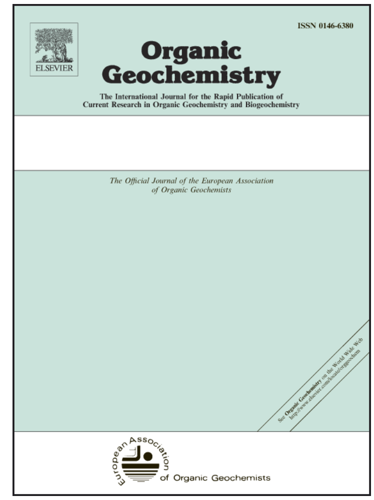
Journal Pre-proofs




# Group 2i Isochrysidales flourishes at exceedingly low growth temperatures (0 to 6 °C)

Sian Liao, Yongsong Huang

PII: S0146-6380(22)00146-2
DOI: https://doi.org/10.1016/j.orggeochem.2022.104512
Reference: OG 104512

To appear in: *Organic Geochemistry*

Received Date: 18 March 2022
Revised Date: 13 August 2022
Accepted Date: 12 October 2022

Please cite this article as: Liao, S., Huang, Y., Group 2i Isochrysidales flourishes at exceedingly low growth temperatures (0 to 6 °C), *Organic Geochemistry* (2022), doi: https://doi.org/10.1016/j.orggeochem.2022.104512

**Group 2i Isochrysidales flourishes at exceedingly low growth temperatures (0 to 6 °C)**

Sian Liao [a,b], Yongsong Huang [c,*]

[a] *Department of Chemistry, Brown University, 324 Brook Street, Providence, Rhode Island 02912, USA*

[b] *Institute at Brown for Environment and Society, Brown University, Providence, RI 02912, USA*

[c] *Department of Earth, Environmental and Planetary Sciences, Brown University, 324 Brook Street, Providence, Rhode Island 02912, USA*

[*] Corresponding author. Tel.: +1 401 863 3822; fax: +1 401 863 2058. E-mail address: yongsong_huang@brown.edu (Y. Huang).

**ABSTRACT**

Group 2i Isochrysidales (Haptophyta) microalgae were recently suggested to be the main producer of the $C_{37:4}$ alkenone in high latitude oceans with seasonal sea ice, enabling potential quantitative sea ice reconstructions using $C_{37:4}$%. However, the conditions favoring the growth of Group 2i are currently unclear. Phylogenetically, Group 2i is indistinguishable from OTU8 and Hap-A previously reported in numerous saline lakes around the world, and has been found to bloom in the early spring season in Lake George, North Dakota. These observations suggest Group 2i may have a preference for low growth temperatures and may grow within or underneath seasonal ice. Here we performed systematic culture experiments of Group 2i RCC5486 (isolated from sea ice of Baffin Bay) at exceedingly low temperatures (0, 3, 6, 9 °C at 31 ppt) and different salinities (15, 21, 26, 31, 38 ppt at 3 °C). We found Group 2i RCC5486 displays unusually fast growth from 0 to 6 °C, but failed to grow at 10.5 and 12 °C. These data confirm that Group 2i is explicitly adapted to low temperatures in both saline lakes and high latitude ocean regions. Salinity, on the other hand, does not significantly influence the growth rates of Group 2i RCC5486. In addition, both growth temperature and salinity show minimal influence on $C_{37:4}$%, which supports the use of $C_{37:4}$% as a proxy for sea ice, rather than temperature or salinity in high latitude oceans.

*Keywords*: Haptophytes; Group 2i Isochrysidales; $C_{37:4}$ alkenone; Culture experiments; Salinity effect; Temperature effect

## 1. Introduction

The Isochrysidales is an order of haptophyte algae. It has been the subject of intense study over the past 40 years due to its capability to produce highly characteristic lipid biomarkers called long-chain alkenones that are excellent for paleoclimate reconstructions in ocean and terrestrial environments (de Leeuw et al., 1980; Brassell et al., 1986; Marlowe et al., 1984; Prahl and Wakeham, 1987; Rechka and Maxwell, 1988; Zheng et al., 2019; Liao et al., 2020). Based on 18S rDNA phylogenetic sequencing data, the Isochrysidales has been classified into three groups with each group varying in preferred growth environments: Group 1 species (e.g., uncultured Isochrysidales from Lake Braya Sø) in fresh to oligohaline environments, Group 2 (e.g., *Isochrysis galbana*) in coastal and saline lake environments and Group 3 (e.g., *Emiliania huxleyi*) in the open ocean (Theroux et al., 2010; Zheng et al., 2019). Alkenones and alkenoates produced by these three groups of Isochrysidales show distinct features that are invaluable for chemotaxonomic classifications (Zheng et al., 2019; Liao et al., 2020, 2021) as well as paleohydrological reconstructions (Zhao et al., 2018; Huang et al., 2021; Yao et al., 2021).

Recently, a subclade of Group 2 Isochrysidales, Group 2i (i refers to sea ice), was defined based on 18S rDNA phylogenetic data obtained from the algal culture RCC5486 isolated within the sea ice on the Baffin Bay (Wang et al., 2021). The small subunit 18S rDNA sequence of RCC5486 was first published by Gérikas Ribeiro et al. (2020) who showed that RCC5486 was a species of *Isochrysis*. Group 2i DNA sequences were also recovered in surface sediment samples where seasonal sea ice occurs (Wang et al., 2021). These surface sediments and associated water column particles also contain unusually high amounts of $C_{37:4}$ alkenones with the percentage of $C_{37:4}$ over total $C_{37}$ alkenones up to 77% (Harada et al., 2003; Bendle et al., 2005; Wang et al., 2021). In fact, a strong correlation between $C_{37:4}$% and sea ice concentration was found for both surface sediments and water column samples in the high latitude north Pacific and Atlantic locations (Harada et al., 2003; Bendle and Rosell-Melé, 2004; Bendle et al., 2005; Harada et al., 2008), suggesting that $C_{37:4}$% could serve as a quantitative proxy for reconstructing past sea ice extent (Wang et al., 2021).

The proposed use of $C_{37:4}$% as a sea ice proxy provides an explanation for the previously observed correlation between $C_{37:4}$% and surface water salinity in the Nordic Sea and Bering Sea (Rosell-Melé, 1998; Ishiwatari et al., 2001; Sicre et al., 2002; Harada et al., 2003; Bendle et al., 2005), as the decrease in surface salinity may have resulted from thawing seasonal sea ice (Wang et al., 2021).

Unlike the sediment concentrations of $C_{25}$ highly branched isoprenoids (HBIs) that were previously developed as a sea ice proxy (Belt and Müller, 2013; Belt, 2018), $C_{37:4}$% is an internal ratio (rather than an absolute concentration), hence its use as a sea ice proxy would be less affected by changes in productivity of various aquatic organisms and sedimenation rates. Importantly, alkenones also have higher diagenetic stability, with the oldest sediments containing alkenones dating back to 120 Ma (Brassell et al., 2004). In comparison, HBIs are likely more susceptible to biotic and abiotic degradations than alkenones due to the presence of terminal C=C bonds (Belt and Müller, 2013; Sanz et al., 2016; Stein et al., 2016; Belt, 2018; Rontani et al., 2018).

Isochrysidales species phylogenetically similar to Group 2i also occur widely in saline lakes. For example, the 18S rDNA small subunit sequence of Group 2i is indistinguishable from OTU8 previously reported from saline lakes around the world

(Theroux et al., 2010, 2020; Yao et al., 2022) and Hap-A from Lake George (Toney et al., 2012). Phylogenetically indistinguishable Isochrysis DNA sequences have also been recovered from waters and sediments of perennially ice-covered Lake Fryxell, Antarctica (Jaraula et al., 2010), and saline lakes in Canada (Plancq et al., 2019; Wang et al., 2021; Yao et al., 2022). Importantly, OTU8 is found to bloom in the water column soon after ice off in the early spring in Lake George, North Dakota, USA and does not seasonally overlap with the growth of OTU7 that blooms in the middle of summer (Theroux et al., 2020). OTU7 occurs widely in Chinese saline lakes, and to be consistent with Group 1-3 classifications (Theroux et al., 2010; Wang et al., 2021), is recently renamed Group 2w1, along with another summer blooming subclade Group 2w2 based on small subunit 18S rDNA data (Yao et al., 2022). In culture, the lacustrine Group 2i strains isolated from Lake George show fast growth at low temperatures from 4 to 10 °C, but growth rate decreases dramatically at higher temperatures (Toney et al., 2012; Theroux et al., 2020). All these data suggest lacustrine Group 2i Isochrysidales are also adapted to grow in cool waters.

The objectives of this research are as follows: (1) To test the growth characteristics of Group 2i strain RCC5486 at exceedingly low temperatures in laboratory cultures, including 0, 3, 6, 9 °C. Such growth characteristics will allow us to evaluate if Group 2i Isochrysidales is indeed particularly adapted to low environmental temperatures, which would shed new light on its association with sea ice, and its early spring bloom season in saline lakes; (2) To grow RCC5486 at salinities ranging from 15 to 38 ppt to examine how $C_{37:4}$% responds to the combined effects of variations in growth temperature and salinity. We are also particularly interested in observing how the $C_{37:4}$% of alkenones might change in response to growth temperatures and salinities.

For $C_{37:4}$% (proposed to be predominantly produced by Group 2i; Wang et al., 2021) as a quantitative sea ice proxy, it is important to determine how temperature and salinity, other than sea ice cover, could change $C_{37:4}$%. During the melting season of sea ice, surface water salinity can experience significant decrease as sea surface water warms up (Rosenblum et al., 2021; Zhao et al., 2022), any influence of salinity and temperature on $C_{37:4}$% produced by Group 2i could thus significantly affect proxy interpretation.

## 2. Materials and methods

### *2.1. Culture experiments*

Group 2i strain RCC5486, isolated from sea ice in Baffin Bay, was purchased from the Roscoff Culture Collection (Gérikas Ribeiro et al., 2020). Culture growth conditions and harvest procedures followed those reported in Liao et al. (2020). RCC5486 was acclimatized for two weeks before the start of the corresponding culture experiments with f/2 medium (Guillard, 1975), which was prepared from seawater collected from Vineyard Sound, Woods Hole, MA, USA at a salinity of 31 ppt (filtered using 0.2 μm Whatman nylon membrane filter and then autoclaved).

To study the influence of temperature on alkenone profiles and growth rates, RCC5486 was cultured at 0, 3, 6, 9, 10.5 and 12 °C at 31 ppt. The culture experiment for RCC5486 at 0 °C was performed in a cold room set at 3 °C, by submerging the culture flask of RCC5486 into a large cooler filled with a mixture of ice and water (the ice was periodically replenished to ensure the temperature was at 0 °C during the entire growth period) (Supplementary Fig. S1). To study the influence of salinity on alkenone profiles, culture experiments of RCC5486 at 0, 6, 15, 21, 26, 31, 38 ppt at 3 °C were performed in triplicate cultures. We did not observe any growth of RCC5486 at 10.5 and 12 °C, or at salinity of 6 and 0 ppt at 3 °C. Cultures were grown under a light:dark cycle set at 16:8. The light intensity was 140 μE $m^{-2}$ $s^{-1}$. All cultures experiments were performed in 165 mL of culture medium.

Cultures were harvested at early stationary phase (monitored using hemocytometer counts (Hausser Scientific, PA, USA)) by filtering onto 0.7 μm glass fiber filters (Merck Millipore, MA, USA). All filters were wrapped with aluminum foil and immediately frozen at –20 °C for further extraction and analysis (Zheng et al., 2016; Liao et al., 2020).

*2.2. Analysis of alkenones*

Analysis of culture samples followed the same procedure reported in Liao et al., 2020. Filters of culture samples were freeze-dried overnight and then sonicated three times with dichloromethane (DCM, 3 × 30 min, 20 mL each time) for lipid extractions. Total extracts of culture samples were divided into three fractions using silica gel (230–400 mesh, 40–63 μm) in glass pipettes, and eluted with hexane, DCM and methanol. Alkenones were in the DCM fraction.

DCM fractions were then analyzed on GC-FID (gas chromatography-flame ionization detection) (Agilent 7890B) and GC–EI-MS (gas chromatography–electron ionization-mass spectrometry) (Agilent 7890B interfaced to 5977 Inert Plus MSD) equipped with RTX-200 columns (105 m × 250 μm × 0.25 μm film thickness) (Zheng et al., 2017). For the analysis on GC-FID, the carrier gas was hydrogen. Samples were injected under pulsed splitless mode at 320 °C. The initial pulse pressure was 35 psi for the first 1 min. Then the purge flow to split vent was 35 mL/min at 1.1 min. The flow rate (constant flow mode) was 1.5 mL/min. The initial oven temperature was 50 °C for 2 min, then increased to 255 °C at 20 °C/min, then increased to 320 °C at 3 °C/min and held for 35 min. For the analysis on GC–EI-MS, samples were injected under pulsed splitless mode at 320 °C. The initial pulse pressure was 35 psi for the first 0.5 min. The purge flow to split vent was 50 mL/min at 1.1 min. The flow rate (constant flow mode) was 1.6 mL/min. The initial oven temperature was 40 °C for 1 min, then increased to 255 °C at 20 °C/min, then increased to 315 °C at 3 °C/min and held for 35 min. Samples were analyzed under full-scan mode. The source temperature was 230 °C. The electron ionization energy was 70 eV. The mass range was from *m*/*z* 50 to 650.

## 3. Results and discussion

### *3.1. Effect of temperature and salinity on the growth rate and cell density of Group 2i RCC5486*

At 31 ppt, RCC5486 shows consistently fast growth from 0 to 6 °C (Fig. 1, Table 1). The growth rates were highest at 3 °C, with mean growth rate at 0.17 $d^{-1}$ and final cell density at 1.18 × $10^7$ cell/mL, respectively. The growth rate and final cell density at 0 °C, however, are only slightly lower (growth rate at 0.11 $d^{-1}$, cell density at 8.0 × $10^6$ cell/mL) than those observed at 3 and 6 °C. The growth rate and final cell density at 9 °C are nearly 50% lower than those observed at 3 and 6 °C. No growth was observed at temperatures of 10.5 °C and 12 °C. The high growth rates of RCC5486 at low temperatures contrast markedly with previous reports of culture experiments with various Isochrysidales, where the minimum growth temperature was 4 °C and the optimal growth temperatures were generally 15–20 °C (Conte et al., 1998; Araie et al., 2018; Zheng et al., 2019; Liao et al., 2020). Our data suggest that the Group 2i RCC5486 strain isolated from sea ice in Baffin Bay brackish water is well adapted to grow at temperatures ≤ 6 °C.

Among five salinity levels (15, 21, 26, 31, 38 ppt) tested, the highest growth rate (~ 0.11 $d^{-1}$) of RCC5486 was observed at 31 ppt at 3 °C. However, the growth rates of RCC5486 appear to be similar in four other salinity levels (0.10, 0.11, 0.11, 0.12 $d^{-1}$ at 15, 21, 26, 38 ppt, respectively). The cell density, on the other hand, is more affected by salinity changes: decreasing salinity from 31 to 15 ppt reduced the final cell density by 52%. No growth was observed at 0 and 6 ppt, consistent with the assessment that RCC5486 may have been actively growing in the micro brine channels of the sea ice (Wang et al., 2021). Our data suggest the optimal growth salinity for RCC5486 may not derivate strongly (i.e., much fresher, <15 ppt) from Arctic Ocean surface waters (annual mean salinity ranging from 23 to 33 ppt) (Shu et al., 2018), although our culture data suggest that RCC5486 can efficiently grow at salinity range between 15 to 38 ppt.

The growth characteristics of RCC5486 above provide new insights on how Group 2i Isochrysidales might have produced high $C_{37:4}$ alkenone that preserves a record of the sea ice extent (Wang et al., 2021). The capability of this Group 2i strain to grow quickly and efficiently at 0 to 3 °C could mean it can bloom rapidly after melting sea ice in the early spring season of the Arctic and sub-Arctic Ocean when relatively high nutrients are available. The slightly fresher surface water conditions would also favor its growth, as it can tolerate a wide range of growth salinity levels.

*3.2. Seasonal bloom dynamics*

In general, diatoms are the first to bloom among various phytoplankton in the high latitude oceans and lakes, with optimal growth temperatures ranging from 1 to 5 °C (Sommer, 1986; Tyrrell and Merico, 2004; Iida et al., 2012; Coello-Camba and Agustí, 2017). However, the ability of RCC5486 to grow exceptionally quickly, even at 0 °C, is surprising. Cultures are grown in nutrient replete conditions and do not involve competition between different algal species. Thus, it is currently unclear if Group 2i might also be able to outcompete diatoms during early season algal successions in the Arctic and sub-Arctic Ocean settings. In particular, $C_{25}$ highly branched isoprenoids (HBIs) have been demonstrated to be effective biomarkers for sea ice (Belt, 2018). Producers of $IP_{25}$ (sympagic diatoms) are suggested to live inside the brine channels inside the sea ice and flourish in cool spring seasons (Belt, 2018).

Alkenones and HBIs have been found in the same site (site M23258-2 located in northeastern Norwegian Sea) (Wang et al., 2021). Group 2i Isochrysidales and HBI-producing diatoms may thus share some similarities in growth environments (e.g., brine channels). However, our culture experiments demonstrate that RCC5486 can also thrive in low salinity (as well as low temperature) sea waters, suggesting the possibility of a bloom after sea ice melt. Phytoplankton time series samples collected across the sea ice covered regions throughout different seasons, especially during the formation and melting of the sea ice, followed by morphological and phylogenetic analyses, would be important to elucidate the detailed bloom patterns of sea ice diatoms and Group 2i Isochrysidales.

The growth succession of lacustrine Group 2i strains observed in Lake George water column time series samples offers additional support for the early spring bloom of Group 2i species in high latitude ocean regions (Theroux et al., 2020). However, in Arctic and sub-Arctic Ocean settings, the dominant summer and fall Isochrysidales blooms are of *E. huxleyi* (a Group 3 species; Theroux et al., 2010; Penot et al., 2022), rather than Group 2w1 (Yao et al., 2022). For example, a large bloom of *E. huxleyi* was observed in September, 2000 in the Bering Sea when the in situ water temperature was ~9 °C (Harada et al., 2003). We also note that the Group 2i strain Hap-A isolated from Lake George, North Dakota could also be grown in culture at higher temperatures from 15 to 20 °C (please also note that the light cycle, or 24:0 light:dark ratio that was used in Theroux et al., 2020, differs from the 16:8 light:dark ratio we used in this study), and much lower salinity at 10 ppt (Toney et al., 2012; Theroux et al., 2020). These suggest that different strains, especially strains isolated from ocean and saline lakes, may have considerable differences in their temperature and salinity tolerance ranges.

### *3.3. Alkenone and alkenoate profiles of Group 2i RCC5486*

Group 2i RCC5486 produces large amounts of tetra-unsaturated alkenones and alkenoates at all temperatures (0, 3, 6 and 9 °C, 31 ppt) and salinities (15, 21, 26, 31 and 38 ppt at 3 °C; Fig. 2, Supplementary Fig. S2). The $C_{37:4}$ alkenone is the dominant compound among all alkenones with an average percentage of 79% over total amounts of $C_{37}$ alkenones (or 41% over total amounts of alkenones and alkenoates; Supplementary Table S1). The abundant production of tetra-unsaturated alkenones and tetra-unsaturated alkenoates in Group 2i species may reflect their adaption to low-temperature environments, which include both high latitude ocean and saline lake settings (Zhao et al., 2018; Wang et al., 2021; Yao et al., 2022).

In the high latitude ocean with mixed alkenone production from Group 2i and Group 3 Isochrysidales (i.e., *E. huxleyi*), $C_{37:4}$% mainly reflects sea ice coverages as *E. huxleyi* generally does not make significant amounts of $C_{37:4}$ alkenone (highest reported $C_{37:4}$% at 10.4% for B92/21 isolated from Norwegian fjord when cultured at 6 °C) (Conte et al., 1998; Zheng et al., 2019). In the case of saline lakes where groups 2i and 2w1 both produce alkenones (Yao et al., 2022), sedimentary $C_{37:4}$% values have been proposed to reflect precipitation during the cold season, which may be augmented by increased precipitation during the spring when increased nutrient supply leads to blooms of Group 2i haptophytes, enhancing their proportional contribution to $C_{37:4}$% in sediments (Zhao et al., 2018).

Group 2i RCC5486 also shows high production of $C_{39:4}$Me alkenone (average percentage at 57% over total $C_{39}$ alkenones; Supplementary Table S1). This is considered a distinct feature of Group 2i Isochrysidales and has been suggested to be a characteristic biomarker for Group 2i species (Yao et al., 2022). Notably, no other cultured Isochrysidales (including Group 1 freshwater species, Group 2 coastal and lacustrine species and Group 3 open ocean species) have been found to make significant amount of $C_{39:4}$Me alkenone, even at relatively low growth temperatures (e.g., 4 °C) (Zheng et al., 2019; Liao et al., 2020).

Another important feature of RCC5486 is the relatively low abundance of $C_{38}$ alkenones and high ratio of $C_{37}/C_{38}$ at 4.9±1.6 (Fig. 2, Supplementary Fig. S4; Supplementary Table S1). Recently, Yao et al. (2022) classified Group 2 species into three different subgroups based on phylogenetic data: Group 2i, Group 2w1 and Group

2w2 with each subgroup varying in its preferred growth conditions (Group 2i in cool conditions whereas Group 2w prefers relatively warm conditions; Supplementary Fig. S5). A high $C_{37}/C_{38}$ ratio has been widely observed in culture/sediment samples where Group 2i is the dominant alkenone producers (Theroux et al., 2020; Wang et al., 2021; Yao et al., 2022). Notably, however, for samples where Group 2w is the dominant alkenone-producers, the $C_{37}/C_{38}$ ratio varies significantly across different sites and cultured Isochrysidales species (e.g., $C_{37}/C_{38}$ ratio of 0.46 for surface sediments from Xiaosugan, a $C_{37}/C_{38}$ ratio of 4.99 for the culture sample of *Isochrysis nuda* RCC1207 at 4 °C) (Liao et al., 2020; Yao et al., 2022). This suggests that the $C_{37}/C_{38}$ ratio, which was previously proposed to differentiate different groups of Isochrysidales (e.g., $C_{37}/C_{38}$ ratio $\geq$ 1.8 suggests Group 2 contribution), may not be valid (Rosell-Melé et al., 1994; Volkman et al., 1995; Conte et al., 1998; Salacup et al., 2019). Combined usage of other group-specific biomarkers (e.g., $C_{41}$Me/$C_{42}$Et alkenones, $C_{38}$OEt alkenoates) will be helpful to constrain alkenone producers (Liao et al., 2020, 2021).

*3.4. Influence of temperature on alkenone profiles of Group 2i RCC5486*

Alkenone profiles change little when growth temperatures changed from 0 to 9 °C. We did not observe a significant correlation between $C_{37:4}$% and temperature (Fig. 3a; Supplementary Table S1). The $C_{37:4}$% values remain nearly constant at 0, 3 and 6 °C (average $C_{37:4}$% over total $C_{37}$ alkenones is 80.4±2.3%). The $C_{37:4}$% slightly decreased from 80.6% at 3 °C to 70.7% at 9 °C. Our results suggest that high $C_{37:4}$% in Group 2i Isochrysidales may reflect a physiologically intrinsic property, rather than a response to low temperatures, as has been found in Group 1 Isochrysidales (Zheng et al., 2019).

Examination of representative alkenone proxies $U^{K}_{37}$, $U^{K'}_{37}$ and $U^{K''}_{37}$ also shows no significant correlations between proxy values and growth temperatures (Fig. 4). Since the $C_{39:4}$Me alkenone appears to be unique to Group 2i species, we also examined temperature sensitivity of $C_{39}$Me alkenones (Supplementary Fig. S3). As with the $C_{37}$ alkenones, no significant correlation was observed between temperature and $U^{K}_{39Me}$, $U^{K'}_{39Me}$ and $U^{K''}_{39Me}$ respectively (Supplementary Fig. S3). Moreover, $U^{K}_{37}$, $U^{K'}_{37}$ and $U^{K''}_{37}$ values of Group 2i RCC5486 at 0 °C were 0.031, 0.013, 0.021 higher (rather than lower), respectively, than the proxy values at 3 °C (Fig. 4). A similar phenomenon (i.e., lower degrees of unsaturation at lower temperature) has previously been observed in culture experiments of *T. lutea* CCMP463, which, based on small subunit 18S rDNA, is classified as a Group 2 Isochrysidales (Zheng et al., 2019). For example, $U^{K'}_{37}$, $U^{K}_{37}$ and $RIE_{36}$ values at 4 °C are higher than those at 14 °C for *T. lutea* CCMP463 (Zheng et al., 2019). To explain such unusual phenomenon, Zheng et al. (2019) hypothesized that *T. lutea* was an evolutionary intermediary between Group 2 and Group 3 species, which displays more Group 3 characteristics at lower temperature (< 15 °C) but more Group 2 characteristics at higher temperature (>15 °C) when different sets of biosynthetic machinery are used to produce alkenones. However, RCC5486 does not display any obvious Group 3 characteristics such as relatively high ratios of $C_{38}$Me to $C_{38}$Et alkenones and very low $C_{37:4}$%, even at low temperatures (Zheng et al., 2019). More experimental data, e.g., culturing of RCC5486 at 1 or 2 °C, may be needed to further ascertain if our preliminary observation of reversed unsaturation ratios is indeed representative of general growth characteristics of Group 2i at low temperatures.

*3.5. Influence of salinity on alkenone profiles of Group 2i RCC5486*

Similar to the influence of temperature, we found alkenone profiles changed little ($C_{37:4}$% was at 79.9±3.4%) when salinity varied from 15 to 38 ppt at 3 °C (Fig. 3b, Supplementary Fig. S2, Supplementary Table S1). We previously published the correlation between $C_{37:4}$% and salinity from single culture experiment at each salinity (Wang et al., 2021). Here, we performed culture experiments at different salinities in triplicate: the results are still similar to our previous observations, i.e., salinity changes do not lead to significant changes in $C_{37:4}$% (Fig. 3b). Sea ice melting has been associated with a local depression in surface sea salinity in high latitude oceans (Rosenblum et al., 2021; Zhao et al., 2022). For example, when sea ice in the Canada Basin starts to melt in May and reaches its minimum coverage in August, the surface salinity decreases from ~28 to 26 ppt (Rosenblum et al., 2021). Therefore, the observed large increases in $C_{37:4}$% in Nordic Sea (Rosell-Melé, 1998) and Bering Sea (Harada et al., 2012) when sea surface salinity (SSS) decreases by 3–4 ppt cannot be attributed to the physiological effect of salinity on alkenone biosynthesis (Bendle et al., 2005; Harada et al., 2012; Wang et al., 2021). The observed correlations between $C_{37:4}$% and SSS in Nordic sea and Bering Sea likely result from sea ice melt induced decrease in SSS and flourish of Group 2i Isochrysidales at low temperatures in seasonally sea ice covered oceans.

## 4. Conclusions

Our culture experiments support the scenario of a mixed alkenone production in high latitude ocean regions by Group 2i and *E. huxleyi* species, with an early bloom of Group 2i in cool ice-melting seasons followed by a bloom of *E. huxleyi* in warmer seasons. Two lines of data support such a scenario: (1) Group 2i RCC5486 grows efficiently at exceedingly low temperatures of 0 to 6 °C, with optimal temperature (highest growth rate and cell density) at 3 °C based on our experimental data. The growth rate and cell density of RCC5486 declined dramatically at 9 °C and failed to grow at 10.5 and 12 °C. Our data suggest that Group 2i may be particularly adapted to grow at low temperatures (≤ 6 °C). 2) Salinity levels from 15 to 38 ppt do not significantly influence the growth rates of Group 2i RCC5486, although no growth was observed at 0 and 6 ppt at 3 °C. The adaptation of Group 2i RCC5486 to low temperature and its tolerance of a broad range of salinity levels suggests that Group 2i Isochrysidales could bloom in regions of Arctic and sub-Arctic Oceans with seasonal sea ice cover, most likely during the early spring. On the other hand, *E. huxleyi*, another dominant alkenone producers in the oceans, is more likely to bloom in warmer seasons. Such successional blooms have also been observed in saline lakes, where a spring bloom of Group 2i species was followed by the bloom of Group 2w species in summer or early fall. Our results also provide new support for the use of $C_{37:4}$% as a quantitative proxy for sea ice reconstructions. Group 2i RCC5486 shows consistently high production of $C_{37:4}$ alkenone ($C_{37:4}$% over total $C_{37}$ alkenones at 79.4±3.8%), regardless of growth temperature (0 to 9 °C) and salinity (15 to 38 ppt). Therefore, the high $C_{37:4}$% in Group 2i is a physiologically intrinsic property of these algae, rather than responses to temperature and salinity changes. Conversely, Group 2i's efficient growth at low temperatures and intolerance of warm conditions may have, at least in part, originated from its inability to produce an alkenone profile with low $C_{37:4}$ and high $C_{37:3}$ and $C_{37:2}$. Mixing of alkenones produced by Group 2i with high $C_{37:4}$% in spring and by Group 3

Isochrysidales with relatively low $C_{37:4}$% in late summer and fall would yield sediment alkenones with a wide range of $C_{37:4}$% values. The greater the sea ice coverage, the more likely the success of Group 2i relative to Group 3 Isochrysidales, resulting in the higher $C_{37:4}$% values in sediments.

## Acknowledgments

This work was supported by the United States National Science Foundation awards to Y.H. (EAR-1762431). We are also grateful for the comments from Dr. Simon Brassell and an anonymous reviewer, which helped us improve the manuscript.

*Associate Editor*-**Ian D. Bull**

## References

Araie, H., Nakamura, H., Toney, J.L., Haig, H.A., Plancq, J., Shiratori, T., Leavitt, P.R., Seki, O., Ishida, K., Sawada, K., Suzuki, I., Shiraiwa, Y., 2018. Novel alkenone-producing strains of genus *Isochrysis* (Haptophyta) isolated from Canadian saline lakes show temperature sensitivity of alkenones and alkenoates. Organic Geochemistry 121, 89-103.

Belt, S.T., 2018. Source-specific biomarkers as proxies for Arctic and Antarctic sea ice. Organic Geochemistry 125, 277-298.

Belt, S.T., Müller, J., 2013. The Arctic sea ice biomarker $IP_{25}$: a review of current understanding, recommendations for future research and applications in palaeo sea ice reconstructions. Quaternary Science Reviews 79, 9-25.

Bendle, J., Rosell-Melé, A., 2004. Distributions of $U^{K}_{37}$ and $U^{K'}_{37}$ in the surface waters and sediments of the Nordic Seas: Implications for paleoceanography. Geochemistry Geophysics Geosystems 5, 19.

Bendle, J., Rosell-Melé, A., Ziveri, P., 2005. Variability of unusual distributions of alkenones in the surface waters of the Nordic seas. Paleoceanography 20, 15.

Brassell, S.C., Dumitrescu, M., the ODP Leg 198 Scientific Party, 2004. Recognition of alkenones in a lower Aptian porcellanite from the west-central Pacific. Organic Geochemistry 35, 181-188.

Brassell, S.C., Eglinton, G., Marlowe, I.T., Pflaumann, U., Sarnthein, M., 1986. Molecular stratigraphy: a new tool for climatic assessment. Nature 320, 129-133.

Coello-Camba, A., Agustí, S., 2017. Thermal thresholds of phytoplankton growth in polar waters and their consequences for a warming polar ocean. Frontiers in Marine Science 4, 168.

Conte, M.H., Thompson, A., Lesley, D., Harris, R.P., 1998. Genetic and physiological influences on the alkenone/alkenoate versus growth temperature relationship in *Emiliania huxleyi* and *Gephyrocapsa oceanica*. Geochimica et Cosmochimica Acta 62, 51-68.

de Leeuw, J., van der Meer, F., Rijpstra, W., Schenck, P., 1980. On the occurrence and structural identification of long chain unsaturated ketones and hydrocarbons in sediments. Physics and Chemistry of the Earth 12, 211-217.

Gérikas Ribeiro, C., Dos Santos, A.L., Gourvil, P., Le Gall, F., Marie, D., Tragin, M., Probert, I., Vaulot, D., Michel, C., 2020. Culturable diversity of Arctic phytoplankton during pack ice melting. Elementa: Science of the Anthropocene 8. DOI: https://doi.org/10.1525/elementa.401

Harada, N., Sato, M., Oguri, K., Hagino, K., Okazaki, Y., Katsuki, K., Tsuji, Y., Shin, K.H., Tadai, O., Saitoh, S.I., 2012. Enhancement of coccolithophorid blooms in the Bering Sea by recent environmental changes. Global Biogeochemical Cycles 26, GB2036.

Harada, N., Sato, M., Sakamoto, T., 2008. Freshwater impacts recorded in tetraunsaturated alkenones and alkenone sea surface temperatures from the Okhotsk Sea across millennial-scale cycles. Paleoceanography 23, PA3201.

Harada, N., Shin, K.H., Murata, A., Uchida, M., Nakatani, T., 2003. Characteristics of alkenones synthesized by a bloom of *Emiliania huxleyi* in the Bering Sea. Geochimica et Cosmochimica Acta 67, 1507-1519.

Huang, Y., Zheng, Y., Heng, P., Giosan, L., Coolen, M.J., 2021. Black Sea paleosalinity evolution since the last deglaciation reconstructed from alkenone-inferred Isochrysidales diversity. Earth and Planetary Science Letters 564, 116881.

Iida, T., Mizobata, K., Saitoh, S.-I., 2012. Interannual variability of coccolithophore *Emiliania huxleyi* blooms in response to changes in water column stability in the eastern Bering Sea. Continental Shelf Research 34, 7-17.

Ishiwatari, R., Houtatsu, M., Okada, H., 2001. Alkenone-sea surface temperatures in the Japan Sea over the past 36 kyr: warm temperatures at the last glacial maximum. Organic Geochemistry 32, 57-67.

Jaraula, C.M.B., Brassell, S.C., Morgan-Kiss, R.M., Doran, P.T., Kenig, F., 2010. Origin and tentative identification of tri to pentaunsaturated ketones in sediments from Lake Fryxell, East Antarctica. Organic Geochemistry 41, 386-397.

Liao, S., Wang, K.J., Huang, Y., 2021. Extended chain length alkenoates differentiate three Isochrysidales groups. Organic Geochemistry, 104303.

Liao, S., Yao, Y., Wang, L., Wang, K.J., Amaral-Zettler, L., Longo, W.M., Huang, Y., 2020. $C_{41}$ methyl and $C_{42}$ ethyl alkenones are biomarkers for Group II Isochrysidales. Organic Geochemistry 147, 104081.

Marlowe, I., Brassell, S., Eglinton, G., Green, J., 1984. Long chain unsaturated ketones and esters in living algae and marine sediments. Organic Geochemistry 6, 135-141.

Penot, M., Dacks, J.B., Read, B., Dorrell, R.G., 2022. Genomic and meta-genomic insights into the functions, diversity and global distribution of haptophyte algae. Applied Phycology 3, 1-20.

Plancq, J., Couto, J.M., Ijaz, U.Z., Leavitt, P.R., Toney, J.L., 2019. Next-generation sequencing to identify lacustrine haptophytes in the Canadian Prairies: Significance for temperature proxy applications. Journal of Geophysical Research: Biogeosciences 124, 2144-2158.

Prahl, F.G., Wakeham, S.G., 1987. Calibration of unsaturation patterns in long-chain ketone compositions for paleotemperature assessment. Nature 330, 367-369.

Rechka, J.A., Maxwell, J.R., 1988. Characterization of alkenone temperature indicators in sediments and organisms. Organic Geochemistry 13, 727-734.

Rontani, J.-F., Belt, S.T., Amiraux, R., 2018. Biotic and abiotic degradation of the sea ice diatom biomarker $IP_{25}$ and selected algal sterols in near-surface Arctic sediments. Organic Geochemistry 118, 73-88.

Rosell-Melé, A., 1998. Interhemispheric appraisal of the value of alkenone indices as temperature and salinity proxies in high-latitude locations. Paleoceanography 13, 694-703.

Rosell-Melé, A., Carter, J., Eglinton, G., 1994. Distributions of long-chain alkenones and alkyl alkenoates in marine surface sediments from the North East Atlantic. Organic Geochemistry 22, 501-509.

Rosenblum, E., Fajber, R., Stroeve, J., Gille, S., Tremblay, L., Carmack, E., 2021. Surface salinity under transitioning ice cover in the Canada Basin: Climate model biases linked to vertical distribution of fresh water. Geophysical Research Letters 48, e2021GL094739.

Salacup, J., Farmer, J., Herbert, T., Prell, W., 2019. Alkenone paleothermometry in coastal settings: evaluating the potential for highly resolved time series of sea surface temperature. Paleoceanography and Paleoclimatology 34, 164-181.

Sanz, P.C., Smik, L., Belt, S.T., 2016. On the stability of various highly branched isoprenoid (HBI) lipids in stored sediments and sediment extracts. Organic Geochemistry 97, 74-77.

Shu, Q., Qiao, F., Song, Z., Zhao, J., Li, X., 2018. Projected freshening of the Arctic Ocean in the 21st century. Journal of Geophysical Research: Oceans 123, 9232-9244.

Sicre, M.A., Bard, E., Ezat, U., Rostek, F., 2002. Alkenone distributions in the North Atlantic and Nordic sea surface waters. Geochemistry Geophysics Geosystems 3, 13.

Sommer, U., 1986. The periodicity of phytoplankton in Lake Constance (Bodensee) in comparison to other deep lakes of central Europe. In: Seasonality of Freshwater Phytoplankton. Springer, pp. 1-7.

Stein, R., Fahl, K., Schreck, M., Knorr, G., Niessen, F., Forwick, M., Gebhardt, C., Jensen, L., Kaminski, M., Kopf, A., 2016. Evidence for ice-free summers in the late Miocene central Arctic Ocean. Nature Communications 7, 1-13.

Theroux, S., D'Andrea, W.J., Toney, J., Amaral-Zettler, L., Huang, Y., 2010. Phylogenetic diversity and evolutionary relatedness of alkenone-producing haptophyte algae in lakes: Implications for continental paleotemperature reconstructions. Earth and Planetary Science Letters 300, 311-320.

Theroux, S., Huang, Y., Toney, J.L., Andersen, R., Nyren, P., Bohn, R., Salacup, J., Murphy, L., Amaral-Zettler, L., 2020. Successional blooms of alkenone-producing haptophytes in Lake George, North Dakota: Implications for continental paleoclimate reconstructions. Limnology and Oceanography 65, 413-425.

Tyrrell, T., Merico, A., 2004. *Emiliania huxleyi*: bloom observations and the conditions that induce them. In: Coccolithophores. Springer, pp. 75-97.

Volkman, J.K., Barrett, S.M., Blackburn, S.I., Sikes, E.L., 1995. Alkenones in *Gephyrocapsa oceanica*: Implications for studies of paleoclimate. Geochimica et Cosmochimica Acta 59, 513-520.

Wang, K.J., Huang, Y., Majaneva, M., Belt, S.T., Liao, S., Novak, J., Kartzinel, T.R., Herbert, T.D., Richter, N., Cabedo-Sanz, P., 2021. Group 2i Isochrysidales produce characteristic alkenones reflecting sea ice distribution. Nature Communications 12, 1-10.

Yao, Y., Huang, Y., Zhao, J., Wang, L., Ran, Y., Liu, W., Cheng, H., 2021. Permafrost thaw induced abrupt changes in hydrology and carbon cycling in Lake Wudalianchi, northeastern China. Geology 49, 1117-1121.

Yao, Y., Zhao, J., Vachula, R.S., Liao, S., Li, G., Pearson, E.J., Huang, Y., 2022. Phylogeny, alkenone profiles and ecology of Isochrysidales subclades in saline lakes: Implications for paleosalinity and paleotemperature reconstructions. Geochimica et Cosmochimica Acta 317, 472-487.

Zhao, J., Thomas, E.K., Yao, Y., DeAraujo, J., Huang, Y., 2018. Major increase in winter and spring precipitation during the Little Ice Age in the westerly dominated northern Qinghai-Tibetan Plateau. Quaternary Science Reviews 199, 30-40.

Zhao, J., Wang, Y., Liu, W., Bi, H., Cokelet, E.D., Mordy, C.W., Lawrence-Slavas, N., Meinig, C., 2022. Sea Surface Salinity Variability in the Bering Sea in 2015–2020. Remote Sensing 14, 758.

Zheng, Y., Tarozo, R., Huang, Y., 2017. Optimizing chromatographic resolution for simultaneous quantification of long chain alkenones, alkenoates and their double bond positional isomers. Organic Geochemistry 111, 136-143.

Zheng, Y.S., Heng, P., Conte, M.H., Vachula, R.S., Huang, Y.S., 2019. Systematic chemotaxonomic profiling and novel paleotemperature indices based on alkenones and alkenoates: Potential for disentangling mixed species input. Organic Geochemistry 128, 26-41.

**Figure captions**

**Fig. 1**. Growth curves for Group 2i strain RCC5486 at 0, 3, 6 and 9 °C at: (a) 31 ppt and at (b): 15, 21, 26, 31, 38 ppt at 3 °C.

**Fig. 2**. Alkenone and alkenoate profiles of Group 2i RCC5486 cultured at 0 (a), 3 (b), 6 (c), 9 (d) °C at 31 ppt.

**Fig. 3**. Influence of temperature (a) and salinity (b) on $C_{37:4}$% values for Group 2i RCC5486 (alkenone data for each culture are provided in Supplementary Table S1).

**Fig. 4**. Influence of temperature on $U^{K}_{37}$ (a), $U^{K\prime}_{37}$ (b) and $U^{K\prime\prime}_{37}$ (c) for Group 2i RCC5486 from 0 to 9 °C (alkenone data for each culture are provided in Supplementary Table S1).

**Table 1**. Average daily growth rate and final cell density for Group 2i strain RCC5486 cultured at different temperatures and salinities.

| Temperature (°C) | Salinity (ppt) | Average growth rates ($d^{-1}$) | Final cell density ($10^6$ cell/mL) |
|---|---|---|---|
| 0 | 31 | 0.11 | 8.0 |
| 3 | 15 | 0.10 | 5.6 |
| 3 | 21 | 0.11 | 7.7 |
| 3 | 26 | 0.11 | 8.4 |
| 3 | 31 | 0.17 | 11.8 |
| 3 | 38 | 0.12 | 11.3 |
| 6 | 31 | 0.12 | 11.3 |
| 9 | 31 | 0.09 | 6.3 |

Highlights

Group 2i RCC5486 thrives at exceedingly low growth temperatures (0–6 °C)

Salinity variation from 15 to 38 ppt had minimal effect on growth rates

Influence of temperature and salinity on $C_{37:4}$% is small

Group 2i is well-adapted to grow in ice-covered aquatic environments

Culture data support the use of $C_{37:4}$% as a sea ice proxy

Fig. 1

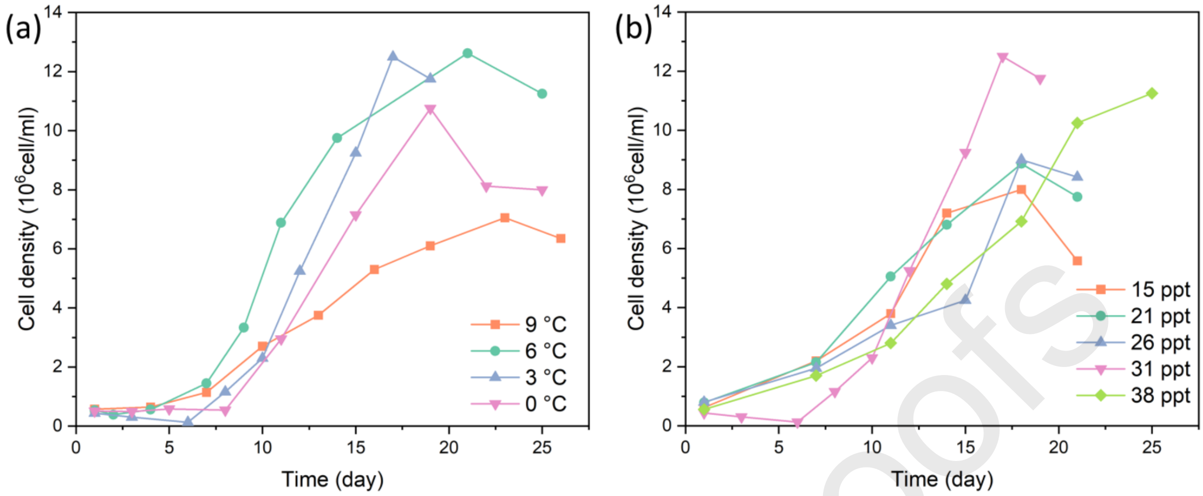

(a)
Cell density ($10^6$cell/ml)
Time (day)
9 °C
6 °C
3 °C
0 °C
(b)
Cell density ($10^6$cell/ml)
Time (day)
15 ppt
21 ppt
26 ppt
31 ppt
38 ppt

Fig. 2

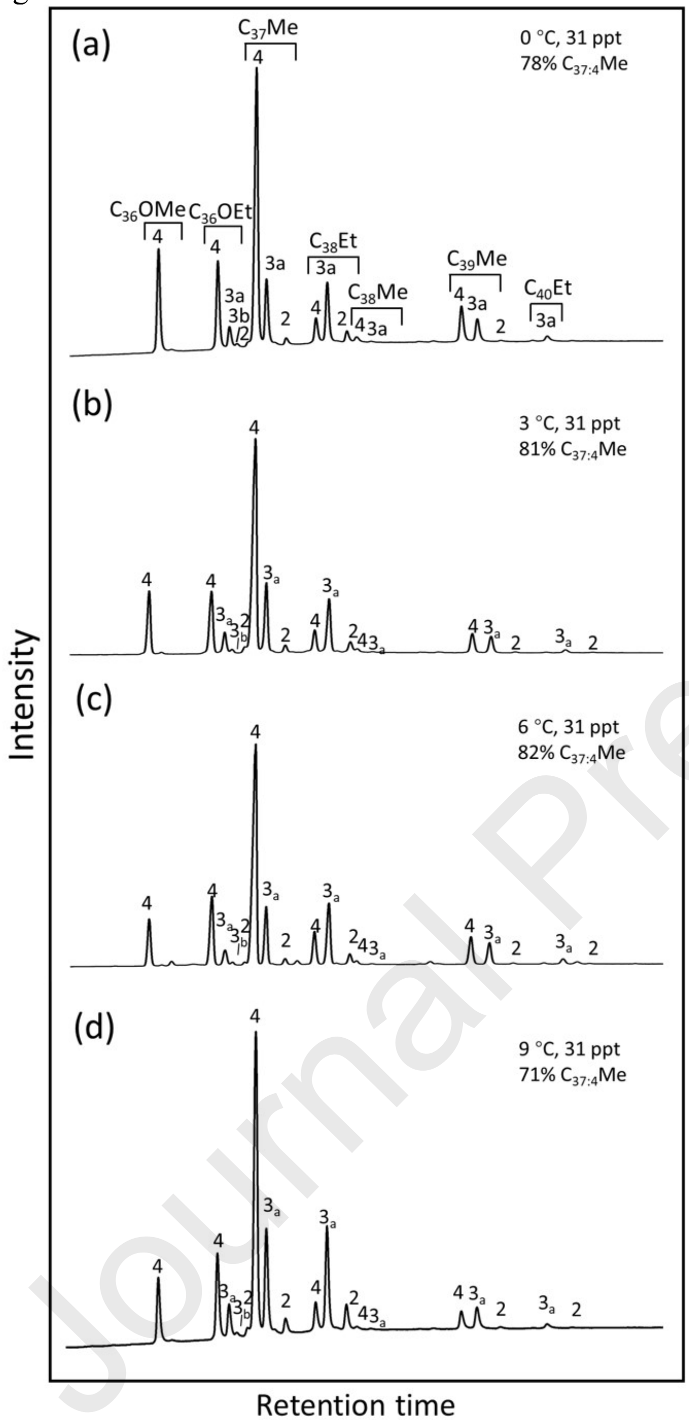

(a)
0 °C, 31 ppt
78% C37:4Me
C37Me
C36OMe
C36OEt
C38Et
C38Me
C39Me
C40Et
(b)
3 °C, 31 ppt
81% C37:4Me
(c)
6 °C, 31 ppt
82% C37:4Me
(d)
9 °C, 31 ppt
71% C37:4Me
Intensity
Retention time

Fig. 3

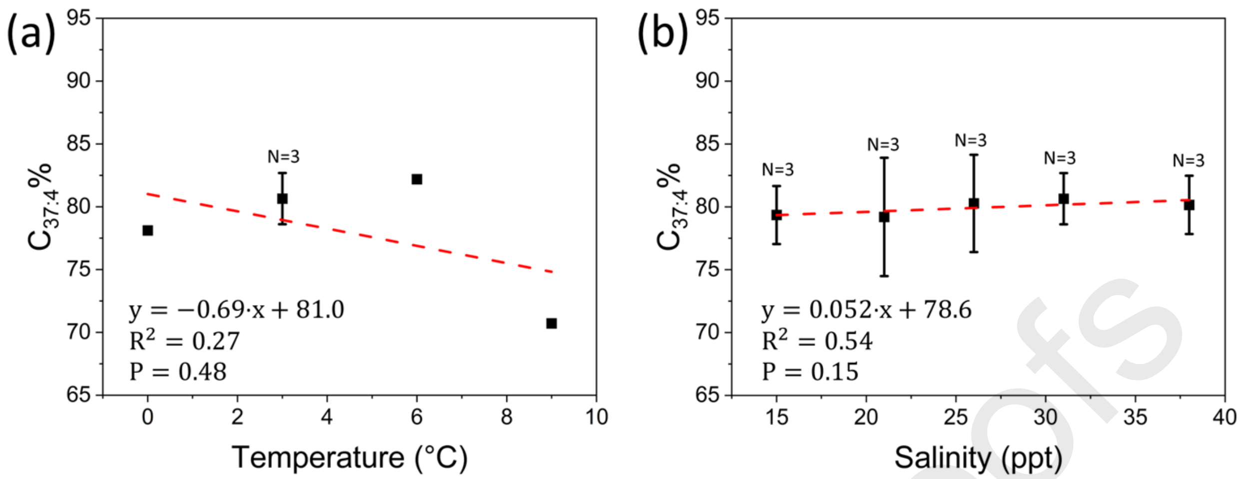
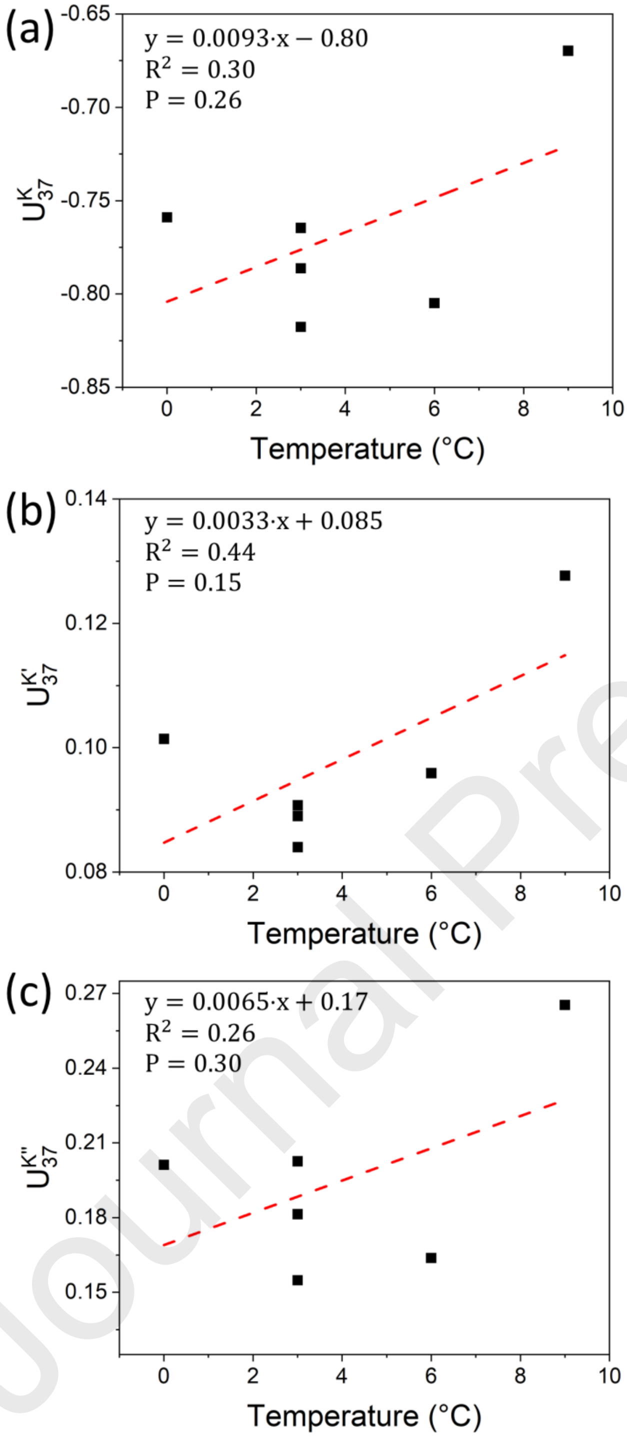

(a)
95
90
85
80
75
70
65
$C_{37:4}$%
N=3
y = −0.69·x + 81.0
$R^2$ = 0.27
P = 0.48
0
2
4
6
8
10
Temperature (°C)
(b)
95
90
85
80
75
70
65
$C_{37:4}$%
N=3
N=3
N=3
N=3
N=3
y = 0.052·x + 78.6
$R^2$ = 0.54
P = 0.15
15
20
25
30
35
40
Salinity (ppt)

Fig. 4






**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: