Different Environmental Stressors and their Effect on TEX_{86} Signature of Marine Ammonia-Oxidizing Archaea

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Abstract

Marine ammonia-oxidizing archaea (AOA) compose their cell membranes with glycerol dialkyl glycerol tetraethers (GDGTs). These lipids are useful biomarkers for developing a paleo-climatological proxy for sea surface temperature. Through the TEX_{86} temperature proxy, GDGTs have been able to provide insight into historic patterns of sea surface temperature through the relationship between annual average sea surface temperature and the composition of GDGTs. To better understand how GDGTs change according to environmental pressures we conducted experiments with varying levels of light, pH, salinity, ammonia and peroxide to test their effect on TEX_{86} on strains of AOA (Qin et. al 2014 & 2015). Light levels influenced the total GDGT composition, where the composition was relatively higher for when the light was present but did not have a significant effect on the calculated TEX_{86} temperature. Salinity concentrations below and above optimal salinity at 25 ppt resulted in lower GDGT compositions and TEX_{86} sea surface temperatures (below 17 °C). As for pH, peroxide and ammonia levels, there appeared to be no major effect on calculated TEX_{86} temperatures with fluctuating concentrations, which suggest the presence of different adaptations that allow for archaea to acclimate to relatively extreme conditions. This will provide more understanding of TEX_{86} and archaeal physiology and more specifically the effect that different environmental stressors have on lipid composition, which ultimately relates to archaeal growth rates. In light of the conducted culture treatments, natural samples from the Northern Pacific Ocean were analyzed for their TEX_{86} temperatures. In situ temperatures did not correlate well with TEX_{86} for surficial nor subsurface temperatures in the water column suggesting that the TEX_{86} proxy may not be as direct of a correlation as originally determined.

Introduction

Archaea Physiology and GDGTs

Marine ammonia-oxidizing archaea (AOA) are abundant microorganisms that are found throughout the entire water column. Although not well-understood, AOA play a key role in the nitrogen cycle. Nitrogen is incorporated into the ocean through N_2-fixation and rivers and then taken up by primary producers, ammonia is released during phytoplankton death and decomposition. This ammonia can then be readily oxidized by archaea and bacteria into nitrite and further oxidized to nitrate and recycled (Stahl and de la Torre, 2012). Due to their importance in the nitrogen cycle, AOA were evaluated by Valentine (2007) for their adaptation to "chronic energy stress", which allows them to survive in an extensive range of environmental variation. Through the cultivation of archaea, it was found that AOA are able to survive in extreme salinity, temperature and pH conditions, which suggests they have adapted a physiological composition to survive a broad range of extreme conditions (Valentine 2007).

In his review paper, Valentine proposed that in order for Archaea to survive in extremely high salinity conditions above 150-200 g/l, they would need to be able to prevent the high concentration of sodium molecules from entering into their cells. An excessive amount of sodium entering cells negatively affects the electrochemical and osmotic gradient for nucleic acids and proteins. This highlights the importance of understanding lipid membrane compositions and the possibility of archaea restructuring their membranes to help them control the internal environment of their cell. Although a reasonable explanation, the effect of salinity on membrane lipid composition in cultured samples has yet to be demonstrated as a majority of these studies do not observe any

direct physiological influence and acclaim any changes in lipid composition to ecological effects (Dawson et al. 2012, Qin et al. 2014, Elling et al. 2015).

Archaea must also be able to prevent too many protons from getting into the cell through a strong proton gradient in acidic conditions because this can affect transmembrane flux. However, in some cases, archaea were found to be able to thrive in a pH below zero (Valentine, 2007). Once again, pH serves as another example of an environmental factor that could lead to changes in membrane composition which protects the internal cell from the external environment.

Many archaea are thermophiles that are known to adjust their membranes as a response to temperature (Wuchter et al. 2004, Qin et al. 2014, Elling et al. 2015). This finding led to the development of the TEX_{86} proxy (Fig. 1) in which the increase in the number of cyclopentane or cyclohexane rings with increasing temperature is related to lipid membrane compositions (Valentine 2007, Pearson and Ingalls 2013).

TEX_{86} and other proxies

With their ability to thrive in a range of conditions, AOA have been key in discovering and understanding different environments in the present as well as the past. Glycerol dialkyl glycerol tetraethers (GDGTs) are the lipid membranes that enclose the cells of AOA. Because they are not easily broken down they are able to provide us with a glimpse of the environment in which they were once living. When archaea die, the lipid remains of archaea sink to the seafloor and are stored and preserved in the sediment uncompromised by time. Since the lipid compositions have been found to increase with temperature, the archaeal lipids that are found in the sediments can provide a record of the temperature in which they were once living. Using the GDGTs found in surficial

sediments and their correlation with sea surface temperatures (SSTs), multiple studies have been performed to calculate historic SSTs dating back to the early Eocene (Zachos et al. 2006, Pearson et al. 2007). This paleotemperature proxy, $TEX_{86,}$ is determined through the relationship between annual average SSTs and GDGT composition in underlying surficial sediments.

Experiments using climate models can use TEX_{86} –derived SSTs from the past to predict future changes in temperature based on the climate conditions that once existed. However, in order for TEX_{86} to do this accurately, the fundamental cause for changes in membrane lipids must be understood. Comparisons between TEX_{86} and other proxies have been conducted, such as U^{k}_{-37}, which is based on long-chain (37 carbons) methyl ketones with two and three double bonds that are found in coccolithophores, called alkenones. Using the same marine sediment cores and measuring TEX_{86} values, it has been determined that TEX_{86} yields SSTs with higher variability than other proxies which may be due to TEX_{86} actually being linked with subsurface instead of surface ocean temperatures (Ho and Laepple 2016). This raises the importance of developing a better understanding of $TEX_{86,}$ its implications, and the factors that influence and affect the proxy.

Core vs Intact Lipids

Since TEX_{86} is assumed to be based on the correlation between surface sediment GDGT composition and SSTs, it is important to recognize the difference between membrane lipids of living and nonliving AOA. Although much focus has been directed towards the GDGTs of nonliving AOA as the main tracer for archaeal biomass to develop the paleo-proxy, recent studies have also begun to examine living AOA represented by

the intact GDGT pool in the marine water column. The key difference between GDGTs of living versus nonliving AOA is that living cells have GDGTs with polar head groups. The polar head group is hydrolyzed after the cell expires (Schouten et al. 2012). In a study conducted by Ingalls et al. (2012), water samples from Puget Sound, Washington State were filtered to determine the distribution of intact and core lipids in the water column. They defined free-living (0.2-0.7 μm), suspended (0.7-60 μm) and aggregate (>60 μm size) fractions to quantify how much of particulate organic matter is composed of intact and core membrane lipids. It was observed that approximately 90% GDGTs in the free-living fraction were intact lipids whereas the suspended and aggregate fractions both had only 29% intact GDGTs. Because particle size increases with depth, their findings suggest that because of their small size as individual particles, living AOA are found in the free-living fraction and once AOA are no longer living, they can be aggregated with other particles and sink to deeper depths. Even though the overall sizes of the fraction pools were different depending on total amount of GDGTs present, they concluded that core and intact pools did vary by fraction size and distribution with depth. These observations led them to suggest that archaea are typically free-living according to a higher proportion of intact GDGTs in the free-living fraction, signifying that once the cells die they are either packaged by grazers or directly aggregated with other particles in the water column before sinking down (Ingalls et al. 2016). This study raises the importance of studying archaeal ecology, where living fractions, which ultimately will aid our understanding of TEX_{86} –derived SSTs from natural samples depending on the environment from which they are extracted.

TEX_{86} in Natural and Culture Experiments

A number of studies, using both natural and cultured samples, have been done to better understand TEX_{86}, one example being a study by Herfort et al. (2006) conducted in the southern North Sea. This location offered a shallow controlled shelf environment with easy access to filter water samples yet dynamic with influence from coastal run-off and freshwater rivers. For a temperate climate, the in situ SSTs for February, April, and August or more generally winter to summer were recorded ranging from 6.5 to 15 ºC whereas the TEX_{86} –derived SSTs were relatively the same for both February and April but had a large inconsistency for August (Herfort et al. 2006). They hypothesized that the discrepancies could be due to a seasonal difference in GDGT composition where different species dominate at different times during the year and have diverse membrane compositions (Herfort et al. 2006, Pearson and Ingalls 2013). Another natural study conducted in the Santa Barbara Basin of California, United States also found inconsistencies between observed and TEX_{86} –derived SSTs and an overall trend of the proxy recording subsurface ocean temperatures as opposed to SSTs in their marine sediment samples (Huguet et al. 2007). A similar conclusion was reached by Xing et al. (2015) in a study of surface sediment samples throughout the Yellow and East China Seas. Huguet et al. (2007) proposed that before attempting to apply TEX_{86}, calibration studies must be done to understand what signal, either subsurface or surface, the proxy is recording.

As for culture studies, a majority focus on a phylum of marine AOA, *Thaumarchaeota* which are convenient for studies because of their wide-spread distribution in environments ranging from polar regions, coastal estuarine systems, to oligotrophic zones in the open ocean and abundance throughout the entire water column

(Pearson and Ingalls 2013). Once they are extracted from an environment, they can be inoculated and grown in laboratories in controlled experiments to see the effect that different parameters have on GDGT composition in isolation as opposed to environmental conditions with many other variables, such as seasonal changeability. Elling et al. (2015) conducted a culture study to observe the effect of environmental factors on GDGT composition and grew different strains of AOA such as *N. maritimus* in varying salinities, temperatures and pH. *N. maritimus* were grown in temperatures of 22 ºC, 25 ºC, and 28 ºC and were observed to have a positive linear correlation with the composition of GDGTs which was a similar finding in other studies focusing on temperature (Wuchter et al. 2004, Schouten et al. 2012, Elling et al. 2015). This strain of AOA also underwent salinity (27-51 ‰) and pH (7.3-7.9) treatments but neither showed a significant effect on TEX_{86} values. This result is unlike other studies that observed a negative correlation with pH and composition of GDGTs (Boyd et al. 2011, Pearson and Ingalls 2013, Elling et al. 2015). Other parameters that appear to influence lipid membrane composition of AOA are oxygen and peroxide concentrations, where calculated TEX_{86} temperatures were found to increase with oxygen limitation (Qin et al. 2015) and peroxide was seen to inhibit ammonia oxidation ultimately decreasing TEX_{86} values (Kim et al. 2016).

In full, these results suggest that there is an ecological influence on TEX_{86} values that are obtained in cultivated and environmental samples. It is important to make clear the effects of parameters such as salinity, pH, and peroxide in lab experiments to understand the effects they have in isolation to expand that knowledge and apply it to the environment where typically many parameters are present in combination.

Testing the Effect of Environmental Factors on TEX_{86}

Existing data suggest that in addition to temperature, other environmental factors might influence TEX_{86} (Qin et. al 2014 & 2015, Hurley et al. 2015). Here, cultured samples of AOA were extracted, inoculated and grown in treatments for salinity, peroxide, pH, ammonia and light to see how TEX_{86} would respond to these parameters. Experiments with varying light, salinity, pH, ammonia and peroxide levels were conducted to test their effect on TEX_{86} on strains of AOA with the hypothesis that these parameters would affect TEX_{86} (Qin et. al 2014 & 2015, Hurley et al. 2015). Then using existing environmental data collected from two transects in the North Pacific Ocean, measured TEX_{86} values were analyzed with findings derived from cultured samples of AOA to interpret the trends observed.

Methods

Culture Growth Experiments

For this study, existing culture samples were used to examine GDGT response to salinity, peroxide, ammonia, pH and light. These data were analyzed in Dr. Ingalls' laboratory at the University of Washington using methods from Qin et al. 2014 & 2015. The AOA were grown in triplicate and in varied conditions of one of the five environmental factors listed above. Treatments for salinity, peroxide and light level were extracted and analyzed in 2015. Cultures grown in treatments for ammonia and pH were extracted in December 2016 and added to the existing GDGT data set (Table. 1). The AOA in cultures were harvested during the early stationary growth phase using 0.22 μm Durapore membrane filters which were later stored at -20 ºC until extraction through acid hydrolysis. The samples were hydrolyzed using 5% Hydrochloric acid in methanol at 70

ºC and then later the methanol was combined with dichloromethane (DCM) and MilliQ in combusted glass vials. The original acid solution was extracted three times and rinsed multiple times with MilliQ and DCM to remove any left-over acid when transferring the rest of the GDGTs in the DCM layer. After rinsing and drying under N_2, the DCM extracts were dissolved with a known amount of internal standard, C_{46} GDGT, and processed using HPLC/MS. GDGT and internal standard peak areas were found by scanning mass range of 730-1350 m/z, where GDGT relative abundance (%) was calculated by dividing the sum of the peak areas of all core GDGTS by the peak area of each lipid (Qin et al. 2014 & 2015). Using these methods, additional cultured samples for ammonia and pH treatments have been extracted and processed for this study.

Environmental Samples

Natural samples were collected from the North Pacific Ocean on the Dimensions of Biodiversity Cruise in August 2013 aboard *R/V Kilo Moana*. Samples were collected at 10 different depths at a total of 16 stations (Fig. 2). Three of the stations: 5, 8, and 13 were selected for analysis in this research study because of the different biogeochemical systems they entail (Table. 2). The samples collected for these stations were stored in the -20 ºC and were extracted along with the culture samples according to Qin et al. 2014 & 2015. Once these samples were processed using HPLC/MS, the results were then analyzed according to trends observed from the culture growth experiments.

Results

Culture Growth Experiments

Peroxide concentrations did not affect calculated TEX_{86} values until concentrations reached 10 µM, which exceeds natural concentrations (Fig. 3). At 10 µM, the depletion in growth rates displayed a significant difference in TEX_{86} temperatures relative to the control (p-value << 0.05). Observations from the ammonia treatment portrayed an inverse relationship such that when ammonia concentrations increased to 1 mM the calculated TEX_{86} temperatures decreased from 13 ºC to approximately 9 ºC (Fig. 4). However, with concentrations above 1 mM, the TEX_{86} temperatures unexpectedly increased to 14.5 ºC at a concentration of 3 mM. The pH treatment appeared to have no effect on TEX_{86} temperatures where it was constant at 12.5 ºC (Fig. 5). With ammonia concentration of 0.2 mM being the control, concentrations above 1 mM were deemed significant (p-value < 0.05). However, when the pH was decreased to 6.4, the calculated TEX_{86} values declined to 9.5 ºC. The difference in TEX_{86} temperatures for pH 6.4 was significant in comparison to the control pH 7.3 at optimum growth but not significant for pH 6.5 with the control. In the culture samples, it was found that TEX_{86} temperatures were highest when the salinity was 25 (Fig. 6). The TEX_{86} temperatures were recorded to be below 17 ºC when outside the optimal salinity range. For the salinity experiments, TEX_{86} values at 40 ppt were calculated to be significantly different relative to the control at 25 ppt. Light levels influenced the GDGT composition where the calculated TEX_{86} values were higher when the cells were exposed to light as opposed to being grown without light (Fig. 7).

Environmental Samples

Depth profiles for TEX_{86} temperatures from Stations 5, 8, and 13 all showed the general trend of decreasing temperatures from surface to depth of nitrification maximum

which is at approximately 100-175 m, while below 175 m TEX_{86} temperatures were found to gradually increase (Fig. 8). Actual temperatures at each of these locations were significantly different than the TEX_{86} inferred temperatures where in situ temperatures were observed to be highest at the surface and then decreased with depth. From the surface down to 300 m, temperatures ranged from 16º to 4 ºC, 15º to 4 ºC, and 26º to 11 ºC for Stations 5, 8 and 13, respectively.

Discussion

Culture Growth Experiments

The factors that directly affect AOA cell growth and physiology and ultimately how TEX_{86} values respond to a changing environment remain poorly understood with contradicting hypotheses circling within the scientific community in regards to whether TEX_{86} provides a record of SST or subsurface temperatures closer to the nitrification maximum or it is also possible that neither of these is correct (Ho and Laepple 2016).

The influence of varying concentrations of peroxide, ammonia, pH, salinity and light on cell growth rates and the TEX_{86} paleotemperature proxy were examined. Hydrogen peroxide (H_2O_2) levels did not affect calculated TEX_{86} temperatures, which suggests the presence of different adaptations that allow for archaea to acclimate to relatively extreme conditions. Kim et al. (2016) conducted a study that focused on the relevance of peroxide as a mechanism affecting the growth of the SCM1 strain of AOA in a culture experiment. The α-keto glutarate found in AOA is an organic compound important in the Krebs cycle. Detoxifying H_2O_2 by undergoing decarboxylation and hydrating the ketone group found in the α-Keto-acid is a crucial step for their cell function. With the removal of α-Keto-acid from the mitochondrial membrane, it was

observed that H_2O_2 levels within cells rose to approximately 4.5 μM which became inhibitory to cell growth suggesting that α-Keto-acid among similar compounds control the levels of H_2O_2 found intracellularly (Kim et al. 2016). The recognition that intracellular levels of H_2O_2 are controlled by extracellular organic compounds could explain our observation of the effect of H_2O_2 on TEX_{86} values, which only changed TEX_{86} temperatures above 10 μM which greatly surpasses any concentration of peroxide found in natural environments (Fig. 3).

The ammonia treatment revealed an inverse relationship where ammonia decreased with increasing TEX_{86} until concentrations reached 1 mM but then increased as concentrations rose to 3 mM (Fig. 4). Since AOA utilize ammonia as a reducing power the trend between 0.2 to 1 mM, although at lower concentrations, was also observed by Hurley et al. (2016) as AOA membranes are expected to become less permeable when ammonia concentrations are higher in order to control intracellular gradients. However, Hurley et al. (2016) have not yet calculated TEX_{86} for ammonia concentrations above 1 mM. These data support the trend that TEX_{86} temperatures are calculated to be higher when AOA are in stressful environmental conditions such as lower concentrations of ammonia. In conditions where ammonia or oxygen availability is considered limiting, AOA electron flow is compromised and may result in increasing lipid membrane permeability. Membrane permeability increases with the increase in cyclopentane ring formation because decreased electron flows causes cyclization of GDGTs. This hypothesis proposed by Hurley et al. (2016) could also explain the higher TEX_{86} temperatures at higher concentrations of ammonia where it is no longer limiting. Excess

amounts of ammonia can be considered another stressful condition but in this case, AOA cell machinery could be compromised or hindered.

There was no significant effect of pH on calculated TEX_{86} temperatures within the range of observed experimental pH values excluding a pH of 6.4 (Fig. 5). There was a change in TEX_{86} temperature when the pH was decreased to 6.4. The calculated TEX_{86} values declined from growth temperatures from about 17.5 ºC to 9.5 ºC but considering that pH in the ocean water column does not reach this low pH this observation is not relevant unless applied to AOA from certain terrestrial or sediment environments. Although the trend with pH for this study is supported by data collected by Elling et al. (2015), this result is unlike other studies that have observed a negative correlation with the composition of GDGTs and pH (Boyd et al. 2011). This inconsistency raises the importance of performing further studies to develop a better understanding of how these factors affect the TEX_{86} proxy and ultimately how it affects GDGT composition.

Similar to Elling et al. (2015), varying concentrations of salinity did not demonstrate any effect on TEX_{86} temperatures unless they were outside the optimal salinity of 25 (Fig. 6). Salinity concentrations above and below 25 resulted in lower calculated TEX_{86} values at 17 °C or below for the HCE1 strain. The only significant difference in TEX_{86} temperatures was found for 40 ppt but further studies will need to be done to confirm and examine effects of salinity above this concentration. A majority of studies, including this one, have detected no effect of salinity on membrane lipid composition in cultured samples. At the current time, if there is any direct physiological influence by salinity it is yet to be demonstrated where any changes in lipid composition

to be attributed to ecological effects (Dawson et al. 2012, Qin et al. 2014, Elling et al. 2015).

The SCM1 strain of AOA were either grown in no light conditions or at an intensity of 15 $\mu e/m^2/s$. The purpose of this treatment was to observe whether photoinhibition affected GDGT composition raising possible implications for trends observed in natural samples. Ammonia oxidation rate in natural samples is mostly attributed to the presence of AOA which in turn could be influenced by depth of light penetration within the water column. From these data collected, it can be seen that TEX_{86} temperatures were higher in the presence of light compared to when AOA were placed in darkness. Variability in ammonia concentrations showed a decrease in TEX_{86} when ammonia increased to 1 mM which displayed a similar trend as observed by Hurley et al. (2016).

Environmental Samples

The three stations were chosen according to their characteristics where Station 5 is representative of a transition between the coastal and open ocean, Station 8 is an open ocean site with higher nutrients relative to Station 13 which is located in the North Pacific gyre and considered as an oligotrophic environment. Examining these data, it can be observed that temperatures derived from the TEX_{86} proxy did not accurately match the measured in situ temperatures (Fig. 8). The decrease in temperature from the surface to the depth where ammonia oxidation rates were highest or in other words the nitrification maximum, was better reflected in the TEX_{86} derived temperatures. But below the AOA rate maximum, TEX_{86} and in situ temperatures, diverged. The depth of maximum nitrification represents the depth at which *Thaumarchaeota* are most abundant, implying

that TEX_{86} values may be sensitive to ammonia oxidation rate as opposed to water temperature, with maximum AO rates associated with maxima in total GDGT concentration and minima in TEX_{86} temperatures (Hurley et al. 2016).

Although the culture studies were conducted on different strains of *Thaumarchaeota* for the purpose of discussing the environmental trends observed it will be assumed the strains would respond relatively similarly until further studies are conducted. Considering the results from the culture treatments, the treatments with no major effect on TEX_{86} values can be disregarded for analysis of the environmental samples. This is because if no effect was observed in more extreme concentration differences found in culture, it can be assumed these effects will not be observed in the North Pacific as it does not experience that level of extremity. Despite not having complete data for ammonia and nitrite concentrations for both Stations 5 and 13, both Stations 5 and 8 started with higher concentrations of ammonia at surface depths, approximately 276 and 203 nM for Stations 5 and 8 respectively (Fig. 8). At the nitrite maximum, the ammonia concentrations decreased to about 87, 12 and 26 nM at the depths 100-115 m for Stations 5, 8 and 13 respectively where TEX_{86} temperatures were at their lowest and ammonia oxidation rates at their highest, as would be expected (Hurley et al. 2016). Isoprenoid or total GDGTs concentration (ng/L) followed the observed trends for ammonia oxidation rate accurately for both Stations 5 and 8 but due to the lack of data for Station 13, it is unclear whether these parameters follow a similar trend.

In terms of understanding the TEX_{86} proxy and the temperatures derived from it, it can be acknowledged that there is a lack of correlation between in situ temperatures and TEX_{86}–derived SSTs. Since the isoprenoid concentration is relatively low in surface

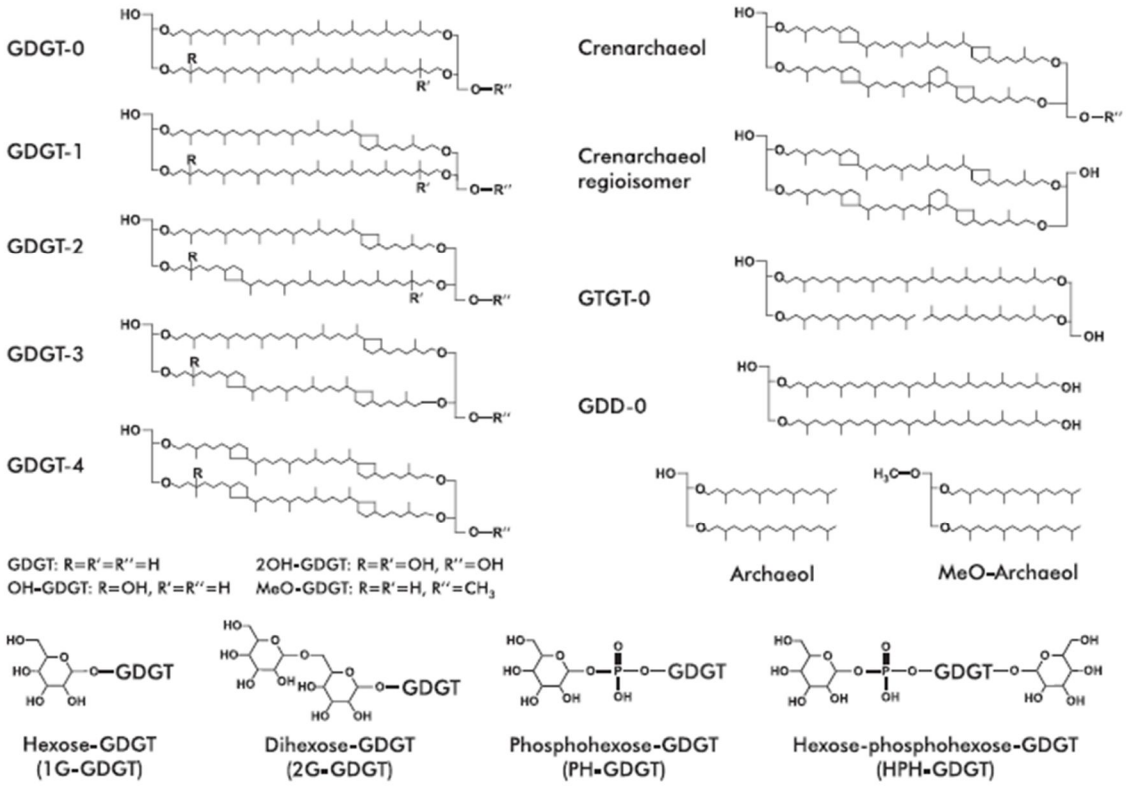
waters and sinking rates, as well as the depth of export, are not well known, this suggests the TEX_{86} proxy may be recording subsurface temperatures instead (Ho and Laepple 2016). Although this is considered to be a reasonable explanation as subsurface depths are composed of the highest concentration of GDGTs, the TEX_{86} temperatures still do not reflect in situ temperatures accurately. It is also possible that neither of these hypotheses is correct, and that the proxy may not be directly measuring temperature but instead measuring a combination of factors that influence GDGTs and result in values that appear to look like SST or subsurface temperatures.

Conclusion

Experiments with varying ammonia, peroxide, salinity, pH and light levels were conducted to test their effect on TEX_{86} values on strains of AOA and relate them to natural samples extracted from the North Pacific. It was found that parameters such as salinity and peroxide respond inversely from increased light whereas pH had no observed effect until a pH of 6.4. Ammonia concentration increased with decreasing TEX_{86} temperatures where higher concentrations are assumed to start inhibiting ammonia oxidation rates and yield warmer TEX_{86} temperatures. These higher TEX_{86} values deviated from in situ temperature profiles for Stations 5, 8 and 13. This study demonstrated that different environmental stressors have an effect on lipid composition. These results raise questions about the implications of using GDGTs as an environment recorder if TEX_{86} temperatures respond differently to the parameters considered. For example, salinity and peroxide responded oppositely from increased light but how to

apply results obtained from culture growth treatments to what occurs in the natural environment has yet to be understood.

Figures and Tables



$$TEX_{86} = \frac{[GDGT-2]+[GDGT-3]+[Cren']}{[GDGT-1]+[GDGT-2]+[GDGT-3]+[Cren']}$$

Fig. 1. Structures of core and intact GDGTs and the TEX_{86} index used to calculate SSTs (Elling et al. 2015).

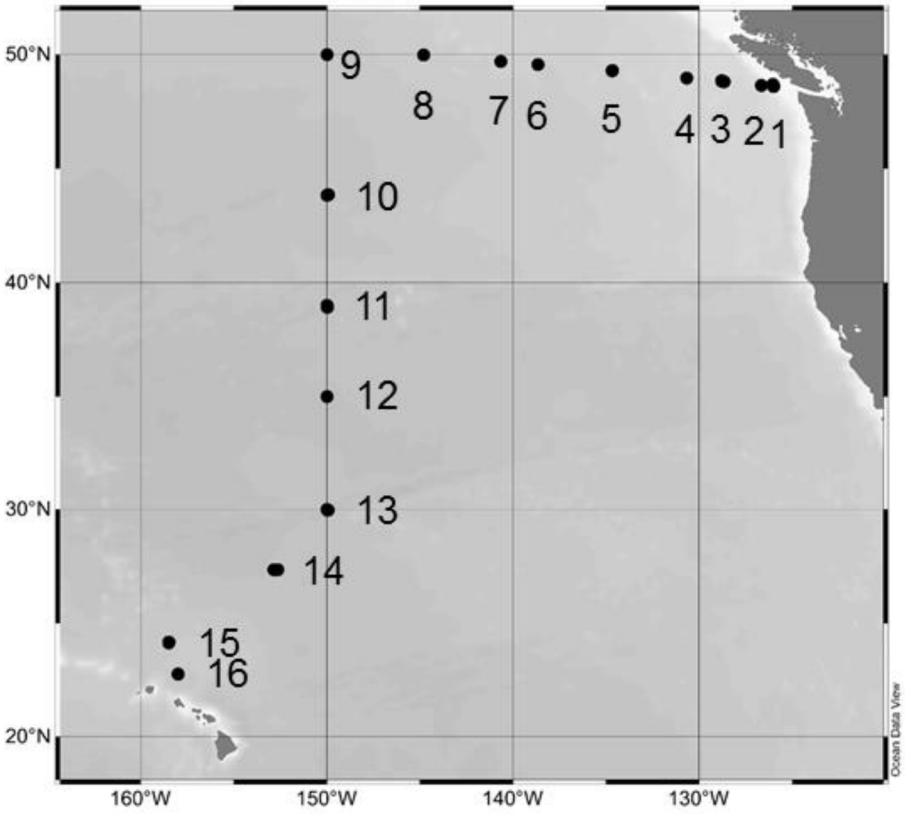


Fig. 2. Map of August 2013 Dimensions of Biodiversity Cruise stations.

Table 1. Five treatments for culture growth experiments.

Treatment				
Salinity	Peroxide	Light Level	pH	Ammonia
15	Control	Light	6.4	0.2 mM
20	50 nM	Dark	6.5	0.5 mM
25	100 nM		6.8	1 mM
35	500 nM		7.3	3 mM
40	1 µM		7.5	5 mM
	5 µM		7.8	10 mM
	10 µM			20 mM

Table 2. August 2013 Dimensions of Biodiversity cruise station coordinates.

Station	Latitude (°N)	Longitude (°W)
5	49.29	225.32
8	49.99	215.19
13	30	210

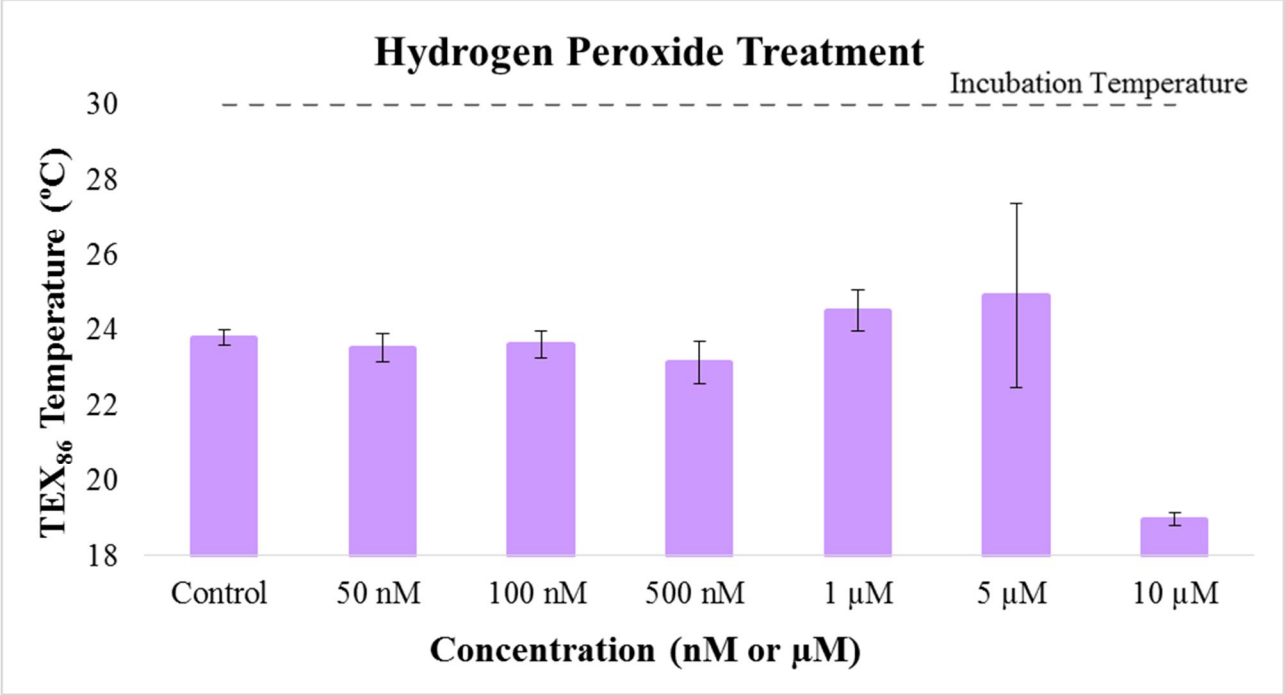


Fig. 3. Peroxide levels did not affect the calculated TEX_{86} temperatures for strain SCM1 until concentrations reached 10 µM where TEX_{86} values rapidly declined. Incubation temperature was at 30 °C.

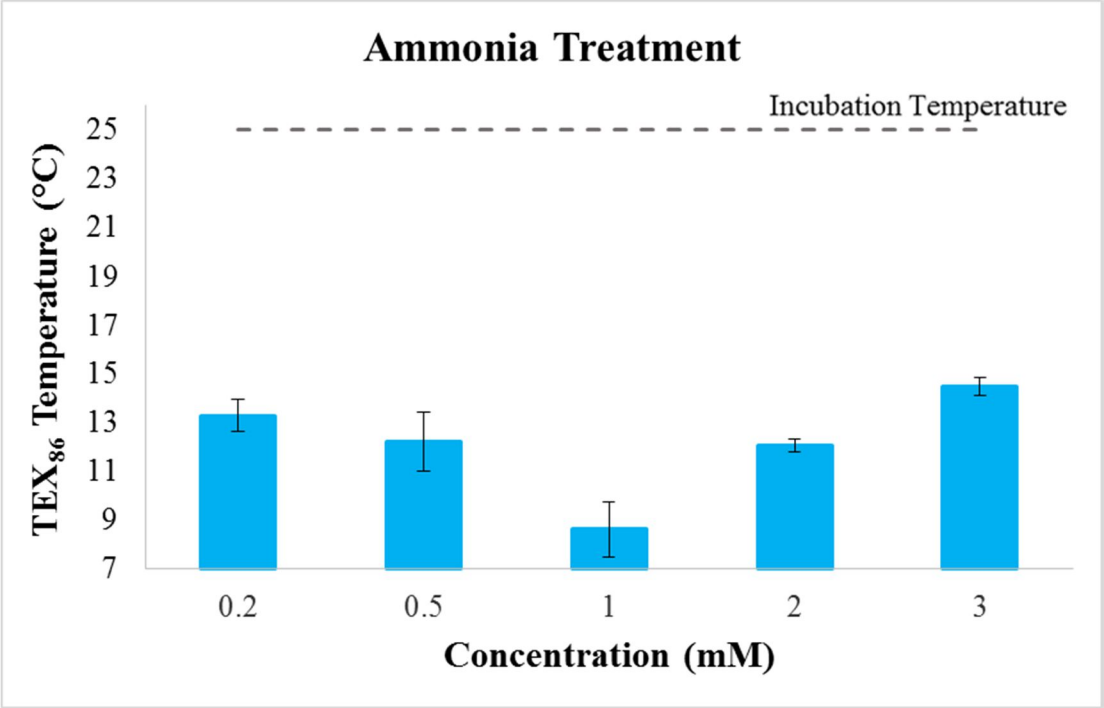


Fig. 4. Ammonia concentrations (mM) decreased from 0.2 to 1 mM. Ammonia concentrations above 1 mM resulted in higher TEX_{86} values (above 9 ºC). Incubation temperature was at 25 °C.

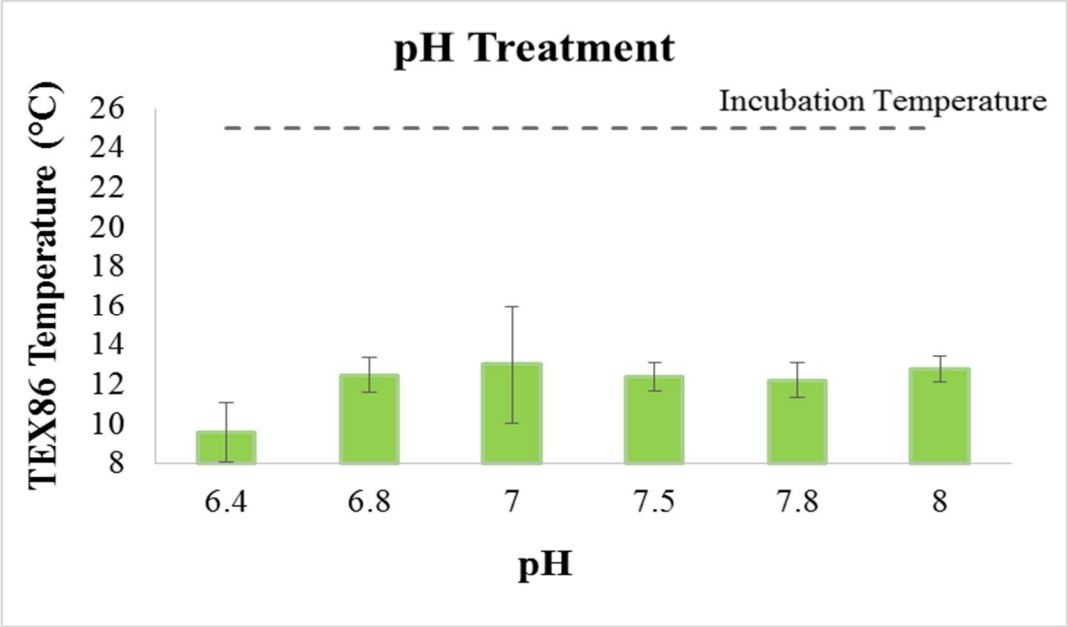


Fig. 5. Calculated TEX_{86} temperatures (ºC) were not affected until pH reached 6.4 where TEX_{86} values declined. Incubation temperature was at 25 °C.

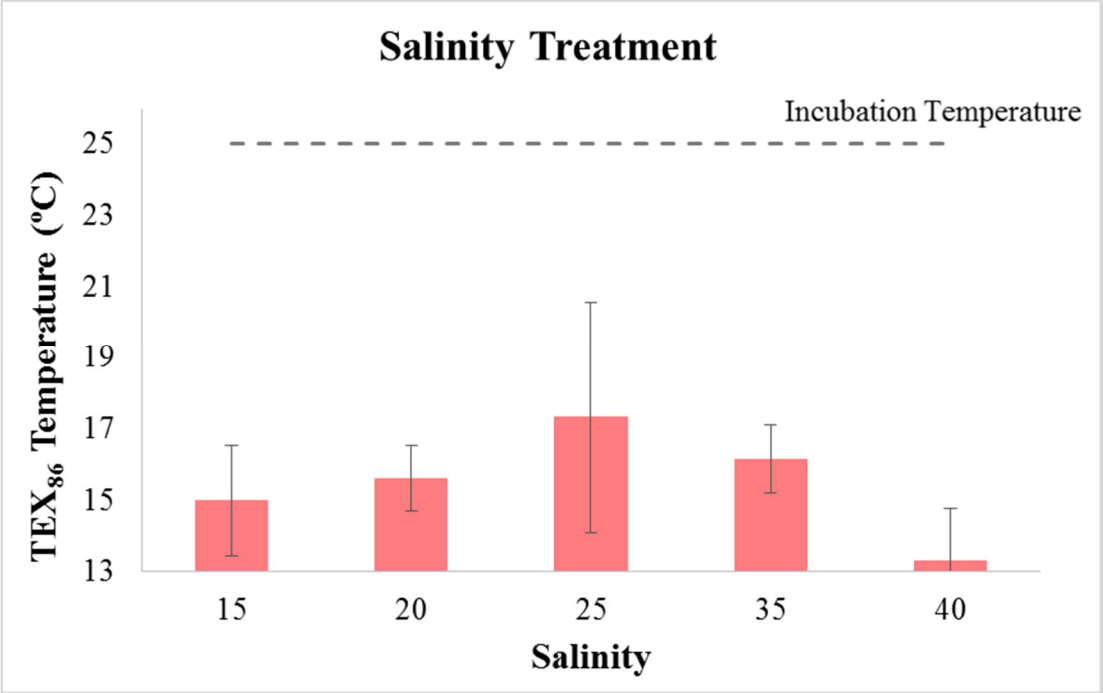


Fig. 6. Salinity concentrations above and below the optimal salinity of 25 resulted in lower calculated TEX_{86} values (below 17 °C). Incubation temperature was at 25 °C.

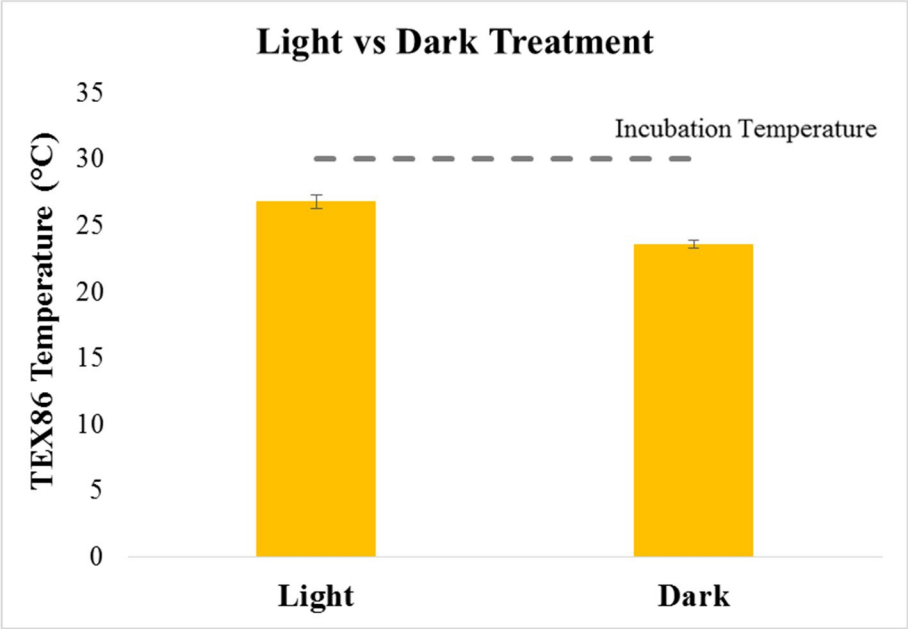
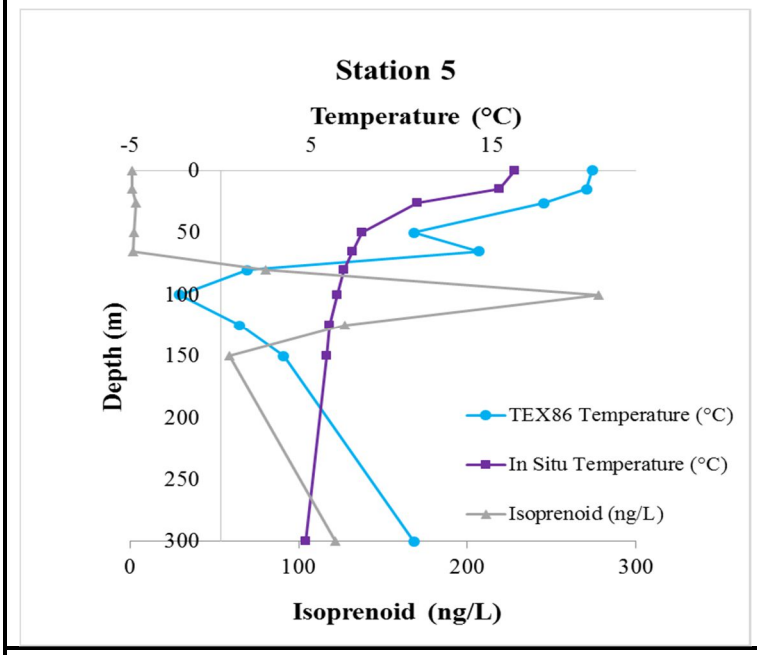
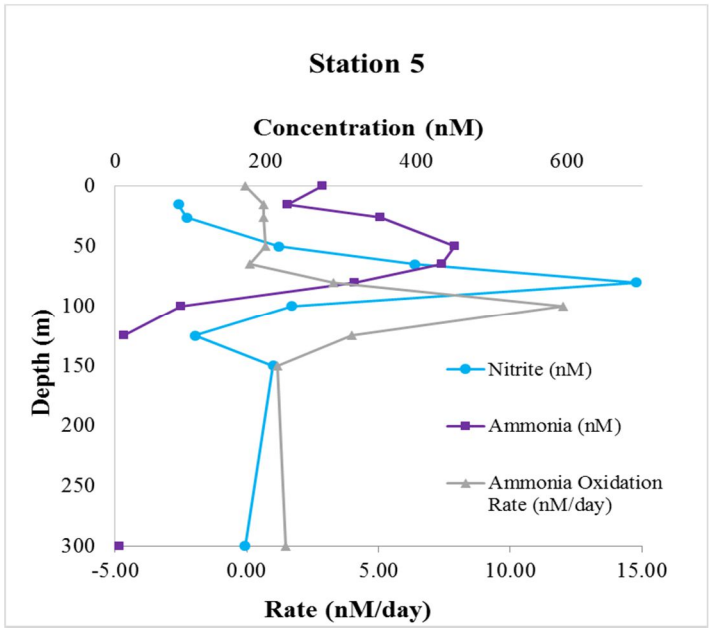


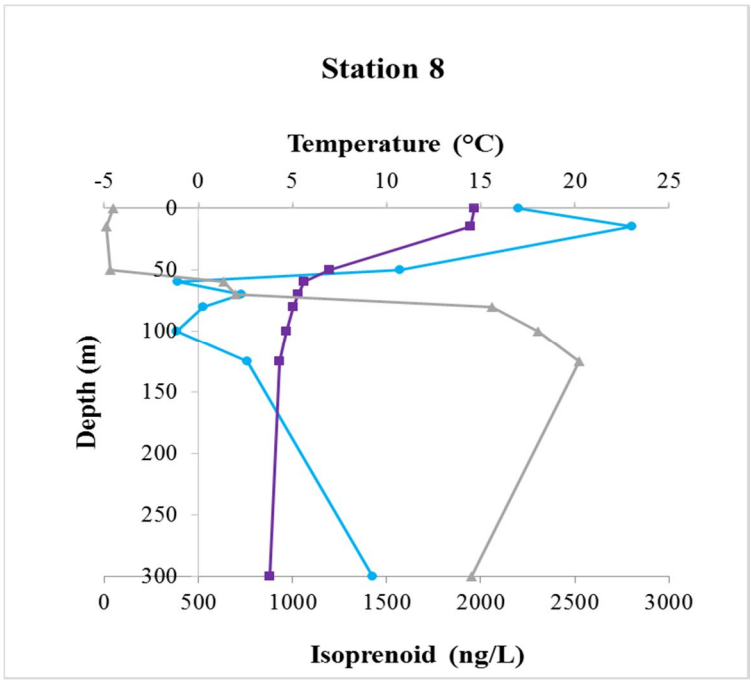
Fig. 7. Light levels influenced the GDGT composition. The calculated TEX_{86} values were higher when the cells were exposed to light. Incubation temperature was at 30 °C for both conditions.

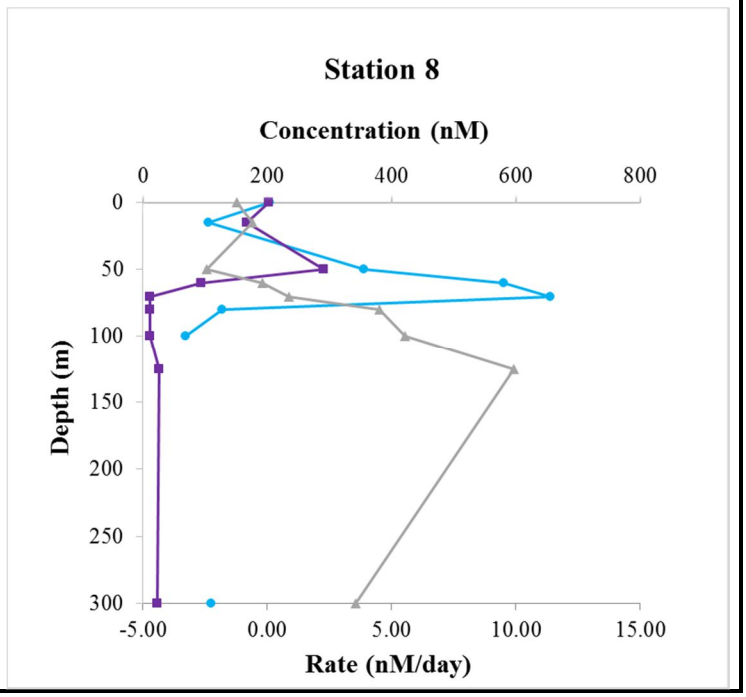
a


Station 5
Temperature (°C)
-5
5
15
0
50
100
150
200
250
300
Depth (m)
TEX86 Temperature (°C)
In Situ Temperature (°C)
Isoprenoid (ng/L)
0
100
200
300
Isoprenoid (ng/L)


Station 5
Concentration (nM)
0
200
400
600
0
50
100
150
200
250
300
Depth (m)
Nitrite (nM)
Ammonia (nM)
Ammonia Oxidation Rate (nM/day)
-5.00
0.00
5.00
10.00
15.00
Rate (nM/day)

b


Station 8
Temperature (°C)
-5
0
5
10
15
20
25
0
50
100
150
200
250
300
Depth (m)
0
500
1000
1500
2000
2500
3000
Isoprenoid (ng/L)


Station 8
Concentration (nM)
0
200
400
600
800
0
50
100
150
200
250
300
Depth (m)
-5.00
0.00
5.00
10.00
15.00
Rate (nM/day)

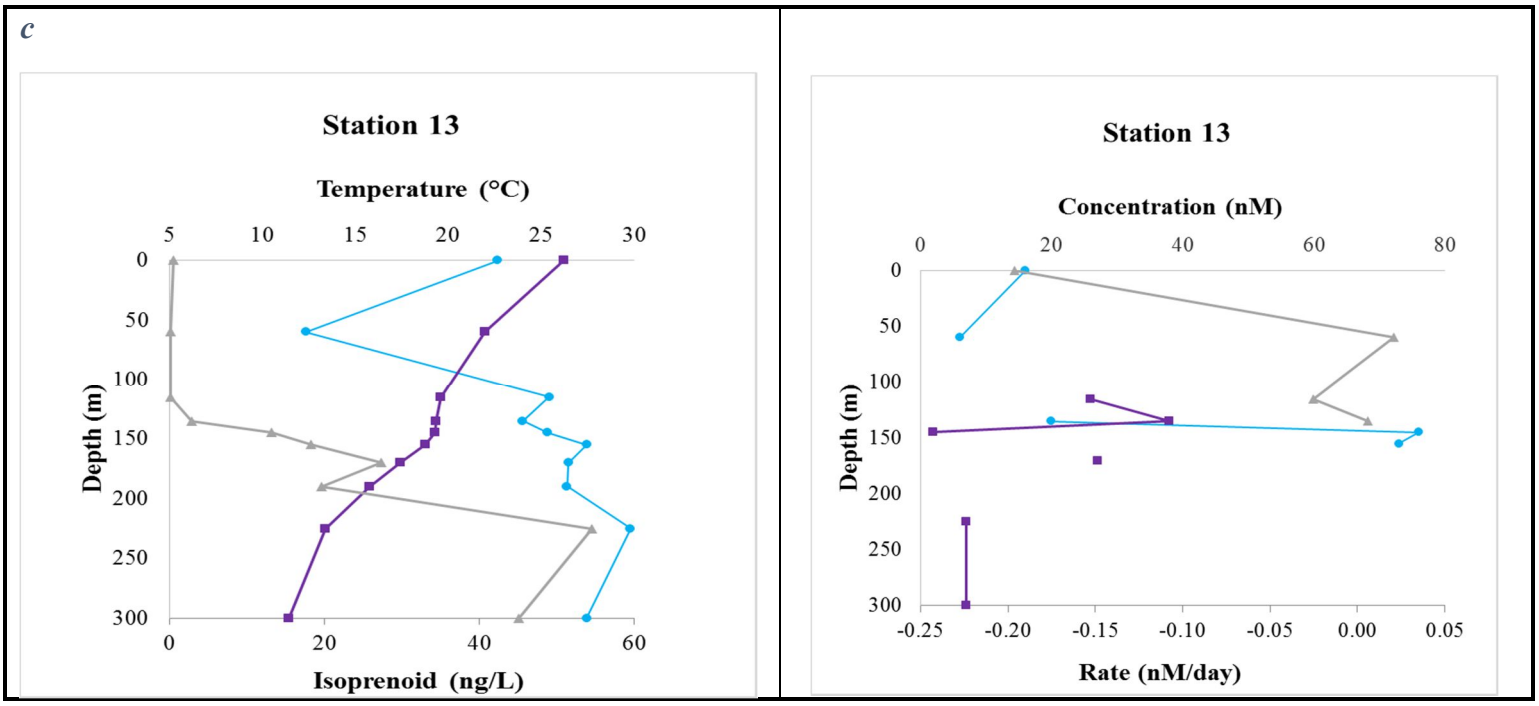


Fig. 8. Depth profiles for each of the stations from the Dimensions of Biodiversity Cruise in August 2013. The TEX_{86} temperatures (°C), in situ temperatures (°C) and total GDGTs concentrations (Isoprenoid ng/L), are portrayed for Station 5 (a), Station 8 (b), and Station 13 (c). The additional panel includes supplementary data from cruise including nitrite (nM) and ammonia (nM) concentrations as well as ammonia oxidation rate (nM/day) for each of the stations in their respective row. Note: supplemental datasets (In Situ Temperature, Ammonia Oxidation Rate, Nitrite, and Ammonia concentrations) for Station 5 and 13 are incomplete.

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