
CH_2Br_2	12.50	174*, 95	95.70%	10 pmol L^{-1}
$CHBrCl_2$	12.57	83*, 85	88.23%	20 pmol L^{-1}
$CHBr_2Cl$	14.26	129*, 127	91.20%	10 pmol L^{-1}
CH_2BrI	14.50	222*, 141	99.77%	10 pmol L^{-1}
$CHBr_3$	15.70	173*, 175	89.89%	10 pmol L^{-1}
CH_2I_2	16.28	268*, 141	77.00%	10 pmol L^{-1}

RT= retention time; ID = identifying ions; * = quantifying ions

3.4 Statistical analysis

All correlation tests were performed using Pearson Product-Moment correlation analysis on normalized data except the correlation between individual seaweed biomass and seawater chlorophyll-a concentration with the mixing ratios of biogenic halocarbon compounds at Cape Rachado, Port Dickson, which was carried out using the Spearman Rank Order Correlations.

One-way ANOVA was used to test the significance ($p < 0.01$) of irradiance on seaweeds F_v/F_m values followed by post-hoc Tukey's test. All correlation analyses were tested to a significance level of $p < 0.05$. These statistical analyses were done using the Statistica 8.0 software.

The Principle Component Analysis (PCA) between the emissions of halocarbon under the different levels of irradiance by the three seaweed species was done with the Canoco 4.5 software.

CHAPTER 4

RESULTS

4.1 Field study

4.1.1 Atmospheric halocarbon

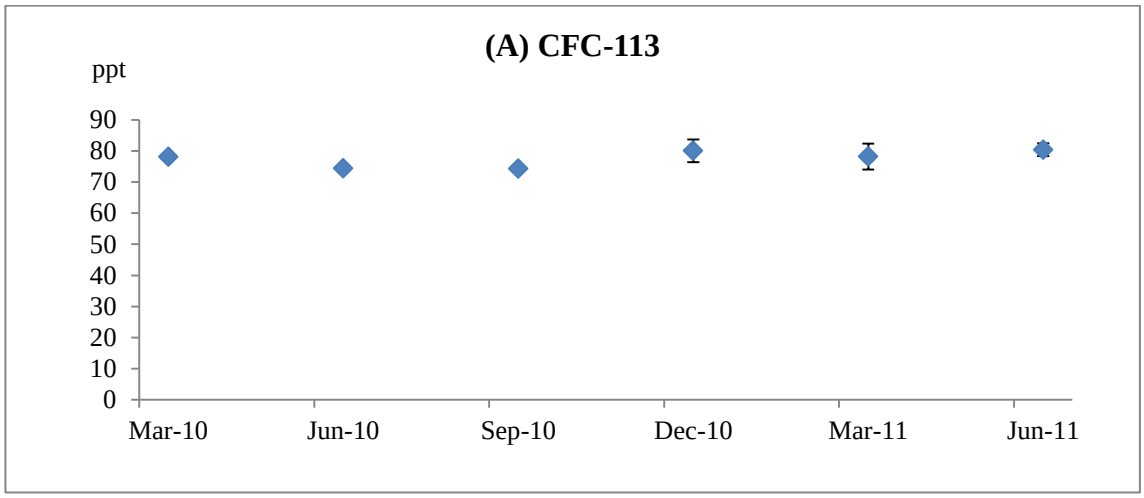
4.1.1.1 Halocarbon compounds measured throughout entire sampling period (March 2010 – June 2011)

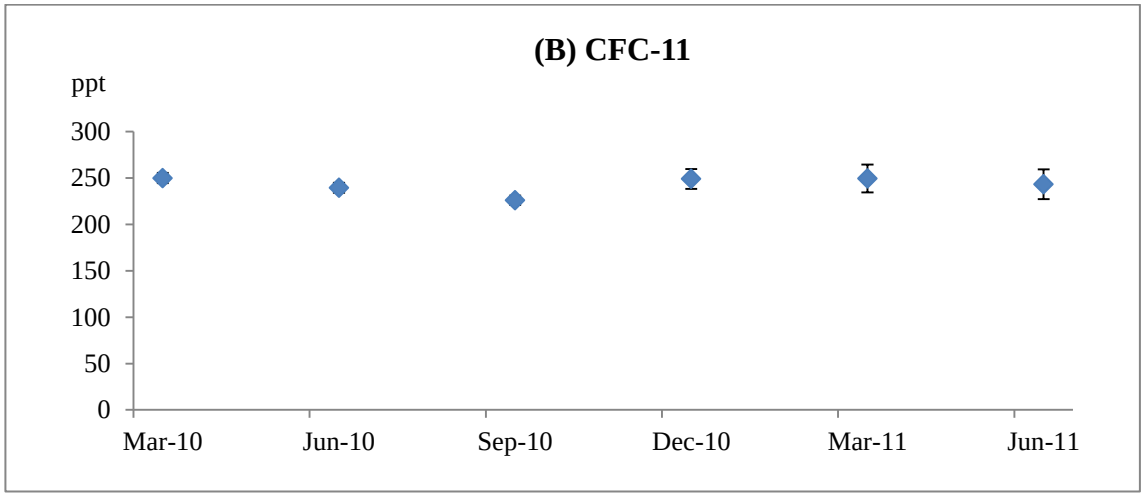
The atmospheric mixing ratios for the long-lived halocarbon compounds namely CFC-113, CFC-11, CCl_4 and CH_3CCl_3 throughout the entire sampling period from March 2010 until June 2011 showed considerably constant values [Fig. 4.1(A) – (D)]. The variation between the mixing ratios measurement during every individual sampling trip for each of these four compounds was less than 10 %. The constant values showed rather big contrast compared to the mixing ratios of those shorter-lived compounds which includes the C_2Cl_4, $CHBrCl_2$ + CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$. The short-lived halocarbon compounds showed much bigger variability in their atmospheric mixing ratios, without displaying any obvious trend throughout the entire sampling period. The differences between the halocarbon mixing ratios observed between each sampling trip for a short-lived halocarbon compound was found to reach as high as 81 % for the C_2Cl_4 peak. Among the short-lived halocarbon compounds, $CHBr_2Cl$ showed the lowest variability between the observed measurements throughout the whole sampling period, which was around 49.14 %. C_2Cl_4 and $CHBr_3$ readings throughout the whole sampling period deviated as much as 81.12 % and 50.98 %.

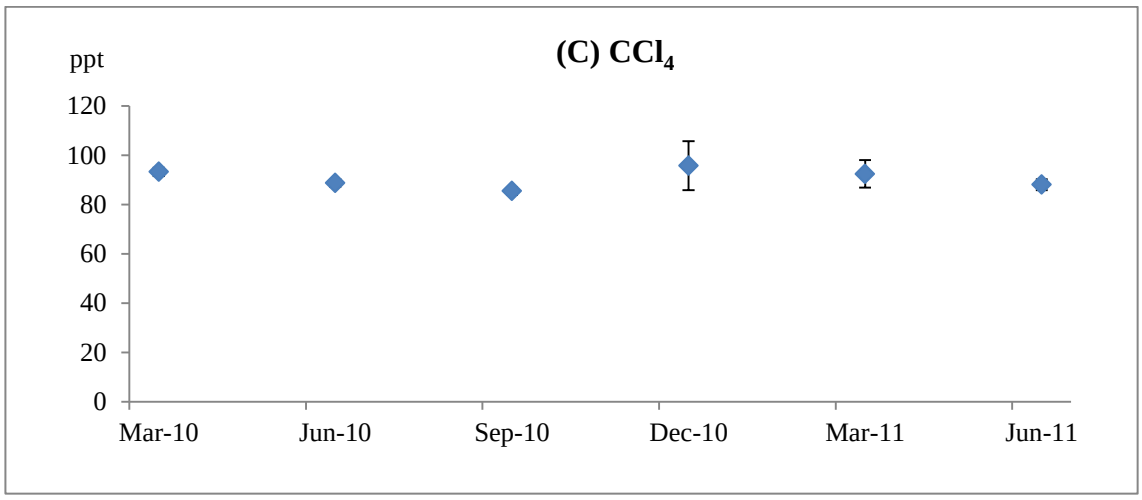
The highest mixing ratio among the halocarbon compounds in the air samples measured at Cape Rachado was that of CFC-11, which has an average value of 243.66 ppt, followed by CCl_4 at a mixing ratio of 90.80 ppt. CFC-113 and CH_3CCl_3 have the averaged atmospheric mixing ratio of 77.62 ppt and 7.89 ppt. On the other hand, lesser

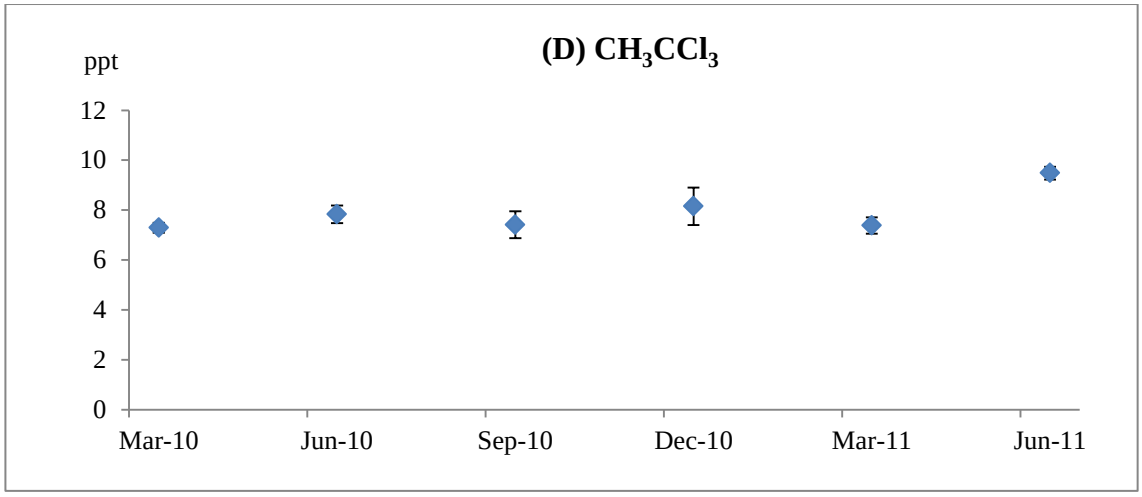
amount of the shorter-lived compounds was detected in the atmospheric air as compared to the long-lived compounds. The average mixing ratio for C_2Cl_4, $CHBrCl_2$ + CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$ was around 5.58, 12.95, 4.78 and 11.92 ppt. The averaged mixing ratio of C_2Cl_4 was the highest among the short-lived compounds while $CHBr_2Cl$ being the lowest found in the air throughout the sampling period.

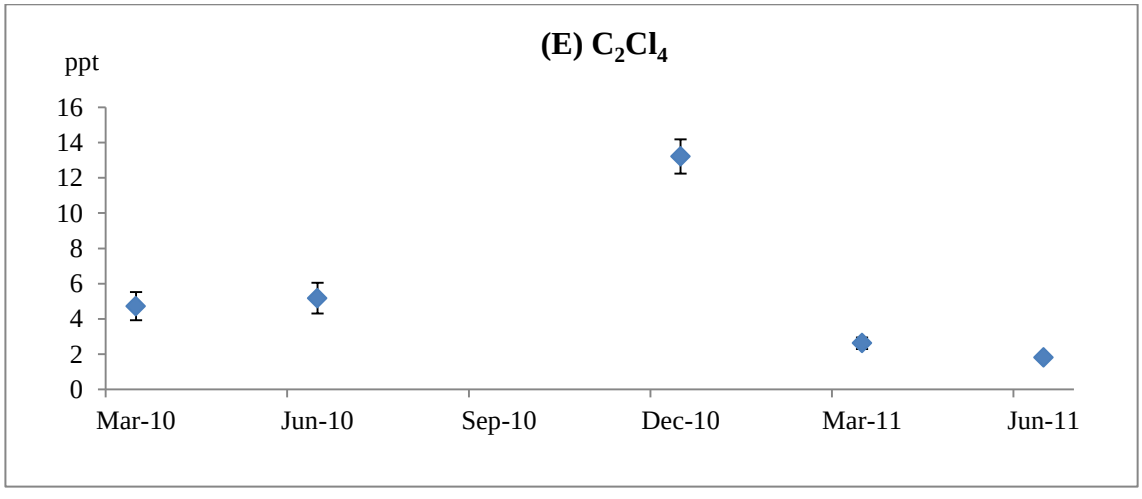
The observed mixing ratios for C_2Cl_4, $CHBrCl_2$ + CH_2Br_2 and $CHBr_2Cl$ achieved a higher value in the December 2010 survey as compared to other time of the survey while the highest mixing ratio for the $CHBr_3$ compound was observed during the September 2010 survey.

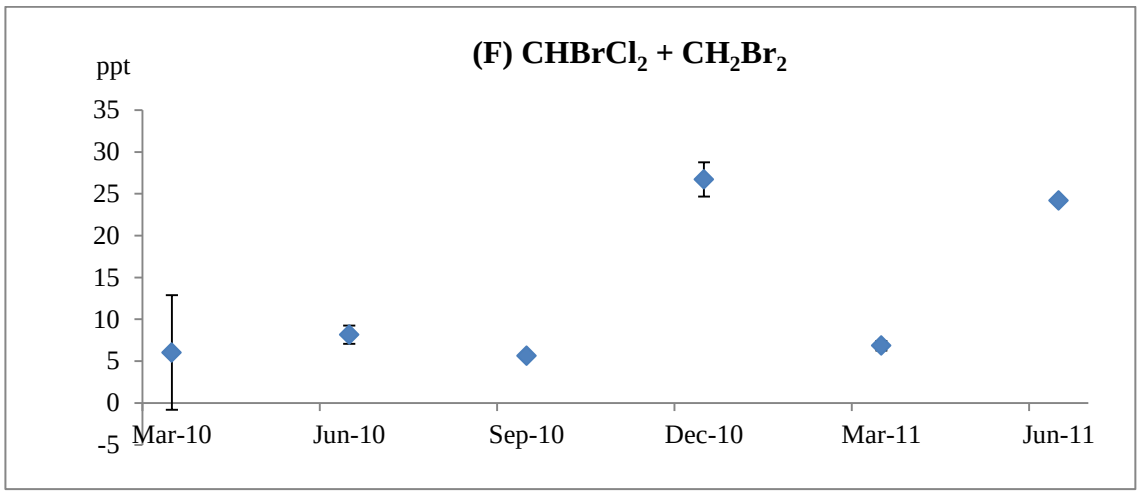


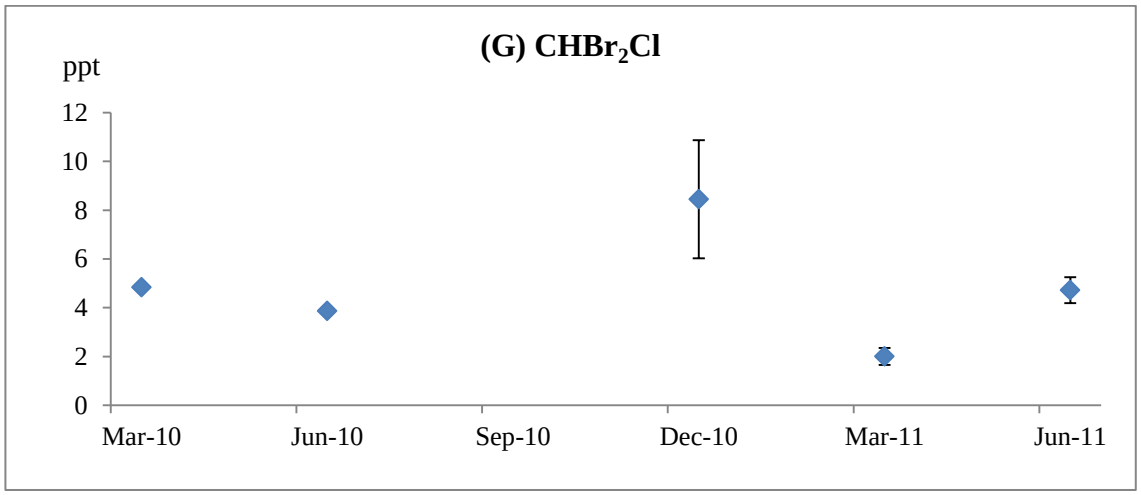



(C) CCl4
ppt
120
100
80
60
40
20
0
Mar-10
Jun-10
Sep-10
Dec-10
Mar-11
Jun-11


(D) CH3CCl3
ppt
12
10
8
6
4
2
0
Mar-10
Jun-10
Sep-10
Dec-10
Mar-11
Jun-11


(E) C2Cl4
ppt
16
14
12
10
8
6
4
2
0
Mar-10
Jun-10
Sep-10
Dec-10
Mar-11
Jun-11





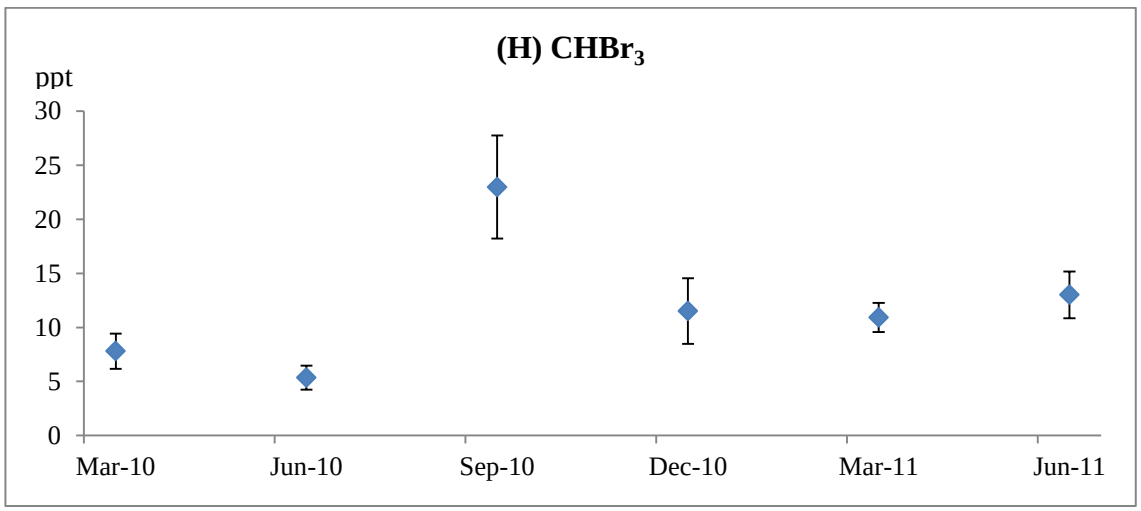


Figure 4.1 (A) – (H): Atmospheric halocarbon mixing ratios for CFC-113, CFC-11, CCl_4, CH_3CCl_3, C_2Cl_4, $CHBrCl_2$ + CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$ at Cape Rachado, Port Dickson throughout the sampling period from March 2010 until June 2011.
Error bar indicates standard deviation of the measurements made during each survey.

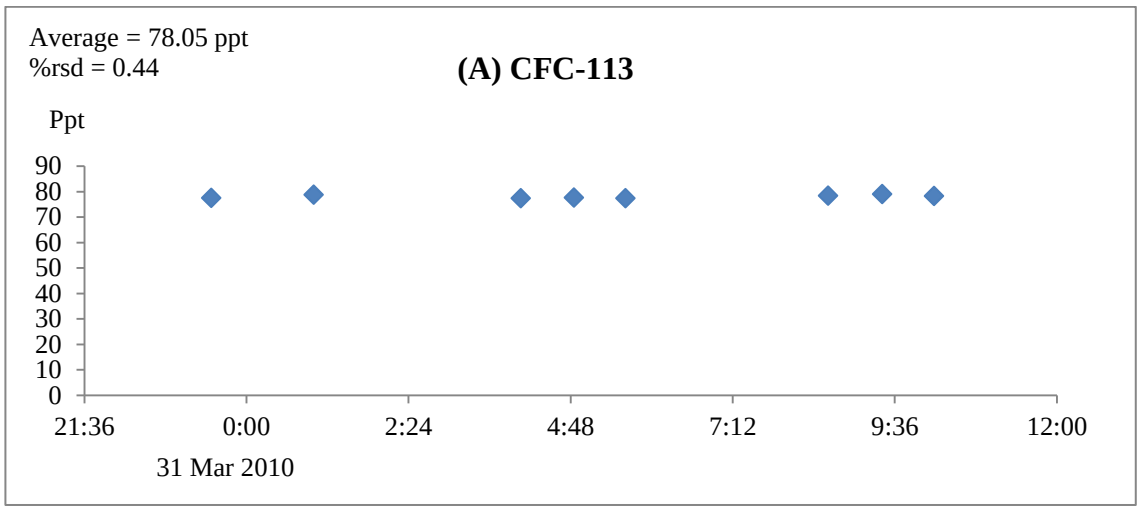
4.1.1.2 Halocarbon compounds measured during each sampling trip

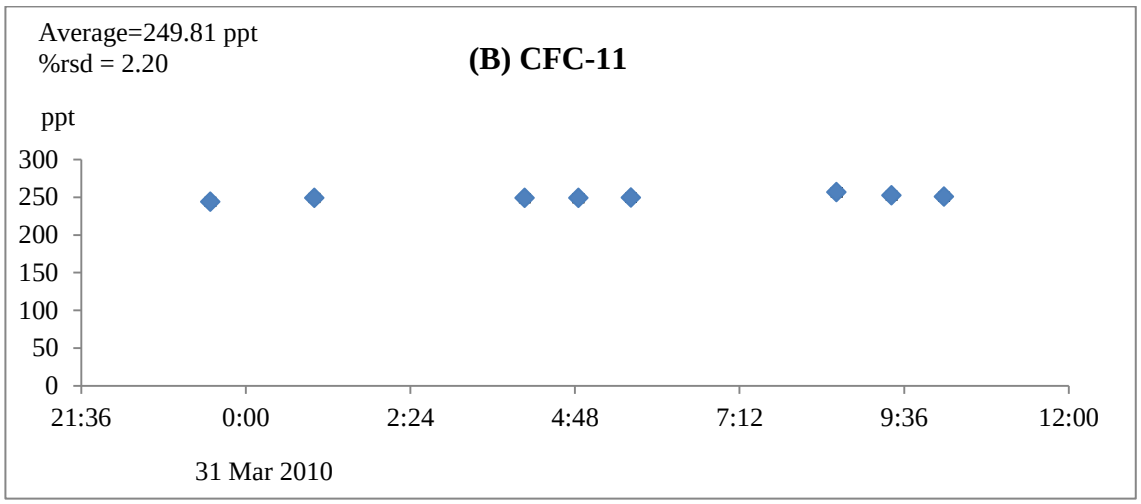
4.1.1.2.1 March 2010

The first site survey started on the 29th to 31st March 2010. As observed, the mixing ratio of the four long-lived halocarbon compounds remained fairly constant with less than 5 % standard deviation between the measured values for each compounds regardless of time of day throughout the days. The averaged mixing ratio for CFC-113, CFC-11, CCl_4, and CH_3CCl_3 was 78.05 ppt, 249.81 ppt, 93.20 ppt, and 7.29 ppt. The mixing ratio of CFC-11 was still the highest among the four long-lived compounds, followed by CCl_4, CFC-113 and CH_3CCl_3.

While long-lived compounds showed rather constant mixing ratios throughout the March 2010 survey, the shorter-lived halocarbon compounds (i.e. C_2Cl_4, $CHBrCl_2$ + CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$) showed much more variations with time as compared to the longer-lived compounds. Also, mixing ratios for C_2Cl_4, $CHBrCl_2$ + CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$ were much lesser than that of CFC-113, CFC-11 and CCl_4. The mixing ratios for the short-lived halocarbon compounds were only comparable to one of the long-lived compound, CH_3CCl_3. The atmospheric mixing ratio for $CHBr_3$ was highest among the short-lived compounds, with an averaged value of 7.32 ppt measured during the March 2010 sampling, followed by CH_2Br_2 + $CHBrCl_2$ at 6.04 ppt, $CHBr_2Cl$ at 5.71 ppt and lastly C_2Cl_4 at 4.72 ppt.

There is no obvious trend in the mixing ratios for the short-lived halocarbon compounds in between the compounds as there were different high-and-low times observed for each of the short-lived compounds [Fig. 4.2 (A) – (H)].


Average = 78.05 ppt
%rsd = 0.44
(A) CFC-113
Ppt
90
80
70
60
50
40
30
20
10
0
21:36
0:00
2:24
4:48
7:12
9:36
12:00
31 Mar 2010


Average=249.81 ppt
%rsd = 2.20
(B) CFC-11
ppt
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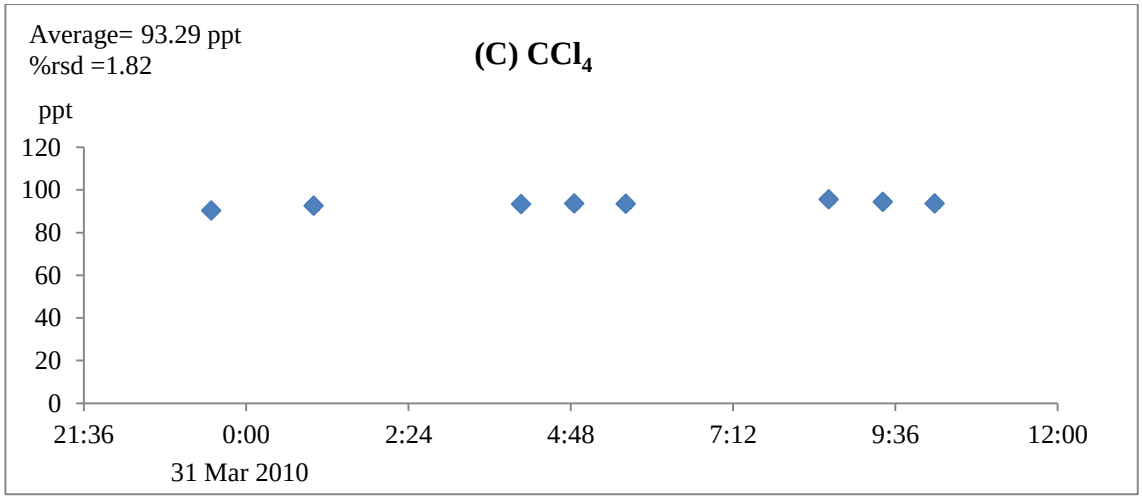
250
200
150
100
50
0
21:36
0:00
2:24
4:48
7:12
9:36
12:00
31 Mar 2010


Average= 93.29 ppt
%rsd =1.82
(C) CCl4
ppt
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100
80
60
40
20
0
21:36
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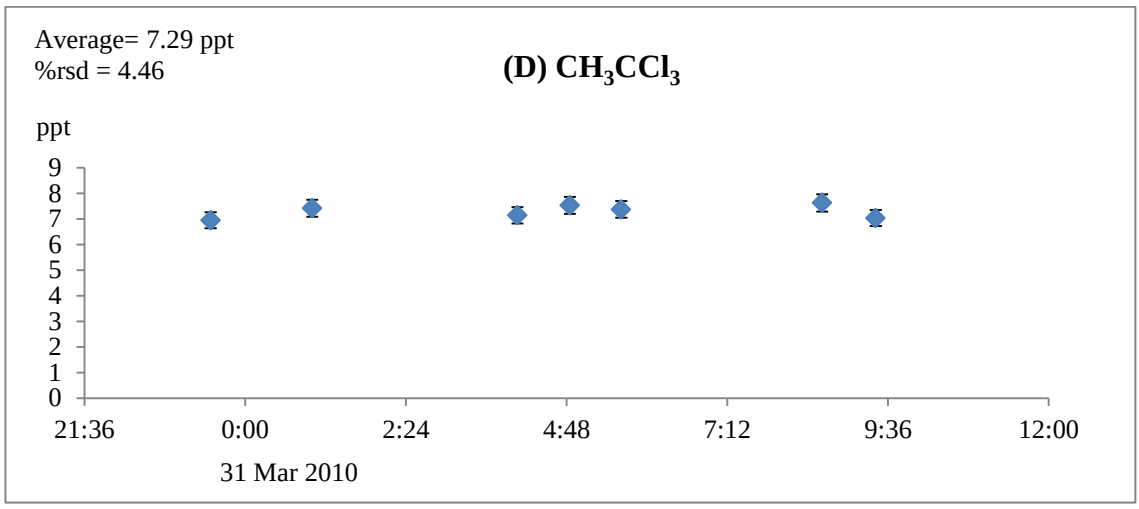
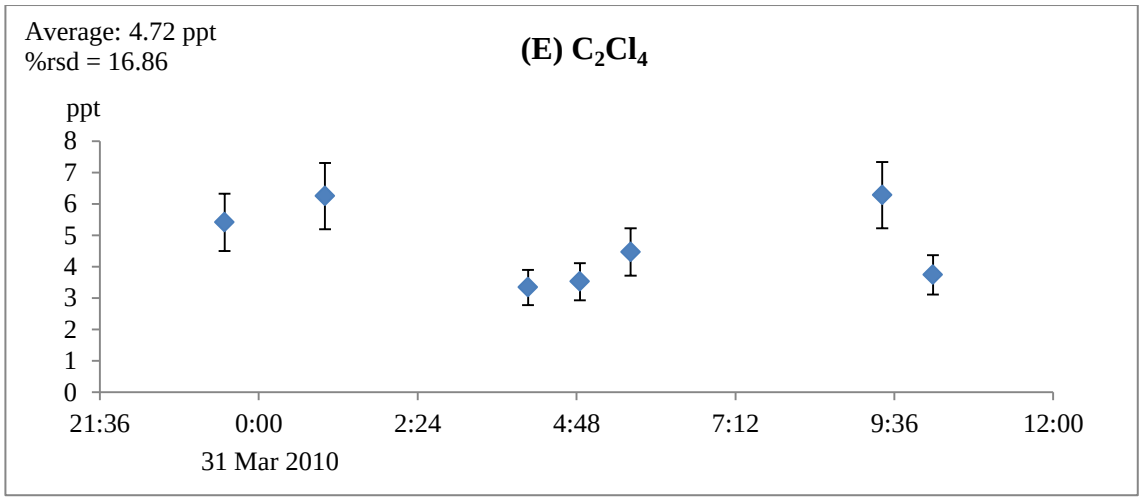
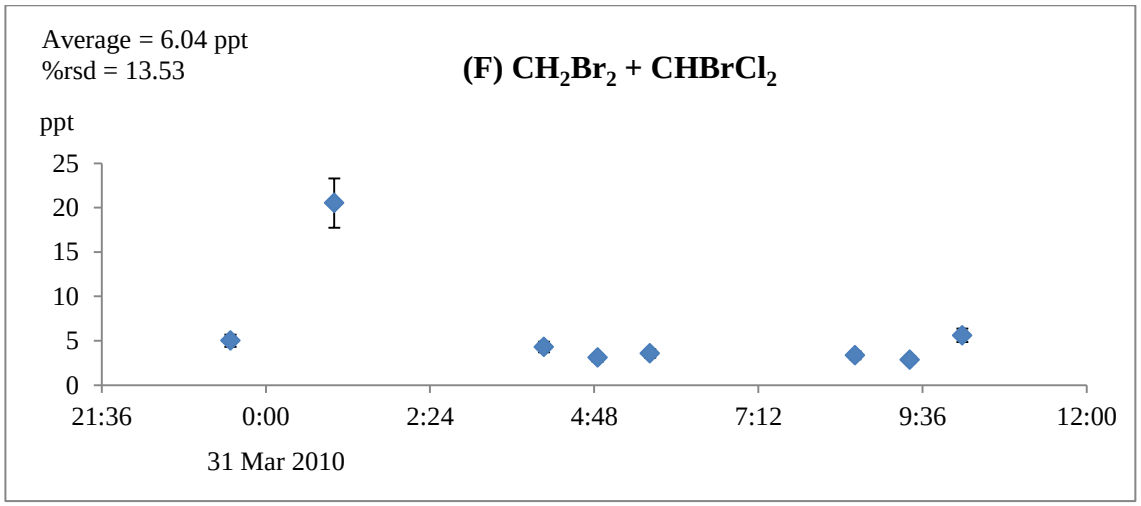
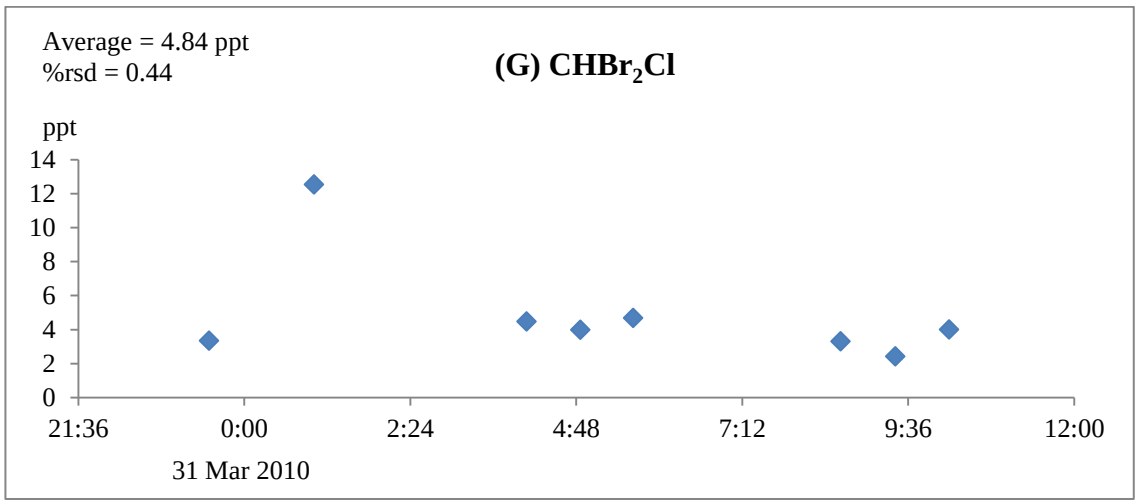
2:24
4:48
7:12
9:36
12:00
31 Mar 2010


Average= 7.29 ppt
%rsd = 4.46
(D) CH3CCl3
ppt
9
8
7
6
5
4
3
2
1
0
21:36
0:00
2:24
4:48
7:12
9:36
12:00
31 Mar 2010


Average: 4.72 ppt
%rsd = 16.86
(E) C2Cl4
ppt
8
7
6
5
4
3
2
1
0
21:36
0:00
2:24
4:48
7:12
9:36
12:00
31 Mar 2010


Average = 6.04 ppt
%rsd = 13.53
(F) CH2Br2 + CHBrCl2
ppt
25
20
15
10
5
0
21:36
0:00
2:24
4:48
7:12
9:36
12:00
31 Mar 2010



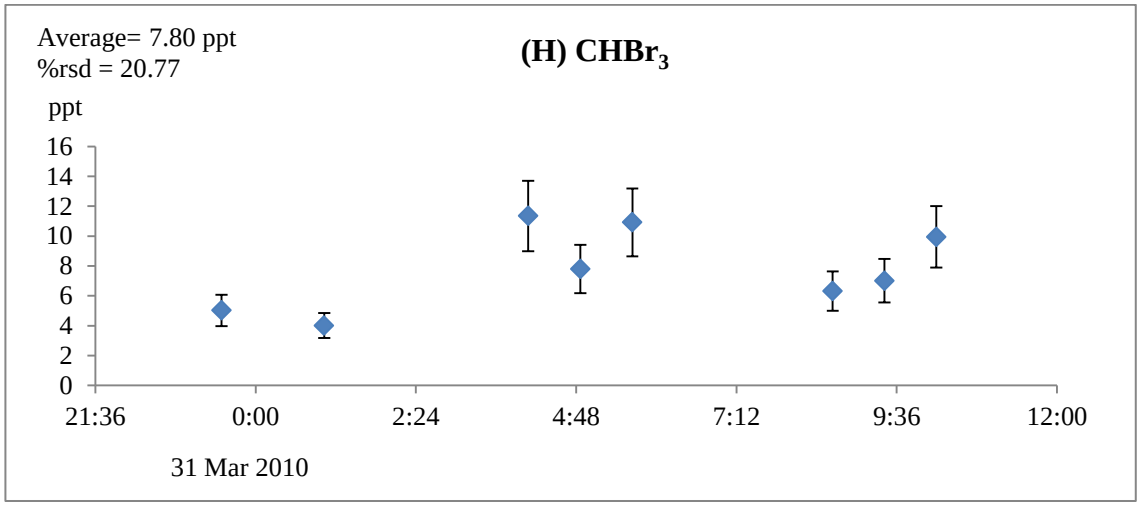


Figure 4.2 (A) – (H): Atmospheric halocarbon mixing ratios for CFC-113, CFC-11, CCl_4, CH_3CCl_3, C_2Cl_4, $CHBrCl_2$ + CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$ at Cape Rachado, Port Dickson during the March 2010 sampling. Error bar indicates standard error of the measurement made relative to the calibration standards.

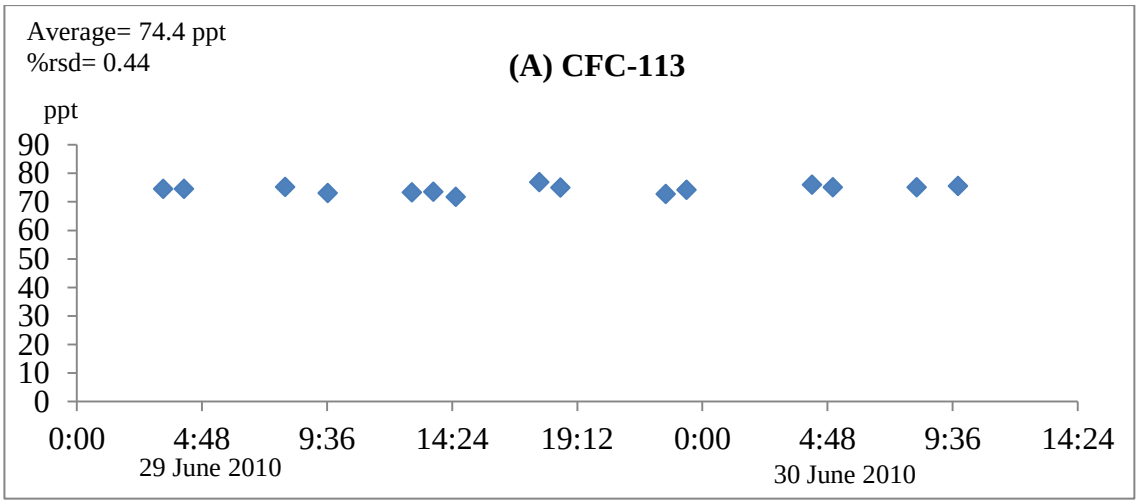
4.1.1.2.2 June 2010

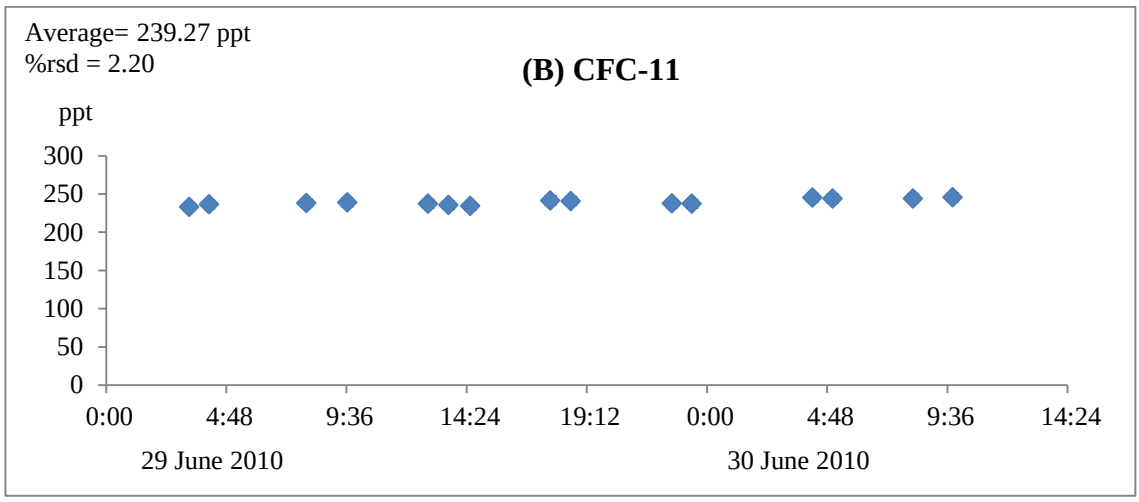
The second site survey was carried out from 28th to 30th June 2010. The total number of samples being picked up this time was more than that of the March 2010 sampling.

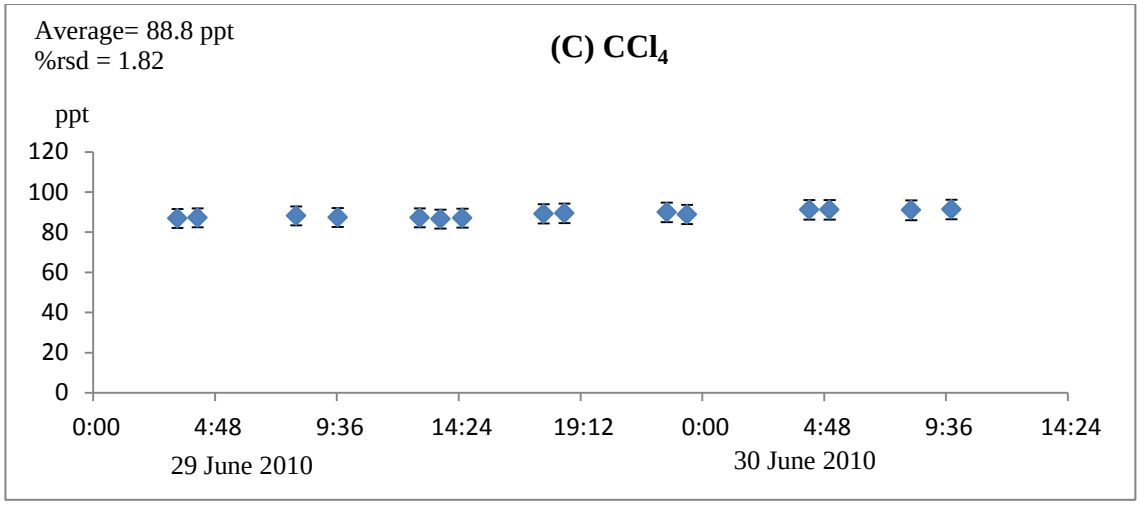
The measured mixing ratios for the long-lived halocarbons stayed quite close to the values observed during the March 2010 survey with the mixing ratio for CFC-113, CFC-11, CCl_4 and CH_3CCl_3 being fairly constant at an average value of 74.4 ppt, 239.24 ppt, 88.8 ppt and 7.83 ppt. The percentage of standard deviation to the average

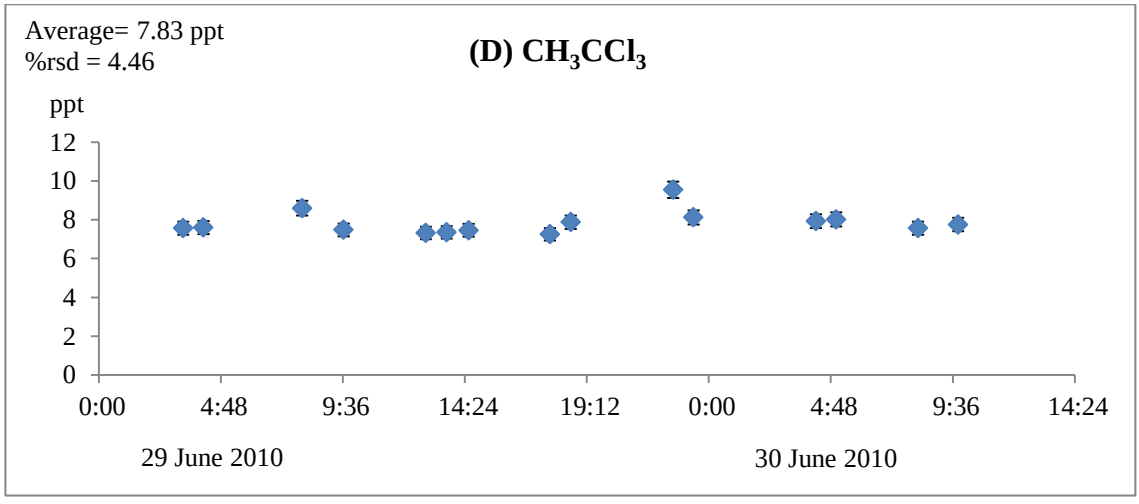
mixing ratio for CFC-113, CFC-11 and CCl_4 throughout the survey was less than 2%, while CH_3CCl_3 having a slightly higher standard deviation percentage of less than 10% between its measured values compared to the average value.

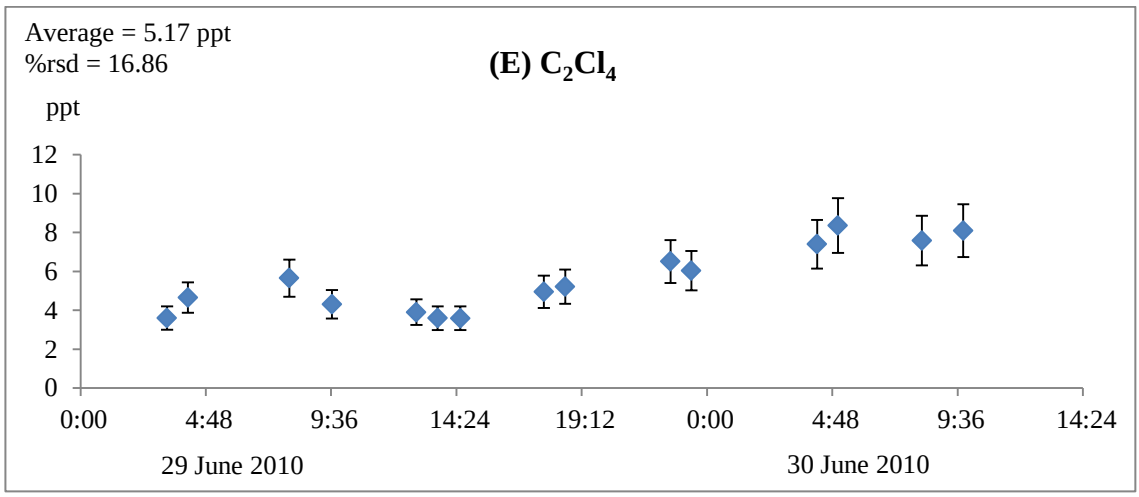
There was some similarity between the trend of mixing ratios of $CHBr_2Cl$ and $CHBr_3$. The measured mixing ratios seemed to increased past midnight 29th June 2010, reached a peak in the morning, and went down in the afternoon until midnight 30th June 2010, after which saw a slight increase in the mixing ratios for both compounds. The average mixing ratios for the short-lived halocarbon were measured to be around 5.17 ppt for C_2Cl_4, 8.l6 ppt for CH_2Br_2 + $CHBrCl_2$, 3.87 ppt for $CHBr_2Cl$ and 5.34 ppt for $CHBr_3$. The short-lived halocarbon having the highest mixing ratio for June 2010 was the double peak compound CH_2Br_2 + $CHBrCl_2$, followed by $CHBr_3$, C_2Cl_4 and $CHBr_2Cl$ [Fig. 4.3(A) – (H)].

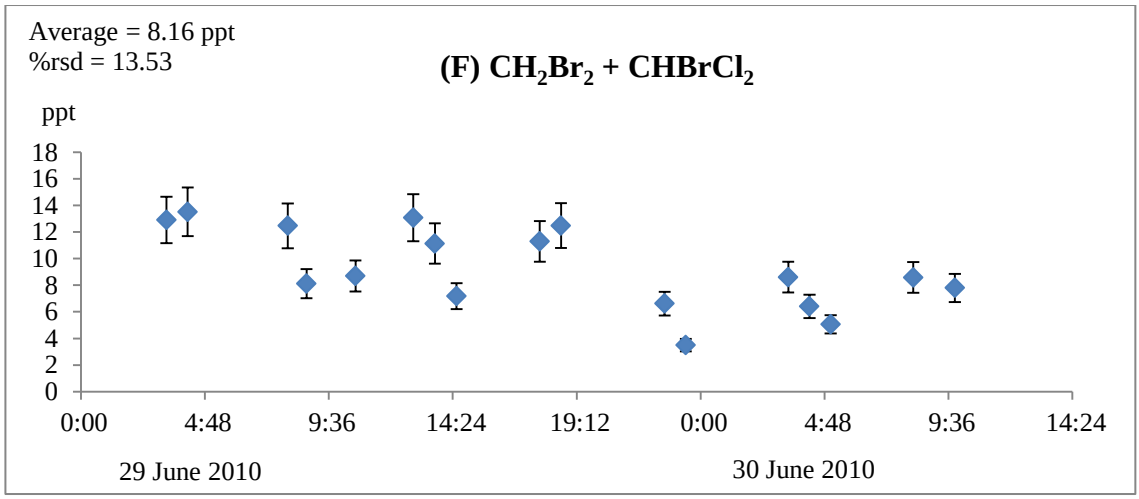


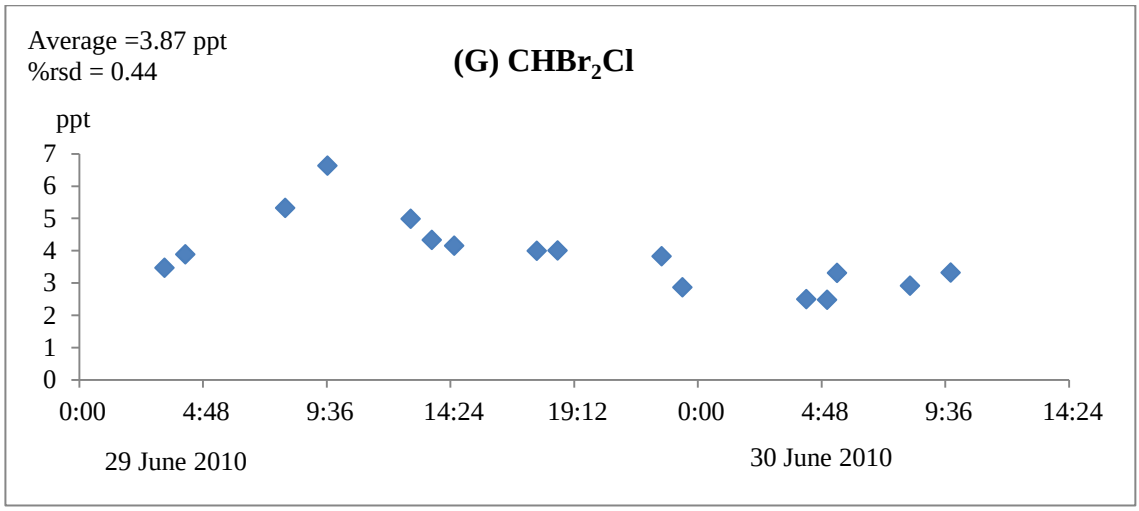

Average= 239.27 ppt
%rsd = 2.20
(B) CFC-11
ppt
300
250
200
150
100
50
0
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
14:24
29 June 2010
30 June 2010


Average= 88.8 ppt
%rsd = 1.82
(C) CCl4
ppt
120
100
80
60
40
20
0
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
14:24
29 June 2010
30 June 2010


Average= 7.83 ppt
%rsd = 4.46
(D) CH3CCl3
ppt
12
10
8
6
4
2
0
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
14:24
29 June 2010
30 June 2010


Average = 5.17 ppt
%rsd = 16.86
(E) C2Cl4
ppt
12
10
8
6
4
2
0
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
14:24
29 June 2010
30 June 2010


Average = 8.16 ppt
%rsd = 13.53
(F) CH2Br2 + CHBrCl2
ppt
18
16
14
12
10
8
6
4
2
0
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
14:24
29 June 2010
30 June 2010


Average =3.87 ppt
%rsd = 0.44
(G) CHBr2Cl
ppt
7
6
5
4
3
2
1
0
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
14:24
29 June 2010
30 June 2010

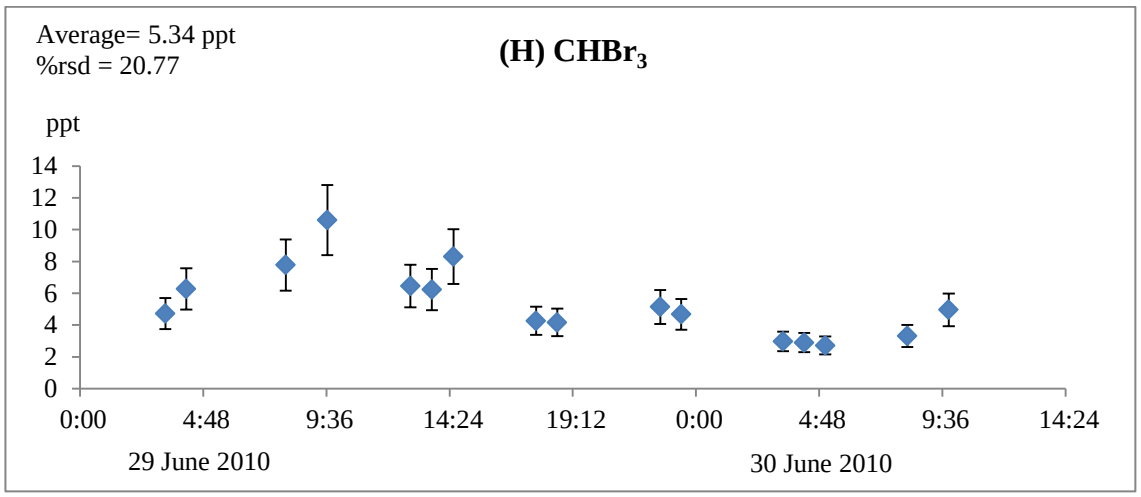
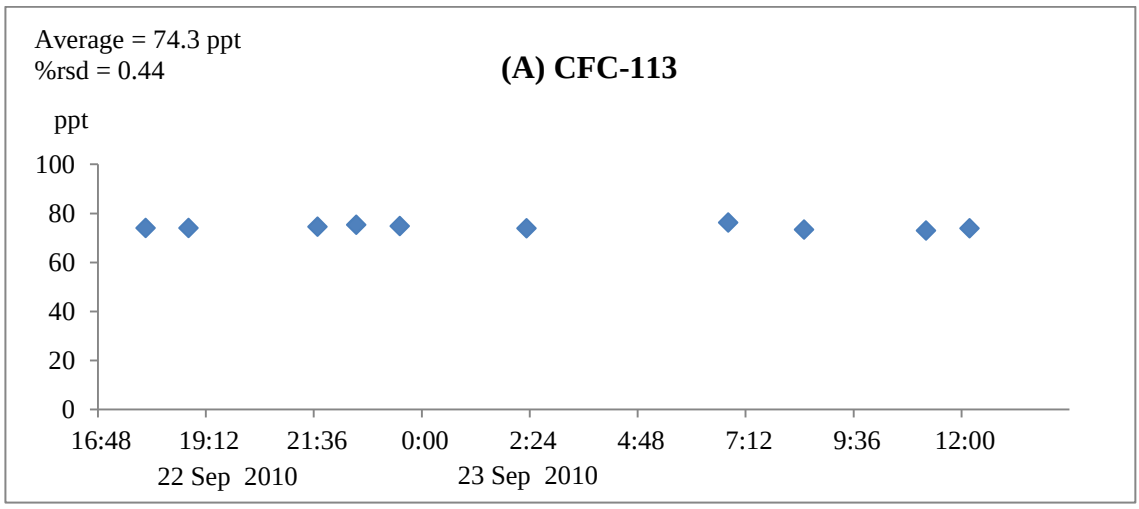


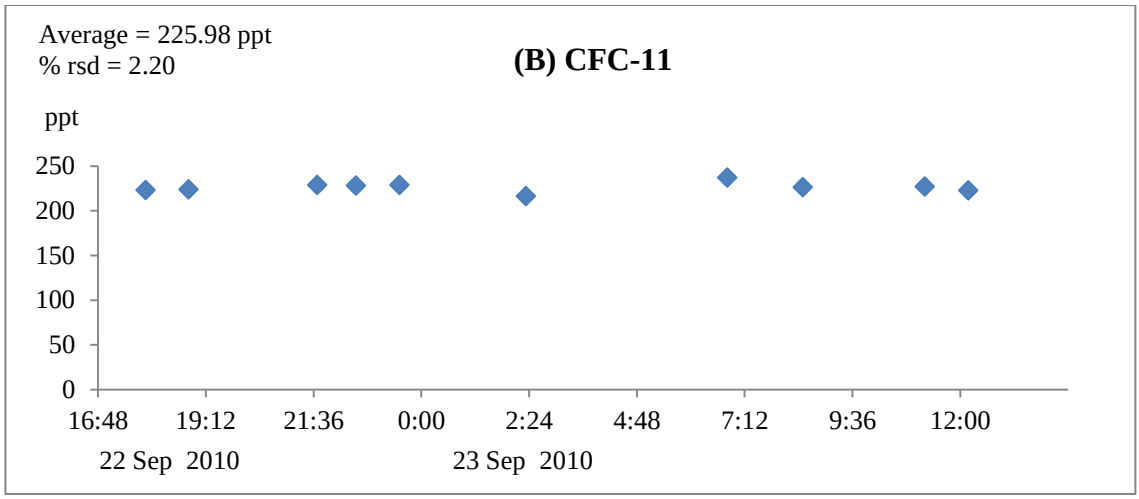
Figure 4.3 (A) – (H): Atmospheric halocarbon mixing ratios for CFC-113, CFC-11, CCl_4, CH_3CCl_3, C_2Cl_4, $CHBrCl_2$ + CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$ at Cape Rachado, Port Dickson during the June 2010 sampling. Error bar indicates standard error of the measurement made relative to the calibration standards.

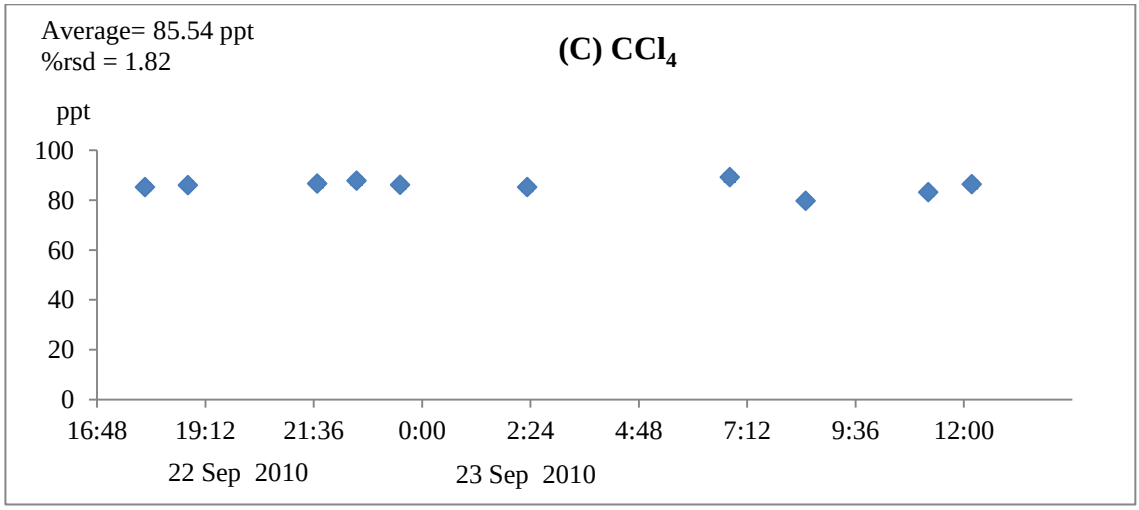
4.1.1.2.3 September 2010

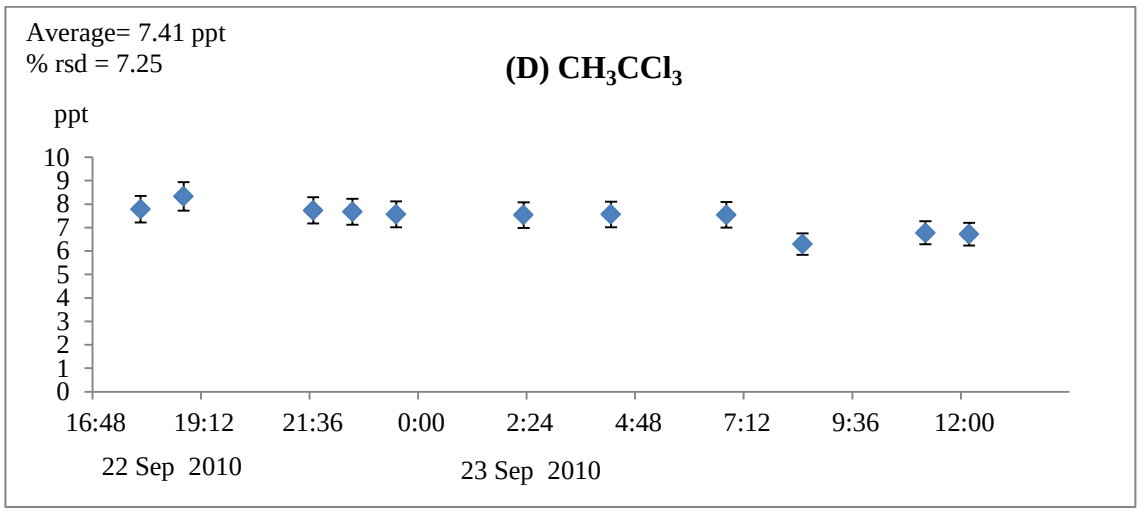
The third site survey was carried out between 21st and 23rd September 2010. As observed in the two previous surveys, the mixing ratios for the long-lived halocarbon compounds were fairly constant throughout the days. The average mixing ratio for CFC-113, CFC-11, CCl_4 and CH_3CCl_3 was 74.3 ppt, 225.98 ppt, 85.54 ppt and 7.41 ppt.

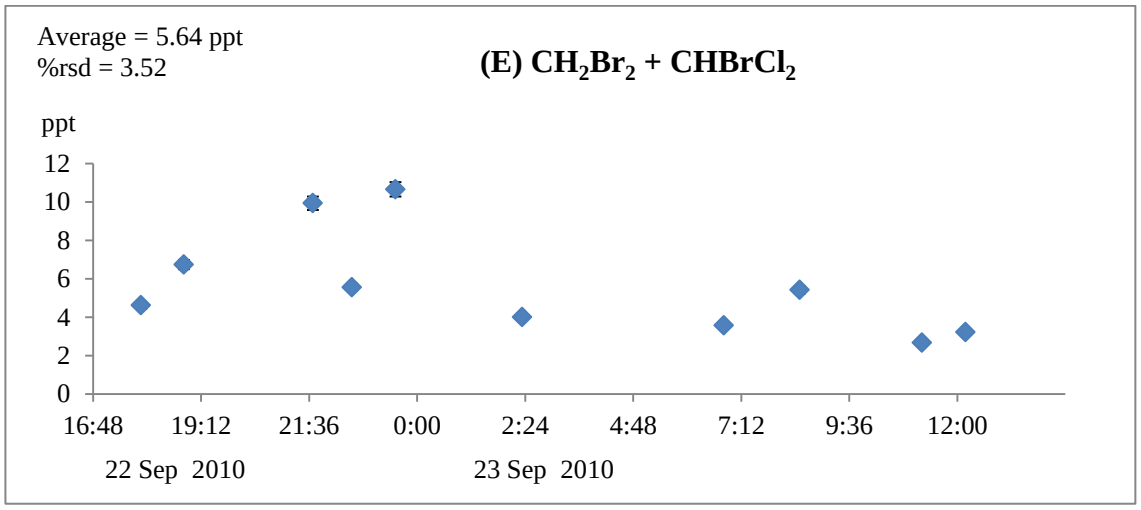
There are only two compounds of the short-lived halocarbons recorded during this survey as the peaks for the C_2Cl_4 and $CHBr_2Cl$ were contaminated by their nearby peaks in the chromatogram which made their peaks near impossible to integrate. The average mixing ratio for CH_2B_{r2} + $CHBrCl_2$ was 5.64 ppt and $CHBr_3$ at 22.97 ppt, which was much highest than what had been observed during the two previous survey [Figure 4.4 (A) – (H)].


Average = 74.3 ppt
%rsd = 0.44
(A) CFC-113
ppt
100
80
60
40
20
0
16:48
19:12
21:36
0:00
2:24
4:48
7:12
9:36
12:00
22 Sep 2010
23 Sep 2010


Average = 225.98 ppt
% rsd = 2.20
(B) CFC-11
ppt
250
200
150
100
50
0
16:48
19:12
21:36
0:00
2:24
4:48
7:12
9:36
12:00
22 Sep 2010
23 Sep 2010


Average= 85.54 ppt
%rsd = 1.82
(C) CCl4
ppt
100
80
60
40
20
0
16:48
19:12
21:36
0:00
2:24
4:48
7:12
9:36
12:00
22 Sep 2010
23 Sep 2010





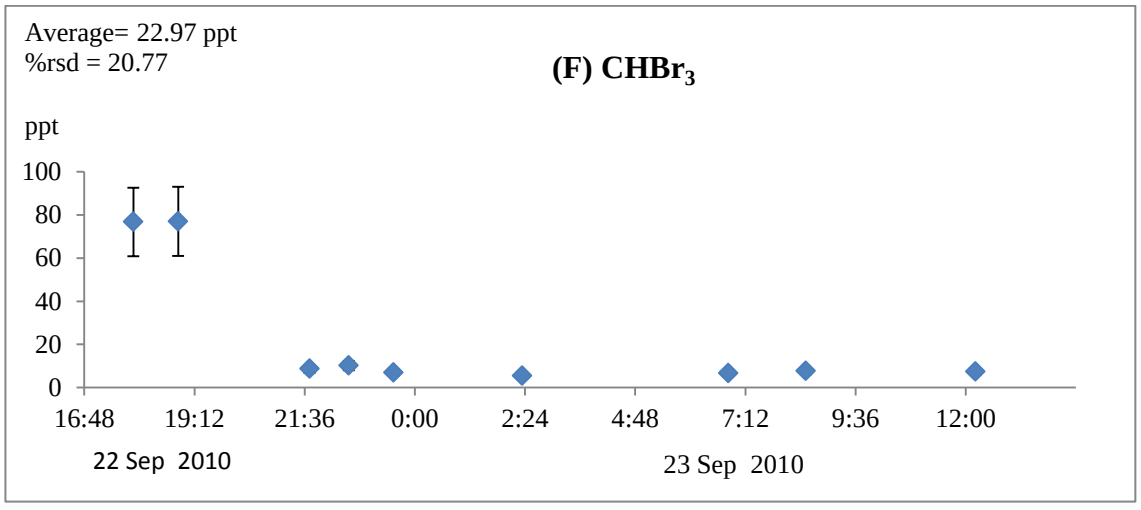
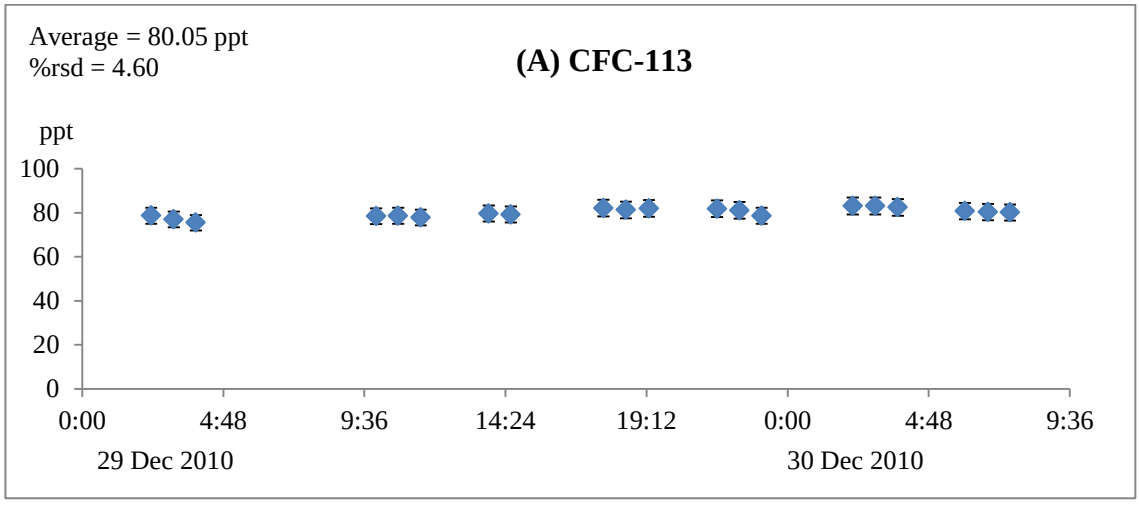


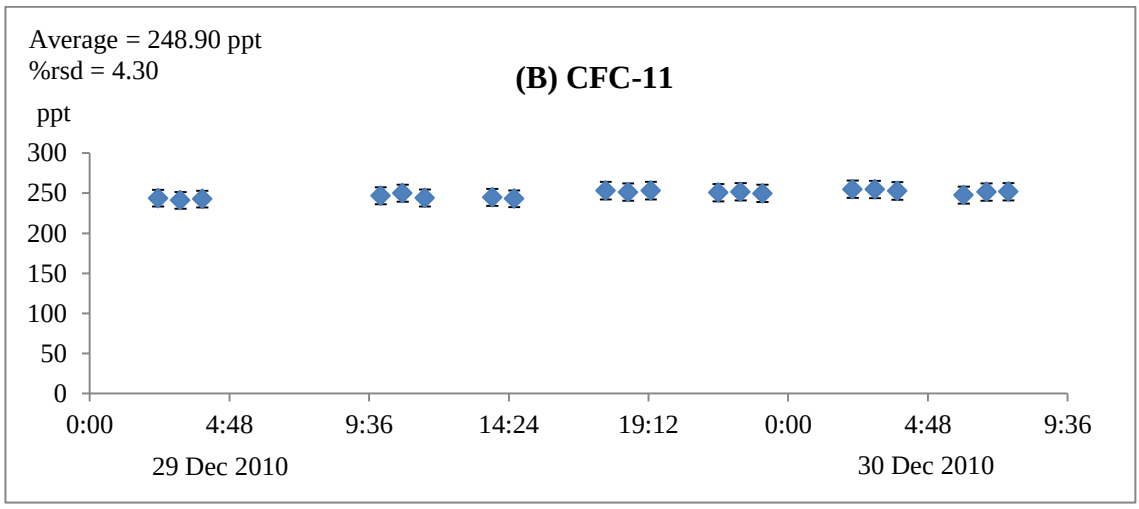
Figure 4.4 (A) – (H): Atmospheric halocarbon mixing ratios for CFC-113, CFC-11, CCl_4, CH_3CCl_3, $CHBrCl_2$ + CH_2Br_2 and $CHBr_3$ at Cape Rachado, Port Dickson during the September 2010 sampling. Error bar indicates standard error of the measurement made relative to the calibration standards.

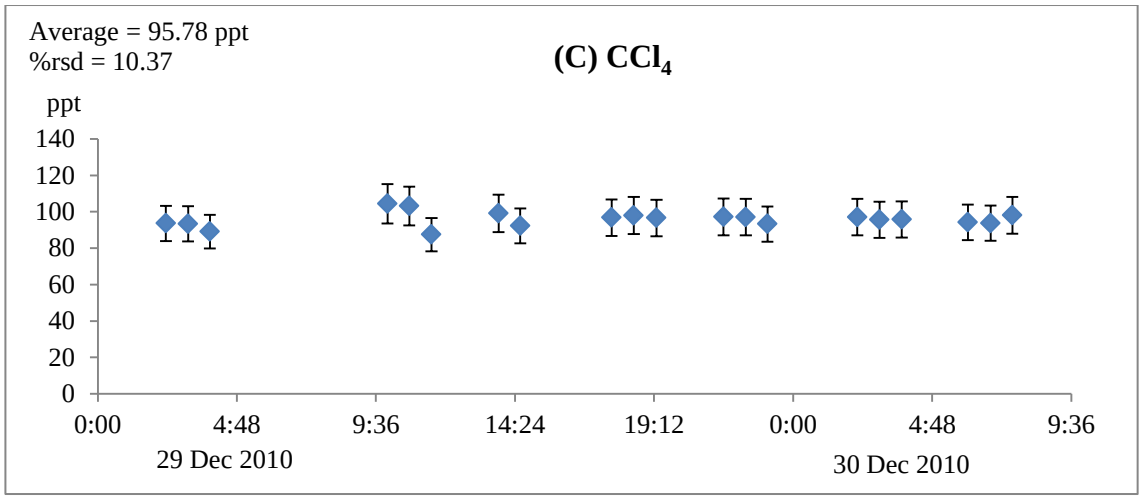
4.1.1.2.4 December 2010

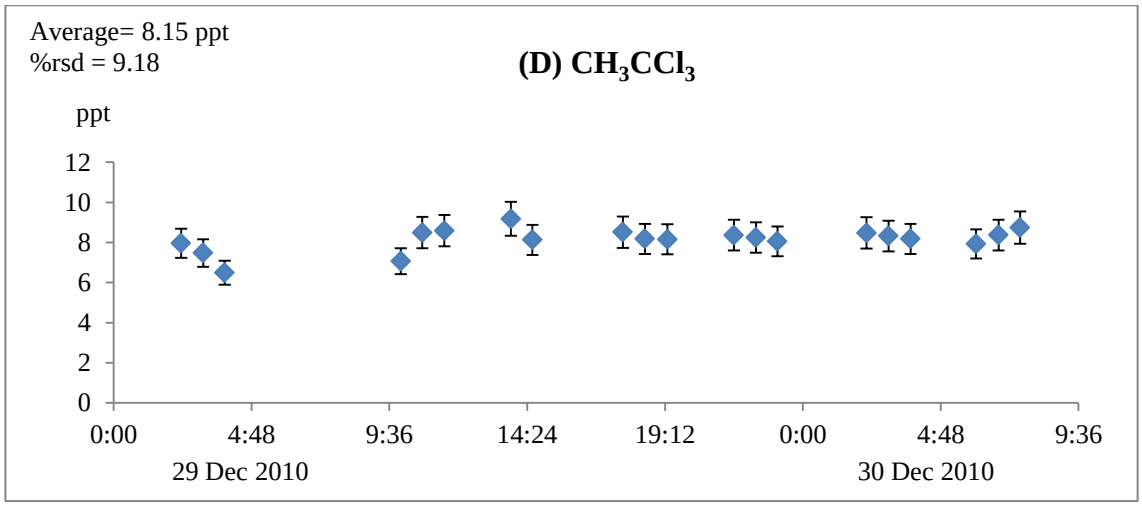
The fourth site survey was carried out from 28th to 30th December 2010. As usual, the long-lived halocarbon compounds displayed almost constant trend throughout the days of survey with little variation between the measurements. The average mixing ratios for CFC-113, CFC-11, CCl_4 and CH_3CCl_3 was 80.05 ppt, 248.90 ppt, 95.78 ppt and 8.15 ppt. There was much bigger variation between the measured mixing ratios throughout the days for both CC_4 and CH_3CCl_3 than compared to the CFCs.

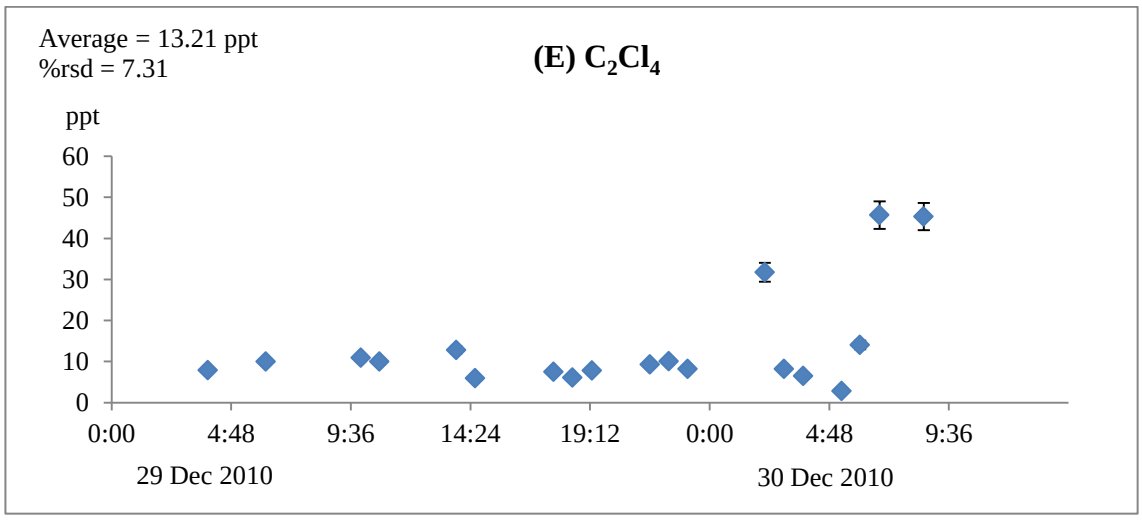
Almost similar trend was observed for the mixing ratios of $CHBrCl_2$ + CH_2Br_2 and $CHBr_2Cl$. However, the effect of the time of day on the measured mixing ratios of these compounds was less obvious. The average mixing ratios for the short-lived halocarbon compounds was 13.21 ppt for C_2Cl_4, 26.71 ppt for $CHBrCl_2$ + CH2Br_2, 8.45 ppt for $CHBr_2Cl$ and 11.51 ppt for $CHBr_3$. The mixing ratios for all of the short-lived compounds apart from $CHBr_3$ showed higher values during the December 2010 sampling as compared to any other sampling period [Figure 4.5(A) – (H)].

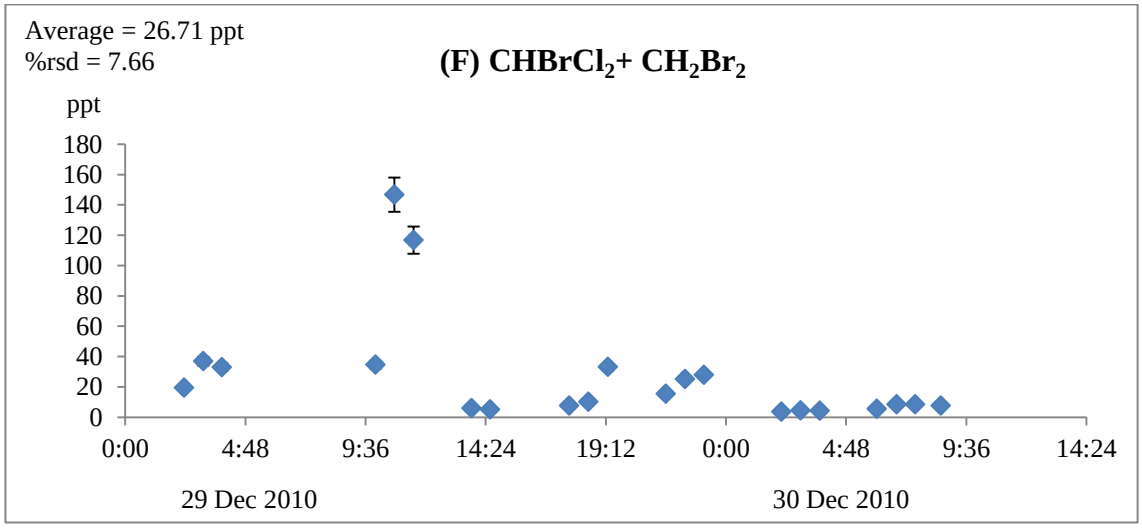


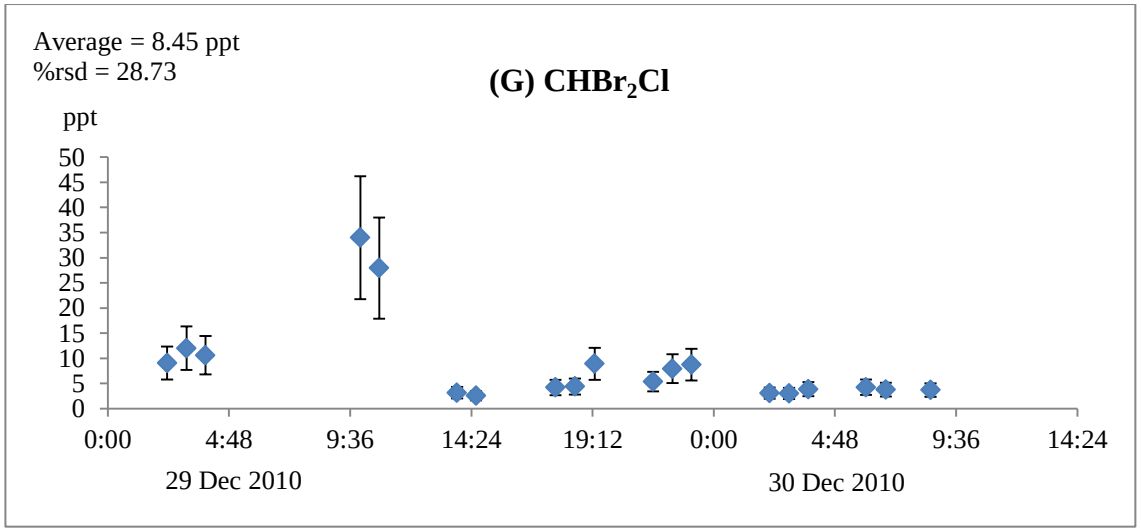

Average = 248.90 ppt
%rsd = 4.30
(B) CFC-11
ppt
300
250
200
150
100
50
0
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
29 Dec 2010
30 Dec 2010


Average = 95.78 ppt
%rsd = 10.37
(C) CCl4
ppt
140
120
100
80
60
40
20
0
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
29 Dec 2010
30 Dec 2010


Average= 8.15 ppt
%rsd = 9.18
(D) CH3CCl3
ppt
12
10
8
6
4
2
0
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
29 Dec 2010
30 Dec 2010


Average = 13.21 ppt
%rsd = 7.31
(E) C_2Cl_4
ppt
60
50
40
30
20
10
0
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
29 Dec 2010
30 Dec 2010


Average = 26.71 ppt
%rsd = 7.66
(F) $CHBrCl_2$+ CH_2Br_2
ppt
180
160
140
120
100
80
60
40
20
0
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
14:24
29 Dec 2010
30 Dec 2010


Average = 8.45 ppt
%rsd = 28.73
(G) $CHBr_2Cl$
ppt
50
45
40
35
30
25
20
15
10
5
0
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
14:24
29 Dec 2010
30 Dec 2010

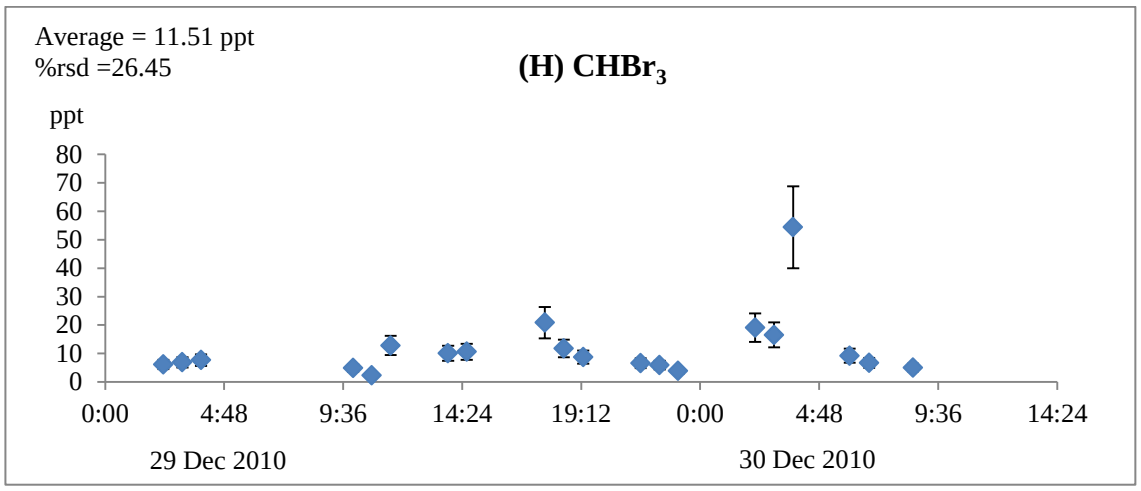


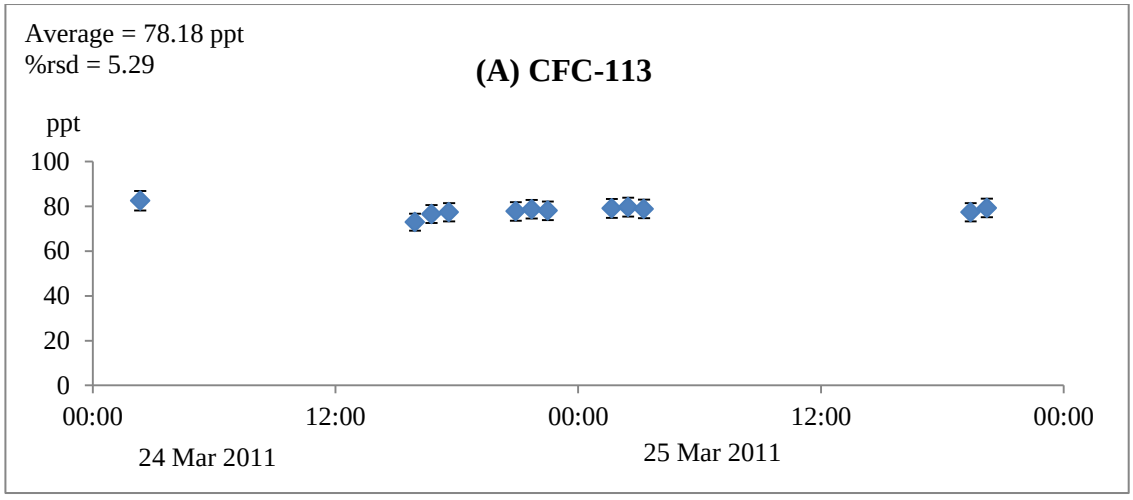
Figure 4.5(A) – (H): Atmospheric halocarbon mixing ratios for CFC-113, CFC-11, CCl_4, CH_3CCl_3, C_2Cl_4, $CHBrCl_2$ + CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$ at Cape Rachado, Port Dickson during the December 2010 sampling.
Error bar indicates standard error of the measurement made relative to the calibration standards.

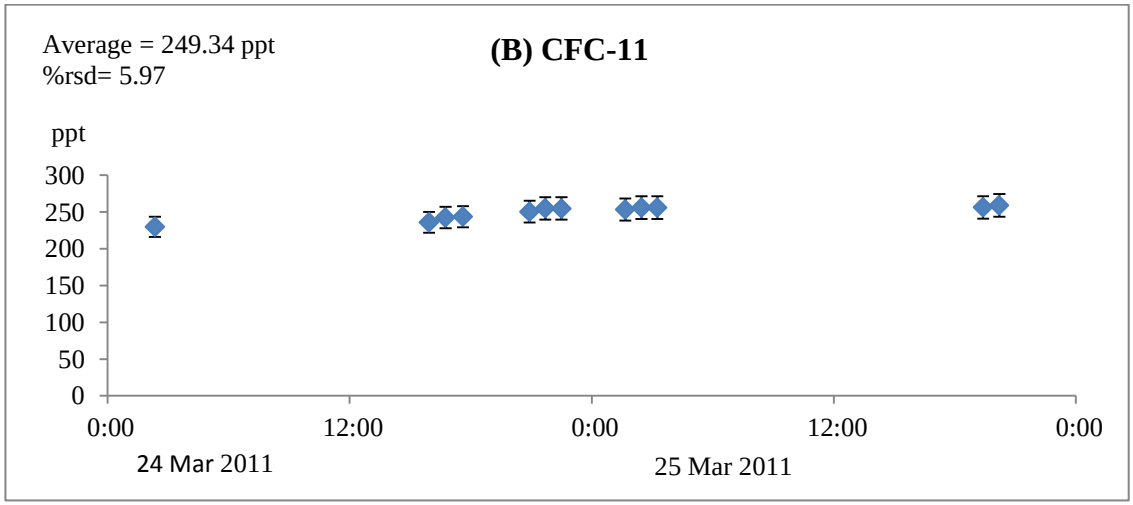
4.1.1.2.5 March 2011

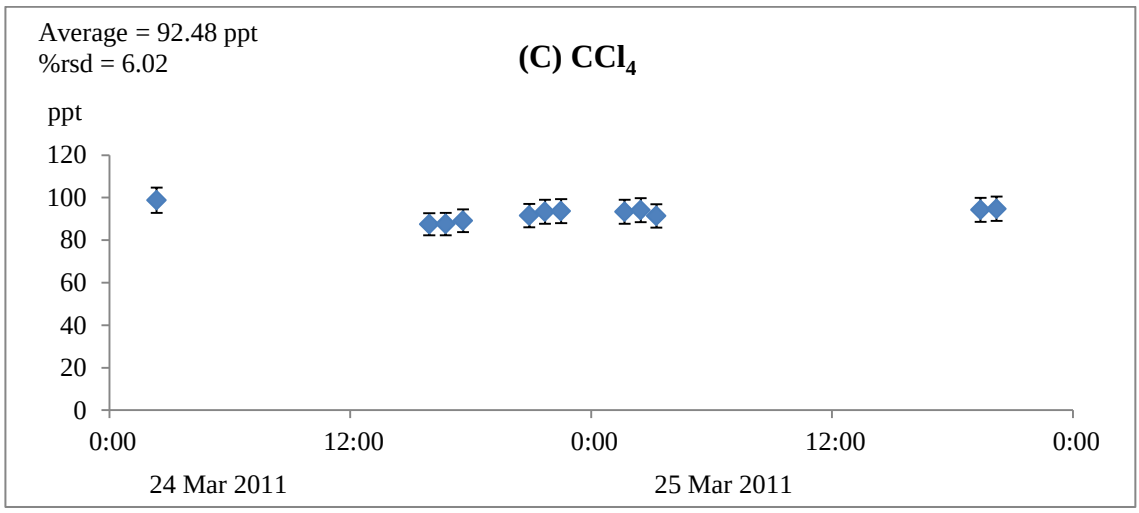
The fifth field survey was carried out from 23 November 2011 to 25 November 2011. The atmospheric mixing ratios for the long-lived halocarbon compounds namely CFC-113, CFC-11, CCl_4 and CH_3CCl_3 throughout the days of measurement showed considerably constant values. The variation between the mixing ratios measurements during every individual sampling trip for each of these four compounds was less than 10 %. This rather constant values showed rather big contrast compared to the mixing ratios of those shorter-lived compounds which includes the C_2Cl_4, $CHBrCl_2$ + CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$. The short-lived halocarbon compounds showed much bigger variability in their atmospheric mixing ratios, without displaying any obvious trend throughout the entire sampling period due to the limited number of measurements taken.

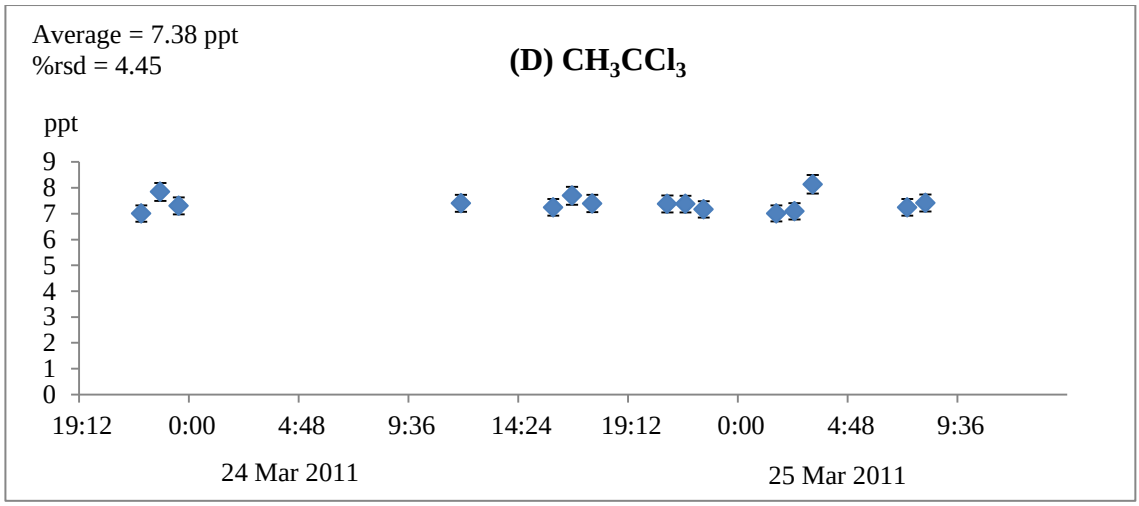
The highest mixing ratio among the halocarbon compounds in the air samples measured at Cape Rachado was that of CFC-11, which has an average value of 249.34 ppt,

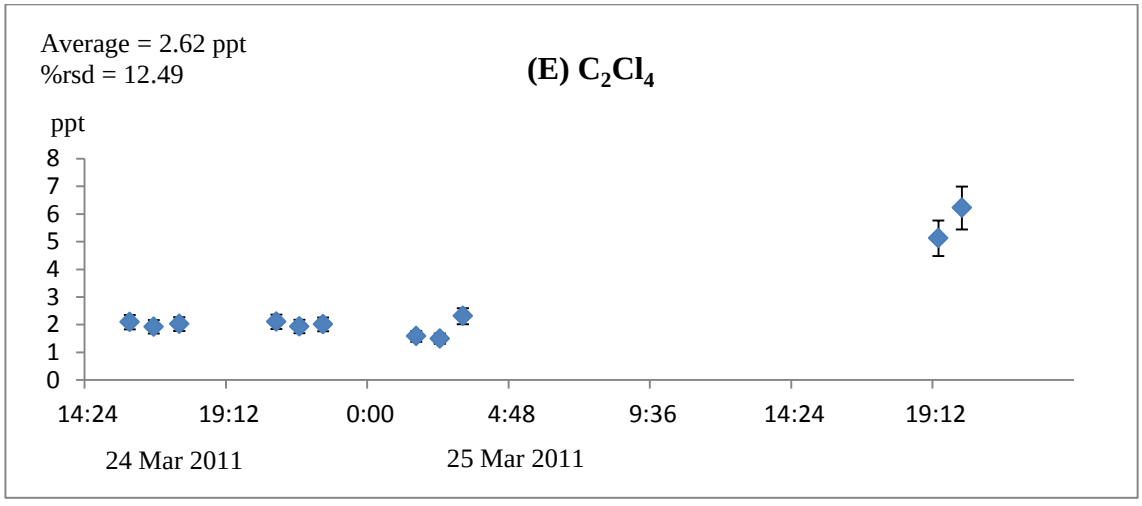
followed by CCl_4 at a mixing ratio of 92.48 ppt. CFC-113 and CH_3CCl_3 have the averaged atmospheric mixing ratio of 78.18 ppt and 7.38 ppt. On the other hand, lesser amount of the shorter-lived compounds was detected in the atmospheric air as compared to the long-lived compounds. The average mixing ratio for C_2Cl_4, $CHBrCl_2$ + CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$ was around 2.62, 6.85, 2.00 and 10.91 ppt, respectively. The averaged mixing ratio of $CHBr_3$ was the highest among the short-lived compounds while $CHBr_2Cl$ being the lowest found in the air throughout the sampling period [Fig. 4.6(A) – (H)].

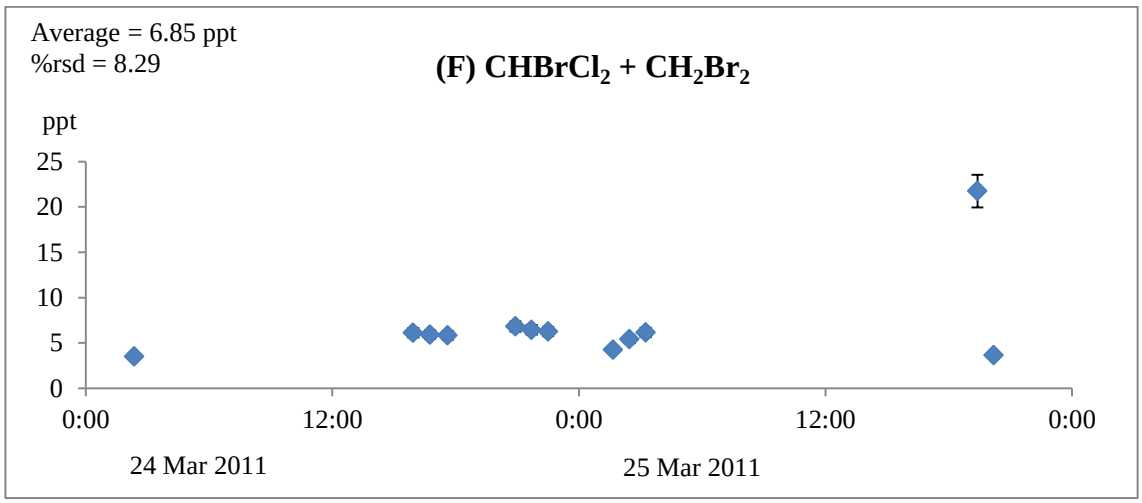


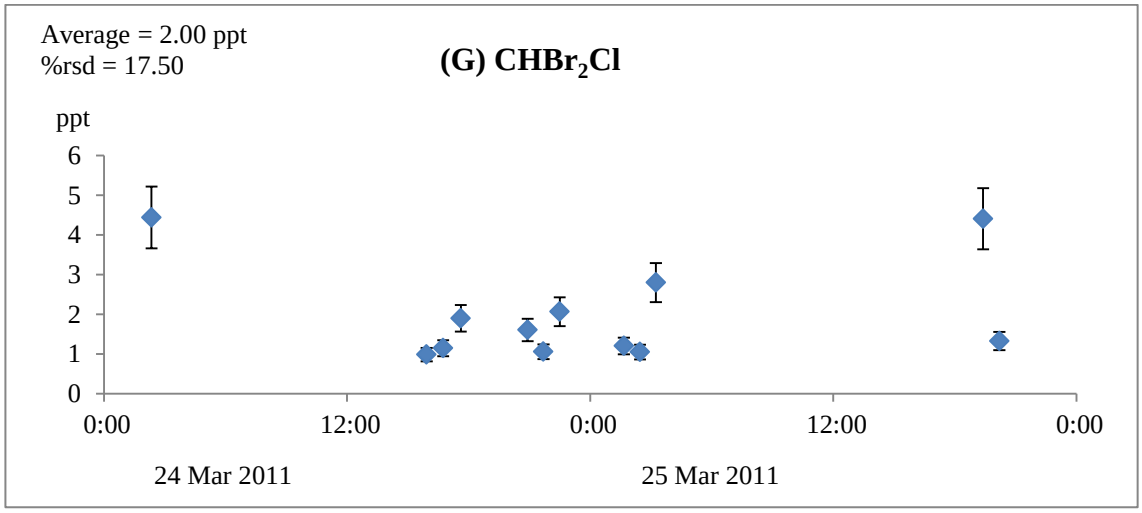



Average = 92.48 ppt
%rsd = 6.02
(C) CCl4
ppt
120
100
80
60
40
20
0
0:00
12:00
0:00
12:00
0:00
24 Mar 2011
25 Mar 2011


Average = 7.38 ppt
%rsd = 4.45
(D) CH3CCl3
ppt
9
8
7
6
5
4
3
2
1
0
19:12
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
24 Mar 2011
25 Mar 2011


Average = 2.62 ppt
%rsd = 12.49
(E) C2Cl4
ppt
8
7
6
5
4
3
2
1
0
14:24
19:12
0:00
4:48
9:36
14:24
19:12
24 Mar 2011
25 Mar 2011





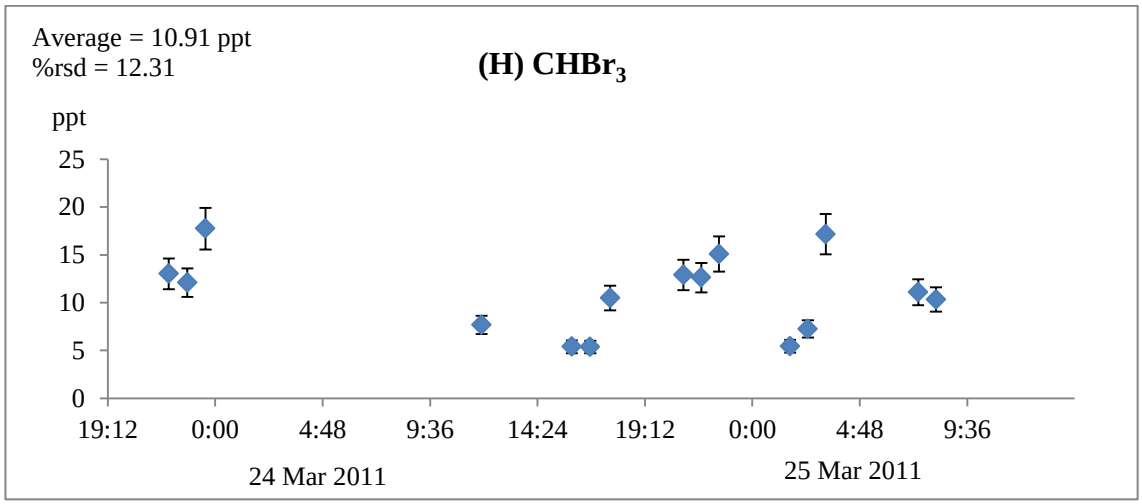
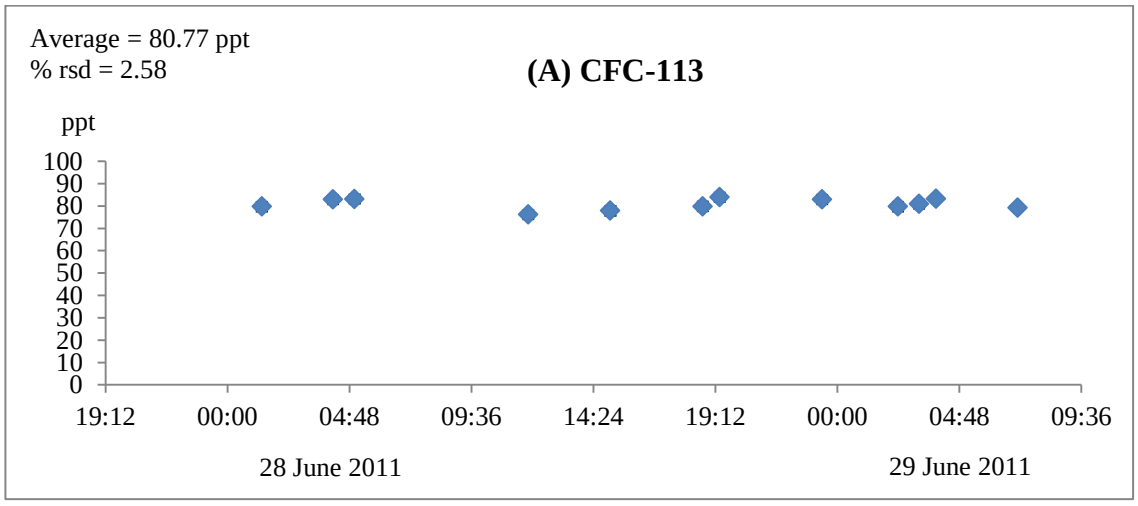


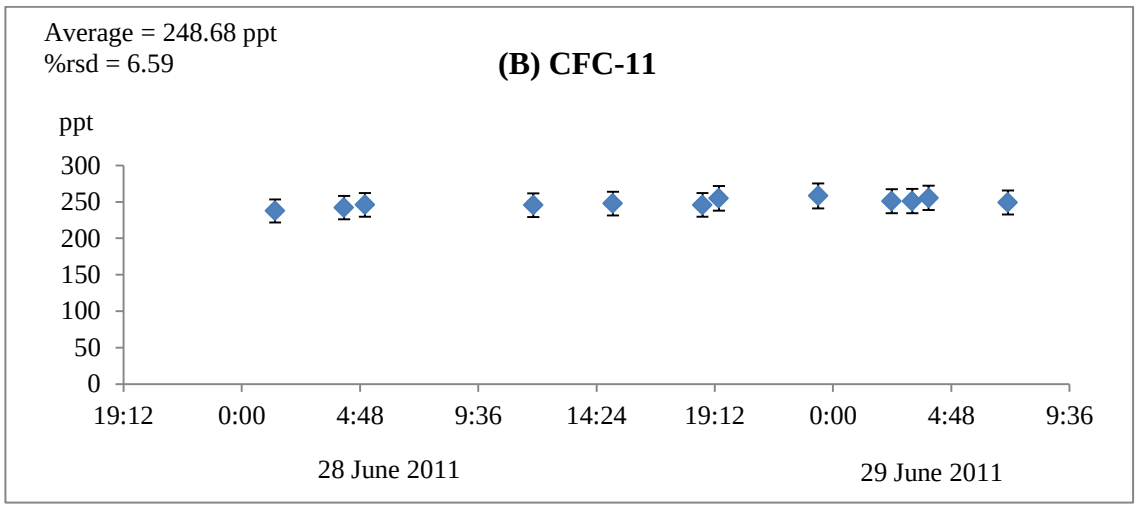
Figure 4.6 (A) – (H): Atmospheric halocarbon mixing ratios for CFC-113, CFC-11, CCl_4, CH_3CCl_3, C_2Cl_4, $CHBrCl_2$ + CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$ at Cape Rachado, Port Dickson during the March 2011 sampling. Error bar indicates standard error of the measurement made relative to the calibration standards.

4.1.1.2.6 June 2011

The sixth and last site survey was done on June 27th to 29th 2011. The measured mixing ratios for the long-lived halocarbons stayed quite close to the values observed during the March 2010 survey with the mixing ratio for CFC-113, CFC-11, CCl_4 and CH_3CCl_3 being fairly constant at an average value of 80.77 ppt, 248.68 ppt, 88.92 ppt and 9.24 ppt. The percentage of standard deviation to the average mixing ratio for CFC-113, CFC-11, CCl_4 and CH_3CCl_3 throughout the survey was less than 10 % between its measured values compared to the average value.

The average mixing ratios for the short-lived halocarbon were measured to be around 1.80 ppt for C_2Cl_4, which was the lowest mixing ratio recorded for this compound throughout the 15-month sampling period, 23.18 ppt for CH_2Br_2 + $CHBrCl_2$, 4.72 ppt for $CHBr_2Cl$ and 13.01 ppt for $CHBr_3$. The short-lived halocarbon having the highest mixing ratio for June 2011 was the double peak compound CH_2Br_2 + $CHBrCl_2$, followed by $CHBr_3$, $CHBr_2Cl$ and C_2Cl_4. The recorded values for the short-lived halocarbons seemed have the highest readings near evening time on the 28th of June 2011. Apart from this similarity, their mixing ratio trends seemed to vary with each compound [Fig. 4.7(A) – (H)].




Average = 248.68 ppt
%rsd = 6.59
(B) CFC-11
ppt
300
250
200
150
100
50
0
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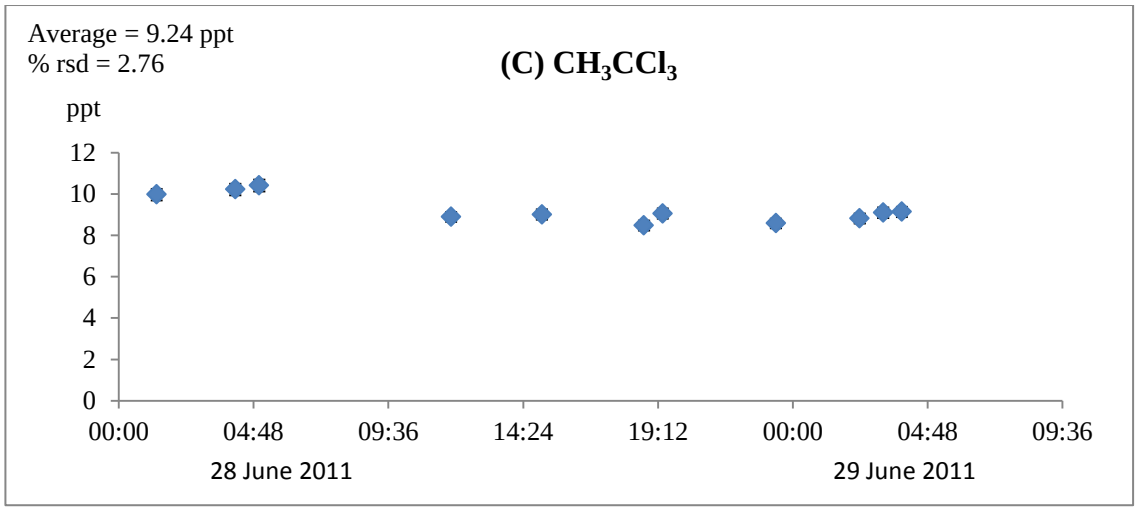
0:00
4:48
9:36
14:24
19:12
0:00
4:48
9:36
28 June 2011
29 June 2011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% rsd = 2.76
(C) CH3CCl3
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10
8
6
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04:48
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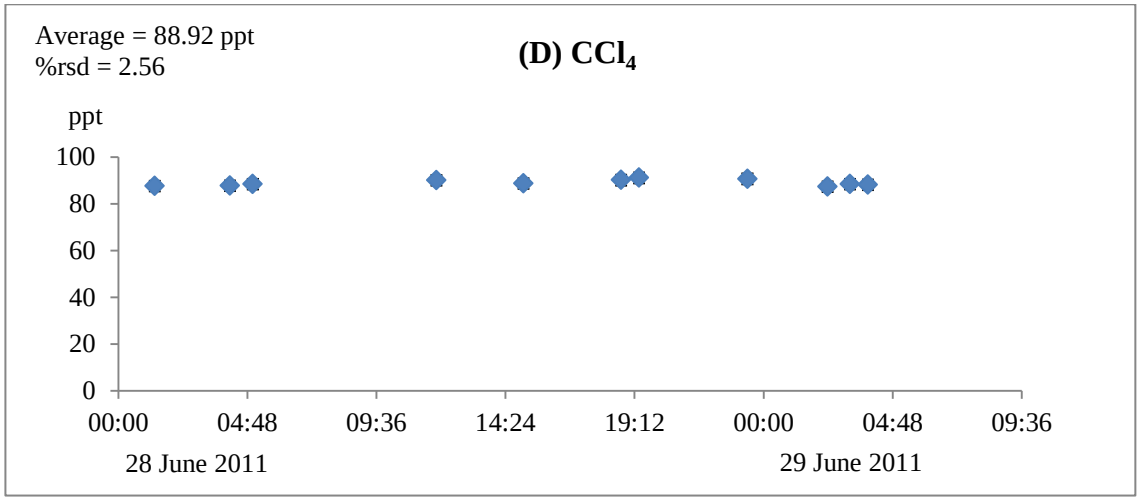
14:24
19:12
00:00
04:48
09:36
28 June 2011
29 June 2011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00:00
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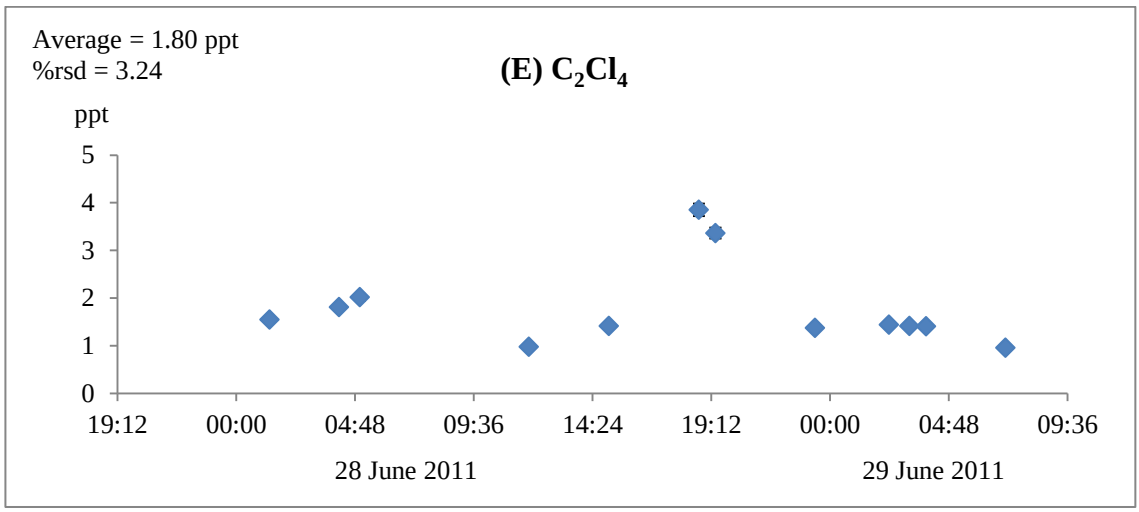
28 June 2011
29 June 2011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4
3
2
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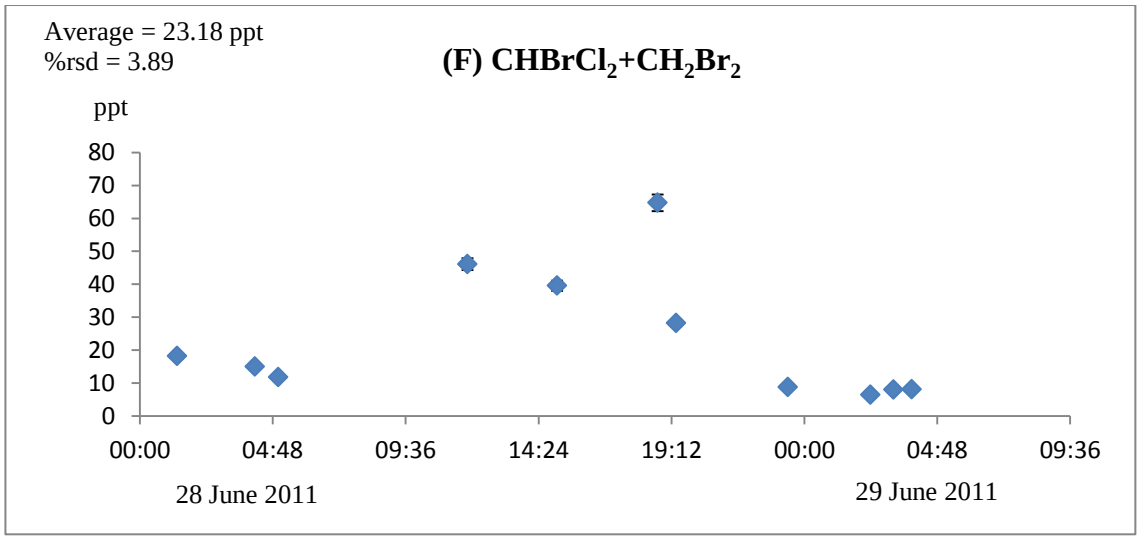
19:12
00:00
04:48
09:36
28 June 2011
29 June 2011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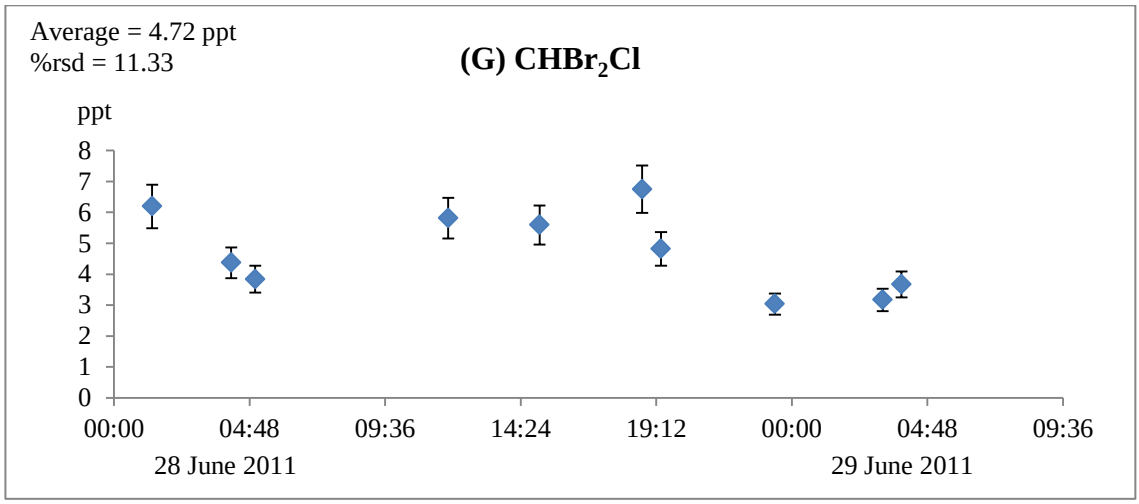
ppt
80
70
60
50
40
30
20
10
0
00:00
04:48
09:36
14:24
19:12
00:00
04:48
09:36
28 June 2011
29 June 2011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%rsd = 11.33
(G) CHBr2Cl
ppt
8
7
6
5
4
3
2
1
0
00:00
04:48
09:36
14:24
19:12
00:00
04:48
09:36
28 June 2011
29 June 2011

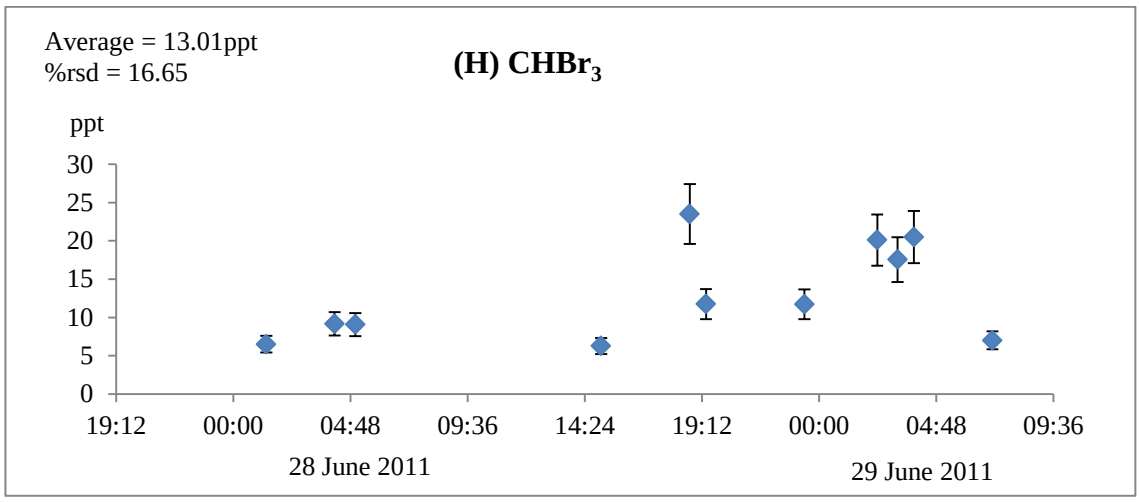

Figure 4.7(A) – (H): Atmospheric halocarbon mixing ratios for CFC-113, CFC-11, CCl_4, CH_3CCl_3, C_2Cl_4, $CHBrCl_2$ + CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$ at Cape Rachado, Port Dickson during the June 2011 sampling. Error bar indicates standard error of the measurement made relative to the calibration standards.

4.1.2 Composition and abundance of seaweeds

4.1.2.1 Seaweed species composition

The seaweed community at Cape Rachado, Port Dickson comprised mainly of the phaeophytes with a percentage of 70.93 ± 7.98 % observed throughout the 15-month sampling period. Of the total seaweed standing biomass at the survey site, 28.67 ± 2.63 % was contributed by the chlorophytes while the rhodophytes were rarely seen, contributing only 0.40 ± 0.01 % of the total seaweed standing biomass at the survey site (Figure 4.8).

The brown seaweeds seemed to contribute to more than half of the total seaweed biomass collected during each sampling trip from March 2010 to June 2011 as shown in Table 4.1 except for the sampling trip in September 2010 which was dominated by the chlorophytes with a percentage of 87.23 ± 29.27 % off the total seaweed biomass. The total biomass observed for the chlorophytes were lesser than that of the phaeophytes for each of the sampling trips except in September 2010 where the chlorophytes contributed

most of the seaweed biomass. The rhodophytes were the least found at the survey site for every sampling trip. They were not found within the quadrats during the September 2010 and June 2011 sampling trips which gave their contribution to the total biomass at the sampling site a value of 0.00 %.

The average value of seaweed standing biomass is shown in Table 4.2. The highest total standing biomass was found during the September 2010 sampling at an average of 163.92 ± 115.35 gm^{-2} where the standing biomass of *Sargassum binderi* dominates over all the other species at 100.02 ± 91.82 gm^{-2}. The lowest standing biomass was recorded during the December 2010 sampling at 47.95 ± 40.77 gm^{-2} where the standing biomass of one of the dominant species, *Sargassum binderi*, dropped to a low of 10.47 ± 9.48 gm^{-2} compared to all the other sampling trips. The standing biomass of *Cauelerpa racemosa* dominated over the biomass of all other species during this period.

All of the seaweed species collected from the sampling site were recorded and shown in Table 4.3. A total of 27 species had been recorded from the sampling sites throughout the sampling period. Of these, eight were chlorophytes, eight rhodophytes and eleven phaeophytes. A preliminary survey carried out in December 2009 recorded three other species that were not observed throughout the survey period. These species included *Caulerpa sertularoides*, *Dictyota bartayresi*, and *Ceratodictyon spongiosum*.

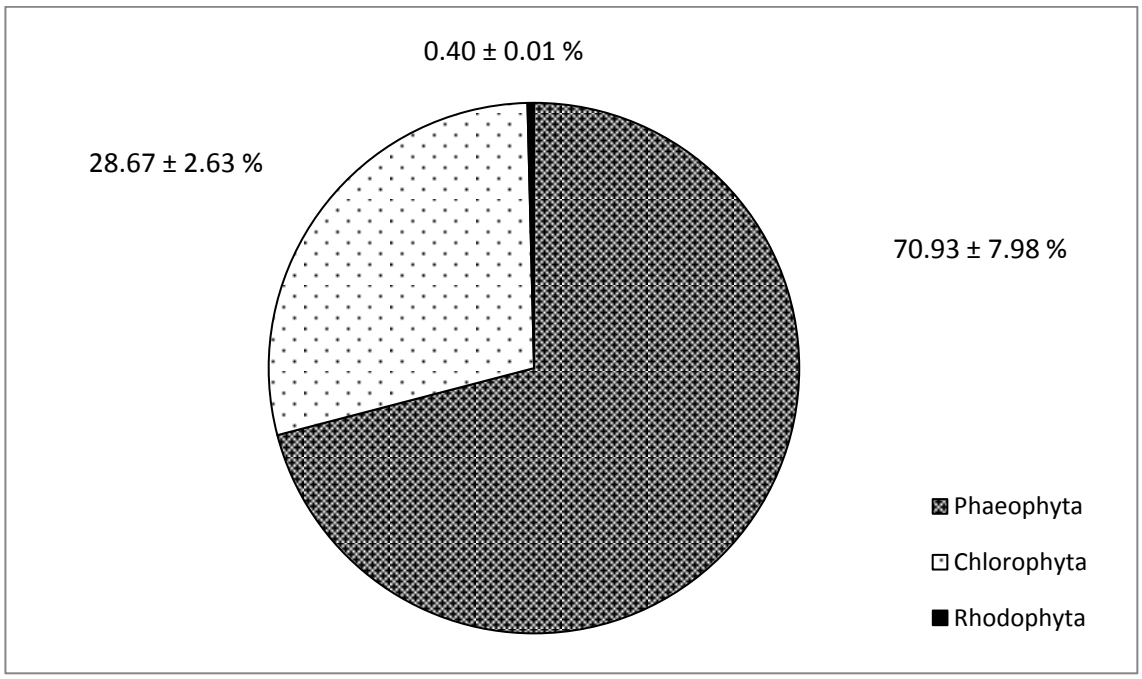

Figure 4.8: Seaweed composition based on the three divisions of seaweeds at Cape Rachado, Port Dickson from March 2010 to June 2011.

Table 4.1: Seaweed composition (percentage %) based on the three divisions of seaweeds at Cape Rachado, Port Dickson during each individual survey trip throughout the 15-month sampling period.

	Total (%)		
	Phaeophyta	Chlorophyta	Rhodophyta
Mar 2010	83.44 ± 55.12	15.51 ± 2.06	1.05 ± 1.98
Jun 2010	92.13 ± 12.32	7.85 ± 2.63	0.02 ± 0.04
Sep 2010	82.45 ± 19.57	16.82 ± 16.14	0.73 ± 0.87
Dec 2010	12.77 ±5.52	87.23 ± 29.27	0.00 ± 0.00
Mar 2011	51.55 ± 41.25	48.20 ± 5.56	0.25 ± 0.22
Jun 2011	67.24 ± 5.05	32.76 ± 3.72	0.00 ± 0.00

Table 4.2: Mean ± standard deviation of the seaweed standing biomass (g m^{-2}) at the sampling site from March 2010 until June 2011.

Seaweeds	March 2010	June 2010	September 2010	December 2010	March 2011	June 2011
Total biomass	81.36 ± 101.06	100.55 ± 66.01	163.82 ± 115.35	47.95 ± 40.77	85.91 ± 85.62	97.70 ± 99.46
Sargassum binderi	62.73 ± 85.74	90.81 ± 76.35	100.02 ± 91.82	10.47 ± 9.48	95.04 ± 99.52	134.69 ± 118.44
Sargassum baccularia	40.86 ± 77.07	38.24 ± 34.72	35.62 ± 22.74	7 ± 5.15	11.31 ± 12.81	23.47 ± 11.36
Turbinaria conoides	11.56 ± 17.19	18.06 ± 20.28	45.33 ± 43.16	5.26 ± 5.08	2.11 ± 1.39	7.94 ± 4.19
Padina australis	8.74 ± 11.99	22.37 ± 28.82	36.42 ± 43.41	2.20 ± 2.70	20.46 ± 31.45	36.69 ± 43.85
Lobophora variegate	9.25 ± 28.84	0.78	3.64 ± 3.62	0.49 ± 0.33	2.13 ± 2.03	2 ± 1.88
Caulerpa sp.	8.77 ± 9.39	17.95 ± 15.16	51.09 ± 84.06	16.03 ± 13.76	13.53 ± 18.35	20.87 ± 21.70
Caulerpa racemosa	12.16 ± 13.77	0	2.85 ± 0.06	30.36 ± 25.88	41.83 ± 45.10	20.71 ± 17.14
Caulerpa lentilifera	38.17 ± 10.29	20.94 ± 2.12	9.81 ± 9.24	23.11 ± 24.95	15.93 ± 18.16	15.41 ± 19.49
Sargassum polycystum	18.94 ± 21.64	49.67 ± 45.70	52.41 ± 46.66	5.78 ± 7.86	13.74 ± 7.70	17.11
Udotea javensis	2.86 ± 3.92	1.85 ± 0.91	4.67 ± 4.70	0	0	6.77 ± 9.39
Gelidiopsis sp.	0.29 ± 0.17	0	0	0	0	0
Jania rubens	9.71 ± 14.62	0	24.39 ± 18.31	0	2.11	0
Sargassum siliquosum	13.82 ± 10.28	0	0	0	2.33	6.78 ± 3.77
Bryopsis pennata	1.11	0	4.56 ± 6.18	9.56 ± 11.35	0	0
Chaetomorpha aerea	0.33	0	0	0	0	0
Gelidiella acerosa	0.67 ± 0.16	0	0	0	0	0
Sargassum oligocystum	19.67	0	6.33	0	0	0
Dictyota dichotoma	0.56	0	0	0	0	0
Amphiroa sp.	0	0.89	0	0	1.78	0
Acanthophora spicifera	0	0	1.44	0	1.44 ± 1.93	0
Dictyopteris deliculata	0	0	76.59 ± 52.01	0	0	0
Dictyota dentata	0	0	2.18 ± 2.53	0	0.39 ± 0.24	0
Pterocladia caerulescens	0	0	0	0	0.22	0

Table 4.3: Seaweed species collected from Cape Rachado, Port Dickson throughout the 15-month survey

Division	**Chlorophyta**	**Rhodophyta**	**Phaeophyta**
Species Name	*Enteromorpha clathrata* (Roth) Greville	*Gelidiella acerosa* (forsskaal) feldmann & Hamel	*Padina australis* Hauck
	Chaetomorpha linum (O.F. Müller) Kützing	*Gelidium* sp.	*Lobophora variegate* (Lamouroux) Womerseley
	Chaetomorpha aerea (Dillwyn) Kützing	*Pterocladia caerulescens* Kützing Santelices	*Dictyota dentata* Lamouroux
	Caulerpa lentilifera J.Agardh	*Amphiroa* sp.	*Dictyota dichotoma* (Hudson) Lamouroux
	Caulerpa racemonsa (Forssakaal) Feldmann & Hamel	*Jania rubens* (Linnaeus) Lamouroux	*Dictyopteris delicatula* Lamouroux
	Caulerpa sp.	*Gelidiopsis* sp.	*Sargassum baccularia* (Mert.) C. Agardh
	Udotea javensis (Montagne) A & E.S. Gepp	*Acanthophora spicifera* (Vahl.) Bergesen	*Sargassum binderi* Sonder ex J.Agardh
	Bryopsis pennata Lamouroux	*Laurencia* sp.	*Sargassum siliquosum* Agardh
			Sargassum polycystum C.Agardh
			Sargassum oligocystum Montagne
			Turbinaria conoides (J.Agardh) Kützing
No. of Species	8	8	11
Total No. of Species			27

4.1.2.2 Frequency and dominance of seaweed species at study site

Table 4.4 shows the frequencies of the top five most commonly occurring species at the sampling site from March 2010 until June 2011. The first two sampling trips were dominated by the phaeophytes *Sargassum baccularia* in terms of frequency, while the frequencies of *Bryopsis pennata*, *Caulerpa lentilifera* and *Caulerpa racemosa* dominated over all other species during the September 2010, March 2011 and June 2011 samplings. The highest frequency was recorded by *Sargassum baccularia* during March 2010 sampling at a value of 75.81 %.

However, as shown in Table 4.5, throughout the entire sampling period, the seaweed species with the dominating frequency was recorded by the chlorophytes *Caulerpa* sp. at 49.81 %, followed by *Turbinaria conoides* (43.35 %), *Sargassum baccularia* (42.21 %), *Caulerpa racemosa* (38.02 %) and *Sargassum binderi* (37.26 %).

Although the frequency of *Sargassum binderi* had not been topping the rank in their frequency percentage during the seaweed surveys, the dominance of this species was the highest in June 2010 (39.52 %), September 2010 (31.98 %), March 2011 (36.88 %) and June 2011 (49.49 %). The March 2010 and December 2010 survey on the other hand was dominated by *Sargassum baccularia* (38.70 %) and *Caulerpa racemosa* (37.70 %) respectively (Table 4.6). *Sargassum binderi* (33.29 %) has the highest dominancy throughout the entire sampling period followed by *Sargassum baccularia* (15.20 %) as shown in Table 4.7.

The importance value index (IVI) was calculated for each species and the top five species having the highest indices during each sampling trip was shown in Table 4.8. *Sargassum binderi* topped the chart during the June 2010 (27.70), September 2010

(23.13), Mach 2011 (23.25) and June 2011 (30.83) sampling while the highest IVI indices for March 2010 and December 2010 sampling was recorded by *Sargassum baccularia* (29.22) and *Caulerpa racemosa* (28.11). *Sargassum binderi* (22.19) has the highest IVI throughout the whole sampling period followed by *Sargassum baccularia* (13.88) as shown in Table 4.9.

Table 4.4: Frequencies (%) of five most commonly occurring seaweed species at Cape Rachado during each sampling trip.

	Species	Frequency (%)
March 2010	*Sargassum baccularia*	75.81
	Caulerpa sp.	62.90
	Turbinaria conoides	61.29
	Caulerpa racemosa	40.32
	Sargassum binderi	33.87
June 2010	*Sargassum baccularia*	64.29
	Turbinaria conoides	52.38
	Sargassum binderi	40.48
	Padina australis	33.33
	Caulerpa sp.	33.33
	Sargassum polycystum	14.29
September 2010	*Sargassum polycystum*	59.52
	Sargassum binderi	52.38
	Turbinaria conoides	52.38
	Caulerpa sp.	45.24
	Sargassum baccularia	30.95
	Caulerpa lentilifera	30.95
	Padina australis	23.81
December 2010	*Bryopsis pennata*	71.79
	Caulerpa racemosa	64.10
	Caulerpa sp.	53.85
	Caulerpa lentilifera	43.59
	Turbinaria conoides	41.03
March 2011	*Caulerpa lentilifera*	61.54
	Caulerpa racemosa	58.97
	Caulerpa sp.	51.28
	Padina australis	33.33
	Sargassum binderi	33.33
	Turbinaria conoides	28.21

June 2011	*Caulerpa racemosa*	61.54
	Caulerpa lentilifera	51.28
	Caulerpa sp.	46.15
	Sargassum binderi	35.90
	Padina australis	25.64
	Sargassum baccularia	25.64
	Udotea javensis	25.64

Table 4.5: Frequencies (%) of the five most commonly occurring seaweed species at Cape Rachado throughout the entire survey.

Species	Percentage (%)
Caulerpa sp.	49.81
Turbinaria conoides	43.35
Sargassum baccularia	42.21
Caulerpa racemosa	38.02
Sargassum binderi	37.26

Table 4.6: Dominance (%) of the five seaweed most commonly occurring seaweed species at Cape Rachado during each sampling trip.

	Species	Dominance (%)
March 2010	*Sargassum baccularia*	38.70
	Sargassum binderi	26.54
	Turbinaria conoides	8.85
	Caulerpa sp.	6.89
	Caulerpa racemosa	6.12
June 2010	*Sargassum binderi*	39.52
	Sargassum baccularia	26.43
	Turbinaria conoides	10.17
	Padina australis	8.02
	Sargassum polycystum	7.63
September 2010	*Sargassum binderi*	31.98
	Sargassum polycystum	19.04
	Turbinaria conoides	14.49
	Sargassum baccularia	6.73
	Padina australis	5.29

December 2010	*Caulerpa racemosa*	37.70
	Caulerpa lentilifera	19.51
	Caulerpa sp.	16.72
	Bryopsis pennata	13.30
	Sargassum binderi	5.72
March 2011	*Sargassum binderi*	36.88
	Caulerpa racemosa	28.71
	Caulerpa lentilifera	11.41
	Caulerpa sp.	8.08
	Padina australis	7.94
June 2011	*Sargassum binderi*	49.49
	Caulerpa racemosa	13.05
	Caulerpa sp.	9.86
	Padina australis	9.63
	Caulerpa lentilifera	8.09

Table 4.7: Dominance (%) of the five most commonly occurring seaweed species at Cape Rachado throughout the entire survey.

Species	Dominance (%)
Sargassum binderi	33.29
Sargassum baccularia	15.20
Caulerpa sp.	10.22
Caulerpa racemosa	10.15
Turbinaria conoides	7.95

Table 4.8: Importance Value Index (IVI) of the five most commonly occurring seaweed species at Cape Rachado during each sampling trip.

	Species	Importance Value Index (IVI)
March 2010	*Sargassum baccularia*	29.22
	Sargassum binderi	17.68
	Turbinaria conoides	12.41
	Caulerpa sp.	11.64
	Caulerpa racemosa	8.31
June 2010	*Sargassum binderi*	27.70
	Sargassum baccularia	25.83
	Turbinaria conoides	15.37
	Padina australis	10.55

	Caulerpa sp.	9.76
September 2010	*Sargassum binderi*	23.13
	Sargassum polycystum	17.64
	Turbinaria conoides	14.39
	Caulerpa sp.	13.22
	Sargassum baccularia	7.59
December 2010	*Caulerpa racemosa*	28.11
	Bryopsis pennata	17.02
	Caulerpa sp.	16.14
	Caulerpa lentilifera	16.05
	Turbinaria conoides	8.02
March 2011	*Sargassum binderi*	23.25
	Caulerpa racemosa	22.88
	Caulerpa lentilifera	14.59
	Caulerpa sp.	11.45
	Padina australis	8.78
June 2011	*Sargassum binderi*	30.83
	Caulerpa racemosa	16.96
	Caulerpa sp.	12.76
	Caulerpa lentilifera	12.74
	Padina australis	9.16

Table 4.9: Importance Value Index (IVI) of the five most commonly occurring seaweed species at Cape Rachado throughout the entire survey.

Species	Importance Value Index (IVI)
Sargassum binderi	22.19
Sargassum baccularia	13.88
Caulerpa sp.	12.52
Caulerpa racemosa	10.73
Turbinaria conoides	10.42

4.1.2.3 Seawater quality

The mean values for the seawater water quality at the sampling sites are shown in Table 4.10. The primary productivity of the seawater was indicated by the chlorophyll-a concentration which was recorded to be between 0.526 – 2.075 µg/L. The four physical parameters of the seawater, namely temperature, salinity, dissolved oxygen (DO), and pH were recorded and the mean values were in the range of 28.45 – 32.50 ℃, 30.00 – 32.55 ppt, 8.48 – 10.89 mg/L and 8.09 – 8.98 respectively. The nutrients measured for the seawater include ammonia (NH_3-N), nitrite (No_2^--N), nitrate (NO_3^--N) and phosphate (O-PO_4) at a range of 0 – 0.66 mg/L, 0.002 – 0.016 mg/L, 0.3 – 0.7 mg/L and 0.03 – 0.17 mg/L respectively. Total suspended solid (TSS) in the seawater was recorded in the range of 0.10 – 0.27 g/L.

Table 4.10: Mean ± standard deviation (S.D) values of the seawater parameters at the sampling site from March 2010 until June 2011.

Parameters	March 2010	June 2010	September 2010	December 2010	March 2011	June 2011
Chlorophyll-a ($\mu g\ L^{-1}$)	1.440 ± 0.998	1.349 ± 0.374	1.349 ± 0.375	2.075 ± 0.749	0.526 ± 0.188	1.139 ± 0.450
Temperature (℃)	31.57 ±0.39	32.50 ± 0.61	29.54 ± 1.77	28.45 ± 0.01	29.40 ± 0.56	32.12 ± 0.18
Salinity (ppt)	32.27 ± 0.33	31.20 ± 0.37	30.08 ± 0.05	32.55 ± 0.03	-	-
Dissolved oxygen ($mg\ L^{-1}$)	8.49 ± 1.18	10.89 ± 1.93	8.69 ± 1.97	8.78 ± 0.19	8.48 ± 1.03	9.20 ± 0.31
pH	8.09 ± 0.14	8.51 ± 0.24	8.33 ± 0.08	8.98 ± 0.01	8.14 ± 0.06	8.71 ± 0.01
Ammonia, NH_3N ($mg\ L^{-1}$)	0.01 ± 0.01	0.01 ± 0	0.01 ± 0.01	0 .00 ± 0	0.66 ± 0.06	0.25 ± 0.09
Nitrite , No_2^--N ($mg\ L^{-1}$)	0.002 ± 0.002	0.004 ± 0.003	0.003 ± 0.001	0.002 ± 0.001	0.016 ± 0.003	0.006 ± 0.004
Nitrate, NO_3^--N ($mg\ L^{-1}$)	0.3 ± 0.1	0.3 ± 0.3	0.7 ± 0.4	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.1
Phosphate, O-PO_4 ($mg\ L^{-1}$)	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.03 ± 0.02	0.17 ± 0.03	0.05 ± 0.04
Total Suspended Solids, TSS ($g\ L^{-1}$)	0.27 ± 0.01	0.16 ± 0.06	0.18 ± 0.05	0.10 ± 0.05	0.13 ± 0.00	0.10 ± 0.05

4.1.3 Statistical analysis

4.1.3.1 Correlation of seaweed biomass

Simple correlation analysis was used to test the correlation between the seaweed occurrences at the sampling site at Cape Rachado throughout the sampling period. The average biomass for several seaweeds with high important value index and dominancy or frequency value was tested. Non-significant correlationship was observed from the analysis result (Table 4.11) apart from the positive correlations between the average biomass of *Sargassum binderi* with *Padina australis* (r = 0.9098; $p < 0.05$), *Caulerpa* sp. with *Turbinaria conoides* (r = 0.9115; $p < 0.05$) and the negative correlation between the average tri-monthly biomass of *Caulerpa racemosa* with *Sargassum baccularia* (r = -0.8903; $p < 0.05$).

4.1.3.2 Correlation of seaweed biomass with seawater quality and rainfall

The seaweed biomass and seawater chlorophyll-a contents were tested against seawater quality and rainfall data from the Malaysian Meteorological Department to determine their correlation and the results shown in Table 4.12. Chlorophyll-a contents in the seawater was found to be negatively correlated to the biomass of *Sargassum binderi* (r = -0.9591; $p < 0.05$) and *Sargassum baccularia* (r = -0.9689; $p < 0.05$) while positively correlated to the biomass of *Caulerpa racemosa* (r = 0.9636; $p < 0.05$).

The biomass of both *Turbinaria conoides* (r = -0.9631; $p < 0.05$) and *Padina australis* (r = -0.9968; $p < 0.01$) were found to be negatively correlated to the salinity of the seawater. The biomass of *Sargassum baccularia* correlated positively (r = 0.9908; $p < 0.01$) with the ammoniacal nitrogen levels in the seawater at the sampling site. Both biomass of *Turbinaria conoides* and *Caulerpa* sp. were found to be positively correlated to the nitrate nitrogen (r = 0.9551; $p < 0.05$) and dissolved orthophosphate (r

= 0.9777; $p < 0.05$) contents in the seawater.

No biomass of the dominant seaweed species or the chlorophyll-a contents in the seawater was found to correlate with temperature, dissolved oxygen, pH, nitrite nitrogen, total suspended solid levels in the seawater and the amount of rainfall.

4.1.3.3 Correlation of biogenic halocarbon compounds with seaweed biomass

Three of the halocarbon compounds with biogenic origin (i.e. $CHBr_3$, $CHBr_2Cl$ and CH_2Br_2 + $CHBrCl_2$) were selected to test their correlation with the major seaweed species found at Cape Rachado (Table 4.13). There was no significant correlation observed between the total seaweed biomass throughout the entire survey with the atmospheric concentration of the three halocarbon compounds. Only very weak significant correlations ($\rho < 0.5$) were observed between $CHBr_3$ and the individual biomass of *Sargassum binderi* (ρ = 0.2370; $p < 0.05$) and *Caulerpa racemosa* (ρ = 0.3928; $p < 0.05$). The chlorophyll-a concentration in the seawater showed a higher coefficient value (ρ) of 0.4642 as compared to seaweeds in terms of their correlation with the atmospheric mixing ratio of $CHBr_2Cl$. The correlation between seaweeds with CH_2Br_2 + $CHBrCl_2$ mixing ratio in the atmosphere was observed with the chlorophyte *Caulerpa* sp. (ρ= 0.2651; $p < 0.05$).

Table 4.11: The Pearson Product-Moment correlation coefficient (*r*) between the seaweed standing biomass at Cape Rachado, Port Dickson

	Sargassum binderi	*Sargassum baccularia*	*Turbinaria conoides*	*Padina australis*	*Caulerpa sp.*	*Caulerpa racemosa*	*Caulerpa lentilifera*
Sargassum binderi		0.3384	0.2394	0.9098*	0.3150	-0.2199	-0.5067
Sargassum baccularia			0.5857	0.3029	0.2511	-0.8903*	0.2758
Turbinaria conoides				0.5330	0.9115*	-0.7334	-0.4053
Padina australis					0.6624	-0.3412	-0.7359
Caulerpa sp.						-0.4641	-0.6981
Caulerpa racemosa							-0.0498

The average biomass of each species from the six sampling trip were tested.

Number of replicates (*n*) = 6; * indicates significance level (*p*) <.0.05

Table 4.12: The Pearson Product-Moment correlation coefficient (*r*) between seaweed biomass and seawater quality and rainfall at Cape Rachado, Port Dickson

	Chl-a	Temp.	Salinity	DO	pH	NH_3-N	No_2^--N	NO_3^--N	O-PO_4	TSS	Rainfall
S. binderi	-0.9591*	0.5694	-0.8573	0.3695	-0.6779	0.9192	0.7228	0.5629	0.5629	0.4471	0.5531
S. baccularia	-0.9689*	0.7980	-0.4938	0.2441	-0.9166	0.9908**	0.4595	0.2195	0.2195	0.1284	0.3280
T. conoides	-0.6206	-0.0716	-0.9631*	-0.0675	-0.3981	0.5589	0.4202	0.9551*	0.9551*	0.1284	0.3280
P. australis	-0.7418	-0.1610	-0.9968**	0.2190	-0.4035	0.6684	0.6614	0.8332	0.8332	0.1180	0.5633
Caulerpa sp.	-0.3398	-0.3637	-0.8914	-0.1550	-0.1079	0.2629	0.3070	0.9777*	0.9777*	-0.1544	0.2880
C. racemosa	0.9636*	-0.6923	0.7796	-0.5062	0.6540	-0.9254	-0.7923	-0.4132	-0.4132	-0.4428	-0.6258
C. lentilifera	0.1185	0.3791	0.7907	-0.1923	-0.2993	-0.0059	-0.5172	-0.7541	-0.7541	0.5606	-0.5930

Number of replicates (*n*) = 6 except salinity (*n*) = 4; * indicates significance level (*p*) <.0.05; ** = (*p*) < 0.01

Table 4.13: The correlations between seaweed biomass and seawater chlorophyll-a concentration with the mixing ratios of biogenic halocarbon compounds at Cape Rachado, Port Dickson

	Total standing biomass	*Sargassum binderi*	*Sargassum baccularia*	*Turbinaria conoides*	*Padina australis*	*Caulerpa* sp.	*Caulerpa racemosa*	*Caulerpa lentilifera*	Chl-a
$CHBr_3$	0.6998	0.2370*	-0.3024*	-0.2992*	0.0546	0.0118	0.3928*	-0.2589	-0.1925
$CHBr_2Cl$	-0.7720	-0.3156*	-0.0608	0.2006	-0.1590	0.1756	-0.2006	0.2929*	0.4642*
CH_2Br_2 +$CHBrCl_2$	-0.5516	-0.0207	-0.2421*	-0.0500	0.0696	0.2651*	-0.0162	-0.0324	0.1008

* indicates significance level (p) <.0.05

Note: The Pearson Product-Moment Correlation (r) was used to test the correlations between total standing biomass and the biogenic halocarbon compounds (n = 6 except $CHBr_2Cl$ where n = 5); The Spearman Rank Order Correlations (ρ) was used to test the correlations between individual seaweed biomass and the biogenic halocarbon compounds (n = 81, 65, 79 for $CHBr_3$, $CHBr_2Cl$ and CH_2Br_2 + $CHBrCl_2$)

4.2 Laboratory study

4.2.1 Halocarbon emissions under different irradiance levels

4.2.1.1 *Sargassum binderi*

The halocarbon emission rates by *Sargassum binderi* are shown in Fig. 4.9 A-H. The average emission rates of CH_3I under the different irradiances ranged between 11.60 and 106. 05 pmol g DW^{-1} hr^{-1}. Another iodinated form, CH_2I_2, was released at a lowest rate of 0.06 pmol g DW^{-1} hr^{-1} in the dark and the highest rate at pmol 12.63 pmol g DW^{-1} hr^{-1}at the irradiance level of 81 µmol photons m^{-2} s^{-1}. The highest rate of CH_2BrCl emissions was 10.16 pmol g DW^{-1} hr^{-1} at the highest irradiance while the lowest rate at 0.66 pmol g DW^{-1} hr^{-1} was observed in the dark. Emission rate of between 10.39 and 44.15 pmol g DW^{-1} hr^{-1} was recorded by CH_2BrI. For the brominated compounds, CH_2Br_2 and $CHBr_3$, emission rates range from 6.95 – 343.21 pmol g DW^{-1} hr^{-1} and 4.37 – 2904.89 pmol g DW^{-1} hr^{-1}. $CHBrCl_2$ and $CHBr_2Cl$ were released at a rate of between 24.16 – 250.18 and 0. 46 – 307. 50 pmol g DW^{-1} hr^{-1}. Overall, the compound $CHBr_3$ was the dominant form of halocarbon released by this seaweed species with its highest average rate of 2904.89 pmol g DW^{-1} hr^{-1} recorded.

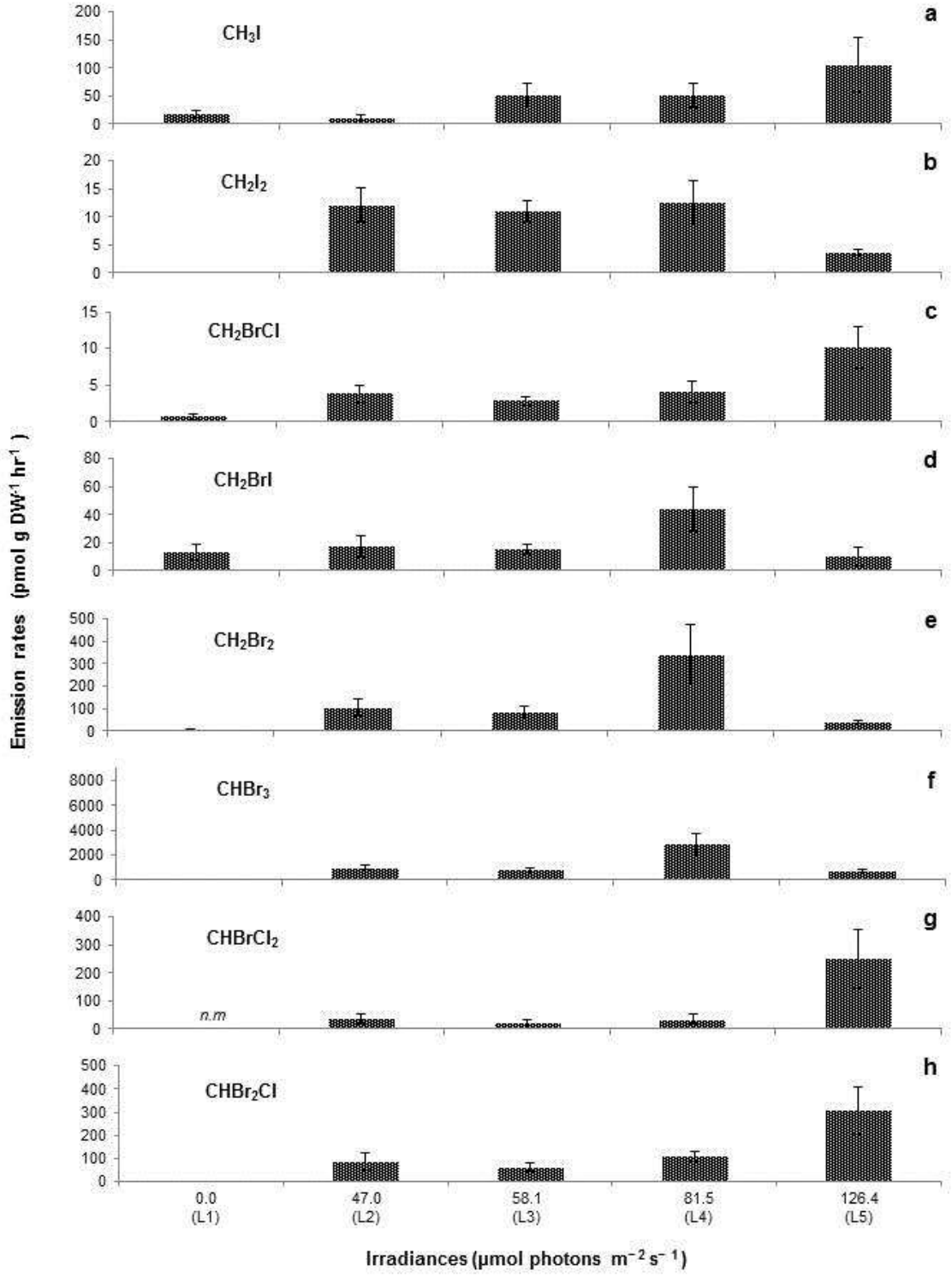

Figure 4.9 A - H: Halocarbon emission rates by *Sargassum binderi* under the different irradiances.
The bar represents the mean (n=5) for each irradiance with their standard error shown.
n.m = not measured

4.2.1.2 *Turbinaria conoides*

The halocarbon emission rates by *Turbinaria conoides* are shown in Fig. 4.10 A-H. The average emission rates of CH_3I under the different irradiances ranged between 11.81 and 27.67 pmol g DW^{-1} hr^{-1}. Another iodinated form, CH_2I_2, was released at a rate of 1.92 pmol g DW^{-1} hr^{-1} in the dark and the highest rate at pmol 167.78 pmol g DW^{-1} hr^{-1}at the irradiance level of 81.48 µmol photons m^{-2} s^{-1}. The highest rate of CH_2BrCl emissions was 9.92 pmol g DW^{-1} hr^{-1} at the light irradiance 46.97 µmol photons m^{-2} s^{-1} while the lowest rate at 0.61 pmol g DW^{-1} hr^{-1} was observed in the dark. Emission rate of between 2.97 and 234.02 pmol g DW^{-1} hr^{-1} was recorded by CH_2BrI. For the brominated compounds, CH_2Br_2 and $CHBr_3$, emission rates range from 19.08 – 619.62 pmol g DW^{-1} hr^{-1} and 279.21 – 6457.37 pmol g DW^{-1} hr^{-1}. $CHBrCl_2$ and $CHBr_2Cl$ were released at a rate of between 1.87 – 9.40 and 16.45 – 175.11 pmol g DW^{-1} hr^{-1}. Overall, the compound $CHBr_3$ was the dominant form of halocarbon released by *Turbinaria conoides* with its highest average rate of 6457.37 pmol g DW^{-1} hr^{-1} recorded.

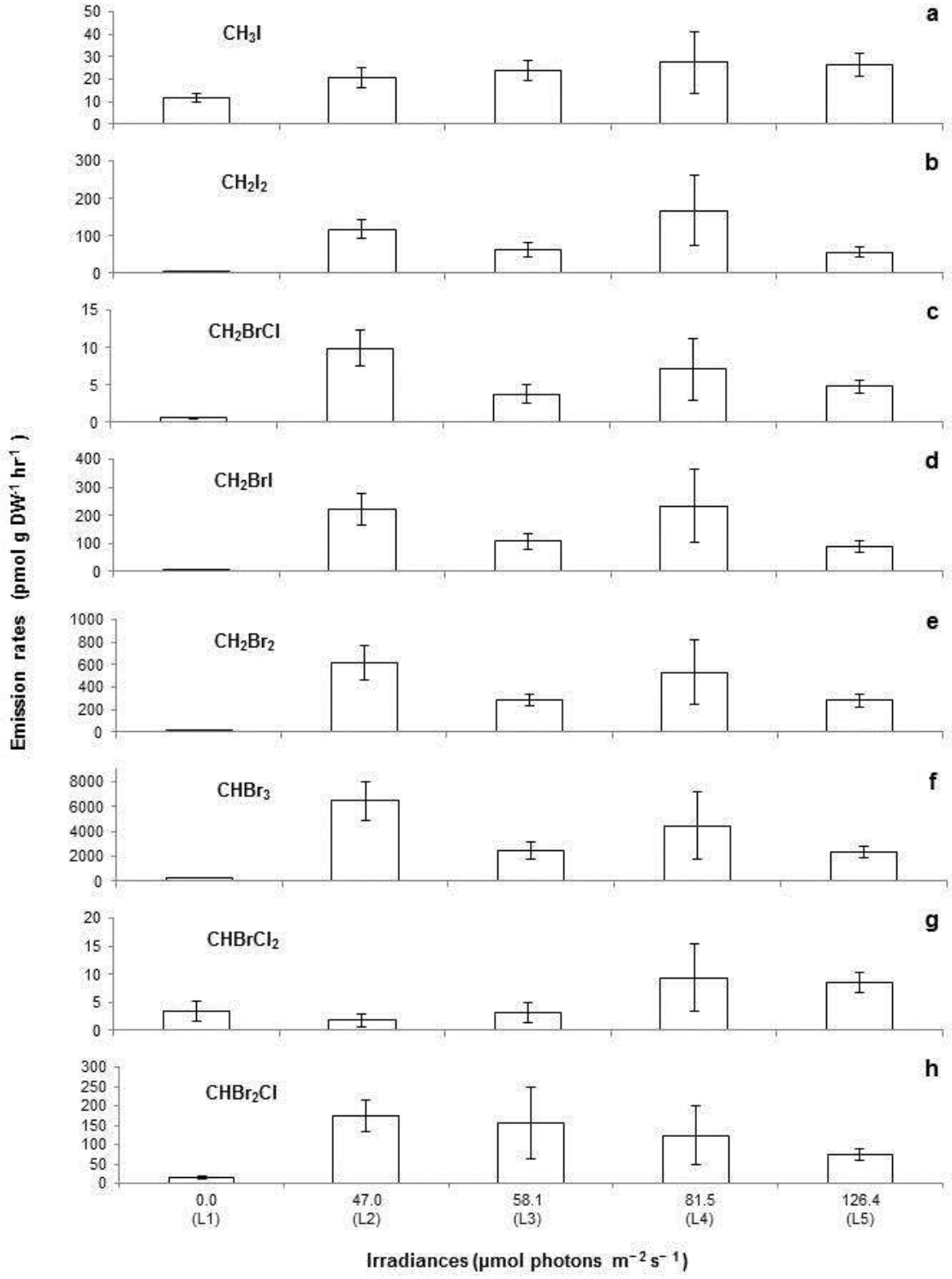

Figure 4.10 A - H: Halocarbon emission rates by *Turbinaria conoides* under the different irradiances.
The bar represents the mean (n=5) for each irradiance with their standard error shown.

4.2.1.3 *Padina australis*

The halocarbon emission rates by *Padina australis* are shown in Fig. 15A - H. The average emission rates of CH_3I under the different light irradiance ranged between 4.43 and 34.67 pmol g DW^{-1} hr^{-1}. Another iodinated form, CH_2I_2, was released at a rate of 0.20 pmol g DW^{-1} hr^{-1} in the dark and the highest rate at 65.72 pmol g DW^{-1} hr^{-1}. The highest rate of CH_2BrCl emissions was 1.15 pmol g DW^{-1} hr^{-1} while the lowest rate at 0.21 pmol g DW^{-1} hr^{-1} was observed in the dark. Emission rate of between 0.00 and 0.70 pmol g DW^{-1} hr^{-1} was recorded by CH_2BrI. For the brominated compounds, CH_2Br_2 and $CHBr_3$, emission rates range from 0.00 – 15.09 pmol g DW^{-1} hr^{-1} and 0.41 – 68.66 pmol g DW^{-1} hr^{-1}. $CHBrCl_2$ and $CHBr_2Cl$ were released at a rate of between 2.24 – 12.50 and 0.12 – 21.13 pmol g DW^{-1} hr^{-1}. Overall, the compound $CHBr_3$ was the dominant form of halocarbon released by this seaweed species with its highest average rate of 68.66 pmol g DW^{-1} hr^{-1} recorded followed by CH_2I_2 at the highest rate of 65.72 pmol g DW^{-1} hr^{-1}.

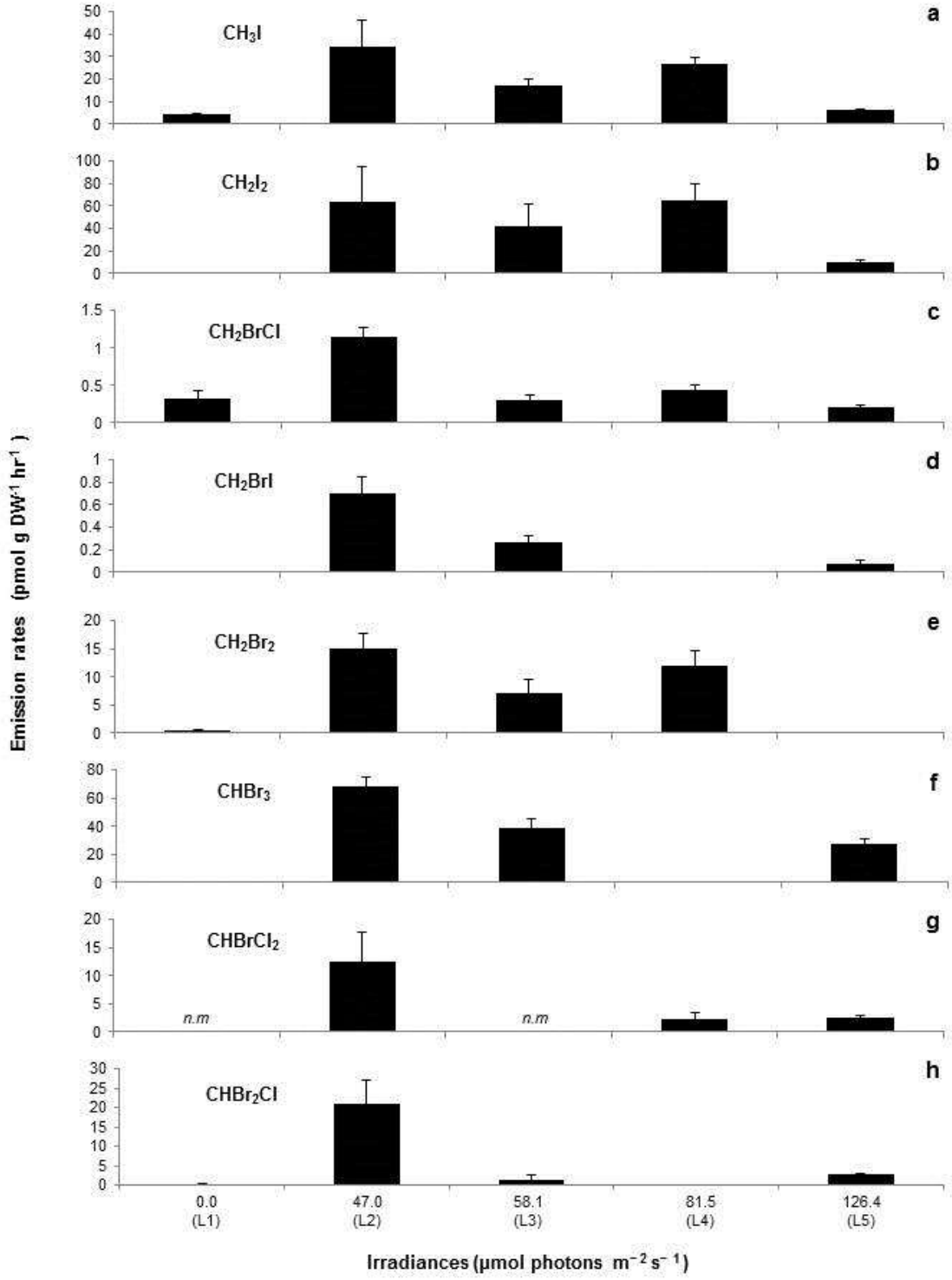

Figure 4.11 A - H: Halocarbon emission rates by *Padina australis* under the different irradiances.
The bar represents the mean (n=5) for each irradiance with their standard error shown.

4.2.1.4 Comparison of halocarbon emissions between the three brown seaweeds selected

All three tropical brown seaweed species used in this study were found to release all eight target halocarbons, although rates of emission varied between halocarbons and seaweed species. The emission rates are shown in Fig. 4.12 A - H. Emissions rates of CH_3I (17.9 – 106.0 pmol g DW^{-1} hr^{-1}) were higher than CH_2I_2 (0.04 – 12.63 pmol g DW^{-1} hr^{-1}) for *Sargassum binderi*. The opposite was true for *Turbinaria conoides* and *Padina australis* which showed higher CH_2I_2 (0.0 – 12.6 pmol g DW^{-1} hr^{-1}) emission rates than CHI_3 (11.8-27.7 pmol g DW^{-1} hr^{-1} & 4.4 – 34.7 pmol g DW^{-1} hr^{-1})

As for the mixed halocarbon compounds, the emission rates of $CHBr_2Cl$ (0.1 – 307.5 pmol g DW^{-1} hr^{-1} & 16.4 – 175.1 pmol g DW^{-1} hr^{-1}) were higher than the emission rates of $CHBrCl_2$ (24.2– 250.2 pmol g DW^{-1} hr^{-1} & 0.9 – 8.6 pmol g DW^{-1} hr^{-1}) in *Sargassum binderi* and *Turbinaria conoides*. The emission rates of the mixed halocarbon compounds by *Sargassum binderi* were in the order of $CHBr_2Cl$ > $CHBrCl_2$ > CH_2BrCl while for *Turbinaria conoides* $CHBr_2Cl$ > $CHBrCl_2$.

Turbinaria conoides was found to be the strongest producer as compared to *Sargassum binderi* and *Padina australis* in terms of CH_2I_2, CH_2Br_2, $CHBr_3$, and CH_2BrI emissions (Fig 4.12 A - H). For this seaweed, the brominated compounds, CH_2Br_2 and $CHBr_3$, were released in higher concentrations than that of CH_2I_2 and CHI_3, the same was observed in *Sargassum binderi*. However, *Padina australis* showed no obvious differences between the iodinated and brominated compounds emitted. $CHBr_3$ is shown to be the dominant brominated halocarbon emitted by *Sargassum binderi* and *Turbinaria conoides* compared to CH_2Br_2. *Padina australis* remained as the weakest producer for both brominated compounds. Generally, $CHBr_3$ and CH_2Br_2 were found to

be the halocarbon compounds with the highest emission rates from both *Sargassum binderi* and *Turbinaria conoides* with the emission rate of $CHBr_3$ (Fig. 4.12 F) up to ten-fold higher than that of CH_2Br_2 (Fig. 4.12 E). For *Padina australis*, the dominant halocarbon compounds released were in the form of $CHBr_3$ and CH_2I_2.

Based on Figure 4.13, the emission rate of $CHBrCl_2$ by *Sargassum binderi* seems to dominate over *Turbinaria conoides* and *Padina australis* while the emissions of $CHBr_3$, CH_2Br_2, CH_2BrI and CH_2I_2 seemed to be dominated by *Turbinaria conoides* as compared to the other two species. The emission of such compounds seemed to be related, with reference to their position in the PCA analysis. The production of $CHBrCl_2$, $CHBr_2Cl$, CH_2BrCl, and emissions of CH_3I by the seaweeds, however, were not closely-related compared to the group of compounds mentioned earlier. There was no big difference between the emissions of halocarbon compounds as observed in *Padina australis*.

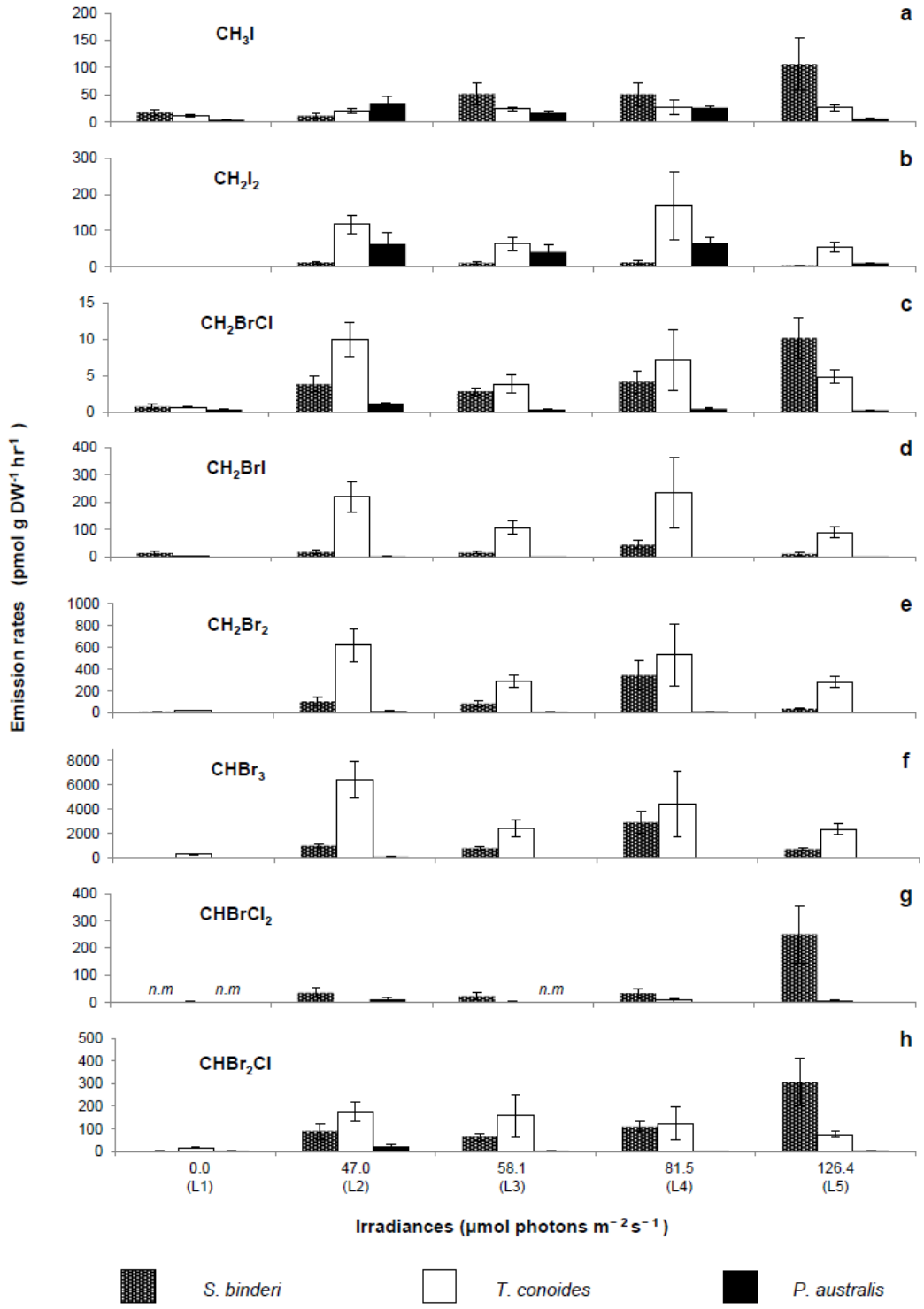

Figure 4.12 A – H: Halocarbon emission rates by the three tropical brown seaweeds under the different irradiances.
The bar represents the mean (n=5) for each irradiance with their standard error shown

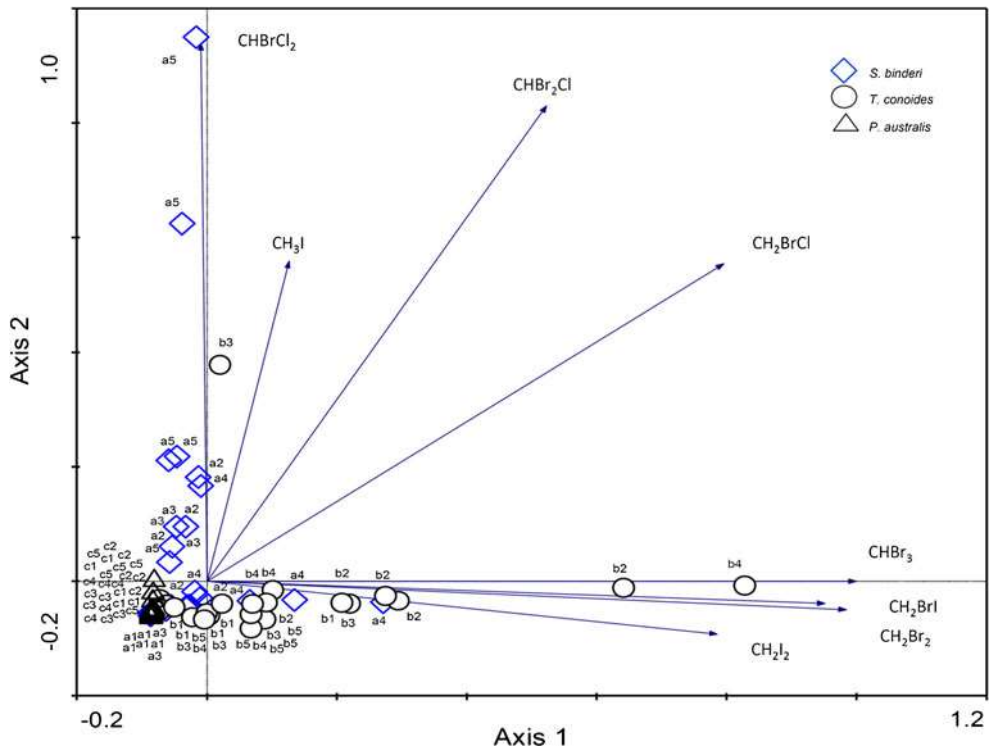

Figure 4.13: The Principle Component Analysis (PCA) between the emissions of halocarbons under the different irradiances by the three seaweed species. [a,b,c] indicate seaweed species. [a] = *Sargassum binderi*, [b]= *Turbinaria conoides*, [c]= *Padina australis*; Light levels L1-L5 indicated by [1,2,3,4,5]

4.2.2 Correlations between halocarbon emissions and irradiance by the three seaweeds

Halocarbon emission by *Sargassum binderi* showed a significant positive correlation between halocarbon emission rate and irradiance (Table 4.14). For *Sargassum binderi* CH_3I, CH_2BrCl, CH_2Br_2, $CHBrCl_2$, $CHBr_2Cl$ and $CHBr_3$ emissions correlated with irradiance (r values 0.40 - 0.79). *Turbinaria conoides* showed significant positive correlation between irradiance and the emission rates of CH_2Br_2, CH_2BrI, $CHBr_3$ and CH_2I_2 ($r < 0.50$; $p < 0.05$). None of the halocarbons emitted by *Padina australis* were significantly correlated with the irradiance levels used in this study.

The emission rates of the brominated compounds, $CHBr_3$ and CH_2Br_2, were the only two to correlate with irradiance by both *Sargassum binderi* and *Turbinaria conoides*, although the correlation coefficients (*r*) were between 0.40 - 0.50. For the mixed compounds, $CHBr_2Cl$ emission rates by *Sargassum binderi* has the highest (r = 0.79) positive correlation to irradiance, followed by $CHBrCl_2$ (r= 0.60) (Table 4.14). The extent to which the emitted compounds were influenced by irradiance varied among the species. The strongest correlation between halocarbon emission rates with irradiance was observed in the release of $CHBr_2Cl$ (r= 0.70*; p*< 0.01*)* by *Sargassum binderi*.

Table 4.14: The Pearson Correlation Coefficient (*r*) between the emissions of volatile halocarbon compounds produced by the three brown tropical seaweeds with irradiance.

Compounds	*Sargassum binderi*	*Turbinaria conoides*	*Padina australis*
CH_3I	0.59^{**}	0.40^{NS}	0.04^{NS}
CH_2BrCl	0.64^{**}	0.16^{NS}	-0.22^{NS}
CH_2Br_2	0.40^{*}	0.45^{*}	-0.06^{NS}
$CHBrCl_2$	0.60^{**}	0.29^{NS}	-0.31^{NS}
$CHBr_2Cl$	0.79^{**}	0.34^{NS}	0.23^{NS}
CH_2BrI	0.06^{NS}	0.45^{*}	0.14^{NS}
$CHBr_3$	0.52^{**}	0.45^{*}	0.09^{NS}
CH_2I_2	0.14^{NS}	0.47^{*}	0.20^{NS}

*= $p < 0.05$; **= $p < 0.01$; NS= Non-significant
Emission rate data were pooled from five replicates for five irradiance levels; n =25, except for emissions of $CHBrCl_2$ by *Sargassum binderi* (n = 20) and *Padina australis* (n= 16)

4.2.3 Correlations between halocarbon emissions and maximal quantum yield of seaweeds

The effect of irradiance on F_v/F_m values of seaweeds was significant ($p < 0.01$) for all three seaweed species (Table 4.15). The mean F_v/F_m values of the three seaweed species were between 0.5 – 0.7 under the different irradiances. The mean F_v/F_m values of *Sargassum binderi* were significantly higher in illuminated condition (L2 – L5) than in the dark (L1), with mean values at L2 and L3 significantly higher than L4 and L5. The mean F_v/F_m values of *Turbinaria conoides* at L5 were significantly lower than L1-L4. Values of F_v/F_m at L4 were lower than those at L1 & L5 and L2 & L3 for *Padina australis*.

The correlations between F_v/F_m values of the seaweeds with halocarbon emission rates are shown in Table 4.16. The emission rates of CH_2Br_2, CH_2BrI and $CHBr_3$ by *Sargassum binderi* were found to be positively correlated to the F_v/F_m values of the seaweeds while the release of $CHBrCl_2$ (r = -0.65) was inversely correlated. However, the release of the same compound by *Padina australis* was positively correlated (r = *0.60)*, together with the emission rates of $CHBr_2Cl$ (r = 0.40), CH_2BrI (r = 0.85) and $CHBr_3$ (r = 0.79). The correlation between the emission rate of CH_2BrI with F_v/F_m values by *Padina australis* was found to be the highest (r = 0.85) followed by $CHBr_3$ (r = 0.78) emission from the same species, while the third highest correlation was of CH_2Br_2 (r = 0.69) emission by *Sargassum binderi* with their F_v/F_m values.

Table 4.15: The mean F_v/F_m ± S.D. values of the seaweeds measured under different irradiance levels in the laboratory experiment. Data was statistically analyzed using One-way ANOVA

Irradiance (µmol photons m^{-2} s^{-1})	*S. binderi*	*T. conoides*	*P. australis*
0.0 (L1)	0.547 ± 0.156^{a}	0.695 ± 0.034^{a}	0.686 ± 0.035^{a}
47.0 (L2)	0.718 ± 0.039^{b}	0.720 ± 0.033^{a}	0.745 ± 0.021^{c}
58.1 (L3)	0.700 ± 0.040^{b}	0.678 ± 0.041^{a}	$0.734 \pm 0.024^{b,c}$
81.5 (L4)	$0.671 \pm 0.053^{b,c}$	0.693 ± 0.044^{a}	0.637 ± 0.067^{d}
126.4 (L5)	$0.604 \pm 0.080^{a,c}$	0.559 ± 0.081^{b}	$0.706 \pm 0.037^{a,b}$

Data presented are mean values of F_v/F_m from a total of 30 replicates (n=30), except for *Padina australis* at L3 (n=28) and L4 (n= 29)
S.D. = standard deviation
a,b,c denote homogenous group ($p<0.01$) according to post-hoc Tukey's test

Table 4.16: The Pearson Correlation Coefficient (*r*) between the maximal quantum yields (F_v/F_m) of the seaweeds and their halocarbon emissions.

Compounds	*Sargassum binderi*	*Turbinaria conoides*	*Padina australis*
CH_3I	0.042^{NS}	-0.161^{NS}	0.056^{NS}
CH_2BrCl	0.026^{NS}	0.096^{NS}	0.274^{NS}
CH_2Br_2	0.692^{**}	0.041^{NS}	0.056^{NS}
$CHBrCl_2$	-0.65^{**}	-0.343^{NS}	0.597^{*}
$CHBr_2Cl$	0.167^{NS}	0.085^{NS}	0.389^{NS}
CH_2BrI	0.355^{NS}	0.066^{NS}	0.847^{**}
$CHBr_3$	0.592^{**}	0.053^{NS}	0.787^{**}
CH_2I_2	0.688^{**}	0.047^{NS}	-0.011^{NS}

*= $p < 0.05$; **= $p < 0.01$; NS= Non-significant
Emission rate data were pooled from five replicates for all F_v/F_m values; n =25, except for emissions of $CHBrCl_2$ by *Sargassum binderi* (n = 20) and *Padina australis* (n= 16)

CHAPTER 5

DISCUSSION

5.1 Halocarbon content in Port Dickson during the survey

The tri-monthly field data taken from the survey site between March 2010 and June 2011 showed constant halocarbon mixing ratios for the long-lived compounds e.g. CFC-11 and CFC-113 (Table 5.1). There is less variability in the mixing ratio measurements observed for the longer-lived anthropogenic compounds having a longer atmospheric lifetime i.e. CFC-113 compared to the long-lived anthropogenic compounds having a shorter atmospheric lifetime i.e. CH_3CCl_3. The variability in the average mixing ratios increases with shorter atmospheric lifetime. This is most probably caused by the long atmospheric lifetime of the compounds. The enactment of the Montreal Protocol 1987 resulted in a drastic reduction in the emission of the long-lived CFC compounds since the end of the 1980s followed by a complete phase-out for the long-lived CFC compounds (Kim *et al*., 2011). Although it had been years since these compounds had been completely phased out, they still linger in the due to their chemical inertness (Kim *et al*., 2011). The only possible sink mechanism for these compounds is their photolysis by the ultraviolet ray in the stratosphere which will result ozone catalytic ozone destruction (Kim *et al*., 2011; Sander *et al*., 2006).

Data acquired through the Advanced Global Atmospheric Gases Experiment (AGAGE) network online saw the average background mixing ratio of the CFC-113 compound at around 75 ppt in year 2011. The measurement was done in Mace Head, Ireland (AGAGE, 2012). In comparison to the AGAGE data, the average mixing ratio of CFC-113 recorded at our survey site at Cape Rachado was 77.62 $\pm$ 2.75 ppt, a slightly higher amount compared to the Mace Head measurement. The differences between the

calibration gases used by the AGAGE network and the calibration gas used in study (NOAA ESRL), the different sensitivity between systems and localized emissions from old refrigerating devices might contribute to the slightly higher readings observed in this study.

The mixing ratio for the compound CFC-11 from the AGAGE network was reported to be around an average of 240 ppt in year 2011 taking into account measurements made from different places worldwide including Ireland, Oregon/California, Barbados, Samoa, and Tasmania. In comparison, the observed mixing for the same compound from Cape Rachado in this study was 243.67 ± 9.53 ppt, which was slightly higher than the readings reported by AGAGE. This might be contributed by the same factors as mentioned in the higher CFC-113 reading.

In addition to the decline in the atmospheric concentration of CFC compounds through the years since the Montreal Protocol, the decline in the atmospheric content of the chlorinated compound, CCl_4 was also observed (AGAGE, 2012). The reported level of CCl_4 in Ireland, Oregon/California, Barbados, Samoa, and Tasmania ranged between 85 – 87 ppt, a slightly lower than the observed reading in this study which has an average of 90.80 ± 3.72 ppt at Cape Rachado, Port Dickson. The direct emission of CCl_4 into the atmosphere due to the major role of the compound as an industrial cleaning solvent (Shao *et al.*, 2011) may contribute to the higher mixing ratio observed in this study. Also, the presence of swimming pools and the water chlorination process that occurs in the vicinity of the sampling site (Ullrich, 1982) may contribute to the higher CCl_4 level observed.

The atmospheric mixing ratio of CH_3CCl_3 reported in countries including Ireland, Oregon/California, Barbados, Samoa, and Tasmania in year 2011 was between 6 – 8 ppt (AGAGE, 2012), which is almost similar from our results of 7.89 ± 0.74 ppt at Cape Rachado.

Although the CFCs and CCl_4 measurements observed in this study were slightly higher than those reported by the AGAGE network, these values still fall below the reported levels in Guangzhou city, China (Chan *et al.*, 2006). The atmospheric mixing ratios for CFC-113, CFC-11 and CCl_4 reported in China were 97, 361 and 138 ppt. Also, the levels of CFC-11 and CCl_4 observed in this study were lower than those reported in Las Vegas, USA which is among one of the places that has recorded the lowest readings. The levels of CFC-11 and CCl_4 were 259 and 99 ppt respectively as reported in Las Vegas (Barletta *et al*., 2006).

Table 5.1: Summary of average mixing ratio for long-lived halocarbon compounds at Cape Rachado, Port Dickson from March 2010 until June 2011

	CFC-113	CFC-11	CCl_4	CH_3CCl_3
Atmospheric Lifetime (WMO 2007)	85 years	45 years	26 years	5 years
Mac 2010	78.05	249.81	93.29	7.29
Jun 2010	74.37	239.27	88.8	7.83
Sep2010	74.30	225.98	85.54	7.41
Dec2010	80.05	248.90	95.78	8.16
Mac 2011	78.18	249.34	92.48	7.38
June 2011	80.77	248.68	88.92	9.24
Average	77.62	243.66	90.80	7.89
S.D	2.75	9.54	3.72	0.74
% Variation	3.55	3.91	4.09	9.40

For the short-lived compounds, their mixing ratios in the atmosphere at the sampling site showed much higher variability compared to the long-lived compounds (< 10%),

which ranges from 49.14 % - 81.12 % (Table 5.3). This high variability was due to the nature of the compounds itself, which are much shorter-lived, and the fresh emission from sources including biogenic and anthropogenic activities. In contrast, some of the long-lived compounds had either been phased-out or under tight regulation such that fresh emission levels were very low.

The atmospheric mixing ratios for C_2Cl_4 and $CHBr_2Cl$ taken during the September 2010 sampling were not recorded due to peak contamination by nearby peaks in the chromatogram therefore making peak integration not possible. The mixing ratios for compounds $CHBrCl_2$ and CH_2Br_2 were recorded as one as their peaks appear very close to each other in the chromatogram until such extent that they are no longer distinguishable and hence, recorded as a double peak. The mixing ratio of the two compounds individually could not be achieved in this study. Nevertheless, the double peak readings are able to provide a rough estimation of the atmospheric content for the two compounds mentioned.

As one of the compound most commonly released by seaweeds, the average mixing ratio of $CHBr_3$ recorded from this study was 11.92 ± 6.08 ppt with the highest level recorded at 22.97 ppt during the September 2010 sampling. This average value of $CHBr_3$ was slightly higher than the background values (2-5 ppt) recorded by Pyle *et al*. (2011) at a bay at Kunak, Sabah, where abundant biomass of macroalgae dominated by the brown seaweeds. The high value recorded in September 2010 was due to the two readings that occurred between 1648 and 1912 on the 22nd of September (Fig. 4.4 (H)) which measured around 80 ppt. These values could be some very localized emissions caused by onshore wind (Pyle *et al*., 2011). Wind speed and wind direction data acquired from the nearest meteorological station at the Atherton Estate (02 ° 33’ N 101°

55’ E) which was about 17 km away from our sampling site recorded a south-west wind at a velocity of 7.3 m/s on the 22nd of September 2010. This onshore wind might have also picked up some $CHBr_3$ released by the oceanic source along its path especially when the biomass study showed the highest average value for the same sampling month.

Table 5.2: Summary of average mixing ratio for short-lived halocarbon compounds at Cape Rachado, Port Dickson from March 2010 until June 2011

	C_2Cl_4	$CHBrCl_2$ + CH_2Br_2	$CHBr_2Cl$	$CHBr_3$
Atmospheric Lifetime (WMO 2007)	99 days	78 / 120 days	69 days	26 days
Mac 2010	4.72	6.04	4.84	7.80
Jun 2010	5.56	9.26	3.87	5.34
Sep2010		5.64		22.97
Dec2010	13.21	26.71	8.45	11.51
Mac 2011	2.62	6.86	2.00	10.91
June 2011	1.80	23.18	4.72	13.01
Average	5.58	12.95	4.78	11.92
S.D	4.53	9.44	2.35	6.08
% Variation	81.12	72.92	49.14	50.98

5.2 Correlation of seaweed standing biomass to selected halocarbons at Port Dickson during the survey

The seaweed biomass recorded from our survey site showed that Cape Rachado was dominated by the brown seaweeds during the entire survey period. The total biomass of the brown seaweeds (71.0 % of total biomass) was more than that of both the green (28.7 %) and red seaweeds (0.4 %) (Fig.4.8). Of these brown seaweed species, the combined biomass of the three common brown seaweeds *Sargassum binderi*, *Padina australis* and *Turbinaria conoides* was found to be high among the seaweed community found at the site, with their combined contribution of more than 50 % of total biomass in the June 2010, September 2010 and June 2011 sampling while their combined

contribution stayed above 40 % during the March 2010 and March 2011 sampling (Fig. 5.1(A)). *Sargassum binderi* seems to be the dominant brown seaweed species at the survey site as compared to the other species with the highest recorded biomass value of 426.15 ± 181.33 g DW m^{-2} during the September 2010 survey (Fig. 5.1 (B)).

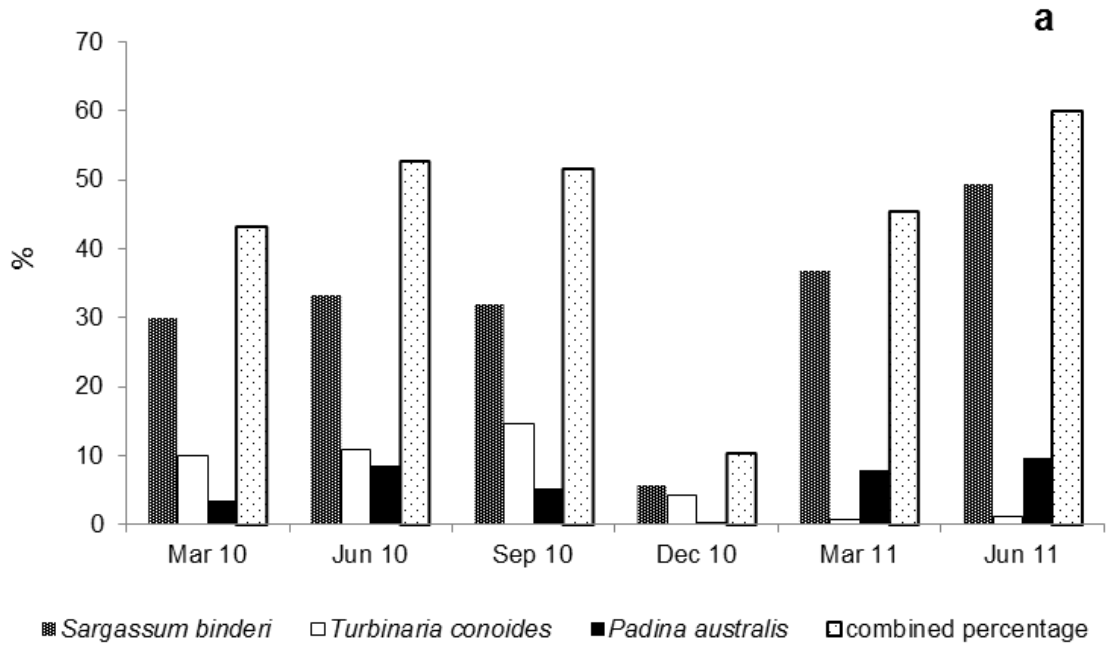

Figure 5.1 (A): Individual and combined percentage of the standing biomass for the three brown seaweed species over total collected seaweed biomass throughout the sampling period at Cape Rachado, Port Dickson.

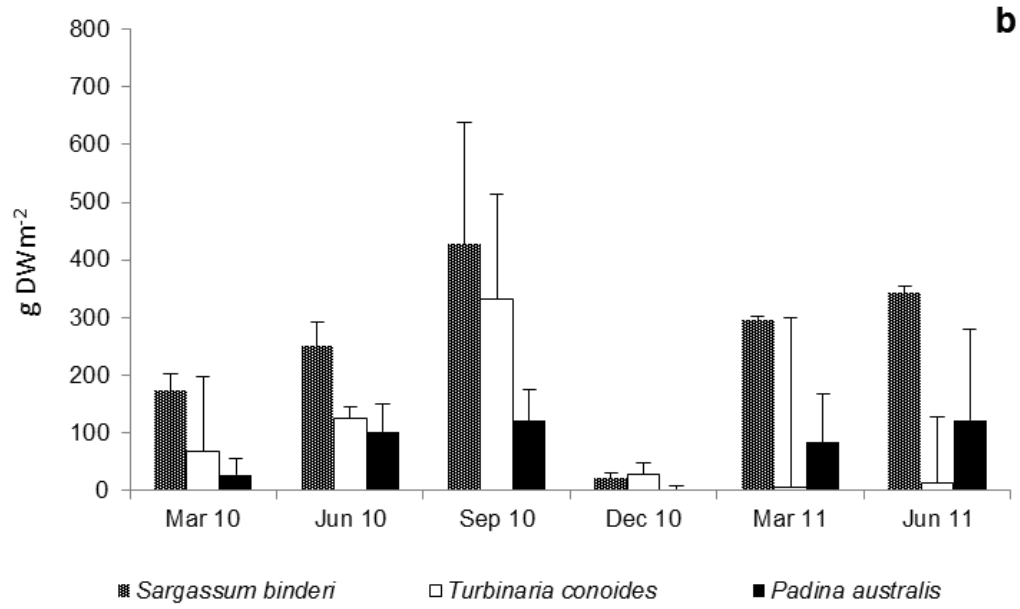

Figure 5.1 (B): Standing biomass for the three brown seaweed species (g DW m^{-2}) at Cape Rachado, Port Dickson throughout the sampling period

Since the emission of the long-lived compounds i.e. CFC-113, CFC-11, CCl_4 and CH_3CCl_3 were of anthropogenic origin; their atmospheric mixing ratios were assumed to be purely related to human activities and therefore not considered for correlation with seaweed biomass. As for the compound C_2Cl_4 which is emitted primarily from human activities (Guo *et al*., 2004; WMO 2007), their atmospheric mixing ratio was not considered for correlation with seaweed biomass as well. Only four compounds, i.e. $CHBrCl_2$, CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$ which are of natural origin or predominantly natural (WMO, 2007) were considered for correlation analysis with seaweed biomass.

Having tested the correlations between the total seaweed biomass and the biogenic halocarbon concentration in the atmosphere (Table 4.13, page 93), it was found that there was no significant correlation between the total seaweed biomass with the atmospheric concentration of the selected halocarbon compounds, despite the fact that 70% of the world $CHBr_3$ emissions was attributed to seaweeds (Carpenter & Liss, 2000).

However, there were few weak correlations ($\rho < 0.5$) observed between the biomass of selected seaweed species with the atmospheric concentration of biogenic or predominantly natural halocarbons compounds. Very weak correlations between the individual biomass of the phaeophyte, *Sargassum binderi* ($\rho = 0.2370$), and the chlorophyte, *Caulerpa racemosa* ($\rho = 3928$) was observed with the $CHBr_3$ content in the atmosphere at Cape Rachado. The biomass of *Caulerpa* sp. was also observed to be weakly correlated to the atmospheric content of CH_2Br_2 + $CHBrCl_2$. Besides, chlorophyll-a content of the seawater, which indicated the presence of phytoplankton, was also found to be weakly correlated ($\rho = 0.4642$) with the $CHBr_2Cl$ contents in the atmosphere. The non-significant correlations of the seaweed biomass to the halocarbon compounds might have been caused by the influence of human activities such as water chlorination, which produce $CHBr_3$ as by-product (Allonier *et al*., 1999) besides the production of chlorinated compounds, changes in onshore breeze (Pyle *et al*., 2011), possible contribution of halocarbon from nearby mangrove (Manley *et al*., 2007), localized emissions and many others. Therefore, in order to have a better understanding on the contribution of the seaweeds in the emission of halocarbons, a laboratory study was followed under a controlled incubation experiment which was able to minimize the uncertainties caused by the many contributing sources in the field.

5.3 Effect of irradiance on halocarbon emissions by selected seaweeds under laboratory condition

Data from the seaweed biomass survey conducted at Cape Rachado, Port Dickson showed the site was dominated by the brown seaweeds. *Sargassum binderi* was found to have the highest dominance value (33.29 %) and was also the species with the highest IVI value of 22.19 throughout the entire survey. Therefore, together with *Turbinaria conoides* and *Padina australis* which were available during the experimental

period, they were selected for laboratory incubation to look at their halocarbon emissions under the influence of irradiance.

The laboratory data acquired through this study showed that irradiance as an environmental factor has positively influenced the emission rates of some volatile halogenated compounds by the brown seaweeds *Sargassum binderi* and *Turbinaria conoides* within the range of irradiance used. Both ultraviolet radiation *a* and *b* were not detected where the incubation flasks were positioned. Though different irradiances had been tested, the findings from this study that irradiance, in general, influences halocarbon emission by seaweeds, agreed with what was suggested by previous studies where some brown, green and red temperate and polar seaweeds had been studied (Carpenter *et al*., 2000; Goodwin *et al*., 1997; Laturnus *et al*., 1998; Laturnus *et al*., 2000; Marshall *et al*., 1999; Mtolera *et al*.,. 1996; Nightingale *et al*., 1995). The production of halocarbons has long been thought to be a defense mechanism, protecting the algae from herbivores, bacteria or even as a chemical defense against oxidative stress. There have been a few suggestions concerning the formation of halogenated compounds by the seaweeds. The emission of methyl halide was suggested to involve the S-adenosylmethionine (SAM): halide ion methyl transferase reactions (Ohsawa *et al*., 2001; Wuosmaa & Hager 1990), while the formation of polyhalogenated compounds by seaweeds could be attributed to the haloperoxidases activities in the seaweeds as a response to the presence of hydrogen peroxide. Most of the correlations in this study, though weak, between the halocarbon compounds with irradiance (Table 4.13) suggest that these halocarbon compounds may be produced from hydrogen peroxide, which is made available by the Mehler reaction or pseudocyclic photophosphorylation during photosynthesis (Manley & Barbero 2001; Pedersén *et al*., 1996), which is highly influenced by light. Other possible sources for production of

hydrogen peroxide includes mitochondrial electron transport chain (Cadenas, 1989) and other cellular sources. The correlation between irradiance and the emission rates of the di- and tri- substituted halomethanes e.g. CH_2Br_2, CH_2I_2, CH_2BrI, $CHBr_3$, $CHBr_2Cl$ by *Sargassum binderi* and *Turbinaria conoides* in this study, showed a possibility of bromoperoxidase involvement in the formation of the compounds mentioned (Goodwin *et al*., 1997; La Barre *et al*., 2010; Manley & Barbero, 2001). Iodinated and brominated compounds are believed to be produced by haloperoxidases through the direct halogenation of organic compounds such as β-keto acids and cyclic β-diketones (Theiler *et al*., 1978). Although suggested to be of a different formation pathway compared to the polyhalogenated compounds, the emission rate of the monohalogenated compound, CH_3I by *Sargassum binderi* was also observed to be influenced by irradiance. We have not found any literature reporting this. However, the highest irradiance achievable for this laboratory study was much below the natural irradiance. The measured irradiance at the sampling site could reach up to 1660 μmol photons m^{-2} s^{-1} during midday.

In comparing the halocarbon emission rates between the three seaweed species, emission of brominated and iodinated compounds by *Sargassum binderi* and *Turbinaria conoides* were observed to be generally higher compared to *Padina australis*. The highest emission rate was recorded by *Turbinaria conoides* in the $CHBr_3$ emission, which is more than 6000 pmol g DW^{-1} h^{-1}. This may be due to the differences in morphology between the seaweed species. *Sargassum binderi* and *Turbinaria conoides* both contain vesicle cells that help in their buoyancy while attaching to a substratum in their natural growth environment. These vesicle cells contain high amounts of bromine and iodine (Wolk 1968), and any breakage in these fragile cells results in the release of free halogens. That, together with the production of hydrogen peroxide from

photosynthesis and the presence of peroxidases in the seaweeds, gives rise to a higher emission of halocarbons (Abrahamsson & Pedersén, 2000).

Since irradiance affects photosynthetic activity, photosynthesis-related mechanisms had been speculated to influence the formation of halocarbons by seaweeds (Goodwin *et al*., 1997). To further investigate the relationship between photosynthesis and halocarbon emission by the seaweeds, the maximal quantum yield ratios for the seaweeds were compared with their respective halocarbon emissions. The use of this technique minimized stress and damage to the plants (Bilger *et al*., 1995), besides taking into consideration the different levels of tolerance of seaweed species towards oxidative stress, which directly interferes with the photosynthetic process (Dummermuth *et al*., 2003). The release of CH_2Br_2, CH_2BrI, $CHBr_3$ and CH_2I_2 by *Sargassum binderi* as well as the release of $CHBr_2Cl$, CH_2BrI and $CHBr_3$ by *Padina conoides* was positively correlated with the F_v/F_m values, which is a sensitive indicator for plant photosynthetic performance. The increase of halocarbon compounds with increased F_v/F_m values might be due to the build-up of photosynthetic hydrogen peroxide in the seaweeds without hydrogen peroxide reaching a level that could cause plant stress or membrane destruction that inhibits photosynthesis and respiration in the plants. $CHBrCl_2$ emission rates of by *Sargassum binderi* was negatively correlated with the F_v/F_m values, in contrast to that observed by *Padina australis*. The general correlation between F_v/F_m values of the seaweeds with the chlorinated mixed halocarbon emissions displayed by these three seaweed species was found to be non-significant, weak, or opposing. The halocarbon compounds that were better correlated ($r > 0.5$) to the F_v/F_m values of the seaweeds apart from $CHBrCl_2$were the di- and tri-substituted halomethanes e.g. CH_2Br_2, CH_2I_2 and $CHBr_3$, and the hetero-substituted form of CH_2BrI. This may suggest the involvement of vanadium-containing bromoperoxidases which are readily found in the

brown seaweeds while vanadium-containing chloroperoxidases were reported to be found only in terrestrial fungi and bacteria (La Barre *et al*., 2010; Winter & Moore 2009). Although hydrogen peroxide produced from the photosynthetic activities of plants was found to contribute to their halocarbon emissions (Goodwin *et al*., 1997; Manley & Barbero 2001), it is important to note that hydrogen peroxide is not only internally produced by the seaweeds via photosynthesis, but also mitochondrial respiration and enzymatic catalysis. It is also present externally in illuminated seawater (Manley & Barbero 2001). Besides, there are also other enzymes involved in destroying hydrogen peroxide in seaweeds apart from the haloperoxidases, among them are the peroxisome-associated catalase (Gross, 1993). These, together with the possible emission of dissolved organic matter (DOM) by the seaweeds used in this experiment (Lin & Manley 2012; Wever *et al*,. 1991) might explain why the correlations between F_v/F_m values of seaweeds with the emissions of some of the halogenated compounds were significant in certain seaweeds while some were not. Only three compounds, CH_3I, CH_2BrCl and $CHBr_2Cl$ were shown to be non-correlated to the F_v/F_m values by all of the three seaweed species. Thus, their formation might not be photosynthetically related.

The results from this study were compared with halocarbon emission rates reported from different species of temperate and polar brown seaweeds (Table 5.3). The emission rates of CH_3I by the tropical seaweeds were generally close to the range reported in the temperate seaweeds except for what was emitted by *Sargassum binderi* at the light irradiance level of 126.4 μmol photons m^{-2} s^{-1} (106 pmol g DW^{-1} hr^{-1}). The emission rates for CH_3I were found to be higher (0.8 – 16.7 pmol g FW^{-1} hr^{-1}) in all the tropical seaweeds than *Adenocystis utricularis*, a polar species (0.03 pmol g FW^{-1} hr^{-1}). The maximum emission rate for brominated compounds, CH_2Br_2 and $CHBr_3$ was found to be higher in tropical brown seaweeds than temperate; up to twice (CH_2Br_2) and five

times ($CHBr_3$) higher. The highest $CHBr_3$ emission rate in tropical *Turbinaria conoides* was up to 500 times higher than what was observed in the polar species. The $CHBr_2Cl$ emission rate by *Ascophyllum nodosum* (70.5pmol g FW^{-1} hr^{-1} was around 1.5 times higher than the highest rate recorded by the tropical *S. binderi* (48.5 pmol g FW^{-1} hr^{-1}). In general, the emission rates of CH_3I, CH_2I_2, CH_2Br_2 and $CHBr_3$ by the polar species (Table 5.3) were lower than that of tropical and temperate species. Meanwhile, the highest emission rates of CH_2Br_2 and $CHBr_3$ recorded by the tropical species was higher than the temperate species, while the emission of $CHBr_2Cl$ was lower in tropical species compared to the temperate species. Giese *et al.* (1999) reported higher emission of both CH_2Br_2 and $CHBr_3$ but decreased emission of iodinated compounds in subtropical species compared to polar species in general. Since emission of halocarbons has been widely reported to be a stress mechanism (Dummermuth *et al.,* 2003; Pedersén *et al.,* 1996), the higher release rate in tropical species seen here may not be necessarily a simple chemical temperature dependency alone as different species are adapted to different temperature in their respective habitat. However, these comparisons were made based on the laboratory incubation of seaweeds instead of an in situ experiment; therefore, there are still many uncertainties that may exist in the natural condition that might influence the emission rates of the tropical seaweeds.

Table 5.3: Comparison of halocarbon emission rates* (pmol g DW/FW $^{-1}$ hr^{-1}) between the tropical, temperate & polar brown seaweeds

Species	Region	Irradiance	CH_3I		CH_2I_2		CH_2Br_2		$CHBr_3$		CH_2BrI		$CHBr_2Cl$		$CHBrCl_2$		CH_2BrCl	
			DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW
Sargassum binderi[a]	Tropical	0	17.9 ±5.5	2.8 ±0.9	0.0 ±0.0	0.0 ±0.0	7.0 ±3.0	1.1 ±0.5	4.7 ±1.6	0.7 ±0.3	13.4 ±5.9	2.1 ±0.9	0.5 ±0.2	0.1 ±0.0	*n.m*	*n.m*	0.7 ±0.4	0.1 ±0.1
		47	11.6 ±4.5	1.8 ±0.1	12.1 ±3.1	1.9 ±0.5	105 ±39.1	16.5 ±6.1	982 ±176	155 ±27.5	17.5 ±7.3	2.8 ±1.1	88.3 ±35.3	13.9 ±5.5	36.3 ±18.4	5.7 ±2.9	3.8 ±1.1	0.6 ±0.2
		58	51.5 ±20.2	8.1 ±3.2	10.9 ±1.9	1.7 ±0.3	86.3 ±27.2	13.6 ±4.2	794 ±143	125 ±22.3	15.2 ±3.7	2.4 ±0.6	64.0 ±17.0	10.1 ±2.7	24.2 ±11.4	3.8 ±1.8	2.8 ±0.5	0.4 ±0.1
		82	50.7 ±22.2	8.0 ±3.5	12.6 ±3.8	2.0 ±0.6	343 ±133	54.1 ±20.7	2.9 x 10^3 ±883	458 ±138	44.2 ±16.1	7.0 ±2.5	110 ±22.0	17.3 ±3.4	34.5 ±17.0	5.4 ±2.7	4.1 ±1.5	0.6 ±0.2
		126	106 ±27.4	16.7 ± 4.3	3.7 ±0.4	0.6 ±0.1	39.4 ±5.4	6.2 ±0.8	714 ±127	113 ±19.9	10.4 ±6.9	1.6 ±1.1	308 ±104	48.5 ±16.2	250 ±104	39.4 ±16.2	10.2 ± 2.8	0.1 ±0.4
Turbinaria conoides[a]	Tropical	0	11.8 ±1.7	2.0 ±0.3	1.9 ±0.5	0.3 ±0.1	19.1 ±0.9	3.3 ±0.2	279 ±32.2	48.2 ±5.5	3.0 ±0.3	0.5 ±0.1	16.4 ±2.9	2.8 ±0.5	3.5 ±1.7	0.6 ±0.3	0.6 ±0.1	1.7 ±0.0
		47	20.7 ±4.4	3.6 ±0.7	118 ±25.1	20.4 ±4.3	620 ±154	107 ±26.1	6.5 x 10^3 ±1.5 x 10^3	1.1 x 10^3 ±256	221 ±56.0	38.1 ±9.6	175 ±41.3	30.3 ±7.0	1.9 ±1.2	0.3 ±0.2	9.9 ±2.4	0.7 ±0.4
		58	24.0 ±4.3	4.1 ±0.7	63.3 ±19.2	10.9 ±3.3	292 ±51.9	50.4 ±8.8	2.4 x 10^3 ±687	417.4 ±117	107 ±25.7	18.5 ±4.4	157 ±93.4	27.0 ±15.9	3.2 ±1.7	0.6 ±0.3	3.8 ±1.3	1.2 ±0.2
		82	27.7 ±13.7	4.8 ±2.3	168 ±93.5	29.0 ±15.9	533 ±285	92.1 ±48.5	4.5 x 10^3 ±2.7 x 10^3	769.4 ±455	234 ±130	40.4 ±22.1	124 ±75.1	21.5 ±12.8	9.4 ±6.0	1.5 ±1.0	7.1 ±4.2	0.8 ±0.7
		126	26.5 ±5.3	4.6 ±0.9	56.0 ±13.6	9.7 ±2.3	285 ±55.5	49.2 ±9.4	2.4 x 10^3 ±452	407.9 ±76.8	89.3 ±19.3	15.4 ±3.3	74.3 ±14.2	12.8 ±2.4	8.6 ±1.9	1.5 ±0.3	4.8 ±0.9	0.8 ±0.2
Padina australis[a]	Tropical	0	4.4 ±0.4	0.8 ±0.1	0.2 ±0.1	0.0 ±0.0	0.4 ±0.2	0.1 ±0.0	0.7 ±0.1	0.1 ±0.0	0.0 ±0.0	0.0 ±0.0	0.3 ±0.2	0.1 ±0.0	*n.m*	*n.m*	0.3 ±0.1	0.1 ±0.0
		47	34.7 ±11.4	6.1 ±2.0	64.1 ±31.0	11.3 ±5.5	15.1 ±2.7	2.7 ±0.5	68.7 ±6.1	12.1 ±1.0	0.7 ±0.2	0.1 ±0.0	21.1 ±6.0	3.7 ±1.0	12.5 ±5.3	2.2 ±0.9	1.1 ±0.1	0.2 ±0.0
		58	17.0 ±3.3	3.0 ±0.6	42.1 ±20.0	7.4 ±3.5	7.2 ±2.5	1.3 ±0.4	39.1 ±6.9	6.9 ±1.2	0.3 ±0.1	0.0 ±0.0	1.3 ±1.3	0.2 ±0.2	*n.m*	*n,m*	0.3 ±0.1	0.1 ±0.0
		82	26.5 ±3.2	4.7 ±0.6	65.7 ±14.5	11.6 ±2.6	12.0 ±2.6	2.1 ±0.5	0.4 ±0.1	0.1 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.0	0.0 ±0.0	2.2 ±1.3	0.4 ±0.2	0.4 ±0.1	0.1 ±0.0
		126	6.1 ±0.6	1.1 ±0.1	10.2 ±1.3	1.8 ±0.2	0.0 ±0.0	0.0 ±0.0	27.8 ±3.8	4.9 ±0.7	0.1 ±0.0	0.0 ±0.0	2.8 ±0.3	0.5 ±0.1	2.4 ±0.4	0.4 ±0.1	0.2 ±0.0	0.0 ±0.0
Laminaria digitata[b]	Temperate	daylight	17.5		67.4		47.9		1.3 x 10^3		14.2		45.2					
Laminaria saccharina[b]	Temperate	daylight	4.0		15.8		190		1.1 x 10^3		6.5		25.6					
Ascophyllum nodosum[b]	Temperate	daylight	0.8		1.1		4.1		28.6		0.4		1.3					
Fucus vesiculosus[b,c,d]	Temperate	daylight	1.1 – 4.8		0.6		4.4	4.43	90.1	79.8	0.2		2.9					
Fucus serratus[b]	Temperate	daylight	0.0		0.1		1.6		32.8		0.1		1.9					
Pelvetia canaliculata[b]	Temperate	daylight	3.0		2.6		47.4		404		2.2		12.5					
Halidrys siliquosa[b]	Temperate	daylight	0.2		0.0		0.7		11.6		0.0		1.8					
Ascophyllum nodosum[d]	Temperate							51.7		237				70.5				
Macrocystis pyrifera[e]	Temperate	artificial						5.29-41.5		4.1-186								
		natural						3.34-21.1		22.4-50.1								
Alaria esculenta[f]	Polar	6-30				0.34[E]		0.2~		10~								
Adenocystis utricularis[f]	Polar	6-30		0.03[E]				0.13~		2.5~								

Irradiance (µmol photons $m^{-2}s^{-1}$); ~Estimated rate from chart reported; n.m= not measured
* Mean emission rates calculated based on dry weight (DW) or fresh weight (FW)
a This study at various artificial light irradiance level
b Carpenter *et al.*, 2000
c Baker *et al.*, 2000
d Gschwend *et al.*, 1985
e Goodwin *et al.*, 1997
f Giese *et al.*, 1999

5.4 Appraisal of Project / Future Areas for Research

This is the first study that closely monitored halocarbons release by Malaysian seaweeds both in the field and in the laboratory. No reports on correlation between seaweed biomass with halocarbon mixing ratio in the atmosphere had been made here in the tropics. The seaweed biomass and their world production are increasing with an annual growth rate of around 7.7% (FAO, 2011) due to their growing economic importance. The tropics is a very important region for such production. Therefore, more studies like this should be carried out to better understand the contribution of the tropical seaweeds towards the regional halocarbon budget since the literatures on halocarbon emissions by tropical seaweeds are really limited currently.

Due the tourist activities found at the sampling site at Cape Rachado, Port Dickson, the monitoring of naturally released halocarbons is interfered. Unaccounted localized emissions from human activities including the burning of biomass, and the emission of anthropogenic halocarbon compounds from swimming pools around the area made interpretation work somehow more complex, not mentioning the influence of environmental factors e.g. on-shore wind have in the determination of the atmospheric mixing ratio of the targeted compounds. Due to these limitations, alternative approaches that remove or reduce other contributing factors i.e. laboratory incubations, grab samples using gas canisters, should be developed for better measurements in the future. The poor correlations between seaweed biomass and the atmospheric halocarbon concentrations could possibly be improved by having a shorter time gap in between samplings e.g. bi-weekly sampling instead of the tri-monthly sampling.

The brown seaweeds especially the *Sargassum* species are important sources for alginic acid (Thomas & Subbaramaiah, 1991; Trono & Lluisma, 1990). With their potential of being exploited and mass cultivated, it is good to have a background knowledge of the influence it might exert on the coastal atmospheric composition should they be mass cultivated in the future. The study of irradiance effect on the halocarbon emission by three of the selected seaweeds provided a glimpse of the halocarbon emissions by seaweeds at their natural habitat when subjected to the different time of day and cloud cover which will affect the irradiation levels. Also, the information on the level of stress the seaweeds encountered that correlated with their halocarbon emission will be useful for future projects concerning the species studied.

Areas for future research:

1. To study the emission of halocarbon by more species of seaweeds especially those with economic importance at their natural habitat under a closed system i.e. using a flux chamber for better characterization of halocarbon emissions.
2. To relate the effect of different environmental factors (pH, temperature, salinity) have on the seaweeds stress level that affects their halocarbon emissions.
3. To establish molecular study that will identify the gene responsible for halocarbon emissions

CHAPTER 6

CONCLUSION

The atmospheric mixing ratio of long-lived halocarbon compounds i.e. CFC-11, CFC-113, CCl_4 and CH_3CCl_3. at Cape Rachado, Port Dickson showed low variability compared to the short-lived halocarbon compounds throughout the sampling period. The atmospheric contents of these long-lived halocarbon compounds were slightly higher than those reported in the AGAGE network but still falls below those reported in China and USA.

The seaweed biomass at Cape Rachado, Port Dickson, was found to be dominated by the brown seaweeds (71.0 %), followed by the green (28.7 %) and the red (0.4 %) throughout the sampling period from March 2010 until June 2011. Among the brown seaweeds, *Sargassum binderi* was found to be the most important species inhabiting the survey site with the highest IVI value of (22.19), followed by *Sargassum baccularia* (13.88) and *Caulerpa* sp. (12.52).

Although there were very weak correlations ($\rho < 0.5$) between the biogenic halocarbon contents (i.e. $CHBrCl_2$, CH_2Br_2, $CHBr_2Cl$ and $CHBr_3$) in the atmosphere with phytoplankton and individual seaweed species including *Sargassum binderi, Caulerpa* sp. and *Caulerpa racemosa*, however, in general, no significant correlation was observed between the total standing biomass of the seaweeds with the atmospheric halocarbon

contents at Cape Rachado throughout the sampling period. Therefore, The H_1 for the first hypothesis is rejected.

The seaweed incubation study in the laboratory suggested that some of the selected halocarbon compounds emitted by the three seaweeds species, namely *Sargassum binderi* Sonder ex J. Agardh, *Padina australis* Hauck, and *Turbinaria conoides* (J. Agardh) Kützing were influenced by irradiance especially the $CHBr_2Cl$ (r = 0.79; p< 0.01) and to some extent, photosynthetic activity, through the correlation between the halocarbon emission rate and the F_v/F_m values of seaweeds. The release of brominated and iodinated compounds especially CH_2BrI (r = 0.85; p < 0.01) and $CHBr_3$ (r = 0.79; p < 0.01) by *Padina australis* was positively correlated to the F_v/F_m values of seaweeds.

$CHBr_3$ was found to be the dominant halocarbon compound released by both *Turbinaria conoides* and *Sargassum binderi*. The emission of halocarbons at different rates suggested by the three seaweed species suggests that emissions of halocarbons by brown seaweeds were species-dependent. The differences in morphology between the seaweed species might influence their halocarbon emissions.

The contributions of these three brown seaweed species towards the local halocarbon pool at Cape Rachado, Port Dickson, are not to be overlooked, as these three species represent the majority of the seaweed community present at the site during our entire survey period. The comparison between the emission rates of tropical, temperate and polar brown

seaweeds showed higher emissions of some halocarbon compounds by the tropical seaweeds. This suggests that it is worthwhile to look further into the actual emission rates under the natural environment in the tropics in the future.

In conclusion, based on the results obtained,

Hypothesis I:

H_1: Atmospheric concentrations of halocarbons are affected by seaweed biomass should be rejected;

For hypothesis II,

H_o: Irradiance does not affect halocarbon emission by seaweeds should be rejected.

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