Effect of time of day on the recovery from light exposure in ice algae from Saroma Ko lagoon, Hokkaido

Andrew McMinn[1*] and Hiroshi Hattori[2]

[1]*Institute of Antarctic and Southern Ocean Studies, Private Bag 77,*
University of Tasmania, Hobart 7001, Australia
[2]*Hokkaido Tokai University, 5–1–1–1, Minamisawa, Minami-ku, Sapporo 005-8601*
[*]*Corresponding author. E-mail: Andrew.mcminn@utas.edu.au*

Abstract: The amount of prior light exposure an algal community receives is likely to determine its ability to recover from exposure to high light levels. Here we investigate the effect of time of day and light on the ability of ice algae to recover from variable light exposure. The ice algae were obtained from Saroma Ko lagoon, a temperate, perennially ice covered lagoon in northern Hokkaido, Japan. Under ice irradiance varied between 0 and 122 μmol photons m^{-2}s^{-1}. Algal biomass, which was dominated by *Nitzschia frigida*, was 272.8±20.2 mg Chl *a* m^{-2}.

There was no consistent relationship between ambient *in situ* irradiance and recovery rates. While the sea ice community showed mild inhibition at the highest irradiance used, 490 μmol photons m^{-2}s^{-1}, after 10 min the quantum yield had recovered to between 90.9% and 112% (average 99.5%) of the original measurement. It is likely that ambient under ice irradiances at Saroma Ko lagoon were never sufficient to cause photoinhibition and consequently had little negative long term effect on photophysiology. The Rapid Light Curves, while providing irradiances of sufficient intensity to cause inhibition, were of too short a duration to cause anything but a short, transient effect. Therefore, it is unclear whether higher *in situ* irradiances would necessitate a longer period of recovery.

key words: sea ice, Hokkaido, recovery photoinhibition

Introduction

At high ambient light levels photosynthesis in marine microalgal communities is often reduced from a maximum rate by processes of photoinhibition. On short time scales photoinhibition is usually associated with the closing of reaction centres and if prolonged can cause permanent damage or bleaching to the photosynthetic apparatus. While most phytoplankton adopt a variety of photoprotective mechanisms, such as changing pigment composition and state transitions (Anderson *et al*., 1997), it appears that much of the photodamage in reaction centres is due to loss of D1 protein, a process which is quickly reversible (Anderson *et al*., 1997). The extent to which natural photoinhibition affects net primary production in marine ecosystems is unclear. In most phytoplankton communities constant mixing is likely to make photoinhibition only a transitory problem (Han *et al*., 2000) as the cells are constantly moving through a sharp light gradient. However, in

many substrate-bound algal communities the individual cells remain in the same position and so are potentially more likely to be effected by inhibition. Photoinhibiton in sea ice algal communities has been documented in Antarctica by McMinn *et al*. (2003, 2005a) and in Arctic communities by Glud *et al*. (2002). Here photoinhibition can be initiated at irradiances as low as 50 μmol photons m^{-2}s^{-1}.

Using variable fluorescence yield (F_v/F_m) to study photosynthesis in marine plants has become widespread since the mid 1990s (Falkowski and Kolber, 1993; Schreiber, 1994). In particular, it has been noticed that there is a decline in F_v/F_m associated with near surface waters (McMinn and Hegseth, 2004) and also with mid day, which is attributed to photoinhibition. Here, we use these techniques to investigate whether natural irradiances cause photoinhibition in a sea ice algal community and whether these irradiances affect the rate of recovery from inhibition.

Materials and methods

Saroma Ko lagoon is a large, shallow lagoon on the Okhotsk Sea coast of northern Hokkaido, Japan. From January to March each year the lagoon is covered by sea ice up to 45 cm thick. Sea ice sampling occurred on 1st and 2nd of March 2006. The samples were collected approximately 1 km off shore at 44°07'07"N, 140°57'56"E with a 7 cm diameter SIPRE corer. Care was taken to avoid exposure to ambient light while sampling.

Surface and under ice light measurements were made simultaneously on a LI1400 PAR meter (LI-COR, Lincoln Nebraska) using a LI-190SA surface sensor and LI193-A underwater sensor (Table 1).

Ice was scrapped from the underside of the ice and placed directly into the measuring cuvette. A WaterPAM (Walz, Effeltrich, Germany) was used to make all measurements. The first measurements were initiated immediately after sampling, the second set was run after the sample had spent approximately 15 to 20 min in the dark. The primary measurements were a set of recovery responses following a rapid light curve (RLC). The RLC and recovery procedure is a preset program controlled by WinControl software

Table 1. Under ice irradiances at Saroma Ko lagoon, Hokkaido. All light measurements are in μmol photons m^{-2} s^{-1}.

Date	Time	Surface PAR	Under ice PAR
1/3/06	11:30	763.7	120.30
1/3/06	14:00	480.9	75.70
1/3/06	16:30	45.3	6.40
2/3/06	9:00	551	3.70
2/3/06	11:30	921.4	6.10
2/3/06	14:00	946.1	6.34
2/3/06	16:40	40.8	0.27
2/3/06	17:00	15.3	0.15
2/3/06	19:40	0.002	0.00

(Walz, Effeltrich, Germany) on a PAMControl unit (Walz, Effeltrich, Germany). All data was logged automatically at the field site. Rapid light Curves are now a well known and accepted method of investigating light adaption in plants (Schreiber, 2003; Ralph and Gademann, 2005; McMinn *et al*., 2005a, b).

PAM fluorometers are able to measure quantum yield ($\Delta F/F_m'$), photosynthetic efficiency (F_v/F_m) and electron transfer rate (ETR) of photosystem II (PSII) in photosynthesis. Using rapid light curves (RLC) it is also possible to gain an insight into the photoadaptive state of the plant and to measure the photoadaptive index, E_k. The PAM method is based on the supply of weak, modulated light pulses (the measuring light) that allows chlorophyll fluorescence to be monitored without inducing photosynthesis. In the dark-adapted state a minimum fluorescence (F_0) is determined when the measuring light is turned on. This mostly results from emissions from the antenna pigments. When the sample is exposed to actinic light, *i.e.* light that induces photosynthesis, a much higher fluorescence results. This fluorescence rapidly peaks and then declines till an equilibrium level, F, is attained. This characteristic behaviour is referred to as the Kautsky curve (Schreiber, 1994). Maximum fluorescence (F_m) is achieved by exposing the dark-adapted sample to a pulse of very intense light. The maximum quantum yield of PSII is defined as

$$\phi_{PSII}= (F_m-F_0)/F_m=F_v/F_m \text{ (Schreiber, 2003)}.$$

If the sample is experiencing additional *in situ* actinic light a similar, saturating pulse will lead to a lower maximum fluorescence, F_m', which is the maximal fluorescence yield of an illuminated sample. The effective quantum yield of charge separation in PSII, ϕ_p is defined as

$$\phi_p= (F_m'-F)/F_m' \text{ or } \Delta F/F_m'.$$

The relative electron transfer rate (rETR) can be calculated from

$$rETR=\phi_p E,$$

where E is the irradiance.

Actinic light levels for the RLCs were preset at 0, 31, 44, 66, 100, 148, 230, 343 and 490 μmol photons m^{-2}s^{-1}. Peak emission wavelengths were 650–660 nm. The photosynthetic parameters, E_k, P_{max} (here equivalent to maximum rETR$_{max}$) and α (here equivalent to ϕ_{PSII}) were estimated for each RLC by undertaking a non-linear regression to the function (Schreiber, 2003).

$$P=P_{max} [1-e^{(-\alpha E/P_{max})}]; \text{ (Webb } et~al., 1974),$$

where P=photosynthesis (here equivalent to rETR), P_{max} is the maximum photosynthetic rate, α_{PSII}=photosynthetic efficiency of the light limiting region of the RLC, E=irradiance. E_k is derived by dividing P_{max} by α. E(inhib), the irradiance at which photoinhibition first occurred, was determined from RLC data, and based on the irradiance at which ETR first declined (Table 2).

At the completion of the RLC the recovery procedure produced additional saturating flashes at 0 s, 30 s, 60 s, 120 s, 300 s and 600 s and measured the quantum yield. The degree of recovery after each time period was normalised to the initial quantum yield at

Table 2. Photosynthetic parameters of sea ice from at Saroma Ko lagoon, Hokkaido. Samples that underwent a 15–20 min dark adaption period are indicated with a '*' in the DA column. F_v/F_m is a dimensionless ratio and ETR and α are relative measurements and so neither have units, Units for E_k (photoadaptive index) and E (inhib) (irradiance of photoinhibition), are μmol photons m^{-2} s^{-1}, % recovery is the recovery of F_v/F_m after a RLC—values are given as a percentage of the pre RLC value, r (rate of recovery) are s^{-1}.

Date	Time	F_v/F_m	DA	$rETR_{max}$	alpha	E_k	E(inhib)	% Recovery	r
1/3/06	10:54	0.448		39.788	0.476	83.6	>490	111.6	3.8E-04
	14:47	0.562		20.209	0.605	33.4	490	97.3	6.0E-04
	14:52	0.584	*	18.119	0.467	38.8	343	90.9	1.3E-03
	16:29	0.573		21.464	0.503	42.7	343	98.6	6.3E-04
	16:51	0.374	*	17.091	0.564	30.3	343	98.6	5.0E-03
2/3/06	9:20	0.508		36.473	0.694	52.6	490	100.5	2.1E-03
	9:40	0.550	*	26.554	0.478	55.6	490	100.4	2.2E-04
	11:08	0.505		25.474	0.356	71.6	490	104.2	3.9E-04
	11:29	0.556	*	31.143	0.629	49.5	490	97.5	5.4E-04
	13:42	0.609		23.14	0.854	27.1	343	101.8	1.2E-03
	14:03	0.603	*	18.456	0.626	29.5	343	99.3	8.7E-04
	16:38	0.479		28.231	0.469	60.2	343	101.0	2.8E-02
	16:58	0.544	*	29.554	0.392	75.4	490	96.7	5.2E-04
	19:31	0.640		18.15	0.684	26.5	343	96.6	2.4E-03
	19:51	0.614	*	20.397	0.544	37.5	343	97.4	1.1E-03

the beginning of the RLC and this is expressed as a percentage (Table 2).

To determine the rate of recovery of F_v/F_m after a RLC the Φ was measured after 10, 30, 60, 120, 300 and 600 s. Using a multiple non linear regression, these were fitted to the function

$$\Phi_t = \Phi_I + (\Phi_M - \Phi_I)\,(1 - e^{-rt}),$$

where Φ_t is the value of F_v/F_m at time t(s), Φ_I is the initial value of F_v/F_m and Φ_M is the maximum value of F_v/F_m taken at night. This calculation of r, the rate of recovery follows Oliver *et al.* (2003) and assumes that the rate of recovery is dependant on the degree of damage as suggested by Kok (1956).

Chlorophyll *a* biomass and taxonomy

Three replicate ice cores were taken for chlorophyll *a* analysis. The bottom 5 cm was removed, melted in filtered seawater in the dark and then filtered on to Whatman GF/F filters. These were then stored in liquid nitrogen for transport back to Sapporo, Hokkaido. Chlorophyll *a* was measured on a Shimadzu MPS-2450 spectrophotometer using the standard method of Jeffrey and Humphrey (1975) after pigments were extracted for 24 hrs with N,N-demethylformamide. As the chlorophyll *a* concentrations were obtained using DMF as a solvent, a correction was made to account for the differing extinc-

tion coefficients of DMF (Moran, 1982). All measurements are presented as mg Chl a m^{-2}.

Sea ice algal species composition was determined on live samples. Ice was scrapped from the bottom of the ice and melted. Examination was on an Olympus CX21 microscope using a 20× objective.

Results

The ice at Saromo Ko was between 35 and 40 cm thick. Initially there was no snow cover on the ice but 20 cm fell over night between the 1st and 2nd of March. Air temperatures were between −10° and +3°C. The water depth was 3 m, the water temperature was −1.5°C and the salinity 30 psu.

Surface light measurements (PAR) varied between a maximum of 926 μmol photons m^{-2}s^{-1} at 11:30 on the 2nd to a minimum of 0.001 μmol photons m^{-2}s^{-1} at 19:40 on the 2nd. Overnight snowfall between the two days drastically reduced the under ice light. Maximum under ice light was approximately 120 μmol photons m^{-2}s^{-1} at 11:34 on the 1st. This represents approximately 15% of the surface irradiance, maximum irradiance after the snow fall was only 6.7 μmol photons m^{-2}s^{-1}, or 0.7%, at 11:30 on the 2nd. Under ice light levels dropped to below 1 μmol photons m^{-2}s^{-1} after approximately 15:00 on the 2nd but were still above 6 μmol photons m^{-2}s^{-1} at 16:40 on the 1st (Table 1).

The sea ice algal biomass was 276.2±20.9 mg Chl a m^{-2} (n=3). The sea ice algal community was overwhelmingly dominated by diatoms. *Nitzschia frigida* (40%) was dominant with *Achnanthes confervacea* (25%) and *Navicula pelagica* (20%) also making up significant proportions.

Photosynthetic parameters

There was no evidence of suppression of quantum yield at the highest under ice irradiances, *i.e.* greater than 80 μmol photons m^{-2}s^{-1}. The effective quantum yield was mostly above 0.5 and showed little variation with irradiance or time of day. After the heavy over night snowfall depressed under ice irradiance levels, quantum yield was generally a little higher, although the difference was not significant (P<0.05) (Table 2).

rETR$_{max}$ values were consistently between a maximum of 39.8 and a minimum of 18.1. There was no apparent relationship between rETR$_{max}$ and time of day or ambient under ice irradiance. Similarly, photosynthetic efficiency, α, varied between 0.694 and 0.356 but showed no systematic relationship with under ice irradiance or time of day. E$_k$ values were likewise uncorrelated and had values between 83 and 27 μmol photons m^{-2}s^{-1}. There was no evidence of photoinhibition at irradiances up to 343 μmol photons m^{-2}s^{-1} but most showed some reduction in ETR at 490 μmol photons m^{-2}s^{-1} (Table 2).

Recovery from light exposure

While ambient light values decreased from a maximum of 122 μmol photons m^{-2}s^{-1} on the first day, there was no significant difference in the recovery response over 600 s. Percentage recovery of quantum yield 10 min after the RLC was between 90.9% and 112% with an average recovery of 99.5% (Table 2). There was no significant correlation (P<0.05) between percentage recovery rate and ambient irradiance. Irradiance for the on-

set of photoinhibition, E(inhib) was always above 343 μmol photons $m^{-2}s^{-1}$ and thus always above ambient under ice irradiance.

A short dark adaption period of approximately 15–20 min had no consistent effect on photosynthetic parameters or recovery rates (Table 2). Even F_v/F_m values did not show a consistent recovery after a period of dark adaption with some values actually dropping.

The recovery rate of F_v/F_m following a RLC, r, was determined by fitting the recovery data to an exponential recovery curve (see Materials and methods). The night-time F_v/F_m value was measured at 0.64. The recovery rates were between 2.2×10^{-4} s^{-1} and 2.8×10^{-2} s^{-1} and likewise did not show a significant correlation with time of day or under ice irradiance (Table 2). There was some evidence of a relationship between recovery rate and F_v/F_m, *i.e.* higher maximum quantum yields lead to faster recovery rates, but the correlation was not significant ($r^2 = 0.20$)

Discussion

Sea ice algal biomass in Saroma Ko lagoon in 2006 was measured at 272.8±20.2 mg Chl a m^{-2}, which is considerably higher than previous measurements, which have included 38.25 mg Chl a m^{-2} (Kudoh *et al*., 1997), 2–119 mg Chl a m^{-2} (Robineau *et al*., 1997) and 14.7 mg Chl a m^{-2} (McMinn *et al*., 2005b). Maximum under ice light levels were approximately 120 μmol photons $m^{-2}s^{-1}$ but decreased to <10 μmol photons $m^{-2}s^{-1}$ after a heavy over night snow fall. E_k values, indicating adaption to ambient light levels, are between 27.1 and 83.6 μmol photons $m^{-2}s^{-1}$ (Table 2) and are thus consistent with measured ambient under ice irradiances and imply a high level of light adaption. These ambient light levels, however, had no significant effect on the ability of the algae to recover from the saturating flashes (3000 μmol photons $m^{-2}s^{-1}$) and actinic light levels (0–490 μmol photons $m^{-2}s^{-1}$) of the rapid light curves. Recovery was almost always complete after 10 min and showed no relationship with the initial ambient irradiance. The rates of recovery were mostly similar between different treatments (2.2×10^{-4} to 2.8×10^{-2} s^{-1}), showed no relationship with ambient irradiance and were consistent with recovery rates measured for freshwater phytoplankton from temperate Australia (4.3×10^{-4} to 9.1×10^{-1} s^{-1}) (Oliver *et al*., 2003).

Ambient under ice irradiances at Saroma Ko lagoon were never sufficient to cause photoinhibition and consequently had little negative long term effect on photophysiology. Photoinhibition, measured from the RLCs (E(inhib), Table 2), did not occur at irradiances below 343 μmol photons $m^{-2}s^{-1}$ and thus measured ambient irradiances were always insufficient to cause inhibition. The RLCs, while providing irradiances of sufficient intensity to cause inhibition, were of too short a duration to cause anything but a short, transient effect. Therefore, it remains unclear whether higher *in situ* irradiances would have necessitated a longer period of recovery.

There was also an inconsistent response to a short period of dark-adaption. F_v/F_m values in some dark-adapted samples actually declined and no significant differences were seen between the photosynthetic parameters of dark and non dark-adapted samples. There was also no significant difference in the rate of recovery of dark-adapted and non dark-adapted samples from high light exposure during the RLCs. Their E_k values, however, suggest that they were all well adapted to their natural ambient light environment.

In the Antarctic, photoinhibition in sea ice algae has been measured at irradiances less than 50 μmol photons m^{-2}s^{-1} (McMinn *et al*., 2003, 2005a). The lack of evidence for photoinhibition in the Saroma Ko lagoon sea ice algal community is most likely a result of thinner ice and much higher average under ice irradiances.

References

Anderson, J.M., Park, Y.I. and Chow, W.S. (1997): Photoinactivation and photoprotection of photosystem II in nature. Physiol. Plant, **100**, 214–223.

Falkowski, P.G. and Kolber, Z. (1993): Estimation of phytoplankton photosynthesis by active fluorescence. ICES Mar. Sci. Symp., **197**, 92–103.

Glud, R.N., Kuhl, M., Wenzhofer, F. and Rysgaard, S. (2002): Benthic diatoms of a high Arctic fjord (Young Sound, NE Greenland): importance for ecosystem primary production. Mar. Ecol. Prog. Ser., **238**, 15–29.

Han, B.-P., Virtanen, M., Koponen, J. and Straskraba, M. (2000): Effect of photoinhibition on algal photosynthesis: a dynamic model. J. Plankton. Res., **22**, 865–885.

Jeffrey, S.W. and Humphrey, G.F. (1975): New spectrophotometric equations for determining chlorphylls *a*, *b*, c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanzen., **167**, 191–194.

Kok, B. (1956): On the inhibition of photosynthesis by intense light. Biochem. Biophys. Acta, **21**, 234–244.

Kudoh, S., Robineau, B., Suzuki, Y., Fujiyoshi, Y. and Takahashi, M. (1997): Photosynthetic acclimation and the estimation of temperate ice algal primary production in Saroma-ko Lagoon, Japan. J. Mar. Sys., **11**, 93–109.

McMinn, A. and Hegseth, E.N. (2004): Quantum yield and photosynthetic parameters of marine microalgae from the southern Arctic Ocean, Svalbard. J. Mar. Biol. Assoc. UK, **85**, 865–871.

McMinn, A., Ryan, K. and Gademann, R. (2003): Photoacclimation of Antarctic fast ice algal communities determined by pulse amplitude modulation (PAM) fluorometry. Mar. Biol., **143**, 359–367.

McMinn, A., Pankowski, A. and Delfatti, T. (2005a): Effect of Hyperoxia on the growth and photosynthesis of polar sea ice algae. J. Phycol., **41**, 732–741.

McMinn, A., Hirawake, T., Hamaoka, T., Hattori, H. and Fukuchi, M. (2005b): Contribution of benthic microalgae to ice covered coastal ecosystems in northern Hokkaido, Japan. J. Mar. Biol. Assoc. UK, **85**, 282−298.

Moran, R. (1982): Formulae for determination of chlorophyllous pigments extracted with N,N-Dimethylformamide. Plant Physiol., **69**, 1376–1381.

Oliver, R.L., Whittington, J., Lorenz, Z. and Webster, I.T. (2003): The influence of vertical mixing on the photoinhibition of variable chlorophyll *a* fluorescence and its inclusion in a model of phytoplankton photosynthesis. J. Plankton Res., **25**, 1107–1129.

Ralph, P.R. and Gademann, R. (2005): Rapid light curves: A powerful tool to assess photosynthetic activity. Aquat. Bot., **82**, 222–227.

Robineau, B., Legendre, L., Kishino, M. and Kudoh, S. (1997): Horizontal heterogeneity of microalgal biomass in the first-year sea ice of Saroma-ko Lagoon (Hokkaido, Japan). J. Mar. Sys., **11**, 81–91.

Schreiber, U. (1994): New emitter detector cuvette assembly for measuring modulated chlorophyll fluorescence of highly diluted suspensions in conjunction with the standard PAM fluorometer. Z. Naturforsch., **49c**, 646–656.

Schreiber, U. (2003): Pulse amplitude (PAM) fluorometry and saturation pulse method. Chlorophyll *a* fluorescence: a signature of photosynthesis. Advances in Photosynthesis and Respiration Series, ed. by G. Papageorgiou and Govindjee. Dordrecht, Kluwer Academic Publishers, 1–41.

Webb, W.L., Newton, M. and Starr, D. (1974): Carbon dioxide exchange of *Alnus rubra*: a mathematical model. Oecologia, **17**, 281–291.