A Large Biomass and the Survival of Phytoplankton beneath the Ice Layer of Lake Akan

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Abstract

A large biomass of phytoplankton was found beneath the ice layer of Lake Akan during 6 years of observation. The winter biomass was higher than that in summer every year. Survival of phytoplankton, which increased in late autumn, was considered as a factor supporting the large biomass beneath the ice layer of Lake Akan. The physiological state of phytoplankton estimated by adenylate energy charge indicated that they could photosynthesize in euphotic zone under the ice in Lake Akan. When water sample was incubated under weak light at 2°C for 130 days, ATP and adenylates quickly decreased and the final ATP concentration was 7 % of the initial ATP in this experiment. The ratio of sugars to proteins decreased slowly over the course of 130 days. The value of adenylate energy charge fell slowly to 0.45 after incubation. When the water temperature gradually reached 15°C from 2°C after 140 days of incubation, the phytoplankton biomass increased and its physiological state rose above initial level. These results suggest that phytoplankton maintains its physiological activity beneath the ice layer, and that survival of phytoplankton supports the large phytoplankton biomass beneath the ice layer.

Key words: adenylate energy charge, ice-covered lake, Lake Akan, phytoplankton biomass

1. Introduction

The ice layer generates a unique environment for phytoplankton in terms of low light intensity and low temperature, and the life of phytoplankton under ice has been of interest in lake ecosystems. There have been several reports of a large phytoplankton biomass beneath the ice layer of lakes in winter (SAIJO and SAKAMOTO, 1964; WRIGHT, 1964; MAEDA and ICHIMURA, 1973; MASAKI and OKINO, 1984). They indicated that the phytoplankton biomass frequently reached an annual maximum value under such conditions.

The large phytoplankton biomass beneath the ice layer can be elucidated by the survival of phytoplankton, which increased its biomass in late autumn, and/or by continuous growth of phytoplankton under ice. SEABURG *et al.* (1983)

reported that the phytoplankton in permanently ice-covered Antarctic lakes was well adapted for performing photosynthesis under extremely low light conditions. Changes of environmental factors should remarkably affect physiological activity and cellular organic component of phytoplankton, yet phytoplankton may well survive and grow under such less advantageous conditions beneath the ice layer.

To clarify the mechanism by which the large biomass of phytoplankton is maintained beneath the ice layer during winter in Lake Akan, the physiological state of phytoplankton was estimated under low temperature and low light intensity from the aspect of the AEC as an index, along with the biomass of phytoplankton under the experimental conditions over a 6-years period.

2. Materials and methods

2-1. Study sites and sampling station

Lake Akan is located in the eastern part of Hokkaido, Japan (43° 27' N, 144° 06' E; altitude 420 m). It is a mesotrophic-eutrophic lake, with the maximum depth of 45 m, the mean depth of 18 m, and the surface area of 13 km² (HRIEP, 1990). This lake is covered with ice from early December to middle April. The sampling station is near the center of the lake at the depth of 38 m.

The biomass of phytoplankton expressed in terms of chlorophyll-*a* (chl-*a*) was measured from May 1982 to May 1988 in Lake Akan. Water samples were collected from depths of 0.5, 2, 5, 10, 15, 20, 25, 30, and 35 m with a 6 liter Van Dorn water sampler. Subsamples for chl-*a* and adenylate analysis were filtered immediately through glass fiber filter (Whatman GF/C, pore size 1.2 μ m). Volume of filtration for chl-*a* and adenylate analysis was 100 ml and 200 ml, respectively. Filters for adenylate analysis were placed in 12 ml polypropylene centrifuge tubes that contained 3 ml of 20 mM Tris-HCl buffer (pH 7.8) and 0.6 ml of 35% perchloric acid. All the samples were carried to the laboratory under freezing condition (-15°C). Samples of net-phytoplankton used for microscopic observation were collected from the surface layer (depth of 0.5 m) by a plankton net (NXXX 25, mesh size 40 μ m).

2-2. Incubation experiments

Water samples for incubation experiments were collected on 22 October 1985 and on 20 October 1987 with a 6 liter Van Dorn water sampler from a depth of 0.5 m. Table 1 shows the water chemistry of samples collected from Lake Akan to be used in the incubation experiments. These samples were sieved to remove

zooplankton by passing through NXX 13 nylon net (mesh size 94 μ m). The sample collected on 22 October 1985 was divided into a pair of 5 liter of glass bottles and incubated under light from fluorescent lamps (2.5 μ Einst·m⁻²·sec⁻¹) with a 16 hr: 8 hr, light and dark cycle at 2°C (Experiment 1). The water sample collected on 20 October 1987 was divided into a pair of 10 liter glass bottles and incubated under weak light provided by fluorescent lamps (4.5 μ Einst·m⁻²·sec⁻¹) with a 12 hr: 12 hr, light and dark cycle. The initial incubation temperature was 10°C, and it fell stepwise to 2°C in 2-degree decrements. After the water sample was incubated at 2°C for 140 days, its temperature was risen up to 14°C in 2-degree increments (Experiment 2).

Each 600 ml of subsample was withdrawn periodically from the two glass bottles. After dividing into 200 ml aliquots, each sample was filtered immediately through Whatman GF/C glass fiber filter for determination of adenylates, proteins, sugars, and chl-*a*.

2-3. Chemical analyses

Chl-*a* was measured with a Turner model 111 fluorometer after extraction from samples on filters with 90% acetone. Nitrate and nitrite nitrogen were measured by the method of Wood *et al.* (1967). Ammonium nitrogen was measured by the Indophenol method (Japan Meteorological Agency, 1970). Total nitrogen was measured by the ultraviolet spectrophotometric method under acid condition (OTSUKI, 1983) after hydrolysis by alkaline potassium persulfate in an autoclave at 121°C for 30 min (SOLORZANO and SHARP, 1980). Phosphate phosphorus was measured by the method of MURPHY and RILEY (1962). Total phosphorus was measured by the same method for phosphate phosphorus after hydrolysis by potassium persulfate

Table 1. The water chemistry of sample collected from Lake Akan.

Date	W. Temp. °C	pH	D. O. mgO ₂ ·l ⁻¹	DIN	T-N μ mole·l ⁻¹	PO ₄ -P	T-P	N/P mole/mole	Chl- <i>a</i> μ g·l ⁻¹
22 Oct. 1985	11.8	7.7	8.8	7.34	17.9	0.26	1.45	12.3	5.31
20 Oct. 1987	12.8	7.6	8.9	9.21	27.9	1.26	1.65	16.9	18.1

D. O., dissolved oxygen; DIN, NH₄-N + NO₂-N + NO₃-N

in an autoclave at 121°C for 30 min. Proteins and sugars on filters were put into glass vials and dissolved in an autoclave at 121°C for 30 min by the same method in a previous paper (HINO and TADA, 1985). Proteins were measured by the method of LOWRY *et al.* (1951) with bovine serum albumin as a standard. Sugars were measured by the anthrone method (SCOTT and MELVIN, 1953) with glucose as a standard. All data of chemical analyses were shown as the mean of the duplicate analyses.

2-4. Measurements of adenylates and determination of AEC value

Adenylates were extracted from the samples on filters by the method of WALKER-SIMMONS and ATKINSON (1977) with a minor modification. The details of adenylate extraction procedure were given in previous papers (HINO, 1988 a, b).

Adenylates were measured before and after conversion of ADP and AMP to ATP by the enzymatic method (BALL and ATKINSON, 1975; HINO, 1988 a, b). ATP was assayed by the luciferin-luciferase reaction with a JRB ATP Photometer, Model 2000. The luciferin, luciferase, myokinase, and pyruvate kinase which used in the assay were purchased from Boehringer Co. Concentrations of ADP and

AMP were calculated from the differences in measured values before and after the conversion to ATP. Conversions of ADP and AMP to ATP were monitored using internal standards of ADP and AMP. The AEC value was determined from the measured concentrations of adenylates by the following equation:

$$\text{AEC} = \{[\text{ATP}] + 1/2[\text{ADP}]\} / \{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]\}, \text{ (by molar)}$$

3. Results and discussion

3-1. Seasonal fluctuations in phytoplankton biomass and dominant species beneath ice layer

The phytoplankton biomass as expressed by chl-*a* was generally lower in summer than other seasons from 1982 to 1988 (Fig. 1). A large phytoplankton biomass was observed in October and February or March in winter when the lake was covered with ice every year. The biomass reached more than 0.5 gchl-*a*·m⁻² under the ice in 1983 and 1988. In contrast, when phytoplankton biomass in October was in low level, the biomass was also in low level under ice in the next year (February 1986 and 1987).

Fig. 1. Seasonal variation in phytoplankton biomass expressed in terms of chlorophyll-*a* in Lake Akan. M: May in 1982-1984 and 1986-1988, and March and May in 1985. -----: average of the standing crop over 7 years.

In Lake Akan, the biomass of net-phytoplankton dominated rather than that of nano-phytoplankton, and it occupied over 80% of the total biomass (HINO, unpublished data). Table 2 shows the dominant species of net-phytoplankton beneath the ice layer in Lake Akan. The dominant diatoms were *Melosira italica*, *Asterionella gracillima*, and *Synedra acus*; and the dominant green algae were *Staurastrum dorsidentiferum*, *Dictyosphaerium pulchellum*, and *Eudorina elegans*. *D. pulchellum* was the dominant species in 1988. These phytoplankton species were also dominant in October in the previous year.

3-2. AEC values of phytoplankton beneath ice layer

Table 3 shows the AEC value of the particle fraction from 1.2 to 94 μm beneath the ice layer. Since phytoplankton was microscopically most dominant in this size fraction, the AEC values represented roughly the physiological state of phytoplankton. The mean AEC values ranged from 0.57 to 0.76 in the euphotic zone under ice, and these values were higher than those in the aphotic zone (ranged 0.45 to 0.56). This result indicated that the physiological state of phytoplankton in the euphotic zone was relatively higher.

3-3. Incubation experiments under low temperature and low intensity

The dominant species of phytoplankton were *A. gracillima*, *S. acus*, and *M. granulata* in the water sample collected on 22 October 1985. In Experiment 1, concentrations of ATP, adenylates, proteins, and sugars rapidly decreased during the first 6 days, and then decreased gradually for 130 days at 2°C under weak light (Fig. 2). The AEC value decreased from 0.83 to 0.74 during 6 days, and to 0.44 after 130 days. The ratio of sugars to proteins (Su/Pro ratio) also eventually fell to 0.38 after 130 days because of decrease of sugars.

In parallel with the drop in water temperature in Experiment 2, the concentration of adenylates, the value of AEC, and the Su/Pro ratio also fell rapidly (Fig. 3). Although the concentration of adenylates decreased gradually for 140 days after an initial 10-day rapid decrease, no decline in AEC value and Su/Pro ratio were observed except for the first 10 days, and the values remained at about 0.4 and 0.35, respectively.

The concentrations of adenylates, the value of AEC, and the Su/Pro ratio increased together with the incubation temperature after the 150th day. At the final stage, these levels exceeded the initial values of this experiment. Chl-*a*

Table 2. Dominant phytoplankton under the ice in Lake Akan.

	1983	1984	1985	1986	1987	1988
<Diatoms>						
<i>Melosira granulata</i>					+	
<i>M. italica</i>		+	+	+	+	+
<i>M. varians</i>						+
<i>Asterionella gracillima</i>			++	+	++	+
<i>Cocconeis</i> spp.	+	+	+			+
<i>Synedra acus</i>	++	tr				
<i>Stephanodiscus astraea</i>			tr			
<Green algae>						
<i>Staurastrum dorsidentiferum</i>	+	+				+
<i>Eudorina elegans</i>			+	+		tr
<i>Dictyosphaerium pulchellum</i>						++
<i>Pandorina</i> sp.						tr
<Blue green algae>						
<i>Anabaena circinalis</i>				tr		
<i>Phormidium</i> sp.						tr

+++ , very frequent; ++ , frequent; + , common; tr , rare

Fig. 2. Time course of changes in levels of adenylate, sugars, proteins, and adenylate energy charge under weak light at 2°C (Experiment 1).

was measured in the sample drawn at the beginning and the end of the incubation, when the concentrations were 18.1 and 21.5 $\mu\text{g}\cdot\text{l}^{-1}$, respectively. The dominant species were *A. gracillima*, *M. granulata*, and *Staurastrum dorsidentiferum* in the initial sample, while the final dominant phytoplankton species were the same as in the initial sample except for *S. dorsidentiferum*.

A large phytoplankton biomass was observed beneath the ice layer of Lake Akan (Fig. 1). Similar observations have been reported in Lake Haruna (SAIJO and SAKAMOTO, 1964; MAEDA and ICIMURA, 1973) and in Lake Suwa (SAIJO and

SAKAMATO, 1964; MASAKI and OKINO, 1984). In Lake Akan, the phytoplankton biomass under the ice should relate closely to its biomass during late autumn in the previous year (Fig. 1).

To support the large biomass, phytoplankton must grow under ice, or they must maintain an appropriate physiological state and survive under the ice after the increase in late autumn. In these cases, phytoplankton should be able to tolerate the low temperature and low light intensity. The recovery of the physiological state and biomass of phytoplankton at the end of Experiment 2 supports this idea although

Fig. 3. Time course of changes in levels of adenylates, organic matter, and adenylate energy charge under weak light at 2°C (Experiment 2). (—●—), adenylate; (---▲---), adenylate energy charge; (---▽---), ratio of sugars to proteins; (—■—), water temperature. Symbols (—●—) of adenylates are as follows: top line, ATP+ADP+AMP; middle line, ATP+ADP; bottom line, ATP.

cellular contents of chl-*a* and proteins likely increased with the lower light intensity.

The AEC values of particles ranged from 1.2 to 94 μm in diameter and mostly consisted of phytoplankton; they exceeded 0.4 in the euphotic zone and were higher than those in the aphotic zone of Lake Akan (Table 3). Since phytoplankton can photosynthesize at an AEC level over 0.35 (HINO, 1988 a), their values indicate that they can photosynthesize in the eu-

photic zone under the ice. Therefore, they should be able to maintain their physiological activities to survive beneath the ice layer of Lake Akan.

As phytoplankton can photosynthesize under the ice in Lake Akan, it may not only survive but also increase its biomass using the energy obtained by photosynthesis under the ice. While such growth was not evident in the present incubation experiments, the possibility

Table 3. Values of the adenylate energy charge (AEC) of phytoplankton fractions beneath the ice layer of Lake Akan.

	5 Mar. 1985	19 Feb. 1986	24 Feb. 1987
	Value of AEC		
Euphotic zone (0-10 m)	0.72-0.79	0.39-0.63	0.61-0.68
(mean)	(0.76)	(0.57)	(0.62)
Aphotic zone (15-35 m)	0.47-0.64	0.35-0.54	0.18-0.65
(mean)	(0.56)	(0.46)	(0.45)
Transparency (m)	5.9	5.5	4.5

should be studied further in ice-covered lakes and its laboratory models.

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摘 要

阿寒湖結氷下での植物プランクトン現存量と その生残

結氷湖沼である阿寒湖の植物プランクトン現存量の変化を調査した結果、結氷下の植物プランクトン現存量は夏期より高く、また一年を通じて最大値を示すことがあることが明らかとなった。この現象を支える要因の一つとして、秋期に増殖した植物プランクトンの結氷下での生残が考えられた。湖沼から得られた植物プランクトン試料の有光層とそれ以深での生理活性度の比較および、室内実験より得られた低温および低照度下での生理活性度や生物量の変化より、低温、低照度下では生物体に由来する有機物やアデニレート量の減少が緩慢に進み130日後にもアデニレートエネルギーチャージは0.45と光合成を行うことが可能な値であること、また水温を上昇させれば生物活性度も再び上昇するなど、結氷下でもその生理活性を十分に保持していることが明らかとなった。

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