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PRODUCTION RATE ESTIMATION OF MYCOSPORINE-LIKE AMINO ACIDS IN TWO ARCTIC MELT PONDS BY STABLE ISOTOPE PROBING WITH $NAH^{13}CO_3$[1]

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The net carbon uptake rate and net production rate of mycosporine-like amino acids (MAAs) were measured in phytoplankton from 2 different melt ponds (MPs; closed and open type pond) in the western Arctic Ocean using a ^{13}C stable isotope tracer technique. The Research Vessel Araon visited ice-covered western-central basins situated at 82°N and 173°E in the summer of 2012, when Arctic sea ice declined to a record minimum. The average net carbon uptake rate of the phytoplankton in polycarbonate (PC) bottles in the closed MP was 3.24 mg C · m^{-3} · h^{-1} (SD = ±1.12 mg C · m^{-3} · h^{-1}), while that in the open MP was 1.3 mg C · m^{-3} · h^{-1} (SD = ±0.05 mg C · m^{-3} · h^{-1}). The net production rate of total MAAs in incubated PC bottles was highest (1.44 (SD = ±0.24) ng C · L^{-1} · h^{-1}) in the open MP and lowest (0.05 (SD = ±0.003) ng C · L^{-1} · h^{-1}) in the closed MP. The net production rate of shinorine and palythine in incubated PC bottles at the open MP presented significantly high values 0.76 (SD = ±0.12) ng C · L^{-1} · $h^{-1}$and 0.53 (SD = ±0.06) ng C · L^{-1} · h^{-1}. Our results showed that high net production rate of MAAs in the open MP was enhanced by a combination of osmotic and UVR stress and that in situ net production rates of individual MAA can be determined using ^{13}C tracer in MPs in Arctic sea ice.

Key index words: **Arctic; melt pond; mycosporine-like amino acids; sea ice; stable isotope probing technique**

***Abbreviations*: AS, asterina-330; MAA, mycosporine-like amino acid; MG, mycosporine-glycine; MP, melt pond; PA, palythine; PC, polycarbonate; SH, shinorine; SIP, stable isotope probing**

In the Arctic, meltwater can be partitioned within different habitats in the sea ice environment (Mundy et al. 2011). In the most recent spring and summer (from late May to mid-August), the sea ice in the Arctic experienced a transformation associated with the increased air temperature, leading to the formation of surface melt ponds (MPs) and a strong reduction in the salinity of multiyear ice (Eicken et al. 2002). The resultant meltwater can be discharged through highly permeable ice or structural flaws and freshwater pooling in surface MPs, which are formed in the Arctic summer by melting snow; surface sea ice is an important feature of summer Arctic sea ice (Lüthje et al. 2006). MPs have numerous impacts on physical factors such as the surface albedo (Perovich et al. 2002) and heat transmission (Inoue et al. 2008). Two different types of MPs can be visually differentiated by their color in a sea ice field. MPs connected to seawater either via holes in ice floes or channels (open MP) are deep blue in color, showing salinities of ~29.0, while freshwater habitats (closed MP) exhibit a salinity of ~0.1 and are light sky blue in color (Gradinger 2002, Lee et al. 2011, 2012).

The compositions of the phytoplankton communities found in ice meltwater have been reported to be characterized by chlorophyte and/or chrysophyte (Bursa 1963, Melnikov et al. 2002, Gradinger et al. 2005) as well as prasinophyte (Gradinger 1996) dominance. Halocline algal communities also can grow in the ice melt seawater-stratified boundary layer (Apollonio 1985). Additionally, Gradinger (2002) reported that bacteria and phototrophic and heterotrophic protists, including *Chlamydomonas nivalis* and flagellated algae, can be found in both types of ponds. In open ponds, higher concentrations of pennate diatoms, such as *Nitzschia frigida* and *Nitzschia grunowii*, are found compared to closed ponds, and an infiltration of diatom dominance has

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been observed in some ponds (Buck et al. 1998, Gradinger 1999), associated with nutrient-rich waters from the underlying water column (Buck et al. 1998).

Increases in the amount of light transmitted through sea ice are caused by the disappearance of snow cover and the development of MPs (Perovich 2005). Hence, the phytoplankton present in surface MPs are subjected to particularly high light levels (Mundy et al. 2011). Although the areal coverage of MPs has recently been estimated to be as high as 80% of the Arctic sea ice area (Lüthje et al. 2006), the photoacclimative response to potentially harmful radiation levels in MP habitats has not been well studied (Uusikivi et al. 2010, Mundy et al. 2011). The active photoprotective strategies of marine microbes include the production of screening and quenching compounds (Kirk 1994, Roy 2000) and the production of carotenoids in ice algae in response to increasing light levels (Kashino et al. 1998, Kudoh et al. 2003, Leu et al. 2010). Furthermore, Uusikivi et al. (2010) reported that mycosporine-like amino acids (MAAs) occur in relatively high quantities in the Baltic Sea. Belzile et al. (2000) indicated that MAAs could occur in first-year ice in an Arctic polynya, and Ryan et al. (2002) detected MAAs in an Antarctic bottom-ice algal community.

The objectives of this study were to measure the net carbon uptake rate and the net production rate of individual MAAs in phytoplankton in both types of Arctic MPs in the western Arctic (Chukchi Sea). To compare the environmental conditions both the MPs, a stable isotope probing (SIP) technique was employed. This is the first report to describe the analysis of in situ net production rate of individual MAAs in different types of MPs on the sea ice floes in the Arctic Ocean.

MATERIALS AND METHODS

Study area. The study station was located on sea ice present in the western Arctic Ocean (Chukchi Sea) in August 2012, during the cruise of R/V ARAON. At the MP stations, salinity was measured with a YSI model 30. Ice thickness was measured a few meters away from the MPs locations after ice cores were extracted (Table 1).

A phytoplankton species composition analysis, including identification and quantification, was performed at each incubation station. The distribution of the phytoplankton species composition in the MPs was determined using a microscope (BX53TR-32FB3F0; Olympus, Inc., Tokyo, Japan) under a combination of light and epifluorescence microscopy at 400× for microplankton and at 1,000× for autotrophic pico- and nanoplankton (Booth 1993).

To measure net carbon uptake rates and net production rates of individual MAAs under natural UV radiation exposure, incubation experiments were conducted at the MPs. The average UV intensities throughout the all incubation periods were measured to be 0.5 $W \cdot m^{-2}$ at 250 nm (ultraviolet B; UVB), 1.88 $W \cdot m^{-2}$ at 320 nm (ultraviolet A; UVA), and 3.14 $W \cdot m^{-2}$ at 395 nm (photosynthetically available radiation; PAR) using a UVX® radiometer (Model UVX-25 and UVX-31; Table 1).

Surface water samples at the MPs sampling stations were collected by basket and transferred to quartz (6 L; HanJin Ouartz Co.: Seoul, Korea) and polycarbonate (PC) bottles (9 L; Nalgene Labware: NY, USA) for incubation. Quartz and PC bottles exhibit different transmission of light wavelengths. PC bottles allow penetration of PAR and UVA and were used as a control for comparison with the results obtained in quartz bottles, which transmit the full solar radiation spectrum, including UVB. Each bottle was incubated in surface MPs water for 30 h at the MPs in situ incubation. ^{13}C $NaHCO_3$ (99 at. % of ^{13}C) was added to the bottles as a tracer. To achieve an increase in the isotopic ratio of particulate organic carbon, the ^{13}C level was brought up to 15 at. % in the dissolved inorganic carbon (DIC) pool. This increase in DIC had little effect on the uptake rate in oceanic environments (Hama et al. 1983), although CO_2 availability can affect carbon fixation and the elemental composition of algal cells (Riebesell et al. 1993, Burkhardt and Riebesell 1997).

Chl a *and net carbon uptake rate analyses.* Samples for the analysis of total Chl *a* were filtered through Whatman GF/F glass fiber filters (24 mm) and stored at −80°C until being analyzed on board. The samples were kept in a freezer (4°C) overnight and then centrifuged following the procedure of Parsons et al. (1984). Chl *a* concentrations were extracted with a 90% Acetone solvent onboard and measured using a Turner design trilogy fluorometer calibrated with a commercially purified Chl *a* standard.

To determine net carbon uptake rates based on stable carbon isotope analysis, $NaH^{13}CO_3$ was added to the each bottle during the incubation period as a tracer. Following incubation, 1 L of each sample was filtered through pre-combusted (450°C, 4 h) glass fiber filters (Whatman GF/F filters; 25 mm) and then stored at −80°C until analysis. The filters were completely dried in a lyophilizer (FDU-2100; EYELA: Tokyo, Japan) and exposed to 1 N HCl fumes overnight to remove inorganic carbon. Neutralization was then performed with NaOH in the desiccator. The filters were preserved for mass spectrometric analysis at Hanyang university using an EA-irMS (EuroEA-Isoprinme IRMS; GV Instruments: Manchester, UK), and the net carbon uptake rate was calculated using the Hama et al. (1983) equation.

Extraction and analysis of the net production rate of MAAs. -MAAs were extracted and their net production rate was calculated according to Sinha et al. (2003) and Ha et al. (2012).

Table 1. Environmental parameters of the melt ponds and open seawater stations. Light intensity unit: $W \cdot m^{-2}$.

Station (Latitude, Longitude)	Date	Name	Pond type	Depth (m)	Diameter (m)	Salinity	Ice thickness (m)	Chl *a* concentration ($mg \cdot m^{-3}$)	UVB (250 nm)	UVA (320 nm)	PAR (395 nm)
Station MP (82°10.75 N, 171°52.10 E)	13 Aug	Closed MP	Closed	0.5	7	0.1	2	0.41	0.48	1.78	2.99
	15 Aug	Open MP	Open	1	15	22	4.2	0.36			

To analyze the MAA contents in the incubated samples, 4 L of each sample was filtered through pre-combusted (450°C, 4 h) glass fiber filters (Whatman GF/F filters; 47 mm) and stored at −80°C until analysis. A 3 mL volume of 100% MeOH was then added to the samples, and an ultrasonicator (30 s, 50 W; Ulsso Hi-Tech: Seoul, Korea) was used to disrupt them. The samples were next placed in a freezer at 4°C overnight, after which the solvent phase was transferred to a 2 mL microtube through a 0.2 μm syringe filter (PTFE 0.2 μm Hydrophobic). A centrifugal evaporator (CVE-200D; EYELA) was employed to completely dry the solvent. The dried sample was then dissolved in 500 μL of distilled water, and 100 μL of chloroform was added to remove lipids and pigment. The sample was subsequently centrifuged for 10 min at 10,000 rpm. A 400 μL aliquot of the supernatant was finally separated and injected into a high-performance liquid chromatograph (HPLC; Agilent Technologies, 1200 series, Wilmington, DE, USA) to quantitatively analyze the MAA contents.

The chromatographic conditions were similar to those described in Sinha et al. 2003. During HPLC analysis, the extracts from the incubated samples were eluted in 100% distilled water with 0.02% acetic acid from a Waters 120DS-AP column (C18; 5 μm; 150 mm × 4.6) at a constant flow rate of 0.8 mL · min^{-1}. An Agilent DAD detector (G1315D) was used at 313 nm (250–750 nm scan), and each MAA compound (shinorine [SH], palythine [PA], asterina-330 [AS], and mycosporine-glycine [MG]) was split using a fraction collector (Agilent analyte (G1364C) FC; Fig. 1). MAA standards from Häder (University of Erlangen Nurnberg, Germany) were employed as standard reference compounds for quantitative analysis of MAAs.

To determine the net production rate of each MAA following the procedure of Ha et al. (2012), the MAA compounds were collected through a tin cap (including the pre-burned filter paper). Once the solvents have been completely removed following the collection of each compound, the ^{13}C value in each compound was measured via EA-irMS (EuroEA-Isoprinme IRMS, GV Instruments). The net production rate of individual MAAs was calculated from each ^{13}C atomic percentage, and the concentration was determined using the modified equation proposed by Hama et al. (1983). The net production rate of each MAA was calculated using the equation described by Ha et al. (2012), and the chl *a*-specific production rate of individual MAAs was obtained through the normalization of each chl *a* concentration.

Statistical analysis. Mean values and standard deviations were obtained for all samples after calculating the values of duplicates. Statistical significance ($P < 0.05$) was determined for all samples using a two-way ANOVA with Tukey's test using the SPSS program, version 18 for Windows (SPSS: Chicago, IL, USA).

RESULTS

Phytoplankton community structure. In general, two types of sea ice MPs, closed-bottom and open-bottom ponds, were found in the Arctic Ocean in 2012. The depth and diameter of the examined closed MPs were 0.5 and 7 m, respectively, and those of the open MP were 1 and 15 m (Table 1). The thickness of the sea ice was 2 and 4.2 m in the closed and open MPs, respectively. The salinity in the closed MP was 0.1, while that in the open MP was higher, at 22 (Table 1).

Small (nano- and pico-sized) phytoplankton generally dominated the phytoplankton communities in the MPs (Table 2). The contributions of these small (nano- and pico-sized) phytoplankton accounted for from 36.6% to 59% and from 48.1% to 39.5% in the closed MP and open MP, respectively. Dinophyceae and Bacillariophyceae also made minor contributions to the total phytoplankton community (Table 2). The total Chl *a* concentration in the closed MP was 0.41 mg Chl *a* · m^{-3} and 0.36 mg Chl *a* · m^{-3} in the open MP, which was slightly lower than that in the closed MP.

Net carbon uptake rates and total MAA concentrations of in situ MPs incubation. The average net carbon uptake rates of phytoplankton in the closed MP was 3.24 mg C · m^{-3} · h^{-1} (SD = ±1.12 mg C · m^{-3} · h^{-1}) in PC bottle incubation and 1.71 mg C · m^{-3} · h^{-1} (SD = ±0.15 mg C · m^{-3} · h^{-1}) ~ in quartz incubation. The average net carbon uptake rates in the open MP was 1.3 mg C · m^{-3} · h^{-1} (SD = ±0.05 mg C · m^{-3} · h^{-1}) exposed PAR+UVA (PC bottles) and 1.21 mg C · m^{-3} · h^{-1} (SD = ±0.06 mg C · m^{-3} · h^{-1})

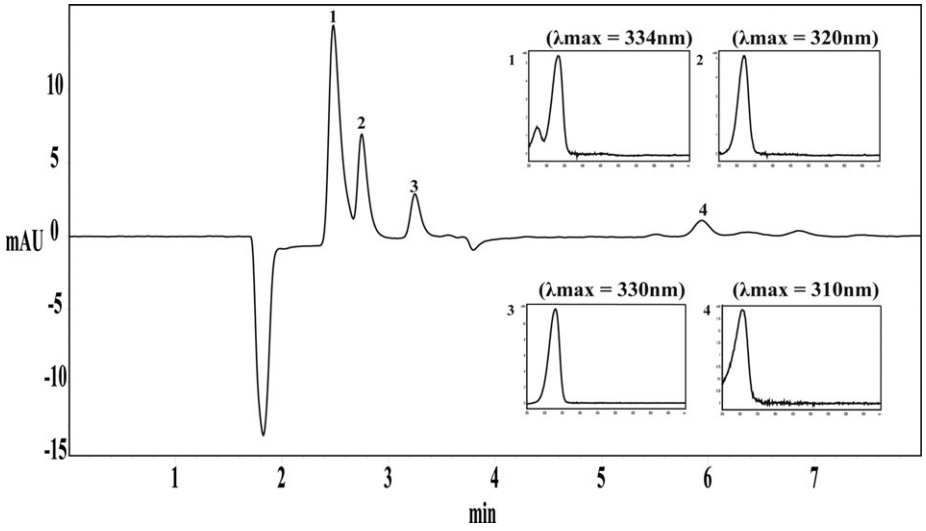

FIG. 1. Typical HPLC-DAD chromatograms and absorption spectra of individual MAAs obtained in this study in melt ponds: 1: shinorine (λ_{max}: 334 nm); 2: palythine (λ_{max}: 320 nm); 3: asterina-330 (λ_{max}: 330 nm); 4: mycosporine-glycine (λ_{max}: 310 nm).

TABLE 2. Relative abundance (%) of phytoplankton communities at the in situ incubation stations.

Taxon	MP01	MP02
Dinophyceae	1.8	1.1
Bacillariophyceae	2.6	11.4
Cryptophyceae		
Chrysophyceae		
Dictyochophyceae		
Prymneosiophyceae		
unidentified sp. (nano size)	36.6	48.1
unidentified sp. (pico size)	59.0	39.5

exposed PAR+UVA+UVB (natural light; quartz bottles), which were lower compared to the closed MP incubation (Fig. 2).

The in situ MAA analysis of both MPs samples revealed four different types of MAAs (SH, PA, AS, and MG; Fig. 1). The initial average total MAA concentration in the closed MP was 1.9 $\mu g \cdot \mu g^{-1}$ Chl *a* (SD = $\pm$0.56 $\mu g \cdot \mu g^{-1}$ Chl *a*), whereas in the open MP it was 10.8 $\mu g \cdot \mu g^{-1}$ Chl *a* (SD = $\pm$0.08 $\mu g \cdot \mu g^{-1}$ Chl *a*; Fig. 3). After in situ incubation of both MPs, the average total MAAs concentration in the closed MP was 1.82 (SD = $\pm$1.19 $\mu g \cdot \mu g^{-1}$ Chl *a*) in PC bottles and 2.58 (SD = $\pm$0.0007 $\mu g \cdot \mu g^{-1}$ Chl *a*) in quartz bottles, which not significantly change between each light conditions. However, the average total MAAs concentration in the open MP was significantly different, depending on light treatments. Under incubated quartz bottles showed the value of 14.59 (SD = $\pm$0.17 $\mu g \cdot \mu g^{-1}$ Chl *a*), while the value of total MAAs in open MP increased 27.74 (SD = $\pm$7.19 $\mu g \cdot \mu g^{-1}$ Chl *a*) under PC bottles incubated (Fig. 3).

Net production rates of MAAs and Chl a*-specific production rates of both MPs.* The net production rates of individual MAA were determined in PC bottles exposed to PAR plus UVA and quartz bottles that transmit natural solar light (PAR+UVA+UVB) in the

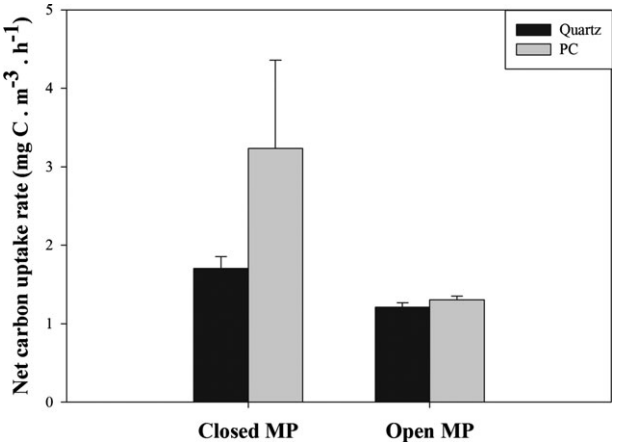

FIG. 2. Net carbon uptake rates in in situ incubations performed at the melt ponds.

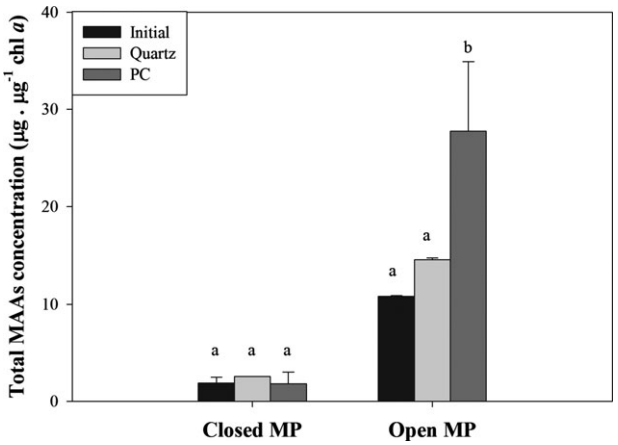

FIG. 3. Total MAA concentrations in melt ponds. Values (mean SD) with different letters (a and b) indicate significant differences over initial values **(Closed MP; $F_{2,\ 6}$ = 0.898; P = 0.456; Open MP; $F_{2,\ 6}$ = 13.750 P = 0.006)**.

MPs. At both MPs, the net production rates of SH, PA, AS, and MG were higher when the samples were incubated in PC bottles compared to quartz bottles (Fig. 4). SH and PA exhibited the highest net production rates of 0.76 ngC $\cdot L^{-1} \cdot h^{-1}$ (SD = $\pm$0.12 ng C $\cdot L^{-1} \cdot h^{-1}$) and 0.53 ng C $\cdot L^{-1} \cdot h^{-1}$ (SD = $\pm$0.06 ng C $\cdot L^{-1} \cdot h^{-1}$), respectively, when samples were incubated in PC bottles at open MPs. The net production rates of AS and MG were 0.06 ng C $\cdot L^{-1} \cdot h^{-1}$ (SD = $\pm$0.05 ng C $\cdot L^{-1} \cdot h^{-1}$) and 0.01 ng C $\cdot L^{-1} \cdot h^{-1}$ (SD = $\pm$0.05 ng C $\cdot L^{-1} \cdot h^{-1}$), respectively, following incubation in PC bottles. The net production rate of SH and PA were 0.5 ng C $\cdot L^{-1} \cdot h^{-1}$ (SD = $\pm$0.05 ng C $\cdot L^{-1} \cdot h^{-1}$) and 0.2 ng C $\cdot L^{-1} \cdot h^{-1}$ (SD = $\pm$0.01 ng C $\cdot L^{-1} \cdot h^{-1}$) incubated in quartz bottles at open MPs. The net production rates of individual MAA at open MP were 10 times higher than at closed MP due to different environmental parameters (Table 1 and Fig. 4). The net production rate of individual MAAs between light treatments (PC vs. quartz bottles) at closed MPs showed no significant distinction. The net production rate of SH was 0.028 ng C $\cdot L^{-1} \cdot h^{-1}$ (SD = $\pm$0.027 ng C $\cdot L^{-1} \cdot h^{-1}$) in PC bottles and 0.027 ng C $\cdot L^{-1} \cdot h^{-1}$ (SD = $\pm$0.001 ng C $\cdot L^{-1} \cdot h^{-1}$) in quartz bottles. The other compounds such as PA, AS, MG also showed a similar trend at closed MPs (Fig. 4).

The Chl *a*-specific production rate of SH was 2.15 ng C $\cdot$ μg Chl $a^{-1} \cdot L^{-1} \cdot h^{-1}$ (SD = $\pm$0.34 ng C $\cdot$ μg Chl $a^{-1} \cdot L^{-1} \cdot h^{-1}$) following incubation in PC bottles and 1.4 ng C $\cdot$ μg Chl $a^{-1} \cdot L^{-1} \cdot h^{-1}$ (SD = $\pm$0.15 ng C $\cdot$ μg Chl $a^{-1} \cdot L^{-1} \cdot h^{-1}$) following incubation in quartz bottles at open MPs, which represented the highest values obtained among the study sites. The net production rate of individual MAAs shows a similar trend to the Chl *a*-specific production rate of individual MAAs at closed MPs (Fig. 4).

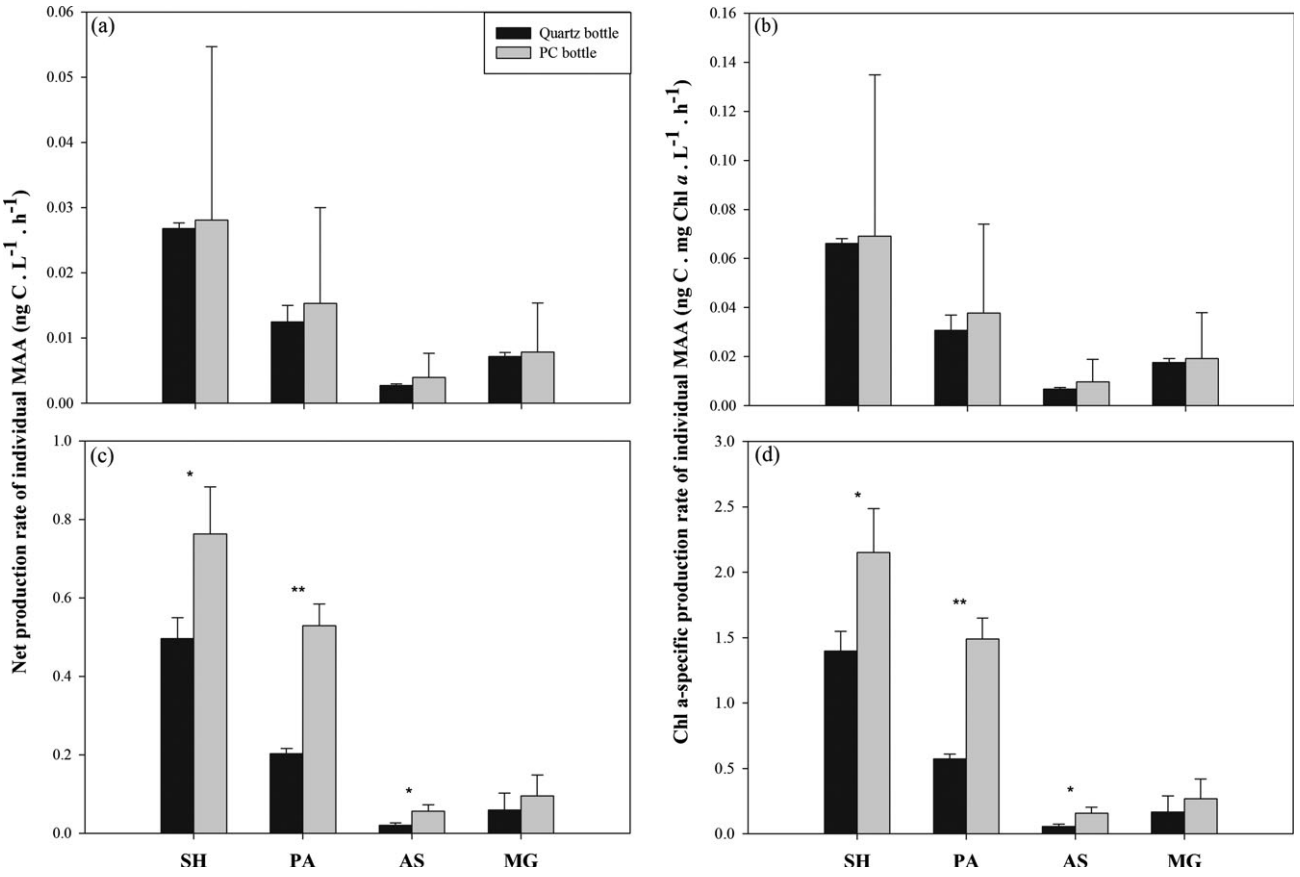

FIG. 4. Individual net production rates and Chl *a*-specific production rates of MAAs in in situ phytoplankton incubations. (a and b): Closed MP, (c and d): Open MP; (a and c): net production rates of individual MAAs, (b and d): Chl *a*-specific production rates of individual MAAs. Black bar: quartz bottle incubation, gray bar: PC bottle incubation. SH (Shinorine); PA (Palythine); AS (Asterina-330); MG (Mycosporine-glycine). Comparative analysis of the incubations in different bottles using the Mann–Whitney test: $*P < 0.05$; $**P < 0.01$.

DISCUSSION

Net carbon uptake rates in MPs. The MPs of the Chukchi Sea examined in this study were located at a different longitude that the MPs in the Canada Basin investigated by Lee et al. (2012), but at a similar latitude, and the in situ incubations were carried out during periods in these two studies (Table 1). Although the Chl *a* concentrations determined in the MPs examined in the present study were similar to or slightly higher than the values reported for the Canada Basin (Lee et al. 2012), the net carbon uptake rates in our MPs were relatively higher than the values from the Canada Basin. The present study revealed high net carbon uptake rates in both MP types than the Canada Basin (Lee et al. 2012). Additionally, the average net carbon uptake rate reported in MPs by Lee et al. (2012) were high compared to the surrounding surface layer, and our data of net carbon uptake rates also had relatively high values compared to the surrounding surface seawater (data not shown).

The daily carbon productivity in the water column in the Arctic Ocean has been suggested to cause large differences in the phytoplankton biomass and photosynthesis rates due to high seasonal variation (Yun et al. 2011, Lee et al. 2012). In this study, the light intensity during the incubations showed similar values at all incubation stations, and the effect of light on the net carbon uptake rates depending on the latitude was considered to be small (Table 1).

The net carbon uptake rate showed a significant difference between the two types of MPs examined in this study. It appeared that the morphological type of the two MPs and the distinct phytoplankton community structures in the ponds affected the recorded net carbon uptake rates. Although the Chl *a* concentrations were not statistically different between the two MPs (Table 1), the closed- MP displayed a low water depth that allowed a relatively greater amount of light to penetrate and a thinner ice layer compared to the open MP. Accordingly, the net carbon uptake rate in closed MP was relatively higher than that in open MP and was lower in the quartz incubations than the PC incubations (Fig. 2). In MPs, thin ice is often formed on the surface water. As the air temperatures decrease, the surface of MPs refreezes more rapidly than that of

the open sea due to the lower salinity and lack of disturbance from waves in the ponds (Lee et al. 2011). This surface ice functions to decrease UVB penetration, which depends on the thickness of surface ice, leading to different transmittance levels (Ehn et al. 2011). Although the surface ice in the MPs was partially protected from UV radiation, the net carbon uptake rate of the phytoplankton in the MPs might have been inhibited by UV radiation. Accordingly, the net carbon uptake rates of the phytoplankton within the MPs were stimulated to a greater extent in PC incubation than in quartz incubation, although this difference was not statistically significant.

Comparison of MAA net production rates between closed MP and open MP. Four types of MAAs were detected in the phytoplankton in the investigated closed and open MPs (Fig. 1). AS was detected rather than porphyra-334 (PR) which is often found in diatoms in open seawater. Although diatoms appeared in the MPs (Table 2), our results did not detect PR in the MPs, as the diatom biomass might be considerably lower than that of other phytoplankton. Small (nano- and pico-sized) flagellates were the most dominant species in the MP habitats (Lee et al. 2011), and small-sized phytoplankton were absolutely dominant in the MPs. The occurrence of diatoms was relatively higher in the open than in the closed MPs, indicating permeation of sea ice algae into the pond.

The induction of MAAs in phytoplankton has been reported to be most strongly affected by UV radiation (Sinha et al. 2003, Häder et al. 2007), and MAAs show a positive correlation induced by light wavelength (Häder et al. 1998). The diatoms and dinoflagellates detected in ponds are known to be the producers of MAAs (Riegger and Robinson 1997, Jeffrey et al. 1999). The MAA concentration shows a higher or similar value in MPs to that observed in the surface layer phytoplankton found in lakes that are transparent to UVR (Sommaruga and Garcia-Pchel 1999, ~ Uusikivi et al. 2010). The high exposure to UVR observed in surface ice resulted in a high MAA concentration (Uusikivi et al. 2010). In the MPs, relatively high albedos were detected compared to those at the open water sites, and the PAR penetration ratio was also observed to be high (Ehn et al. 2011). Mundy et al. (2011) reported that the much greater peak of UV absorption observed in average ice meltwater spectra suggested enhanced accumulation of MAAs in the phytoplankton community compared to the interior ice community and that the ice meltwater community responds actively to the higher UVR levels.

In fact, the individual MAA net production rates and Chl *a*-specific MAA production rates observed in the phytoplankton incubated in the PC bottles were higher than those recorded in the phytoplankton incubated in quartz bottles in open MP (Fig. 4). The MAA concentrations and net production rates in phytoplankton cells were increased not only by UVR but also by environmental factors such as osmotic pressure and nutrient conditions to allow survival in open MP. According to Oren (1997), MAAs are produced as osmolytes in some algae such as halophilic cyanobacteria, and MAA assimilation in the cell is reinforced by the combination of UV radiation and osmotic stress (Portwich and Garcia-Pichel 1999, Oren and Gunde-Cimerman 2007). Because algae in the sea ice interior and the sea ice surface experience dramatic osmotic shifts related to the sea ice brine salinity, they can display high MAA contents as a strategy to achieve osmotic balance (Arrigo and Thomas 2004). MAAs may be involved in the adaptation of sea ice algae to osmotic changes when variations in ambient temperature cause large changes in sea ice brine salinity (Arrigo and Thomas 2004). Therefore, the value of chl *a*-specific MAA production rates in open MPs was higher value than that in closed MPs due to the inflowing salt shock from the surrounding seawater in open MPs (Fig. 4).

The phytoplankton assemblage in the closed MPs experienced lower osmolyte pressure compared to the phytoplankton assemblage in the open MPs and exhibited constant cellular contents due to the Chl *a*-specific MAA production rates and concentrations of MAA in the closed MP. Thus, the MAA acclimation was shown to be relatively lower in the closed MP than in the open MP incubation. The relative UVR and PAR light intensities recorded during the incubation period in the MPs were found to be similar in this study (Table 1). However, the environmental differences among closed MP and open MP resulted in osmotic stress on phytoplankton, which would affect their MAA net production rates (Table 1).

This study measured the MAA net production rate, and the modulation of this parameter represents a survival strategy of phytoplankton in new habitats (MPs) in the Arctic. The phytoplankton assemblage in both open and closed MPs showed similar net carbon uptake rates, but the net production rate of MAAs in the open MPs was higher than in the closed MPs. The phytoplankton in open MPs, experiencing UV radiation and osmotic stress simultaneously, appear to exhibit higher UV-absorbing compound production compared to other closed MPs. The Arctic Ocean, which is experiencing climate change, will exhibit a high contribution of MP organisms to primary production (Lee et al. 2012), and the changes in biochemical compounds according to the environmental changes within MPs will affect the ecology of animals at higher trophic levels in the Arctic Ocean.

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