Seasonal Change of Chlorophyll-*a* and Bacteriochlorophyll in Lake Fukami-ike

Akihiko YAGI, Isami SHIMODAIRA, Hisayoshi
TERAI and Yatsuka SAIJO

Abstract

Lake Fukami-ike is a small lake having a maximum depth of 8.5 m, and is well protected from the wind. Lake water stratified from March to October and circulated from November to March. Anoxic condition prevailed below 4-5 m depth from April to October. From 1956 to 1957, SHIMODAIRA found that the amount of chlorophyll-*a* in the euphotic layer was low during the stagnation period, and high during the circulation period. The same pattern of seasonal change in chlorophyll-*a* was observed in this study. It was suggested that such a seasonal change in chlorophyll-*a* amount might be caused by 1) the interruption of upwards nutrient transportation from the tropholytic layer due to a distinct stagnation of water and the consumption of nutrients by photosynthetic sulfur bacteria growing near the top of anoxic layer in summer, and 2) the sequential change of circulation and stagnation of water which gives a favorable condition for phytoplankton growth in winter.

1. Introduction

Lake Fukami-ike is a small lake in central Japan. The lake is located at the bottom of a basin and is well protected from the wind.

From 1956 to 1957, SHIMODAIRA (unpublished) studied the seasonal change of chlorophyll-*a* content, and found that the amount of chlorophyll-*a* in the euphotic layer was low during the stagnation period, from April to September, and high during the circulation period, from October to March.

This study was undertaken to extend our knowledge of the organic matter production in this lake, and especially to clarify the reason for such a seasonal change of chlorophyll.

2. Area Studied and Method

Lake Fukami-ike locates 484 m above sea level and has an area of 2.2 ha and a maximum depth of 8.5 m (Fig. 1). There is no inflowing stream in this lake, but it receives the runoff water from paddy fields, orchards and a certain amount of domestic sewage. The lake water is used as an

Fig. 1 Bathymetrical map of Lake Fukami-ike.

irrigation water for paddy fields, but the yearly change of water level is generally within ± 0.5 m. The observation was generally carried out two times every month from June 1979 till August 1980 and afterwards once every month at a station locating near the center of lake.

Water temperature was measured with a thermistor thermometer. For underwater light, a Li-Cor quanta meter was used. Water samples were taken with a vinyl tube and a hand pump from every 0.5 m or sometimes every 0.25 m. For nutrient

analysis and chlorophyll measurement, the sample water was filtered through Whatman GF/C glass-fiber filter immediately after the sampling and the filters and filtrates were stored at -20°C until chemical analysis in the laboratory. Dissolved oxygen was determined by the Winkler technique, the total carbon dioxide by SATAKE *et al.* (1972) with infrared CO_2 analyzer and the hydrogen sulfide by iodometry after precipitated with cadmium carbonate. Nitrate was measured by WOOD *et al.* (1967), nitrite after BENDSCHNEIDER and ROBINSON (1952) and ammonia by the method of SAGI (1966). Inorganic phosphate was determined by MURPHY and RILEY (1962). Chlorophyll-*a* was measured by the fluorometric method of HOLM-HANSEN *et al.* (1965). Relative values of bacteriochlorophyll were determined by measuring the difference of *in vivo* absorbance between 720 and 650 nm for the concentrated suspension of lake water with Nuclepore filter (pore size $0.4\ \mu\text{m}$). The absolute amount of bacteriochlorophyll-*c* and -*d* was determined by the method of TAKAHASHI and ICHIMURA (1968). However, this technique is not reliable when the sample contains chlorophyll-*a*. Therefore, to calculate the absolute value of bacteriochlorophyll from *in vivo* data, the sample which contains spectrophotometrically negligible amount of chlorophyll-*a* was determined with both techniques, and the conversion factor was obtained. Here, only the values of bacteriochlorophyll-*c* were cited to show the distribution of green sulfur bacteria, because the bacteriochlorophyll-*c* and -*d* gave same pattern of distribution by our technique. Although dominant species of green sulfur bacteria was not isolated as pure culture, morphologically very likely to *Prosthecochloris* by direct microscopic observation after BERGEY's Manual (1974) and GORLENKO (1970).

In situ gross production was determined by light and dark bottle oxygen method. The production was measured from the surface to 3 m depth. Carbon uptake was measured by ^{14}C technique of STEEMANN NIELSEN (1952).

3. Results

3-1. General features

1) Light: Secchi disk depth was deep (about 1 m) during the summer stagnation period and shallow (about 0.5 m) during the circulation period. One percent optical depths ranged from 2.5 to 3.2 m in summer and 1.8 to 2.5 m in other seasons.

2) Water temperature (Fig. 2): Lake water stratified from March to October forming a distinct thermocline between 3 and 5 m depth, and circulated from the end of November to March. In August, the temperatures of the surface and near the bottom were about 28° and 10° , respectively. On the other hand, in January the water temperature was about 4° to 6° throughout the water column. In cold winter, the lake was covered with thin ice from the end of January to early February.

3) Dissolved oxygen and hydrogen sulfide (Fig. 2): In this lake, the dissolved oxygen concentration near the surface often exceeded $14\ \text{mg}\cdot\text{l}^{-1}$ in spring and summer. The maximum value reached $17.7\ \text{mg}\cdot\text{l}^{-1}$ on 8 April 1979. On the other hand, in deeper layer, an anoxic condition prevailed from April to October. The top of the anoxic layer was about 4 to 5 m depth in summer.

It is noteworthy that in winter the dissolved oxygen concentrations often showed a slight decrease with depth, though the water temperature was almost constant throughout the water column. For instance, on 14 January 1979, the water temperature ranged 4.5° to 4.4° from the surface down to the bottom, but the dissolved oxygen concentrations at 0, 5 and 8 m depths were 12.40, 11.11 and $9.86\ \text{mg}\cdot\text{l}^{-1}$, respectively.

Seasonal change of hydrogen sulfide in 1979 was given in Fig. 2. The highest H_2S concentrations, $8\text{--}9\ \text{mg}\cdot\text{l}^{-1}$, were found in August. It is interesting to note that the hydrogen sulfide was often detected even above the upper limit of anoxic layer, as already described by SOROKIN (1970) and others.

3-2. Nutrient

Higher NO_3^- concentrations, ranging from

Fig. 2. Seasonal changes of water temperature, dissolved oxygen and hydrogen sulfide in Lake Fukami-ike. Sampling intervals were denoted at the topmost of each figure.

Fig. 3. Seasonal changes of nitrate, ammonia and phosphate in Lake Fukami-ike.

10 to 50 $\mu\text{g at. N}\cdot\text{l}^{-1}$, were generally observed from October to May, and lower ones of 1 $\mu\text{g at. N}\cdot\text{l}^{-1}$ or so were observed from August to early October (Fig. 3). High concentrations of NO_3^- , about 50 $\mu\text{g at. N}\cdot\text{l}^{-1}$, were observed from the end of June to early July, probably because of the runoff from the surrounding area in the rainy season. NO_2^- concentration was also relatively high (1 to 2 $\mu\text{g at. N}\cdot\text{l}^{-1}$) during the circulation period, and low (0.2 to 0.8 $\mu\text{g at. N}\cdot\text{l}^{-1}$) during the stagnation period. However, remarkably high values (3 to 5 $\mu\text{g at. N}\cdot\text{l}^{-1}$) were found above the anoxic layer in April and June. In the upper layer, NH_4^+ and PO_4^{3-} (Fig. 3) were generally high during the circulation period (2 to 10 $\mu\text{g at. N}\cdot\text{l}^{-1}$ and 0.5 to 3 $\mu\text{g at. P}\cdot\text{l}^{-1}$) and low during the stagnation period (below 1 $\mu\text{g at. N}\cdot\text{l}^{-1}$ and below 0.5 $\mu\text{g at. P}\cdot\text{l}^{-1}$). NH_4^+ and PO_4^{3-} in the anoxic layer reached 500 $\mu\text{g at. N}\cdot\text{l}^{-1}$ and 60–70 $\mu\text{g at. P}\cdot\text{l}^{-1}$, respectively.

3-3. Chlorophyll and production

Values of chlorophyll-*a* from 50 to 100 $\mu\text{g}\cdot\text{l}^{-1}$ or more were observed during the circulation period, whereas values of only 20 $\mu\text{g}\cdot\text{l}^{-1}$ or so were obtained in the upper layer during the stagnation period (Fig. 4).

If we compare the seasonal change of chlorophyll-*a* amounts in the water column from the surface down to 3 m depth during 1978–1979 with that during 1956–1957, there is no difference in seasonally changing pattern between both periods. However, the chlorophyll-*a* amounts in 1978–1979 were higher than in 1956–1957 (Fig. 5). The reason for such a difference would be the difference in the technique for preservation of the sample and the progress of eutrophication in this lake. In the case of earlier study, the filtered sample was stored at the room temperature rather for long time. From 1979 to 1980, chlorophyll-*a* values in the circulation period were considerably low except in December, though an exact comparison is difficult because of the difference in frequency of the observation.

Seasonal change of bacteriochlorophyll was shown in Fig. 6 as bacteriochlorophyll-*c*. The highest values of bacteriochlorophyll-*c*, about 140 and 120 $\mu\text{g}\cdot\text{l}^{-1}$, were found near the top of anoxic layer in summer of 1978 and 1979, respectively. Consequently, the major part of high chlorophyll-*a* values below the middle layer during the stratification period in Fig. 4 might be not the real chlorophyll-*a* but the bacteriochlorophyll. However, the correction of chloro-

Fig. 4. Seasonal change of chlorophyll-*a* in Lake Fukami-ike in summer stagnation period. The chlorophyll-*a* concentrations below 4 m depth included the bacteriochlorophyll.

phyll-*a* value in Fig. 4 was not attempted because of the low accuracy of *in vivo* technique employed for bacteriochlorophyll determination.

Results of gross production measurement by the oxygen technique, from the surface down to 3 m depth, were shown in Fig. 7 with the data obtained from 1956 to 1957. The period of *in situ* exposure in this study was different from that of 1957-1958.

In the case of 1956-1957, the exposure period was from noon to noon (24 hours), and in this study, from 11 a.m. to 3 p.m. (4 hours). If we consider the difference in exposing time, the daily gross production in 1978-1980 might be about two times that of 1956-1957. This suggests the progress of eutrophication in this lake. In spite of above difference in values, there was no marked difference in the patterns of seasonal change in gross production between both periods.

To clarify the significance of photosynthetic sulfur bacteria in the photosynthetic production in this lake, $^{14}\text{CO}_2$ uptake was determined. Results of *in situ* $^{14}\text{CO}_2$ uptake measurement on 29 July 1979, were shown in Fig. 8. A high carbon uptake by phytoplankton ($1.6 \text{ mg C} \cdot \text{l}^{-1} \cdot \text{day}^{-1}$) was observed at the surface, but the carbon uptake by green sulfur bacteria was very

Fig. 5. Seasonal changes of chlorophyll-*a* amount in the water column from surface to 3 m depth in 1956-1957 and in 1978-1980.

Fig. 6. Seasonal changes of bacteriochlorophyll-*c* in Lake Fukami-ike.

Fig. 7. Seasonal changes of gross production in the water column from surface to 3 m depth in 1956-1957 and in 1978-1980.

low ($0.04 \text{ mg C} \cdot \text{l}^{-1}$ at 4 m depth), in spite of its high standing crop. In addition to above experiment, the dependency of bacterial photosynthetic activity to the light intensity was determined by exposing the sample water of 4.8 m depth, where the bacteriochlorophyll concentration was the highest, at 0, 2, 4, 4.5, 4.75, 5, 6 and 8 m depths after adding ^{14}C . As clear from this figure green sulfur bacteria showed the highest carbon uptake ($0.33 \text{ mg C} \cdot \text{l}^{-1} \cdot \text{day}^{-1}$) at the surface. Considering the results of this experiment, on 24 September 1979, the sample waters taken from 0, 1.5, 3, 3.5, 4, 4.5, 4.75 and 5.5 m depths were exposed at 3.5 m depth, the layer of 3% optical depth, from sunrise to sunset (Fig. 9). In this case, the maximum carbon uptake rates in the light and in the dark were obtained for the sample of 5.0 m where the bacterio-

chlorophyll concentration was the highest.

4. Discussion

The occurrence of high chlorophyll-*a* content in this lake during the circulation period from late autumn to early spring was reconfirmed by this observation, especially from 1978 to 1979. The first reason for such a high standing crop of phytoplankton would be the abundance of the nutrients in lake water during that period. However, even the amount of nutrients is enough for the phytoplankton growth, if the vertical mixing of water continues, the net production will become very low or negative, because the depth of mixing will exceed the depth of the euphotic layer (2 m or so), and the total amount of respiration in the water column will become equal or exceed the amount of photosynthesis

Fig. 8. Vertical profile of *in situ* photosynthetic activity on 29 July 1979.

- (a) Water temperature (●), D.O. (○) and H₂S (▲).
 (b) Chl. *a* (○) and B-Chl. *c* (●).
 (c) *In situ* ¹⁴CO₂-uptake during 3 hr incubation in light bottle (○), in dark bottle (●) and light dependency of ¹⁴CO₂ uptake (3 hr incubation in light bottle) with the sample water taken at 4.8 m depth (□).

Fig. 9. Comparison of photosynthetic activity at lower light intensity (3.5 m depth) on 24 September 1979.

- (a) Water temperature (●), D.O. (○) and H₂S (▲).
 (b) Chl. *a* (○) and B-Chl. *c* (●).
 (c) Relative light intensity (△) and comparison of ¹⁴CO₂-uptake in dim light (incubated at 3.5 m depth from sunrise to sunset) among the water sample taken from indicated depths; (○) light bottle, (●) dark bottle.

as discussed by SVERDRUP (1953) and others as the concept of critical depth. This lake, however, is well protected from the wind. Thus, the water occasionally circulates down to the bottom under a rather strong wind, but after that the lake water stagnates

during a certain period though the water temperature is almost constant throughout the water column. A slight decrease of dissolved oxygen concentration with depth on 14 January 1979 (see page 284) suggests such a temporal stagnation of water. It is

probable that during the temporal stagnation period, a positive production of phytoplankton takes place using sufficient nutrients in upper layer.

This second reason, the temporal water stagnation, would be the major factor causing such a high standing crop of phytoplankton in winter in this lake. Change of chlorophyll-*a* content in winter, as shown in Fig. 5, also seems to suggest such a mechanism. In winter, the primary production was comparable with that of summer though the standing crop of phytoplankton was several times as high. This high standing crop might be produced or maintained by the low rates of respiration and/or of decomposition under the low water temperature. In the summer stagnation period, the amount of chlorophyll-*a* would be limited by the nutrients, especially by the phosphate, because no phytoplankton blooming was observed in summers 1978 and 1979, though considerably high nitrate concentrations, 20 to 50 $\mu\text{gat. N}\cdot\text{l}^{-1}$, were determined in the upper layer from end of June to July in both year. The reason of such a low concentration of nutrients in the upper layer during the summer stagnation period would be caused by a very stable stratification of lake water and the consumption of nutrients by the photosynthetic sulfur bacteria which grew extensively at the boundary layer of the oxic and anoxic layer. These two factors seem strongly prevent the supply of nutrients from the lower anoxic layer to the upper oxic layer.

There are many informations describing the significant contribution of photosynthetic bacteria in the carbon uptake in lakes (SOROKIN, 1970; TAKAHASHI and ICHIMURA, 1968; MATSUYAMA and SAIJO, 1971). In this lake, however, the *in situ* photosynthetic activity of photosynthetic bacteria was very low compared with that of phytoplankton, in spite of a large population of green sulfur bacteria. This might be caused by the insufficiency of light at the dense layer of green sulfur bacteria when the *in situ* photosynthesis was determined, while con-

siderably high photosynthetic activities were obtained when the deep sample waters (from 4.5 and 5 m depths) containing high amounts of bacteriochlorophyll were exposed to a little stronger light (at 3.5 m depth) as shown by the experiment on 24 September 1979. Consequently, it can be surmised that an accumulation of green sulfur bacteria took place in early summer when the underwater light condition was more favorable for the photosynthesis, *i.e.* under a little more transparent condition of lake water.

Acknowledgements

We would like express our thanks to Town authorities of Anan-cho, Shimoina-gun, Nagano Prefecture for providing the facilities to our research.

This study was supported in part by a Grant-in-Aid for Co-operative Research (No. 438034) from the Ministry of Education, Science and Culture.

摘 要

深見池は長野県下伊那郡阿南町にある最大深度 8.5 m の小池で、凹地に位置しているために風の影響を受けにくい。湖水は 3 月から 10 月まで成層し、11 月から 3 月まで循環する。4 月から 10 月まで 4-5 m 以深は無酸素状態になる。下平は 1956-1957 年に生産層のクロロフィル *a* 量が、成層期に低く循環期に高いことを見出した。同様のクロロフィル *a* 量の季節変化は本研究においても観察された。このようなクロロフィル *a* 量の季節変化は、夏季における顕著な成層と無酸素層上部付近に多量に生育している光合成硫黄細菌による栄養塩の消費のための上層への栄養塩の供給の阻害、ならびに冬季に湖水の循環と停滞が交互にくりかえされることにより、植物プランクトンの増殖に好都合な条件が与えられるためと考えられる。

References

- BENDSCHNEIDER, K. and R. J. ROBINSON (1952) : A new spectrophotometric method for determination of nitrite in sea water. *J. Mar. Res.*, 11: 87-96.
- BUCHANAN, R. E. and N. E. GIBBONS (Eds.) (1974) : *Bergey's Manual of Determinative Bacteriology*, 8th ed., p 55, Williams & Wilkins Co., Baltimore.
- GORLENKO, V. M. (1970) : A new phototrophic green sulphur bacterium *Prosthecochloris aestuarii* nov. gen. nov. spec. *Z. Allg. Mikrobiol.*, 10: 147-149.

- HOLM-HANSEN, O., C. J. LORENZEN, R. W. HOLMS and J. D. H. STRICKLAND (1965) : Fluorometric determination of chlorophyll. *J. Conseil. Perm. Intern. Explor. Mer*, 30: 3-15.
- MATSUYAMA, M. and S. SAIJO (1971) : Studies on biological metabolism in meromictic Lake Suigetsu. *J. Oceanogr. Soc. Jap.*, 27: 197-206.
- MURPHY, J. and J. P. RILEY (1962) : A modified single solution method for the determination of phosphate in natural waters. *Anal. Chem. Acta*, 27: 31-36.
- SAGI, T. (1966) : Determination of ammonia in sea water by the indophenol method and its application to the coastal and off-shore waters. *Oceanogr. Mag.*, 18: 43-51.
- SATAKE, K., Y. SAIJO and H. TOMINAGA (1972) : Determination of small quantities of carbon dioxide in natural waters. *Jap. J. Limnol.*, 33: 16-36.
- SOROKIN, YU. I. (1970) : Interrelations between sulphur and carbon turnover in meromictic lakes. *Arch. Hydrobiol.*, 66: 391-446.
- STEEMANN NIELSEN, E. (1952) : The use of radioactive carbon (^{14}C) for measuring organic production in the sea. *J. Conseil. Intern. Explor. Mer*, 18: 117-140.
- SVERDRUP, H. U. (1953) : On conditions for the vernal blooming of phytoplankton. *J. Conseil. Intern. Explor. Mer*, 18: 287-295.
- TAKAHASHI, M. and S. ICHIMURA (1968) : Vertical distribution and organic matter production of photosynthetic sulfur bacteria in Japanese lakes. *Limnol. Oceanogr.*, 13: 644-655.
- WOOD, E. D., F. A. J. ARMSTRONG and F. A. RICHARDS (1967) : Determination of nitrate in sea water by cadmium-copper reduction to nitrite. *J. Mar. Biol. Ass. U. K.*, 47: 23-31.
- (著者：八木明彦, 467 名古屋市瑞穂区, 名古屋女子大学家政学部；下平勇, 395-06 長野県下伊那郡平谷村, 平谷小中学校；寺井久慈, 西條八束, 464 名古屋市千種区, 名古屋大学水圏科学研究所；Akihiko YAGI, Faculty of Home Economics, Nagoya Women's University, Mizuho-ku, Nagoya 467；Isami SHIMODAIRA, Hiraya Junior High School, Hirayamura, Shimo-Ina-Gun, Nagano Prefecture 395-06；Hisayoshi TERAI and Yatsuka SAIJO, Water Research Institute, Nagoya University, Chikusaku, Nagoya 464)

Accepted: 11 April 1983