Halocarbon Emissions by Selected Tropical Seaweeds Exposed to Different Temperatures

Fiona Seh-Lin Keng[1,2], Siew-Moi Phang[1,3*], Noorsaadah Abd Rahman[4], Hui-Yin Yeong[1], Gill Malin[5], Emma Leedham Elvidge[6], William Sturges[5]

[1] Institute of Ocean and Earth Sciences (IOES), University of Malaya, 50603 Kuala Lumpur, Malaysia.

[2] Institute for Advanced Studies, University of Malaya, 50603 Kuala Lumpur, Malaysia.

[3] Faculty of Applied Sciences, UCSI University, Cheras, 56000, Kuala Lumpur, Malaysia.

[4] Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

[5] Centre for Ocean and Atmospheric Sciences, School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom.

[6]Faculty of Science, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, United Kingdom.

*Corresponding author: Email: *phang@um.edu.my*

ABSTRACT

Four tropical seaweeds, *Gracilaria manilaensis* Yamamoto & Trono, *Ulva reticulata* Forsskål, *Kappaphycus alvarezii* (Doty) L.M.Liao and *Turbinaria conoides* (J.Agardh) Kützing, collected from various habitats throughout Malaysia, were subjected to temperatures of 40, 35, 30, 25 and 20 °C in the laboratory. An exposure range of 21 – 38 °C is reported for Malaysian waters. The effect of the temperature exposures on the halocarbon emissions of the seaweeds were determined 4 and 28 h after treatment. The emission rates for a suite of six halocarbons commonly emitted by seaweeds, bromoform ($CHBr_3$), dibromomethane (CH_2Br_2), diiodomethane (CH_2I_2), iodomethane (CH_3I), dibromochloromethane ($CHBr_2Cl$) and dichlorobromomethane ($CHBrCl_2$), were measured using a cryogenic purge-and-trap sample preparation system coupled to a gas chromatography–mass spectrometry. The emission rate of $CHBr_3$ was the highest of the six halocarbons for all the seaweeds under all the temperatures tested, followed by CH_2Br_2, and CH_2I_2. The emission rates were affected by temperature change and exposure duration, but overall responses were unique to each seaweed species. Larger decreases in the emissions of $CHBr_3$, CH_2Br_2, CH_2I_2 and $CHBr_2Cl$ were found for *K. alvarezii* and *T. conoides* after 4 h at 40 °C. In both cases there was a >90% ($p < 0.05$) reduction in the F_v/F_m value suggesting that photosynthetic actitivity was severely compromised. After a 28 h exposure period, strong negative correlations ($r = -0.69$ to -0.95; $p < 0.01$) were observed between temperature and the emission of $CHBr_3$, CH_2Br_2 and CH_2I_2 for *U. reticulata, K. alvarezii* and *T. conoides.* This suggests a potential decrease in the halocarbon emissions from these tropical seaweeds, especially where the temperature increase is a prolonged event. Strong correlations were also seen between seaweed chlorophyll and carotenoid pigment contents and the emission rates for $CHBr_3$, CH_2Br_2 and CH_2I_2 ($r = 0.48$ to 0.96 and -0.49 to -0.96; $p < 0.05$). These results suggest that the regulation of halocarbon production versus reactive oxygen species production in seaweeds is an area worthy of further exploration.

Keywords

Gracilaria manilaensis (Gracilariaceae), *Ulva reticulata* (Ulvaceae), *Kappaphycus alvarezii* (Solieriaceae), *Turbinaria conoides* (Sargassaceae), Tropical Seaweeds, Climate Change, Temperature, Halocarbon Emissions, Bromoform

1. Introduction

Seaweeds are an important source of revenue in the tropics and especially within the Coral Triangle. The latest UN FAO statistics show an increase in seaweed cultivation with a three-fold increase in global production from 10.6 million tonnes in 2000 to 32.4 million tonnes in 2018 (FAO, 2020). Whilst economically important, seaweeds are also responsible for the emission of trace gases, including short-lived volatile halocarbons (Keng et al., 2013; Leedham et al., 2013; Mithoo-Singh et al., 2017; WMO, 2018), which are often implicated in affecting the tropospheric oxidizing capacity, contributing to stratospheric ozone loss and cloud nuclei formation, affecting radiative forcing and local climate (Read et al., 2008; Carpenter et al., 2013; Hossaini et al., 2016; Willis et al., 2016). Reactive bromine constituted around 5 (3–7) ppt, or 25%, of the total

stratosphere bromine recorded in 2016, with a large contribution from oceanic sources such as seaweeds, especially from emissions in the tropics (Ziska et al., 2013; WMO 2018). Iodine occurs in the shortest-lived gases and is thought to mostly influence tropospheric chemistry by virtue of this short lifetime, while the contribution of chlorine from these short-lived gases is thought to be small compared with other sources (Yvon-Lewis & Butler, 2015)

Many seaweeds occur in intertidal zone habitats where they are constantly exposed to diurnal and seasonal changes in temperature. Temperature changes could affect biochemical processes including general enzymatic and photosynthetic activities and the growth of seaweeds. A more than two-fold increase in photosynthetic rate was reported for the seaweed *Gracilariopsis lemaneiformis* (Bory) E.Y.Dawson, Acleto & Foldvik (Gracilariaceae) when temperature was increased from 12°C to 26 °C (Zou & Gao, 2014). A modeling study suggested significant increases in the photosynthetic activity of seaweeds with increases of 2 and 4°C in seawater temperature (Colvard et al., 2014). In Malaysia daily temperature fluctuations at coastal stations range between 5 and 10°C, and sea surface temperatures ranged from 25.7 to 33.9 °C (Tan et al., 2002; MMD, 2019). In the face of climate change, seaweeds are rendered vulnerable to further potential increases in air and sea surface temperatures. The ocean acts as a sink for heat energy in the Earth system with the 0–700 m and 700–2000 m ocean layers recording absorbtion rates of 5.31 ± 0.48 and 4.02 ± 0.97 ZJ $year^{-1}$ from 2005 to 2017 (Bindoff et al., 2019). The consequent ongoing changes in temperature could trigger ecosystem reshuffling and further changes in seaweed community composition in the future climate (Brodie et al., 2014), thereby affecting regional halocarbon budgets.

The emission of halocarbons by seaweeds is often affected by environmental changes in irradiance, pH, desiccation and temperature (Laturnus et al., 2000; Abrahamsson et al., 2003; Keng et al., 2013, 2020; Leedham Elvidge et al., 2015; Mithoo-Singh et al., 2017). Changes and fluctuations in temperature can result in elevated production of reactive oxygen species (ROS) in seaweeds. The production of halocarbons is proposed to arise from stress-related metabolic changes where H_2O_2 activates seaweed haloperoxidases (Almeida et al., 2001; Ohsawa et al., 2001; Baharum et al., 2013) to produce halocarbons such as $CHBr_3$ through the reaction of HOBr with dissolved organic matter (Lin & Manley, 2012; Liu et al., 2015).

There are several reports on the emission of halocarbons by seaweeds, but nothing has yet been published on the effect of temperature on emissions from tropical seaweeds. For temperate and polar seaweeds no obvious trends have been established between the emission of halocarbon and temperature change although increases in CH_2I_2 (Abrahamsson et al., 2003) and $CHBr_3$ (Laturnus et al., 2000) have been seen with higher temperature exposure of the brackish water-living *Cladophora glomerata* (Linnaeus) Kützing (Cladophoraceae) and the Antarctic species *Gymnogongrus antarcticus* Skottsberg (Phyllophoraceae) respectively. In this paper, we present the first dedicated study on the effect of temperature changes in the tropics on halocarbon emissions by *Ulva reticulata* Forsskål (Ulvaceae, Chlorophyta) and *Turbinaria conoides* (J.Agardh) Kützing (Sargassaceae, Ochrophyta) from wild populations and the economically important

tropical seaweeds *Gracilaria manilaensis* Yamamoto & Trono (Gracilariaceae, Rhodophyta) and *Kappaphycus alvarezii* (Doty) L.M.Liao (Solieriaceae, Rhodophyta). We compared halocarbon emission rates at five different temperatures (40, 35, 30, 25 and 20 °C) with exposure durations of 4 and 28 h. Seaweed pigments were also extracted 28 h post-treatment to investigate the possible connection between pigment content and halocarbon emissions for the selected seaweeds.

2. Results and Discussion

2.1. Emissions of halocarbons by the selected seaweeds

The compound with the highest recorded emission rates at temperatures between 20 and 40 °C throughout the whole study was $CHBr_3$, followed by CH_2Br_2, CH_2I_2, $CHBr_2Cl$, $CHBrCl_2$ and CH_3I (Fig. 1). A Kruskal–Wallis H test showed that distributions of halocarbon emission rates for all compounds were similar for all four seaweeds. Median of the specific halocarbon compound emission rates were statistically significantly different among the four seaweeds, with H(3) = 123, 104, 39, 115, 78 and 54; $p < 0.005$ for $CHBr_3$, CH_2Br_2, CH_2I_2, CH_3I, $CHBr_2Cl$ and $CHBrCl_2$. All four seaweeds showed higher tendency towards the emission of brominated compounds i.e. $CHBr_3$ and CH_2Br_2 at all temperature treatments compared to the other compounds (Fig. 2). For *K. alvarezii* the emissions of $CHBr_3$ and CH_2Br_2 throughout the experiment were closely associated, while in *T. conoides* it was the emissions of CH_2Br_2 and CH_2I_2 (Fig. 2).

Although a maximum single emission rate of 800 pmol gFW^{-1} hr^{-1} was observed for *U. reticulata,* both *K. alvarezii* (red) and *T. conoides* (brown seaweed) emitted higher amounts of $CHBr_3$ based on the median of the pooled emission rate data (Supplementary Table S1). This corresponds well with the findings of Leedham et al., (2013), who reported that red seaweeds including *K. alvarezii* produced higher amounts of $CHBr_3$, followed by the brown seaweed *T. conoides.* Red seaweeds including *Asparagopsis armata* Harvey (Bonnemaisoniaceae) from the temperate region and the tropical Gracilariales, such as *Gracilaria changii* (B.M.Xia & I.A.Abbott) I.A.Abbott, J.Zhang & B.M.Xia (Gracilariaceae), and *Gracilaria salicornia* (C.Agardh) E.Y.Dawson (Gracilariaceae),, are known to produce higher quantities of halocarbons than brown and green seaweeds (Carpenter and Liss, 2000; Leedham et al., 2013; Keng et al., 2020). This is the first study on the halocarbon emissions of *G. manilaensis* so no comparative data are available for comparison. However, a previous report on the production of halocarbons showed that emissions from green seaweeds, such as *U. reticulata* from the tropics and the temperate *Ulva intestinalis* Linnaeus (Ulvaceae), could surpass those of the brown seaweeds *Sargassum baccularia* (Mertens) C.Agardh (Sargassaceae) (Leedham et al., 2013) and *Ascophyllum nodosum* (Linnaeus) Le Jolis (Fucaceae) (Carpenter and Liss, 2000). Therefore, there is some precedent for the emission by *U. reticulata* to exceed (Fig. 1) that of *G. manilaensis* though the reasons behind this observation are not fully elucidated. Different physiological states of the seaweeds when collected from the natural environment are a possibility. *G. manilaensis* was found growing nearer to the shore than *U. reticulata* and was completely exposed during tidal ebb and so may have been exposed to larger twice-

daily temperature fluctuations. In contrast, *U. reticulata* was growing in an area where it was always fully submerged.

T. conoides was the most dominant emitter of CH_2I_2 among the four seaweeds. *T. conoides* belongs to the brown seaweeds which are known to store iodine in vesicles. The physode-like vesicles of temperate brown seaweeds *Laminaria digitata* (Hudson) J.V.Lamouroux (Laminariaceae) can release iodide as an inorganic antioxidant which leads to an elevated production of iodinated halocarbons (Küpper et al., 2008). This could be a factor contributing to the higher emission of halogenated compounds, especially CH_2I_2, by *T. conoides.* The highest emission of CH_2I_2 by *T. conoides* in this study agrees well with previous findings for tropical seaweeds where the highest emission rate for CH_2I_2 was found for *T. conoides* when compared to various other brown seaweeds (Leedham et al., 2013; Mithoo-Singh et al., 2017). A simple comparison of data for brown seaweeds from tropical and temperate regions confirms that *T. conoides* is a prolific producer of CH_2I_2 (Table 1).

2.2.Effect of temperature exposure duration on halocarbon emissions by the selected seaweeds

The emission trends for $CHBr_3$, CH_2Br_2 and CH_2I_2 for exposure periods of 4 and 28 h at the higher temperatures of 30 – 40 °C were noticeably different to those for the lower temperatures of 20 and 25 °C, especially for *K. alvarezii* and *T. conoides* (Fig. 3), indicating the possible influence of higher temperatures on the halocarbon emissions of these seaweeds. It was observed that with exposure at 40 °C, these two seaweeds, which emit high amounts of $CHBr_3$, had a bigger reduction in halocarbon emission rate (Fig. 3) compared to *G. manilaensis* and *U. reticulata.* This reduction, together with a large decrease in the F_v/F_m values after 4 h treatment at the high temperature (>90% at 40 °C; Fig. 4; Supplementary Table S2), could indicate that *G. manilaensis* and *U. reticulata* are more heat tolerant than *K. alvarezii* and *T. conoides* under a rapid temperature change over 4 h. Meanwhile, inherent biological variabilities are common in seaweed halocarbon studies (Carpenter et al., 2000; Leedham et al., 2013; Mithoo Singh et al., 2017). These could be caused by, but not limited to algae age and the different physiological state of the seaweeds and contributed to the high standard deviations in the emission rates (Fig. 3). Since the influence of algae age in the seaweeds could be insignificant as they were either seeded and harvested at the same time or are seasonal and were collected in a same batch from the collection sites, the high deviations could probably be related to the differences in the physiological state of the seaweeds or other intrinsic factors that could have contributed to this.

High temperatures can affect protein structure and enzymatic function, including those needed for photosynthetic carbon fixation, photophosphorylation and electron transport, which could then be reflected in a reduced F_v/F_m value in seaweeds (Borlongan et al., 2016). It is generally accepted that the threshold value for a healthy state of a seaweed is $F_v/F_m = 0.5$, although lower values have been reported for seaweeds in the natural environment (Li et al., 2016; Rabiei et al., 2016; Wang et al., 2016). While they could be exposed to stresses nearing tolerance limits during tidal ebb, intertidal seaweeds have been shown to display a high level

of plasticity in the photosynthetic response towards temperature change (McCoy and Widdicombe, 2019). The ability to tolerate thermal stress by both *G. manilaensis* and *U. reticulata* at higher temperature could be due to the ability of the seaweeds to quickly activate molecular defence mechanisms through upregulation of the expression of heat shock proteins (Smolina et al., 2016), through the protection of photosystem II against ROS by detoxifying enzymes, or stabilizing photosynthetic performance by the accumulation of osmolytes (Allakhverdiev et al., 2008). This could lead to a smaller decrease in F_v/F_m values at higher temperatures after a short exposure period (4 h) in *G. manilaensis* and *U. reticulata* compared with *K. alvarezii* and *T. conoides,* which both also showed signs of tip discolouration or bleaching of some thallus tips.

The large standard deviations in the emissions of $CHBr_3$ and CH_2Br_2 from *K. alvarezii* observed at 35 °C (Fig. 3) compared with lower temperatures, could be attributed to the low resilience of this seaweed at temperatures above than 30 °C. At high temperatures, stress of the seaweeds was indicated by the large decrease in the F_v/F_m values (Fig. 4). A longer duration of exposure to 40 °C led to the emission rates of $CHBr_3$, CH_2Br_2 and CH_2I_2 by all four seaweeds diminishing (Fig. 3), with all average F_v/F_m values below 0.1 (Fig. 4), where the thermal tolerance limit of the seaweeds could have been met or exceeded.

At the lowest temperature of 20 °C, emission trends were rather species-specific. *G. manilaensis* showed a similar trend of decreasing emission rates for all compounds except $CHBrCl_2$ and CH_3I for 4 and 28 h treatments, and similar trend were observed at 25 °C (Fig. 3). *U. reticulata* showed increased emission rates for all the halocarbon compounds followed by decreased emission when the temperature was decreased from 25 °C (ambient) to 20 °C for 4 h followed by 28 h. *K. alvarezii* showed significantly ($p < 0.05$) decreased emission rates for $CHBr_3$, CH_2Br_2 and CH_2I_2 4 h after exposure at 20 °C, while no significant differences ($p < 0.05$) were observed in emissions of the other compounds. At the same temperature, the emission rates of all compounds except for CH_3I by *T. conoides* did not differ significantly ($p < 0.05$) between exposure period of 4 and 28 h (Fig. 3). The higher emission rates for compounds such as $CHBr_3$ and $CHBr_2Cl$ seen for *G. manilaensis* at 25 °C ambient compared with that of the experiments at 25 °C (Fig. 3) could be due to higher photosynthetic rate during the initial exposure and subsequent adaptation over time.

The results from this study also showed that $CHBr_3$, CH_2Br_2, CH_2I_2 and $CHBr_2Cl$ emission responses were similar for the four compounds during exposure to higher temperatures. For example, for *G. manilaensis* there was no significant change in the emissions rate of any of the halocarbon compounds during the first 4 h of incubation at the higher temperatures, but there was a significant decrease in all the compounds after 28 h (Fig. 3). *U. reticulata* responded by showing an increase in the emission of $CHBr_3$ and CH_2Br_2 after 4 h, followed by a decrease after 28 h. The emission trends, however, were unique to each of the seaweeds (Fig. 3).

Different responses in the halocarbon emissions of polar seaweeds to different periods of temperature exposure were published previously. An initial doubling of $CHBr_3$ emissions from *G. antarcticus* was recorded when the temperature was raised from 0 to 10 °C for 24 h, but the emission rates decreased to values

lower than those of the control culture when the exposure period was extended to two months (Laturnus et al., 2000). Although exposure duration and temperature change could affect the halocarbon emissions by seaweeds, the response towards these factors is difficult to ascertain. A similar short-term study with 6 and 10 h incubation periods was conducted on temperate brackish water seaweeds, but no general response pattern was observed (Abrahamsson et al., 2003). Their 10 h cross-incubation experiment showed no significant changes in the emissions of $CHBr_3$, CH_2I_2 and $CHCl_3$ from the temperate green algae *C. glomerata* and *Ulva linza* Linnaeus (Ulvaceae). The cross-incubation involved incubation of seaweeds at 23 °C in the laboratory after collection from the field at 12 °C, and vice versa (23 °C in field and then 12 °C in the laboratory) (Abrahamsson et al., 2003).

2.3. Correlation between halocarbon emissions and temperature

Significant correlations were observed between the emission of certain halocarbon compounds with temperature (Table 2). While significant positive correlations ($p < 0.01$) occurred between the emissions by *G. manilaensis* and temperature during the first 4 h of temperature treatment, the emission rates of halocarbons by the four seaweeds were mainly negatively ($p < 0.05$) correlated to temperature change between 20 and 40 °C.

With the shorter 4 h duration temperature exposure, the emission rates for $CHBr_3$, CH_3I and $CHBr_2Cl$ by *G. manilaensis* showed a significant positive correlation ($0.59 \leq r \leq 0.81$; $p<0.01$) with temperature change (Table 2). Compounds with a strong positive correlation to temperature change included $CHBr_3$ ($r = 0.64$; $p < 0.01$) and $CHBr_2Cl$ ($r = 0.81$; $p < 0.01$). The emissions of all halocarbons by *U. reticulata,* however, did not correspond well with temperature change in the first 4 h of exposure. At the same exposure duration, the emissions of CH_3I by *K. alvarezii* and *T. conoides* showed strong ($r = 0.72$ and 0.75; $p < 0.01$) positive correlation with temperature change, while emissions of $CHBr_3$ by the two seaweeds were negatively correlated to temperature ($r = -0.69$ and -0.63; $p < 0.01$). A short-term increase in temperature therefore enhanced the emissions of some halocarbon compounds but this varied with seaweed species and the halocarbon compounds. This could happen during the daily diurnal change, for example Carpenter and Liss (2000) reported higher halocarbon emission rates from temperate rockpool seaweeds around midday. However, the direct effect of temperature on the increased emissions cannot be ascertained because varying factors such as irradiance and photosynthesis that could influence halocarbon emissions co-exist (Keng et al., 2013).

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

temperature at 28 h (r = −0.80 to −0.95; $p < 0.01$). *U. reticulata, K. alvarezii* and *T. conoides* also showed strong negative correlations (r = −0.69 to −0.95; $p < 0.01$) in the emissions of $CHBr_3$, CH_2Br_2 and CH_2I_2 with increased temperature at 28 h. The decrease in halocarbon emissions with increasing temperature and prolonged exposure could possibly be caused by damage of the photosynthetic apparatus due to the accumulation of ROS in the seaweeds, indicated by the low F_v/F_m values (Fig. 4; Supplementary Table S2). In addition, there is possibility that the respiration apparatus of the mitochondria could have been compromised for the same reason. Both the chloroplast and the mitochondria are major sources of ROS in plant cells due to the intense rate of electron flow within these organelles (Gill and Tuteja, 2010). At a temperature of 35 °C *K. alvarezii* showed signs of bleaching. This was more evident at 40 °C and after 28 h exposure the seaweeds were completely bleached suggesting a generalized biochemical stress response to temperature change leading to cell death. On the other hand, 20–30 °C temperature treatments had little effect on the F_v/F_m values of the seaweeds. This coincides with findings for another tropical brown seaweed, *Sargassum polycystum* C.Agardh (Sargassaceae) where changes of F_v/F_m values were insignificant between temperatures of 15 and 30 °C (Zou et al., 2018). While the bromoperoxidases involved in halocarbon production could remain active at temperatures as high as 50 °C (Kongkiattikajorn & Ruenwongsa, 2006), the decrease in the production of $CHBr_3$, CH_2Br_2, CH_2I_2 and $CHBr_2Cl$ seemed to be affected by the photosynthetic yield of the seaweeds as shown in our study, especially at 40 °C (Fig. 3; Supplementary Table S2). Exposure to 40 °C causes stress and strongly affects seaweed health as indicated by the large percentage reduction in the F_v/F_m values after just 4 h exposure compared with pre-exposure values at the ambient temperature of 25 °C, and the near-zero values at 28 h (Fig. 4; Supplementary Table S2). The near-zero values suggest that the seaweeds were essentially dead due to prolonged exposure to high temperature.

2.4. Temperature effect on pigment contents of seaweeds

The pigment contents of the seaweeds showed different responses to the temperature treatments after 28 h exposure (Fig. 5). *U. reticulata* had the highest and *K. alvarezii* the lowest amounts of chlorophyll-*a* (Chl-*a*) and carotenoids (Car). Significantly lowered ratios of Chl-*a*: Car (C:C) were observed in *G. manilaensis* (0.1 ± 0.1 μg g^{-1}) and *K. alvarezii* (2.3 ± 0.0 μg g^{-1}) at 40 °C. Studies have shown that the concentration of Car can increase during temperature stress, while chlorophyll content decreases, with differential responses of individual seaweeds observed when subjected to moderate and high temperatures (Ismail & Osman, 2016; de Silva & Asaeda, 2017; Kumar et al., 2020). The results show significantly lower Chl-*a* content in *G. manilaensis* (9.2 ± 3.5 μg g^{-1}) and *K. alvarezii* (1.4 ± 0.1 μg g^{-1}) at 40 °C and significantly higher carotenoid content in *G. manilaensis* at 40 °C (74 ± 11 μg g^{-1}) and *U. reticulata* (160–195 μg g^{-1}) at 40, 35 and 20 °C i.e. at higher and lower temperatures (Fig. 5). These data relate well to the previous findings and indicate stressful conditions for the seaweeds at these temperatures. Stressful conditions such as high temperature could disturb the existing equilibrium between antioxidants and the ROS in the seaweeds (Gill & Tuteja, 2010). As antioxidants, higher amounts of Car are commonly produced under heat stress for the deactivation of ROS as overproduction destroys cellular constituents leading to programmed cell death (Sharma et al., 2012; Gill &

Tuteja 2010; de Silva et al., 2017). The low carotenoid content in *K. alvarezii* at 40 °C could be due to the bleaching of the seaweed.

When halocarbon emission rates for all temperature levels at 28 h exposure were plotted against the pigment contents of the seaweeds, positive correlations were found between the Chl-*a* contents of *G. manilaensis* and *K. alvarezii* with the emission rates of $CHBr_3$, CH_2Br_2 and CH_2I_2 (r = 0.48–0.90, $p < 0.05$; Table 3). Stronger positive correlations were observed between the same compounds with carotenoids by *K. alvarezii* while *G. manilaensis* showed stronger negative correlations. Chl-*a* content in *U. reticulata* and *K. alvarezii* (r = −0.50 to −0.66; 0.63 to 0.90, $p < 0.05$) showed stronger correlations with the emission of halocarbons than with the Car contents and C:C ratios (Table 3). However, the emission of halocarbons was negatively correlated to the pigment contents in *U. reticulata* while positively correlated for *K. alvarezii.* Pigment contents were not strongly correlated to the emission rates of any of the halocarbon compounds with *T. conoides* (Table 3). These differences suggest that the general effect of the pigment contents on the emission of halocarbons could be hard to determine, although species-specific responses of pigment contents towards temperature changes have been reported (de Silva & Asaeda, 2017). This could be due to the varying degree of antioxidant enhancement in the seaweeds (Raja et al., 2016) under the various temperature treatments. Nevertheless, the strong correlations found in *K. alvarezii* between pigment content and halocarbon emission could serve as a starting point for future research into the relationships between pigments, temperature and halocarbon emissions in seaweeds.

3. Conclusions

The emission rates of $CHBr_3$ by *G. manilaensis, U. reticulata, K. alvarezii* and *T. conoides* were the highest amongst the six halocarbon compounds investigated, with the highest median emission rate observed for *K. alvarezii* and *T. conoides.* Changes in temperature and exposure duration affected the emissions of halocarbons by all four seaweeds. Larger decreases in the emissions of $CHBr_3$, CH_2Br_2, CH_2I_2 and $CHBr_2Cl$ by *K. alvarezii* and *T. conoides,* coupled with significant ($p < 0.05$) reductions in F_v/F_m values (>90%) were notable after 4 h exposure at 40 °C, compared with *G. manilaensis* and *U. reticulata.* Strong negative correlations (r = −0.69 to −0.95; $p < 0.01$) were seen in the emissions of $CHBr_3$, CH_2Br_2 and CH_2I_2 by *U. reticulata, K. alvarezii* and *T. conoides* versus temperature after 28 h exposure, indicating decreasing emissions of these compounds with increasing temperature after a longer duration exposure. This would be worth further investigation using a longer term mesocosm approach because if the trend is observed in a future climate the contribution of seaweeds to total coastal halocarbon emissions could be substantially reduced. Tropical seaweeds and corals are currently at or close to their lethal temperature (Bartsch et al., 2012), with a temperature threshold of 27.5–32 °C reported for coral bleaching, and 30–37 °C for tropical seaweeds (Liu et al., 2009; Bartsch et al., 2012). Therefore, a small climate-related rise in temperature could also result in a change in the abundance and distribution of tropical seaweeds, thereby affecting the regional halocarbon pool.

The responses of seaweeds towards temperature change could involve changes in both enzymatic and chemical reactions leading to halocarbon production or changes to other aspects of physiology that subsequently affect the halocarbon production in the seaweeds. This means that factors such as the resilience capacity of individual seaweeds could contribute to the variability of the acquired results. It is therefore crucial for more studies to explore this area, especially the underlying formation pathway for halocarbons. Inherent variabilities among biological samples could probably be reduced by refining similar investigation through the standardisation of biological and geographical factors such as tissue age and sample collection point. Meanwhile, in view of future climate scenarios, a more holistic mesocosm approach incorporating multiple environmental factors (Boyd et al., 2018; Hopkins et al., 2020) and using a longer exposure duration could help to reduce the uncertainties in halocarbon production prediction capacity arising from the scarcity of data in this area.

4. Experimental

4.1. Sample collection

Two tropical seaweeds, *Gracilaria manilaensis* Yamamoto & Trono (Gracilariaceae, Rhodophyta), and *Ulva reticulata* Forsskål (Ulvaceae, Chlorophyta) were collected from Tanjong Kupang, Johor, West Peninsular Malaysia, at a sandy/muddy beach area in the vicinity of a land reclamation project. The area was dominated by *U. reticulata* and seagrass meadows. *G. manilaensis* and *U. reticulata* were the visually dominant seaweeds at the sampling site at the time of sampling. *Kappaphycus alvarezii* (Doty) L.M.Liao (Solieriaceae, Rhodophyta), a commercially important red seaweed, was purchased from a seaweed farm off Semporna, Sabah, East Malaysia. *Turbinaria conoides* (J.Agardh) Kützing (Sargassaceae, Ochrophyta) was collected from a fringing coral reef at Port Dickson, West Peninsular Malaysia, where it is one of the dominant seaweed species present (Keng et al*.,* 2013). Table 4 gives collection dates and site co-ordinates. The farmed seaweeds, *K. alvarezii,* were seeded at the same time and were all later harvested together, while *G. manilaensis, U. reticulata* and *T. conoides* are seasonal seaweeds and were collected at the same time in a single batch. Samples of approximately the same size and height were selected whenever possible. All seaweeds were packed in cool boxes wrapped in paper towel soaked in seawater to prevent moisture loss during transportation. The seaweeds were then brought back to the University of Malaya hatchery within 4–8 hours of collection (including air transit for KA) and maintained in a flowing seawater system at an average temperature of 27.4 (25–29.7) °C for no longer than eight weeks.

4.2. Experimental setup

The treatment of seaweeds at five different seawater temperatures, 40, 35, 30, 25 and 20 °C, started with an 'ambient' treatment where seaweeds were incubated in custom-made stoppered flasks (with a Luer port at the bottom) for 4 h at the laboratory seawater temperature of 25 ± 2 °C, to determine the halocarbon emissions

prior to temperature treatment. This temperature was denoted as the 'ambient' for comparison purposes between the starting temperature and the treatment temperature conditions. It was close to the average seawater temperature of 27.4 (25–29.7) °C in the hatchery tanks with flowing seawater where the seaweeds were maintained (HOBO logger). It falls within previously reported sea surface temperatures of 25.7–33.9 °C for Malaysia (Tan et al., 2002), and also within the temperature range of 20.9–33.5 °C reported for Port Dickson, (Hamzah et al., 2011), where one of the seaweed samples was collected. Temperatures of 20 and 40 °C were included as the extremes of the temperature series and we note that exposure of seaweeds during tidal ebb at Morib can already reach 38.2 ± 1.1°C.

Prior to exposure to the temperature treatments the seaweeds were transported from the hatchery to the laboratory, visible epiphytes were removed and the seaweeds were acclimatized to the laboratory conditions in filtered seawater with a constant air supply on a shaking incubator (HiPoint 600SR; gentle setting of 30 rpm) for between 16 and 20 h. The incubator was set at an irradiance level of 81 ± 7 μmol photons m^{-2} s^{-1} (LICOR, Inc. LI-250A light meter with LI-190SA quantum sensor). Natural sunlight at Malaysian coastal sites can go up to 3000 μmol photons m^{-2} s^{-1} at midday in air but the light reaching seaweeds is attenuated by water coverage and overlapping fronds. The incubation flasks were filled with seawater allowing no headspace. Control flasks with seawater alone were also included to enable the subsequent determination of seaweed halocarbon emissions. At 4 and 28 h a 100 mL gas-tight glass syringe was used to remove 40 mL of seawater from all flasks (n = 4 for each temperature treatment and the control flasks) and promptly injected into the Purge-and-Trap (P&T) sample preparation system coupled to the GCMS.

Upon completion of the ambient treatment, seaweeds were returned to their previous vessels and acclimatized back to the laboratory conditions mentioned earlier, until the next day, when the same seaweeds were subjected to another temperature regime. For this, seaweeds were again incubated at that temperature for four h, again in the custom-made stoppered flask filled to the top with pre-filtered seawater without headspace (Fig. 6). Control flasks were also prepared and subjected to the same treatment. Seawater from the flasks was then extracted using a glass syringe. Upon completion of this 4-h exposure treatment, the seaweeds were returned to their acclimatization vessels and maintained at the same conditions, as would the acclimatization condition, except this time at the respective treatment temperatures for a further 20 h.

The same batches of seaweeds were once again placed into the incubation flasks, 24 h after their first exposure to the temperature treatment. Seawater was extracted from the respective flasks immediately after another 4-hr incubation for halocarbon analyses. These steps were repeated at all temperature treatments i.e. 40, 35, 30, 25, and 20 ± 2 °C for profiling of halocarbon emissions at the various temperature, and to determine the correlations between temperature change and emission rates at 4 and 28 h of exposure.

Halocarbons emitted by the seaweeds were derived from the net difference in seawater halocarbon concentrations between seawater-filled flasks containing seaweeds and seawater-filled flasks without

seaweeds (control). The seawater used in this experiment was sourced naturally from Port Dickson and was filtered (0.7 μm GF/F, Whatman) prior to experimental use. The pH of the seawater throughout the entire experiment ranged between 7.83 and 8.00.

Seaweed biomasses used for each flask were between 10 g and 15 g and were weighed prior to the start of each temperature treatment (Day 1). To determine the moisture content (Table 5) of the seaweeds, the dry weights of seaweeds were determined after 72 h of drying in the oven at 60 °C. The dry weight (DW) to fresh weight (FW) ratio of the seaweeds is useful for future comparisons of halocarbon emission rates.

4.3. Halocarbon analysis

Seawater samples extracted from the incubation flasks were swiftly injected into a custom-made P&T system (Keng et al., 2013; Leedham et al., 2013; Mithoo-Singh et al., 2017). Each collection of seawater from the flasks was timed 30 minutes apart to enable direct injection of the seawater into analytical system to avoid photochemical-related changes with time. The seawater samples were injected into the sampling vessel and purged with oxygen-free nitrogen at a rate of 40 mL min^{-1} for 15 mins. The purged gas was channelled through a glass tube fitted with glass wool in series with a Nafion dryer (Perma Pure) with an oxygen free nitrogen counter-flow rate of 100 mL min^{-1} to remove aerosol particles and water vapour. The analytes were than trapped and concentrated in a stainless-steel sampling loop attached to a six-port two-way valve (VICI®) using liquid nitrogen maintained at –150 °C through a thermostatic liquid nitrogen boiler (custom made by the University of East Anglia).

At 15 mins after purging, the position of the six-port valve was changed from 'trap' to 'inject'. With a quick switch between liquid nitrogen and boiling water at the sampling loop, the trapped analytes were desorbed at around 96 °C using high purity helium (Linde Malaysia) at 1 mL min^{-1} to sweep them into a gas chromatography (GC) system (Agilent Technologies, 7890B), through a heated transfer line maintained at 91 ± 2 °C. The GC system was fitted with a 60 m capillary column (J&W DB-VRX, film thickness 1.40 μm; internal diameter 0.25 mm). The GC oven was programmed to hold the temperature at 40 °C for 4 mins and ramp up to 200 °C at a rate of 20 °C min^{-1} and held for 2 mins, followed by a ramp up of 40 °C min^{-1} until 240 °C and held for 5 mins. The detection and quantification of analytes were done by the mass spectrometry system (Agilent Technologies, 5977B MSD) coupled to the GC.

A total of six compounds were monitored in this study, through the Single Ion Monitoring mode. These include the brominated compounds, i.e. bromoform ($CHBr_3$) and dibromomethane (CH_2Br_2), the iodinated compounds, diiodomethane (CH_2I_2) and iodomethane (CH_3I), and the mixed compounds dibromochloromethane ($CHBr_2Cl$) and bromodichloromethane ($CHBrCl_2$). Each compound was identified using two target ions and the pre-identified retention time from the analysis of the commercial standards (Supplementary Table S3). Concentration of halocarbons were determined by fitting the relative abundance of

each compound into the equations derived from the calibration curves. The relative abundance of each peak was determined through manual integration of peak area against the baseline.

Concentrations of target compounds were determined through five-point calibration curves of compound standards (Sigma-Aldrich, Merck) at a temperature of 25 ± 1 °C. The commercially available neat (apart from $CHBr_2Cl$) liquid standards were gravimetrically prepared and diluted in methanol (Fisher Scientific, HPLC grade) for this purpose into concentrations in the range of 40 – 6000 pmol L^{-1}. Surrogate analytes, i.e. deuterated iodomethane (CD_3I) and deuterated diiodomethane (CD_2I_2), were added to each of the samples prior to P&T injection to monitor for system drift. These surrogate analytes were chosen due to their extremely low natural concentrations in the seawater and had been proven with trial runs of seawater prior to the experiment. Peak areas were corrected according to the purging efficiencies at temperatures of 40, 35, 30 and 20 °C relative to 25 °C determined through our system. The detection limits of the system for each compound were determined from the standard deviation (S.D) of the blanks (three times S.D) (Abrahamsson and Pedersén, 2000). The detection limits for each halocarbon compound was 10 pmol L^{-1}.

4.4. F_v/F_m measurements

The maximum quantum yield (F_v/F_m) of the seaweeds pre- and post-incubation was determined using the Pulse Amplitude Modulated Chlorophyll Flurescence (Walz Inc., DIVING PAM). Photosynthetic parameters such as the maximum quantum yield (F_v/F_m) are useful for indicating the photosynthetic performance of the seaweeds. F_v/F_m was used in this experiment as a measure of the physiological health of the seaweed. Seaweeds were first dark adapted for at least 15 min using the dark leaf clips prior to measurement. A weak modulating light beam (0.15 m^{-2} s^{-1}) was then applied for the determination of the ground fluorescence (F_0), followed by a saturation pulse of 800 µmol photons m^{-2} s^{-1} for 0.6 s to determine the maximal fluorescence (F_m). F_v/F_m was then determined through the formula $F_v/F_m = (F_m – F_0)/F_m$. A single F_v/F_m value was derived from three technical replicates for the same seaweed sample.

4.5. Determination of pigments' content in seaweeds

The pigment contents of the seaweeds were determined after each temperature treatment was complete (n = 12). Between 0.3–3 g of seaweeds were ground in acetone using a mortar and pestle on ice under close to dark conditions. The extracts were then transferred into a centrifuge tube and topped up with acetone to 20 mL and stored in the dark at 4 °C overnight. The extracts were then centrifuged at 3000 rpm for 10 mins. Around 4 mL of extract supernatant was then pipetted into a quartz cuvette and all pigment extracts were read at wavelengths of 665, 645, 630, and 452 nm with a spectrophotometer (Shimadzu UV-1800 UV spectrophotometer). The Chl-*a* and carotenoid contents of the seaweeds were determined through Equations 1 and 2 (Strickland and Parsons, 1968).

Equation 1

$$\text{Chl} - a = \frac{\text{Ca} \times \text{Volume of Acetone (mL)}}{\text{Seaweed fr wt (g)}}$$

where $\text{Ca} = 11.6\ (\text{OD } 665\text{nm}) - 1.31\ (\text{OD } 645\text{nm}) - 0.14\ (\text{OD } 630\text{nm})$; Chl-*a* is given in $\mu g\ g^{-1}$

Equation 2

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

where Carotenoid is given in $\mu g\ g^{-1}$

4.6. Statistical analysis

A Kruskal–Wallis H test was used to determine if there were differences in the emission of all six halocarbon compounds between the four seaweed species. A one-way ANOVA was conducted to test the difference between halocarbon emission rates, F_v/F_m values and pigment levels at different incubation durations and temperature exposures (Figs 3, 4, 5; Supplementary Table 2). The relationships between halocarbon emissions and temperature change (Table 2), and between halocarbon emissions and pigment content (Table 3) were determined using Pearson's Product-Moment Correlation. All halocarbon emission rates were fourth root transformed prior to the ANOVA and correlation testing. The variables were normally distributed, as assessed by Shapiro–Wilk test ($p > 0.05$, and the homogeneity of variances was met according to Levene's test for equality of variances. All statistical analyses except the Principle Component Analysis (PCA) were carried out using SPSS Statistics software (IBM, Version 22). The PCA (Fig. 2) was done with CANOCO 4.5 software to investigate correlations between the abundance of halocarbons emitted by the different algal species and the temperature treatments. All halocarbon emission rates were converted to their logarithm to the base ten prior to analysis. The factor loadings or eigenvectors indicated that the first two axes derived from PCA explained approximately 98.5% of the total variance in the treatment data.

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Figures

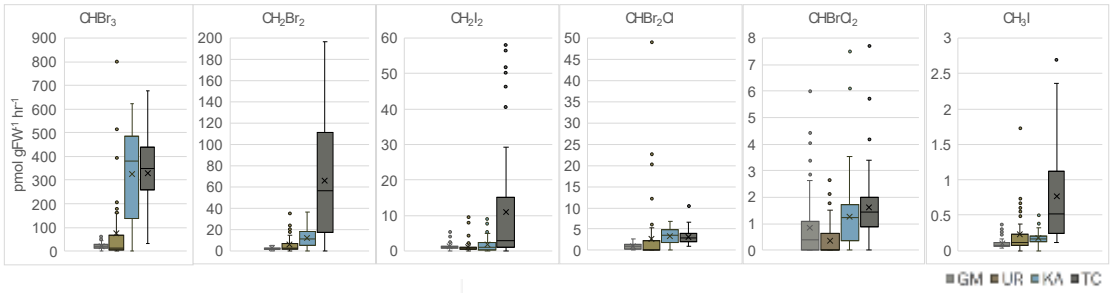

Fig. 1.

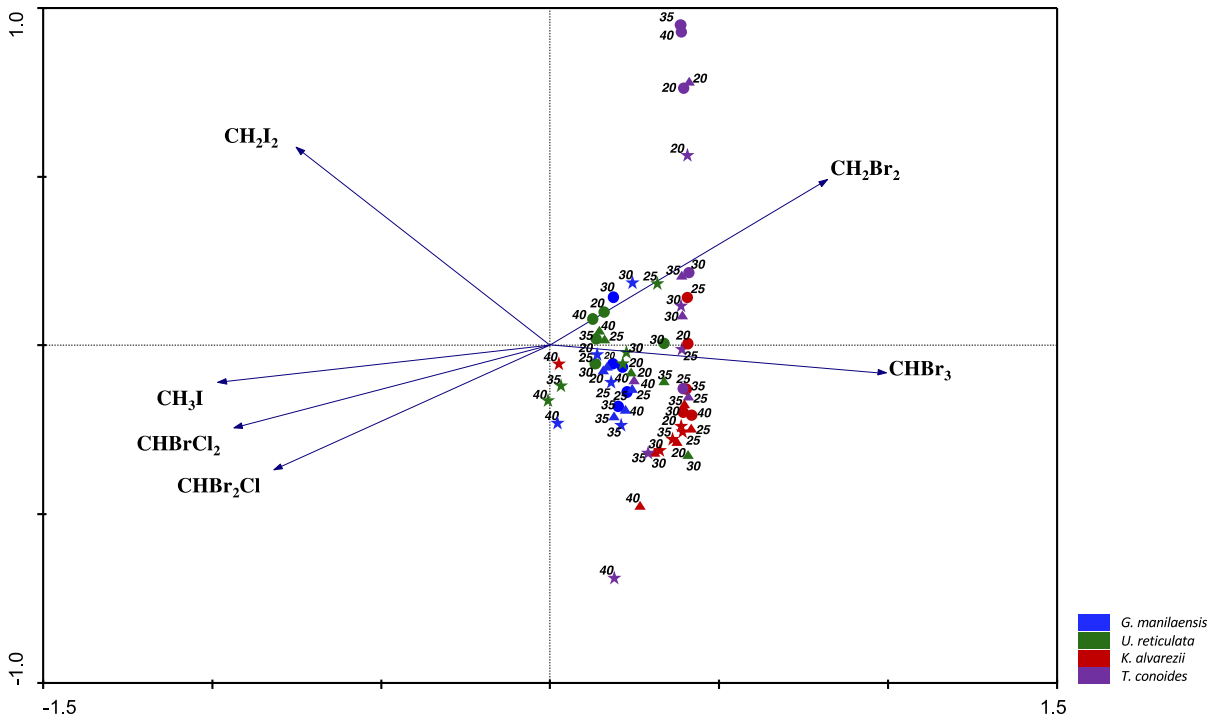

Fig. 2.

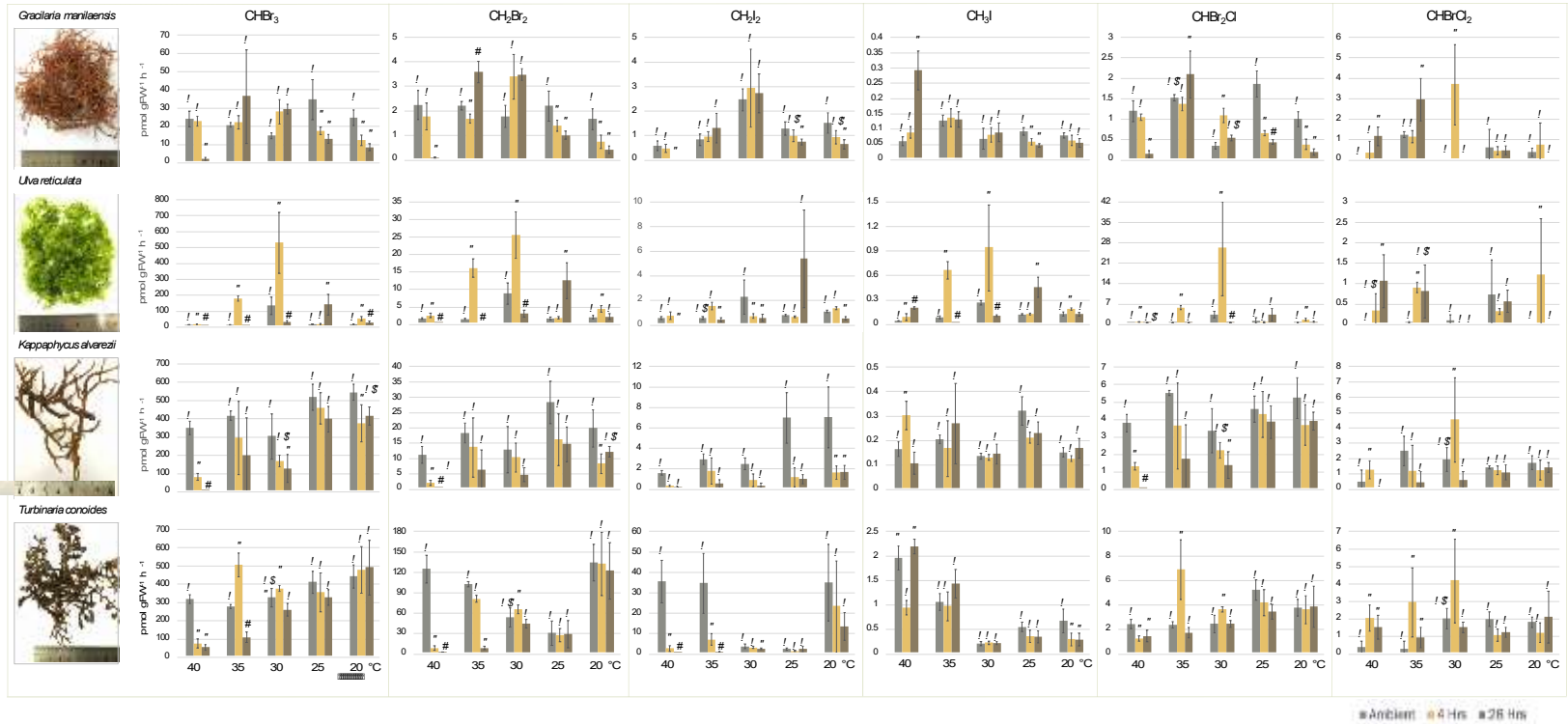
Gracilaria manilaensis
Ulva reticulata
Kappaphycus alvarezii
Turbinaria conoides
CHBr3
CH2Br2
CH2I2
CH3I
CHBr2Cl
CHBrCl2
pmol gFW-1 h-1
40 35 30 25 20 °C
Ambient
4 Hrs
26 Hrs

Fig. 3.

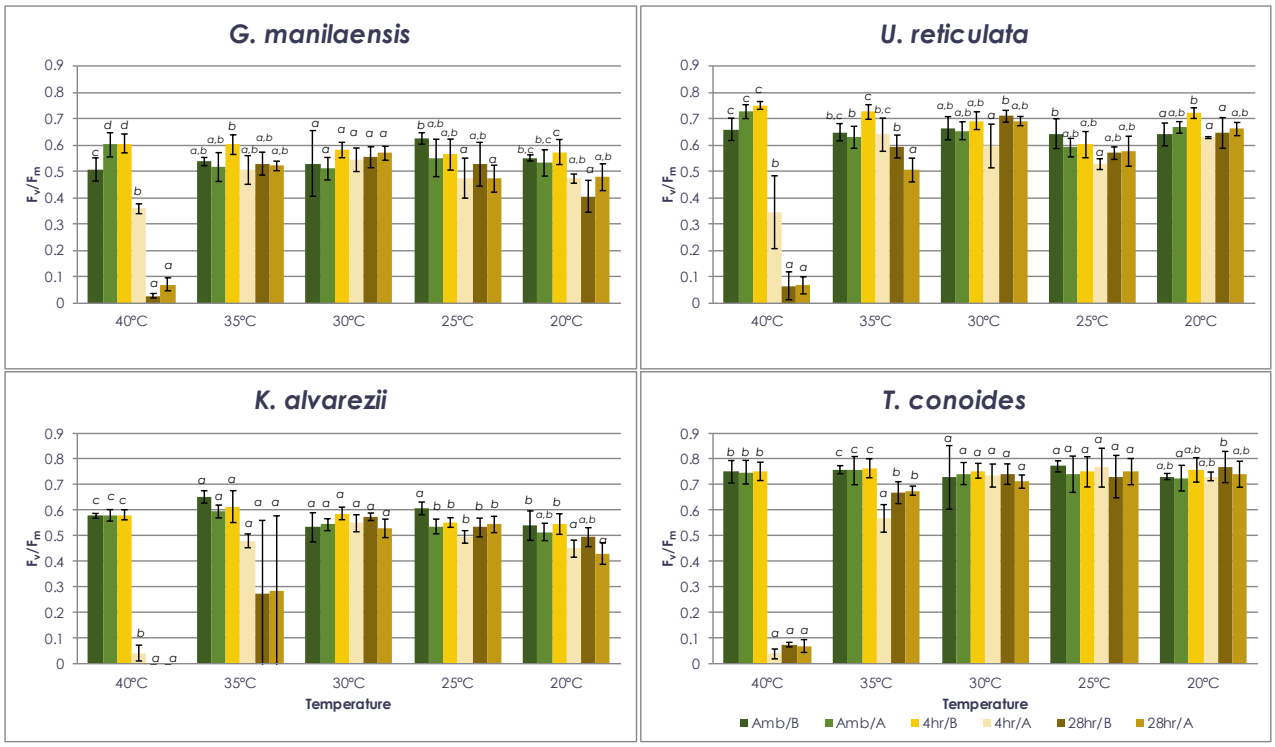

Fig. 4.

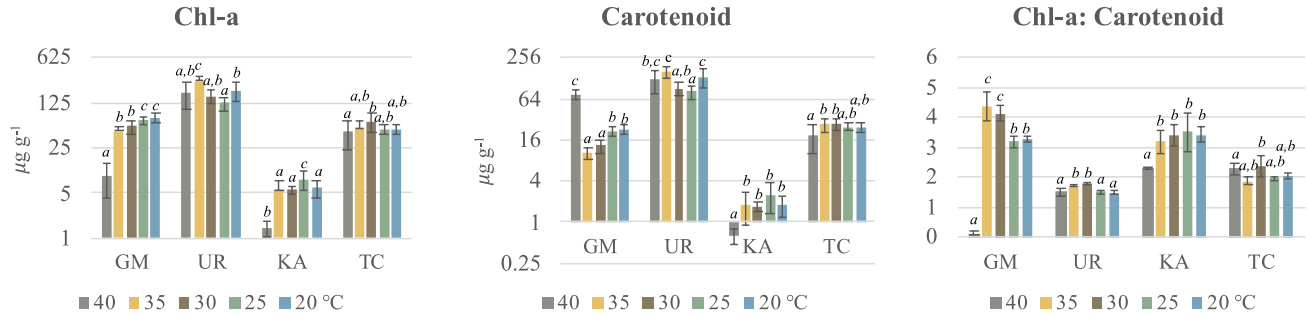

Fig. 5.

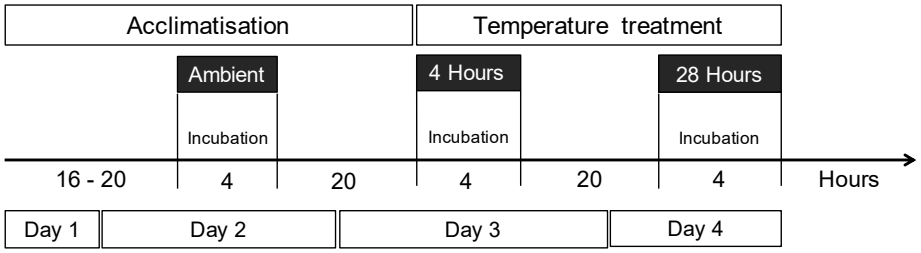

Fig. 6.

564
565 **Table 1**
566 The emission rates of CH_2I_2 (pmol gFW^{-1} h^{-1}) for *T. conoides* and other brown seaweeds.
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T. conoides	All other brown seaweeds	
	Tropical	Temperate
39–265 [1*]	0–2 [1]	0–10.3 [5]
485–502 [2]	2–18 [2]	
0–29 [3]	0–12 [3]	
0–58 [4]	-	

568 [1] Mithoo-Singh et al., 2017; [2] Leedham et al., 2013; [3] Keng et al., 2013; [4] This
569 study; [5] Carpenter and Liss, 2000; [*]Converted based on the assumption of
570 moisture content of ~83% for *T. conoides*, ~84% for *S. binderi,* and ~82% for
571 *P. australis (*Keng et al., 2020)
572
573
574 **Table 2**
575 Pearson Product-Moment Correlation coefficient, *r*, for the emission rates of the six halocarbon compounds
576 versus changes in temperature between 40 and 20 °C for 4 and 28 h exposures.
577

	G. manilaensis		***U. reticulata***		***K. alvarezii***		***T. conoides***	
	4 h	28 h	4 h	28 h	4 h	28 h	4 h	28 h
$CHBr_3$	0.64			−0.73	−0.69	−0.83	−0.63	−0.95
CH_2Br_2				−0.74		−0.81	−0.65	−0.89
CH_2I_2		−0.52*		−0.69		−0.80		−0.93
CH_3I	0.59	0.89			0.72		0.75	0.82
$CHBr_2Cl$	0.81			−0.50*	−0.54*	−0.83		−0.82
$CHBrCl_2$		0.67		0.62		−0.85		

578 [*] Correlation significant at 0.05 level ($p < 0.05$; 2-tailed). All others are significant at the 0.01
579 level ($p < 0.01$; 2-tailed); $n = 20$ for all correlations.
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Table 3

Pearson Product-Moment Correlation coefficient, *r*, of the emission rates of the halocarbon compounds to the chlorophyll-*a* (Chl-a), carotenoids (Car) contents and the Chl-*a*: Car ratio (C:C) of the seaweeds extracted at the end of the 28 h exposure experiment.

	G. manilaensis			***U. reticulata***			***K. alvarezii***			***T. conoides***		
	Chl-*a*	Car	C:C	Chl-*a*	Car	C:C	Chl-*a*	Car	C:C	Chl-*a*	Car	C:C
$CHBr_3$	0.48*	−0.81	−0.84	−0.50*	−0.49*		0.90	0.80	0.59			
CH_2Br_2	0.63	−0.92	0.96	−0.63*	−0.59		0.89	0.77	0.66			
CH_2I_2	0.73	−0.95	−0.96				0.73	0.64				
CH_3I	−0.95	0.69	−0.64	−0.66	−0.53*	−0.60	0.63	0.62				
$CHBr_2Cl$		−0.57*	−0.62				0.88	0.78	0.59			
$CHBrCl_2$	−0.51*	−0.52*	−0.49*				0.76	0.66	0.54*			

* Correlation significant at 0.05 level ($p < 0.05$; 2-tailed). All others are significant at the 0.01 level ($p < 0.01$; 2-tailed); $n = 20$ for all correlations.

Table 4

Location and date of collection for seaweed samples used in this study.

Specimen No.	Seaweed	Abbreviations	Coordinates and Location	Collection Date
PSM 13041	*Gracilaria manilaensis* Yamamoto & Trono (Gracilariaceae, Rhodophyta)	GM	1°20'26" N 103°36'16" E Tanjong Kupang, Johor	22.5.2017
PSM 13037	*Ulva reticulata* Forsskål (Ulvaceae, Chlorophyta)	UR		22.5.2017
PSM 13038	*Kappaphycus alvarezii* (Doty) L.M.Liao (Solieriaceae, Rhodophyta)	KA	4°30'6" N 118°37'40" E Semporna, Sabah	17.7.2017
PSM 13039	*Turbinaria conoides* (J.Agardh) Kützing (Sargassaceae, Ochrophyta)	TC	4°30'6" N 118°37'40" E Cape Rachado, Port Dickson	9.8.2017

Table 5

Moisture content and dry weight (DW) and fresh weight (FW) analysis of the selected seaweed species.

Seaweed	Moisture content (%)[1]	DW: FW ratio
G. manilaensis	85.50 ± 1.08	0.15
U. reticulata	81.91 ± 1.17	0.18
K. alvarezii	90.45 ± 0.26	0.10
T. conoides	84.97 ± 4.96	0.15

[1](mean ± standard deviation, %; $n = 7$ for *G. manilaensis* and *U. reticulata*; $n = 3$ for *K. alvarezii* and *T. conoides*).

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865 **List of Figure Legends**
866
867
868 **Fig. 1.** Total emission rates for halocarbons (pmol $gFW^{-1}\,h^{-1}$; $n = 60$) for the four seaweeds, *G. manilaensis*
869 (GM), *U. reticulata* (UR), *K. alvarezii* (KA) and *T. conoides* (TC) for all experimental temperatures and treatment
870 durations. In this, the horizontal bar represents the median value, the box gives the upper and lower quartile range
871 and the error bar shows the spread of the data. The circles denote excluded outlier data (+ between 1.5 and 3 box
872 lengths from the box edges) and extreme cases (/ > 3 box lengths from the box edges).
873
874 **Fig. 2.** PCA analysis based on $\log_{10}$ of the halocarbon emissions by the four selected seaweeds, *G.*
875 *manilaensis*, *U. reticulata*, *K. alvarezii* and *T. conoides*, exposed to ambient temperature (circles), and
876 exposed to treatment temperatures of 40, 35, 30, 25 and 20 °C for 4 (triangles) and 28 (stars) h. Numerals next
877 to the coloured symbols indicate temperature levels.

878 **Fig. 3.** Average emission rates of halocarbons ± standard deviation (pmol $gFW^{-1}\,h^{-1}$; $n = 4$) for the four
879 seaweeds, *G. manilaensis, U. reticulata, K. alvareii* and *T. conoides* after 4- and 28-h exposure to the 25 °C
880 ambient temperature or the treatment temperature levels of 40, 35, 30 and 20 °C. ^{a,b,c} indicate homogeneous
881 groups based on Tukey's post hoc test ($p < 0.05$).
882 **Fig. 4.** Averaged F_v/F_m values (with standard deviation; $n = 4$) of seaweeds measured before (/B) and after
883 (/A) incubation, under the ambient 25 °C condition and the temperature treatments of 40, 35, 30 and 20 °C, after 4
884 and 28 h exposure. ^{a,b,c,} indicate homogenous groups based on Tukey's post hoc test ($p < 0.05$).
885
886 **Fig. 5.** Chl-*a* (μg g^{-1}), carotenoid (μg g^{-1}) contents and the Chl-*a*: carotenoid ratios (average ± standard
887 deviation; $n = 12$) of the four seaweeds, *G. manilaensis* (GM), *U. reticulata* (UR), *K. alvarezii* (KA) and *T.*
888 *conoides* (TC), measured after a 28h exposure to temperatures of 40, 35, 30, 25 and 20°C. Data were analysed
889 using a one-way ANOVA. ^{a,b,c,} indicate homogeneous groups across temperature based on Tukey's post hoc test
890 ($p < 0.05$).
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892 **Fig. 6.** Treatment of seaweeds at a particular temperature exposure.
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Supplementary Table S1

Average ± standard deviation; median and range (bracketed) of the emission rates (pmol gFW^{-1} h^{-1}; n = 60) of halocarbons released by *G. manilaensis*, *U. reticulata*, *K. alvarezii* and *T. conoides* recorded throughout the experiment.

	G. manilaensis	*U. reticulata*	*K. alvarezii*	*T. conoides*
$CHBr_3$	21 ± 12; 22	75 ± 143; 11	325 ± 188; 382	330 ± 159; 347
	(1–62)	(0–800)	(0–624)	(32–677)
CH_2Br_2	2 ± 1; 2	6 ± 7; 2	11 ± 9; 11	66 ± 54; 56
	(0–5)	(0–35)	(0–36)	(0–196)
CH_2I_2	1 ± 1; 1	1 ± 2; 1	2 ± 2; 1	11 ±16; 3
	(0–5)	(0–9)	(0–9)	(0–58)
CH_3I	0.1 ± 0.1; 0.1	0.2 ± 0.3; 0.1	0.2 ± 0.1; 0.2	0.8 ± 0.7; 0.5
	(0–0.4)	(0 – 1.7)	(0 – 0.5)	(0.1 – 2.7)
$CHBr_2Cl$	1 ± 1; 1	3 ± 8; 0	3 ± 2; 4	3 ± 2; 3
	(0 – 3)	(0–49)	(0–7)	(1–10)
$CHBrCl_2$	1 ± 1; 0	0 ± 1; 0	1 ± 1; 1	2 ± 1; 1
	(0–6)	(0–3)	(0–7)	(0–8)

Emission rates were derived from the net difference in total halocarbons between the control flask and flask containing seaweeds; Zero emission rate was assumed when halocarbon from the seaweed-containing flask did not surpass that of the control

Supplementary Table S2

Averaged $F_v/F_m \pm$ standard deviation values at the ambient temperature of 25 °C prior to experimentation and after 28 h exposure of the four seaweeds at 40, 35, 30, 25 and 20°C. The percentage change in F_v/F_m values after 4h is also given. The data were statistically analysed using one-way ANOVA. The letters [a,b,c,] indicate homogeneous groups across the temperature treatments based on Tukey's post-hoc test ($p < 0.05$; $n = 4$).

Temperature	Ambient	4 h	28 h
G. manilaensis			
40 °C	0.55 ± 0.03[a]	−40.73 ± 4.61[a]	0.05 ± 0.01[a]
35 °C	0.53 ± 0.04[a]	−15.54 ± 12.49[b]	0.53 ± 0.03[c]
30 °C	0.52 ± 0.08[a]	-6.30 ± 6.87[b]	0.56 ± 0.03[c]
25 °C	0.59 ± 0.03[a]	−14.38 ± 19.85[b]	0.50 ± 0.05[b,c]
20 °C	0.54 ± 0.03[a]	−17.34 ± 4.57[a,b]	0.44 ± 0.05[b]
U. reticulata			
40 °C	0.69 ± 0.03[b]	−54.00 ± 18.40[a]	0.07 ± 0.03[a]
35 °C	0.64 ± 0.03[a,b]	−11.58 ± 11.34[b]	0.55 ± 0.03[b]
30 °C	0.66 ± 0.01[a,b]	−14.08 ± 9.70[b]	0.70 ± 0.06[c]
25 °C	0.62 ± 0.05[a]	−11.75 ± 8.85[b]	0.57 ± 0.02[b]
20 °C	0.65 ± 0.02[a,b]	−12.82 ± 2.70[b]	0.65 ± 0.04[c]
K. alvarezii			
40 °C	0.58 ± 0.02[a,b]	−92.99 ± 5.06[a]	0.00 ± 0.00[a]
35 °C	0.62 ± 0.02[b]	−21.42 ± 5.38[b]	0.28 ± 0.29[a,b]
30 °C	0.54 ± 0.03[a]	−6.42 ± 6.96 [c]	0.55 ± 0.02[b]
25 °C	0.57 ± 0.01[a,b]	−10.14 ± 3.12[b,c]	0.54 ± 0.03[b]
20 °C	0.53 ± 0.05[a]	−17.58 ± 4.68[b,c]	0.46 ± 0.02[b]
T. conoides			
40 °C	0.75 ± 0.03[a]	−94.95 ± 4.61[a]	0.07 ± 0.01[a]
35 °C	0.76 ± 0.04[a]	−25.50 ± 12.49[b]	0.67 ± 0.03[b]
30 °C	0.74 ± 0.08[a]	−2.47 ± 6.96[c]	0.73 ± 0.03[b,c]
25 °C	0.76 ± 0.03[a]	2.24 ± 19.75[c]	0.74 ± 0.05[c]
20 °C	0.73 ± 0.03[a]	−3.29 ± 4.58[c]	0.75 ± 0.05[c]

940 **Supplementary Table S3**

941 Halocarbon compounds quantified and the surrogate analytes used in the experiment.

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Halocarbon	Chemical formula	Retention time (min)	Target ions (m/z)	Purity %
CH_3I	Iodomethane	8.4	142, 141	≥ 99
CH_2Br_2	Dibromomethane	11.2	174, 93	≥ 99
$CHBrCl_2$	Bromodichloromethane	11.3	83, 85	98.5
$CHBr_2Cl$	Dibromochloromethane	12.5	129, 127	95
$CHBr_3$	Bromoform	13.7	173, 171	≥ 99
CH_2I_2	Diiodomethane	14.2	268, 141	≥ 99
Surrogate analytes				
CD_3I	Deuterated iodomethane	8.4	145, 143	≥ 99
CD_2I_2	Deuterated diiodomethane	14.2	270, 143	≥ 99

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