The effects of photoirradiation on changes in the fluorescence intensities, dissolved organic carbon (DOC) concentrations, and molecular weights of dissolved organic matter (DOM) in Lake Biwa and its surrounding rivers were evaluated and compared with the results of humic substances and algal DOM. During the completely stratified period (summer), the fluorescence intensities of fulvic-like fluorophores in the surface water in the northern basin of Lake Biwa decreased and were lower than those in other months and in bottom water. The fluorescence quenching and degradation of high-molecular substances by further solar irradiation were hardly observed in the surface water samples but were significantly observed in bottom water samples. On the other hand, changes in the DOC concentrations in all samples were relatively small with solar irradiation. These results suggest that in the northern basin of Lake Biwa, the susceptibility of fulvic-like fluorophores to degradation by further solar irradiation is dependent on the water depth collected during the stratified period (summer), but the rest of fulvic-like fluorophores might be resistant to further photochemical degradation regardless of the water depth. Furthermore, the effects of the wavelength region on the characteristics of DOM and fluorophores in Lake Biwa and its surrounding rivers were examined by Xe lamp irradiation using two kinds of wavelength cut filters. From these results, it is considered that wavelengths between 290 and 495 nm and below 290 nm might largely affect the characteristics of fulvic-like fluorophores and protein-like fluorophores, respectively.

Keywords: photoirradiation, wavelength cut filter, photodegradation, dissolved organic matter, fulvic-like fluorophore, protein-like fluorophore, environmental waters

1. Introduction

Dissolved organic matter (DOM) is the main component of the organic substances in natural water. Hence, DOM dynamics and characteristics can affect multiple biogeochemical processes in aquatic environments, including light penetration, pH buffering, oxygen consumption, nutrient availability, and toxicity of pesticides and metals. Many studies have evaluated the characteristics and sources of DOM in Lake Biwa using chemical fractionation methods, the natural carbon stable isotope ratio, and spectroscopic analyses. From our results of the fractionation of DOM in the northern basin of Lake Biwa collected from 2002 to 2004, hydrophobic acids (humic substances: HS), hydrophobic neutral DOM, and hydrophilic DOM were estimated to be about 20–25%, 15–20%, and 55–65%, respectively. The dominant fractions of HS were fulvic acids (FA), and some hydrophobic DOM and hydrophilic DOM may be produced by phytoplankton. The fulvic-like fluorophores (Ex/Em = 320-350/430-450 nm, 240-260/430-450 nm) and a protein-like fluorophore (Ex/Em = 280-290/320-330 nm) were always detected in Lake Biwa using a three-dimensional excitation-emission matrix (3-DEEM). The dynamics of DOM and fluorophores in Lake Biwa were evaluated, and it was found that the fluorescence intensities of fulvic-like fluorophores in surface water during the completely stratified period (July-September) tend to be lower than those during other months and in bottom water, even though DOC concentrations in surface water tend to be higher than those of others due to high primary production. These phenomena might be due to the photolysis of fulvic-like fluorophores in surface water during the stratified period. It has been reported that the fluorescence of DOM in the lake has sources other than epilimnetic primary production, and it is degraded by solar radiation in the epilimnion during summer. Furthermore, seasonal changes in the UV absorbance of lake water were different from those of DOC. The clearly positive correlation between UV absorbance and HS was observed in an inner bay (Akanoi); however, the correlation in offshore water was weaker than in the inner bay. Therefore, it is necessary to analyze the behavior and photoirradiation effects of both allochthonous and autochthonous DOM separately. Many studies of the photochemical degradation of DOM and aquatic HS have been performed. However, few studies about the effects of photoirradiation on the characteristics of algal DOM have been conducted.

In the present paper, the effects of photoirradiation...
(solar and Xe-lamp irradiation) on changes in the fluorescence intensities, DOC concentrations, and molecular weights of DOM in Lake Biwa and its surrounding rivers were evaluated and compared with the results of soil humic acid (HA), FA, lake FA, and algal DOM. Furthermore, influences of wavelength regions on the characteristics of DOM were examined by Xe-lamp irradiation, using two kinds of wavelength cut filters.

2. Materials and methods

2.1 Reagents and apparatus

Aldrich HA (extracted from peat soil: Aldrich Chemicals), Dando HA and FA (Dystric Cambisol, Dando, Aichi, Japan),20) were used as soil HS. Aldrich HA was purified in accordance with the procedures of a previous paper.13) Dando HA and FA, and Lake Biwa FA (LBFA)21) were supplied by the Japan Humic Substances Society (JHSS) and used without further purification.

Three kinds of phytoplankton—Microcystis aeruginosa (blue-green algae NIES-109, Lake Yogo, Shiga), Staurastrum dorsidentiferum (green algae NIES-665, Lake Biwa, Shiga), and Cryptomonas ovata (dark brown whip-hair algae NIES-275, Tsuchiura, Ibaraki)—were supplied by the National Institute for Environmental Studies, were cultivated in accordance with the procedures of the previous paper.13)14) These phytoplankton were selected as the predominant algal species in Lake Biwa.14) All other chemicals were of the best commercial grade. Pure water was prepared by a Millipore Milli-Q water purification system.

Three incubators—an Iwaki LIB-302, an Iwaki ICB-142L, and an NIK LH-100SP—were used to cultivate and biodegrade plankton. A TOMY BS-305 autoclave was used for sterilization. An Olympus IX71N-22PH-D microscope was used to count the number of algal cells. A TOC meter (Shimadzu TOC-V CSH) was used for the determination of DOC concentration. A Kubota KN-70 centrifuge and a Hitachi Koki Himac CR20G II refrigerated centrifuge were used for the fractionation and concentration of algal DOM, respectively. A Horiba F-51 pH meter and a TOA CM-60S EC meter were used for the measurement of pH and electric conductivity in environmental water samples, respectively.

2.2 Procedure for the characterization of DOM and humic substances in environmental water samples

Environmental water samples were collected from Lake Biwa and its surrounding rivers (Fig. 1). The water samples of Lake Biwa were monthly collected at Imazu in the northern basin (St. 17B, 35°23'41N, 136°07'57E) using a Van Dorn water sampler. Water temperature and other basic parameters were measured by a Hydrolab DS5 water quality instrument. River water samples were collected from the Katsura, Kizu, and Yodo Rivers.11)12) In the laboratory, all water samples were filtered through a membrane filter (0.45 μm, Millipore) to avoid biodegradation, stored in a refrigerator and used in the experiment as soon as possible. Membrane filters were used after washing with 1 M hydrochloric acid and distilled water. Dissolved organic substances were analyzed by using gel chromatography with a fluorescence detector that was developed to simultaneously determine the concentration and molecular weight of humic substances.22) The apparatus used for gel chromatography was a Shimadzu LC-20AD chromatography pump equipped with a Shimadzu RF-20A XS fluorescence detector and a Shimadzu SPD-20AV UV-VIS detector. The test samples (100 μL) were applied to a gel filtration column, Superox HR10/30 (300 x 10 mm i.d.; GE Healthcare), and a Shimadzu C-R7A Chromatopac or a Shimadzu LC solution was used for data analysis. A 0.01 M sodium hydroxide solution was used as an eluent at a flow rate of 0.4 ml min⁻¹. The fluorescence properties of DOM were measured with three-dimensional excitation-emission matrix (3-DEEM), using a Shimadzu RF-5300PC fluorescence spectrophotometer, as previously reported.13)14) Fluorescence readings were normalized by fluorescence intensity (Ex=345 nm/Em=450 nm) of 10 μg/l quinine sulfate (0.05 M H₂SO₄ solution) 10 QSU. The values were treated as relative fluorescence intensity (RFI).

2.3 Cultivation of phytoplankton13)14)

The three kinds of phytoplankton were cultivated in an improved VT medium as previously reported.13)14) Microcystis aeruginosa and S. dorsidentiferum were grown in one-liter (1 L) flasks at 20°C and 2000 lux under a 12 h : 12 h light/dark cycle. Cryptomonas ovata was grown in a 1 L triangle flask at 15°C and 2000 lux under the same light/dark cycle.

2.4 Photoirradiation of HS, algal DOM, and DOM in environmental water by sun and a Xe lamp

A 40 ml water sample (HS, algal DOM or environmental water) adjusted to pH 8 with NaOH in a 50 ml quartz glass container (60 mm high with a diameter of 40 mm,
the quartz glass about 2mm thick) was irradiated inside darkness-controlled chamber at 25°C using a Ushio Xe lamp (150 W). The distance between a quartz glass container and the Xe-lamp was fixed to 10 cm. As quartz glass transmits nearly 100% of light in the ultraviolet-visible region, quartz glass containers were used for photoirradiation. The solutions (2.0-2.5 mg/L) of HS such as Aldrich HA, Dando HA, Dando FA, and LBFA were prepared. Algal DOM solutions were prepared from the medium of three kinds of phytoplankton by filtration through a membrane filter (0.45 μm, Millipore). Time changes in the fluorescence properties (3-DEEM) and DOC of these sample solutions were measured after 1, 3 and 5 h by Xe lamp irradiation. Furthermore, the effects of the wavelength region on the characteristics of DOM in these samples were examined by Xe lamp irradiation for 5 h using two kinds of wavelength cut filters, AGC Techno glass UV-29 (cut below 290 nm), or HOYA W-Y 495 (cut below 495 nm). Time changes in the fluorescence properties (3-DEEM) and DOC concentrations of the same samples in a 50 ml quartz glass container were measured after 1, 3 and 5 h by solar radiation on the roof of the No. 6 building (Kyoto Institute of Technology). The amounts of solar radiation were measured with an illuminometer (Delta OHM PYRA03) during the irradiation experiment.

3. Results and discussion

3.1 Behavior of DOM and fluorophores in Lake Biwa during stratified and circulation periods

Vertical distributions in the water temperature and concentrations of DOC at Imazu (St. 17B) in Lake Biwa during the completely stratified period (July–September) and the circulation period (January–March) in 2015 and 2016 are shown in Fig. 2 (a) and (b), respectively. During the stratified period, when the thermocline was formed in Lake Biwa, the DOC concentrations at a water depth of 0.5 m were 1.27–1.36 mgC/l and tended to be higher than those at a water depth of 90 m (ca. 1.0 mg/L). The DOC concentrations during the circulation period were about 1.0 mgC/l regardless of the water depth, and their seasonal changes were relatively small.

In the water samples of Lake Biwa, two fulvic-like fluorescence peaks, peak A (Ex/Em = 320-350/430-450 nm) and peak B (Ex/Em = 240-260/430-450 nm), and a protein-like fluorescence peak, peak C (Ex/Em = 280-290/320-330 nm) were observed by 3-DEEM. As an example, the 3-DEEM contour plot of surface water at St. 17B in Lake Biwa (December 2015) is shown in Fig. 3. The vertical distributions in the RFI values of peak A at St. 17B during the circulation period (January–March) and the completely stratified period (July–September) in 2015 and 2016 are shown in Fig. 4 (a) and (b), respectively. The RFI values of peak A of surface waters during the stratified periods in 2015 and 2016 were 1.81–2.41 QSU and 2.05–2.55 QSU, respectively, which were lower than those of bottom waters. Furthermore, vertical changes in the gel chromatograms of fulvic-like DOM (peak A, Ex/Em = 340/435 nm) in Lake Biwa collected in February and July, 2016, are shown in Fig. 5 (a) and (b), respectively. Four peaks, peak h (RT = 16 min), peak 1 (RT = 29–30 min), peak 2 (RT = 32 min), and peak 3 (RT = 35 min) were detected. It has been reported that the peaks 1 and 2 correspond to the peaks of soil FA, and the peaks 2 and 3 correspond to the peaks of algal DOM and degradation of FA. The fluorescence values (peak area) of these peaks are listed in Table 1. The
Fig. 4  Vertical distributions of the fluorescent intensities of peak A at Imazu (St. 17B) in the northern basin of Lake Biwa in 2015 (a) and 2016 (b) circulation period: ○ January, △ February, □ March stratified period: ● July, ▲ August, ■ September

Fig. 5  Vertical changes in the gel chromatograms of fulvic-like DOM (peak A) in Lake Biwa (Ex/Em = 340/435 nm) (a) February 2016, (b) July 2016 water depth: I 0.5 m, II 10 m, III 20 m, IV 80 m

Table 1  Vertical changes in the fluorescence of peaks h, 1, 2 and 3 in the gel chromatograms of fulvic-like DOM in Lake Biwa (Ex/Em=340/435 nm)

<table>
<thead>
<tr>
<th>Water depth (m)</th>
<th>Fluorescence</th>
<th>Fluorescence</th>
<th>Fluorescence</th>
<th>Fluorescence</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>peak h</td>
<td>peak 1</td>
<td>peak 2</td>
<td>peak 3</td>
</tr>
<tr>
<td></td>
<td>(RT=16 min)</td>
<td>(RT=29-30 min)</td>
<td>(RT=32 min)</td>
<td>(RT=35 min)</td>
</tr>
<tr>
<td>February 2016</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>17.0</td>
<td>89.1</td>
<td>101.2</td>
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</tr>
<tr>
<td>10</td>
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<td>20</td>
<td>29.1</td>
<td>89.3</td>
<td>102.1</td>
<td>139.9</td>
</tr>
<tr>
<td>80</td>
<td>29.0</td>
<td>94.5</td>
<td>102.6</td>
<td>136.0</td>
</tr>
<tr>
<td>July 2016</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>10.4</td>
<td>58.9</td>
<td>87.7</td>
<td>108.4</td>
</tr>
<tr>
<td>10</td>
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<tr>
<td>20</td>
<td>17.3</td>
<td>81.0</td>
<td>103.9</td>
<td>158.4</td>
</tr>
<tr>
<td>80</td>
<td>26.3</td>
<td>131.9</td>
<td>142.4</td>
<td>167.7</td>
</tr>
</tbody>
</table>
molecular distributions were similar in February regardless of the water depth, while in July, the fluorescence intensities of four peaks at a water depth of 0.5 m were lower than those at other water depths. The fluorescence intensities of peaks 1 and 2 at 80 m were higher than those at 0.5-20 m. It was reported that the RFI and RFI/DOC values of peaks A and B of the bottom waters, which were higher than those of the surface waters during the stratified period, might be due to the elution of HS from the sediment.\textsuperscript{26} Then, the dynamic analysis of chemical components in sediment cores and bottom water samples collected at St. 17B in Lake Biwa was carried out under incubation experiments, and the results show that fulvic-like fluorescence DOM in bottom water may be regularly released from lake sediment.\textsuperscript{27} On the other hand, the fluorescence quenching and degradation of high-molecular fulvic-like fluorophores in surface water might occur by solar irradiation. Hayakawa et al. reported that 1% attenuation of PAR (photosynthetically active radiation) and ultraviolet radiation (340nm) was at water depth of 16.1 m and 4.6 m in Lake Biwa (St. 17B), respectively.\textsuperscript{28} Therefore, changes in the fluorescence properties, DOC concentrations, and molecular weights of HS and algal DOM by light irradiation were investigated because HS and algal DOM are known to be the principal DOM in Lake Biwa.

3.2 Effects of photoirradiation on the characteristics of HS and algal DOM

Two fluorescence maxima were observed in the Aldrich HA and Dando HA by 3-DEEM: one at Ex/Em values of 460/510 nm (peak H1), and the other at Ex/Em values of 260/480 nm (peak H2).\textsuperscript{29} Meanwhile, two fluorescence maxima were observed in the Dando FA and LBFA: one at Ex/Em values of 320/440 nm (peak A), and the other at Ex/Em values of 260/450 nm (peak B). The effects of photoirradiation on the fluorescence properties of humic acids (Aldrich HA, Dando HA) and fulvic acids (Dando FA, LBFA) were examined. Time changes in the fluorescent intensities (I/I\textsubscript{0}) of peaks H1 and H2 of Aldrich HA and Dando HA during photoirradiation by the sun and a Xe-lamp are shown in Fig. 6 (a) and (b), respectively. During 5 h of solar radiation, the I/I\textsubscript{0} values of peaks H1 and H2 of Aldrich HA decreased down to 0.376 and 0.609, and those of Dando HA decreased down to 0.459 and 0.685 by solar radiation, respectively. After 5 h of photoirradiation by a Xe-lamp, the I/I\textsubscript{0} values of peaks H1 and H2 of Aldrich HA decreased down to 0.615 and 0.762, and those of Dando HA decreased down to 0.755 and 0.894, respectively. These results suggest that the fluorescence of Aldrich HA and Dando HA may become extinct during photoirradiation, and their fluorescence quenching by solar irradiation may be larger than that by Xe-lamp irradiation.

Time changes in the fluorescence intensity (I/I\textsubscript{0}) values of peaks A and B of Dando FA and LBFA during photoirradiation by the sun and a Xe-lamp are shown in Fig. 7 (a) and (b), respectively. After 5 h of solar radiation, the I/I\textsubscript{0} values of peaks A and B of Dando FA decreased down to 0.487 and 0.571, and those of LBFA decreased down to 0.511 and 0.563, respectively. After 5 h of Xe-lamp irradiation, the fluorescent intensities of peaks A and B of Dando FA decreased down to 0.487 and 0.571, and those of LBFA decreased down to 0.511 and 0.563, respectively. After 5 h of Xe-lamp irradiation, the fluorescent intensities of peaks A and B of Dando FA decreased down to 0.487 and 0.571, and those of LBFA decreased down to 0.511 and 0.563, respectively. After 5 h of Xe-lamp irradiation.
radiation, the $I/I_0$ values of peaks A and B of Dando FA decreased down to 0.819 and 0.833, and those of LBFA decreased down to 0.743 and 0.747, respectively. The decreasing trends of FA fluorescence during solar irradiation were also larger than those during Xe-lamp irradiation, which matched the HA results. The results indicate that the fluorescence of HA and FA may become extinct during photoirradiation, and their fluorescence quenching by solar irradiation may be larger than that by Xe-lamp irradiation.

Three fluorescence maxima were observed in the cultivation of three kinds of phytoplankton: two fulvic-like fluorescence peaks (A and B), and a protein-like fluorescence peak (C). Photoirradiation effects by the sun and a Xe-lamp on the time changes in the fluorescent intensities ($I/I_0$) of peaks A, B, and C of algal DOM from $M. aeruginosa$, $S. dorsidentiferum$, and $C. ovata$ were examined, and the results are shown in Fig. 8 (a), (b), and (c), respectively. The $I/I_0$ values of fulvic-like fluorophores in $M. aeruginosa$ after 5 h of solar and Xe-lamp irradiation decreased down to 0.249 and 0.289 for peak A and 0.501 and 0.513 for peak B, respectively, while those in $S. dorsidentiferum$ decreased down to 0.255 and 0.286 for peak A and 0.463 and 0.488 for peak B, respectively. In the case of $M. aeruginosa$ and $S. dorsidentiferum$, the decreasing trends of fluorescence for peak A were larger than those for peak B; however, the difference in the photoirradiation effect (sun and Xe-lamp) on the fluorescence of peaks A and B was confirmed to be small. Meanwhile, in the case of $C. ovata$, the $I/I_0$ values of peaks A and B after 5 h of solar irradiation decreased down to 0.39 and 0.532, and those after 5 h of Xe-lamp irradiation decreased down to 0.783 and 0.933, respectively. The decreasing fluorescence trends of peaks A and B in $C. ovata$ during solar irradiation were considerably larger than those during Xe-lamp irradiation, which were different from those in $M. aeruginosa$ and $S. dorsidentiferum$.

Meanwhile, the $I/I_0$ values of protein-like fluorophores (peak C) in $M. aeruginosa$ after solar and Xe-lamp irradiation for 5 h decreased down to 0.813 and 0.448, respectively, and those of peak C in $S. dorsidentiferum$ decreased down to 0.563 and 0.379, respectively. The $I/I_0$ values of peak C in $C. ovata$ after 5 h of solar and Xe-lamp irradiation decreased down to 0.532 and 0.265, respectively. During Xe-lamp irradiation, the fluorescence quenching of protein-like fluorophores (peak C) in $C. ovata$ was largest, followed in order by $S. dorsidentiferum$ and $M. aeruginosa$, while the fluorescence quenching of fulvic-like fluorophores (peaks A and B) in $C. ovata$ was small. These results suggest that the effects of ultraviolet radiation on the fluorescence quenching of protein-like fluorophores (peak C) may be large because the relative intensities (<400 nm) of a Xe-lamp light are larger than those of solar light.22

Furthermore, the effects of photoirradiation on the DOC concentrations of HS (Dando HA, Dando FA, and LBFA) and algal DOM released from $M. aeruginosa$ were investigated, and the results are listed in Table 2. The DOC concentrations of these HS after photoirradiation for 5 h decreased by 2–10%, while that of algal DOM released from $M. aeruginosa$ decreased by 1–3%. The decrease in the DOC concentrations of these substances by photoirradiation was relatively small.

Next, the effects of photoirradiation on the molecular weight distributions of HS and algal DOM were investigated by gel chromatography with a fluorescence detector (Ex/Em = 340/435 nm or Ex/Em = 280/320 nm). The results of Dando FA and algal DOM from $M. aeruginosa$ are shown in Fig. 9. In the case of Dando FA, the peak (RT = 29 min) decreased, and the peaks (RT = 30, 32, and 35 min) increased after Xe-lamp irradiation. It is believed that low-molecular substances may be produced from high-molecular substances of FA. Furthermore, in the case of algal DOM from $M. aeruginosa$, the peaks (RT = 35, 39 min) of fulvic-like fluorophores (peak A) were larger than the peaks (RT = 29–30, 32 min) observed in soil FA, and all peaks decreased
after photoirradiation. Meanwhile, in the case of protein-like fluorophores (peak C), a large peak (RT = 16–17 min) and several peaks (RT = 23–25, 30, 35, 40, and 47 min) were observed, and the peak (RT = 16–17 min) considerably decreased after photoirradiation. These results suggest that light irradiation may influence both fluorescence quenching and the degrading of high-molecular substances of HS and algal DOM.

### 3.3 Effects of photoirradiation on the characteristics of DOM in Lake Biwa and its surrounding rivers

The effects of photoirradiation on the DOC concentrations, fluorescence properties, and molecular weight distributions of DOM in Lake Biwa and its surrounding rivers were investigated. Figure 10 shows the monthly changes in the RFI values of fulvic-like fluorophores (peak A) in Lake Biwa (St. 17B) before and after solar irradiation, respectively. The RFI values of peak A at a water depth of 0.5 m during August–October 2015 and June–August 2016 were especially low as compared to those in other months and at a water depth of 80 m.

The RFI values of peak A at 0.5 m during August–October 2015 and June–August 2016 were virtually unchanged (3-12%) after solar irradiation; however, they declined by 30–45% in other months. Meanwhile, the RFI values of peak A at 80 m were larger than those at 0.5 m and

### Table 2 Effects of photoirradiation on the DOC concentrations of Dando HA, Dando FA, LBFA, and algal DOM from *M. aeruginosa*

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Solar irradiation</th>
<th></th>
<th>Xe-lamp irradiation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DHA</td>
<td>DFA</td>
<td>LBFA</td>
<td>M</td>
</tr>
<tr>
<td>0</td>
<td>1.09</td>
<td>1.15</td>
<td>1.44</td>
<td>37.9</td>
</tr>
<tr>
<td>1</td>
<td>1.05</td>
<td>1.16</td>
<td>1.40</td>
<td>38.2</td>
</tr>
<tr>
<td>3</td>
<td>1.04</td>
<td>1.12</td>
<td>1.38</td>
<td>37.7</td>
</tr>
<tr>
<td>5</td>
<td>1.07</td>
<td>1.04</td>
<td>1.34</td>
<td>37.5</td>
</tr>
</tbody>
</table>

DHA: Dando humic acid, DFA: Dando fulvic acid, LBFA: fulvic acid of Lake Biwa M: algal DOM from *M. aeruginosa* during cultivation for 26 days

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**Fig. 9** Effects of Xe-lamp irradiation on the molecular weight distributions of Dando FA and algal DOM from *M. aeruginosa* (M-1 and M-2) by gel chromatography with a fluorescence detector (Ex/Em = 340/435 nm for Dando FA and M-1, Ex/Em = 280/320 nm for M-2)

■ before irradiation, □ after irradiation
results of fulvic-like fluorophores in river waters were similar to the results of Dando FA and LBFA (Fig. 7), but they were different from the results of peak A of algal DOM released from three kinds of phytoplankton (Fig. 8). These results were consistent with the results that 30-60% of DOM in these rivers were fulvic acids by fractionation analysis.31 On the other hand, the RFI values of peak C in rivers during Xe-lamp irradiation declined by 60-65%, while those during solar irradiation declined by 14-40% (Table 3). The changes in the DOC concentrations in rivers by light irradiation were also small (3-9%). The photodegradation of DOM in river waters in the Lake Biwa watershed has demonstrated that fulvic-like fluorescence DOM is more susceptible to photodegradation than those of protein-like substances and DOC31; this is consistent with our results from Lake Biwa. It is believed that the effects of visible radiation on the fluorescence quenching of fulvic-like fluorophores may be large, while the effects of ultra-violet radiation may be larger on protein-like fluorophores.

The effects of the wavelength region on the characteristics of DOM and fluorophores in environmental waters were examined by Xe-lamp irradiation using two kinds of wavelength cut filters and compared with those of soil FA and algal DOM (Fig. 11). The I/I0 values of peak A of soil FA (Dando FA), algal DOM from M. aeruginosa and aquatic DOM in Kizu River (No. 31, October 2016) by Xe-lamp irradiation decreased down to 0.76, 0.47 and 0.77, respectively, and those by Xe-lamp irradiation with a cut filter (UV-29) decreased down to 0.75, 0.48 and 0.76, respectively. The decrease in the RFI values of peak A by Xe-lamp irradiation with a cut filter (UV-29) was similar to that in the values by Xe-lamp only in the case of soil FA, algal DOM, and aquatic DOM. Moreover, the changes in the RFI values of peak A by Xe-lamp irradiation with a cut filter (W-Y495) became smaller because the I/I0 values of peak A of Dando FA, algal DOM from M. aeruginosa and aquatic DOM in Kizu River by Xe-lamp irradiation with a cut filter (W-Y495) were 0.95, 1.00 and 0.96, respectively, while, the I/I0 values of peak C of algal DOM from M. aeruginosa and aquatic DOM in Kizu River by Xe-lamp irradiation decreased down to 0.25 and 0.34, respectively,

<table>
<thead>
<tr>
<th>River</th>
<th>Irradiation</th>
<th>Peak A (QSU)</th>
<th>Peak C (QSU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I0</td>
<td>I</td>
<td>I/I0</td>
</tr>
<tr>
<td>Katsuura (No. 11)*</td>
<td>solar</td>
<td>7.14</td>
<td>3.34</td>
</tr>
<tr>
<td>Kizu (No. 31)**</td>
<td>solar</td>
<td>16.9</td>
<td>8.41</td>
</tr>
<tr>
<td>Kizu (No. 31)**</td>
<td>solar</td>
<td>16.4</td>
<td>8.54</td>
</tr>
<tr>
<td>Kizu (No. 31)**</td>
<td>Xe</td>
<td>16.4</td>
<td>13.6</td>
</tr>
<tr>
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<td>18.8</td>
<td>9.28</td>
</tr>
<tr>
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<td>Xe</td>
<td>20.1</td>
<td>10.2</td>
</tr>
<tr>
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<td>Xe</td>
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<tr>
<td>Yodo (No. 41)*</td>
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<td>13.1</td>
<td>6.42</td>
</tr>
</tbody>
</table>

* collected in August, 2015, average amount of solar radiation 609 W/m²
** collected in August, 2016, average amount of solar radiation 419 W/m²
Fig. 11 Effects of wavelength regions on the fluorescence intensities of peaks A and C by Xe-lamp irradiation using two kinds of wavelength cut filters
FA: Dando FA, M: algal DOM from M. aeruginosa, River: aquatic DOM in Kizu River (No. 31, October 2016)
■ before irradiation, □ Xe-lamp only, ▪ Xe-lamp with UV-29, □ Xe-lamp with W-Y495

and those by Xe-lamp irradiation with a cut filter (UV-29) decreased down to 0.85 and 0.76, respectively. The decrease in the RFI values of peak C in algal DOM and aquatic DOM became smaller when using a cut filter (UV-29). These results indicate that wavelengths between 290 and 495 nm and below 290 nm may largely affect the characteristics of fulvic-like fluorescence DOM and protein-like fluorescence DOM, respectively.

From these results, it is considered that strong solar irradiation in summer may influence both the light quenching and degradation of fulvic-like fluorophores in surface water of the northern basin of Lake Biwa. As a result, their molecular distributions may converge to relatively stable conditions.

4. Conclusion

The decrease in the fluorescence intensities of HS and algal DOM from three kinds of phytoplankton was observed by both types of photoirradiation (solar and Xe-lamp irradiation), and the fluorescence quenching by solar irradiation of HS and fulvic-like fluorophores released from phytoplankton was larger than that by Xe-lamp irradiation, while the fluorescence quenching by Xe-lamp irradiation of protein-like fluorophores released from phytoplankton was larger than that by solar irradiation. On the other hand, the decrease in the DOC values of these substances by photoirradiation was relatively small.

We clarified by Xe-lamp irradiation experiment using two kinds of wavelength cut filters that fulvic-like fluorophore might be more sensitive to visible light irradiation than protein-like fluorophore, while protein-like fluorophore might be sensitive to UV irradiation. Therefore, the fluorescence quenching and degradation of fulvic-like fluorophores attributed to soil FA and algal DOM in surface water of Lake Biwa might occur by strong solar irradiation in summer. Furthermore, the rest of fulvic-like fluorophores might be resistant to further photochemical degradation. However, we cannot conclude the effects of photoirradiation on protein-like fluorophores in Lake Biwa because their fluorescence intensities were low and fluctuated by microbiological production in Lake Biwa. Then, further study to clarify this is needed.

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