Measurements, sources and sinks of photoformed reactive oxygen species in Japanese rivers

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Reactive oxygen species (ROS) are photochemically generated in sunlit natural water and are involved in degradation of organic matter, redox reactions, and biological processes. Hydroxyl radicals ($\bullet\text{OH}$), nitric oxide radicals ($\text{NO}\bullet$), and singlet oxygen ($^1\text{O}_2$) are some of the dominant ROS in natural water. In this study, these three ROS were measured in samples collected from nine rivers across 65 stations along the west to east axis of Japan. Quantification of $\bullet\text{OH}$, $\text{NO}\bullet$, and $^1\text{O}_2$ was performed by High-Performance Liquid Chromatography using benzene, 4,5-diaminofluorescein-2, and furfuryl alcohol as chemical probes, respectively. The absorption coefficient at 300 nm (a_{300} , m^{-1}), which ranged from 2.44 to 36.2 m^{-1} , was used to investigate the chromophoric dissolved organic matter (CDOM) properties of the rivers. The photoformation rate ranges were $(13.9\text{--}944) \times 10^{-12} \text{ M s}^{-1}$ for $\bullet\text{OH}$, $(2.76\text{--}2610) \times 10^{-12} \text{ M s}^{-1}$ for $\text{NO}\bullet$, and $(9.48\text{--}133) \times 10^{-9} \text{ M s}^{-1}$ for $^1\text{O}_2$. The steady-state concentration ranges were $(1.53\text{--}16) \times 10^{-16} \text{ M}$ for $\bullet\text{OH}$, $(10.2\text{--}1520) \times 10^{-12} \text{ M}$ for $\text{NO}\bullet$, and $(3.79\text{--}53.4) \times 10^{-14} \text{ M}$ for $^1\text{O}_2$. The results showed that nitrite was a major source for both $\bullet\text{OH}$ and $\text{NO}\bullet$, and CDOM was a major source for $^1\text{O}_2$ across all the rivers. According to significant relationships with these sources, models were generated to predict the formation rates of the ROS (in M s^{-1}) from known concentrations of source compounds using the equations $R_{\bullet\text{OH}} (10^{-12}) = 19.2 [\text{NO}_2^-] - \mu\text{M} + 36.9$, $R_{\text{NO}\bullet} (10^{-12}) = 41.4 [\text{NO}_2^-] - \mu\text{M} + 44$, and $R_{^1\text{O}_2} (10^{-9}) = 3.52 (a_{300}) - \text{m}^{-1} + 1.61$. Dissolved organic matter, escape to the atmosphere, and water molecules were the major sinks for river $\bullet\text{OH}$, $\text{NO}\bullet$, and $^1\text{O}_2$, respectively. A general scavenging rate constant of $\bullet\text{OH}$ as a function of the dissolved organic carbon concentration was obtained [$k_{\text{C,OH}} = [(7.5 \pm 6.8) \times 10^8 \text{ L (mol C)}^{-1} \text{ s}^{-1}]$]. These models will allow for easy prediction of ROS concentrations on a large-scale.

Keywords: hydroxyl radical, nitric oxide radical, singlet oxygen, photochemistry, river water

INTRODUCTION

Natural water can absorb solar radiation to produce some transitory species, such as reactive oxygen species (ROS). These species include hydrogen peroxide (H_2O_2) (Cooper *et al.*, 1988; Sakugawa *et al.*, 1990; Mostofa and Sakugawa, 2009), hydroxyl radicals ($\bullet\text{OH}$) (Mopper and Zhou, 1990; Vaughan and Blough, 1998), singlet oxygen ($^1\text{O}_2$) (Zepp *et al.*, 1977; Al Housari *et al.*, 2010), nitric oxide radicals ($\text{NO}\bullet$) (Zafiriou *et al.*, 1980; Olasehinde *et al.*, 2009), superoxide anions ($\text{O}_2^{\bullet-}$), (Voelker *et al.*, 2000; Anifowose *et al.*, 2015a), and hydrated electrons ($\text{e}^-(\text{aq})$) (Zepp *et al.*, 1992). These species are reactive

and are involved in biological and chemical processes that occur in natural water. They facilitate the photochemical degradation of natural organic matter and organic pollutants, biological reactions, and redox reactions (Takeda *et al.*, 2004). In natural environmental systems, ROS are often generated by photolysis, energy transfer, or electron transfer reactions (Zafiriou *et al.*, 1984; Lu *et al.*, 2006) as shown in Eqs. (1)–(10) and are generally present in picomolar (10^{-12} M) to micromolar (10^{-6} M) concentrations.

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Dissolved organic matter (DOM) in natural water is a complex mixture of macromolecules derived from the partial decomposition of organic materials. It can either be autochthonous (i.e., from phytoplankton and microbial) or allochthonous (i.e., from anthropogenic or terrestrial inputs) in origin (Blough and Del Vecchio, 2002). A sub-fraction of DOM is chromophoric (Latch and McNeill, 2006). Chromophoric DOM (CDOM) is an important component in natural water because it can absorb light (Eq. (1)) and change the water's colour (Das *et al.*, 2017). Most of the light CDOM absorbs is in the ultraviolet (UV) region (Coble, 1996; Liang *et al.*, 2014) and this helps prevent damage to phytoplankton and other biota in coastal ecosystems (Blough and Zepp, 1990).

In sunlit natural water, processes such as photolysis of CDOM (Eq. (2)) (Mopper and Zhou, 1990; Vione *et al.*, 2006), NO_3^- (Eq. (3)) (Zafiriou and True, 1979a), NO_2^- (Eq. (4)) (Zafiriou and True, 1979b), and H_2O_2 (Eq. (5)) (Takeda *et al.*, 2004) produce $\bullet\text{OH}$. Another source of $\bullet\text{OH}$ in natural water is the photo-Fenton reaction (Eqs. (6) and (7)) (Zepp *et al.*, 1992; Nakatani *et al.*, 2007a; Mostofa and Sakugawa, 2016). The fates of natural and anthropogenic compounds discharged into water can be strongly influenced by $\bullet\text{OH}$. Advanced oxidation processes are a series of methods that use $\bullet\text{OH}$ for oxidative destruction of organic pollutants. Pharmaceutical compounds, pesticides, and dyes are effectively degraded by these methods (Wang and Xu, 2012).

$\text{NO}\bullet$ is a reactive atmospheric trace gas that plays an important role in tropospheric chemistry. It regulates the photochemical production of tropospheric ozone and is also an intermediate of both the terrestrial and marine nitrogen cycles. Biological activities involving nitrification and denitrification processes in natural water often result in production of $\text{NO}\bullet$ (Ward and Zafiriou, 1988; Liu *et al.*, 2017). Common sources of this radical are NO_2^- (Eq. (4)) and, at low pH, nitrous acid (HNO_2) (Venterea *et al.*, 2005). During the nitrogen cycle in the water column, NO_2^- is produced as an important intermediate (Carlucci *et al.*, 1970) but it is photochemically unstable

because it significantly absorbs solar radiation at $\sim 300\text{--}410\text{ nm}$, ($\lambda_{\text{max}} = 356\text{ nm}$), which leads to the photolytic generation of $\text{NO}\bullet$ (Zafiriou and True, 1979b; Zafiriou and McFarland, 1981).

Formation of $^1\text{O}_2$ occurs when CDOM absorbs sunlight and is photo-excited to its triplet state, and subsequently transfers energy to the triplet ground-state oxygen molecule (Eq. (8)). CDOM absorbs UV and visible light and plays a vital role in photochemistry in natural water, where it is known to be the main source of $^1\text{O}_2$ (Zepp *et al.*, 1977; Peterson *et al.*, 2012). In the environment, $^1\text{O}_2$ is very reactive towards organic contaminants, especially those containing heterocyclic, phenolic, sulfidic, and olefinic functional groups (Foote and Peters, 1971; Haag and Hoigne, 1986; Zeinali *et al.*, 2019).

Several sinks of ROS have been reported (Blough and Zafiriou, 1985; Vione *et al.*, 2006; Zielonka *et al.*, 2010; Arakaki *et al.*, 2013). Inorganic anions including chloride (Cl^-), bromide (Br^-), NO_2^- , NO_3^- , sulfate (SO_4^{2-}), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) consume $\bullet\text{OH}$ to various levels in natural water (Arakaki and Faust, 1998; Vione *et al.*, 2006). The main scavenger of $\bullet\text{OH}$ in seawater and brackish water is Br^- . A general equation to represent $\bullet\text{OH}$ loss by inorganic anions (A^-) is as follows:

The main sink of $\bullet\text{OH}$ in surface freshwater is DOM (Arakaki *et al.*, 2013; Vione *et al.*, 2014).

DOM has a poorly defined chemical structure and its sink with $\bullet\text{OH}$ is represented as follows:

Previous studies suggest the atmosphere as a possible sink for $\text{NO}\bullet$ in natural water (Zafiriou *et al.*, 1980; Olasehinde *et al.*, 2010; Anifowose and Sakugawa, 2017). Another sink mechanism for $\text{NO}\bullet$ in natural water is via radical-radical interactions. Photochemically generated ROS like $\bullet\text{OH}$ and $\text{O}_2^{\bullet-}$ (produced via Eq. (9)) can react with $\text{NO}\bullet$ in a diffusion-controlled manner to produce nitrous acid and peroxynitrite according to the following equations:

$^1\text{O}_2$ may undergo quenching via physical or chemical processes. Physical quenching occurs via triplet energy

transfer, whereas in chemical quenching, the quencher is oxidized by oxygen to form a new product (Matheson *et al.*, 1974; Haag and Hoigne, 1986; DeRosa and Crutchley, 2002). $^1\text{O}_2$ in natural water could be scavenged by water molecules via physical quenching to triplet ground state oxygen as represented by the following equation:

An example of chemical quenching is the reaction of $^1\text{O}_2$ with furfuryl alcohol (FFA) to yield 6-hydroxy-2H-pyran-3(6H)-one as shown below:

Several studies on the photogeneration and quantification of ROS such as $\bullet\text{OH}$, $\text{NO}\bullet$, and $^1\text{O}_2$ have been carried out in both fresh and marine water environments worldwide (Zepp *et al.*, 1977; Takeda *et al.*, 2004; Olasehinde *et al.*, 2010; Peterson *et al.*, 2012).

The aim of this study was to investigate the photochemical generation of these ROS in different rivers located along the west-east axis of Japan. To date, only a few studies have quantified ROS in rivers throughout Japan. To the best of our knowledge, this is the first nationwide and comprehensive study on river water ROS in Japan, and perhaps in the world. Our objectives were to 1) measure the photogeneration rates of ROS in the same batch of samples across different seasons over a 2-year period, 2) quantify the source contributions of some organic and inorganic compounds to the photogenerated ROS, and 3) generate models that could be useful for predicting ROS production from known source compounds in the future. Concerted measurements of ROS and their source compounds in the same batch of samples could help generate meaningful information on the distributions of these ROS in rivers in Japan.

EXPERIMENTAL

Reagents and chemicals

Analytical grade reagents were used without further purification and are described in detail under Supplementary Materials.

Study area and water sampling

Sampling was carried out from spring through autumn in 2018 and 2019 along the west-east axis of Japan located between $33^\circ 31' 8.26'' \text{N}$ – $35^\circ 46' 43.38'' \text{N}$ latitude and

Fig. 1. Map of sampling locations.

$130^\circ 26' 21.53'' \text{E}$ – $139^\circ 56' 11.67'' \text{E}$ longitude (Fig. 1). Samples were collected from 65 stations in nine rivers (Mikasa, Ohta, Kurose, Ashida, Yodo, Yamato, Shonai-Yada, Urui, and Kokubu). Supplementary Table S1 shows the average annual flow rates, lengths, and catchment areas of the rivers while each river location details are given in Supplementary Table S2.

River water samples were collected with polyethylene bucket and filtered immediately on site into a pre-cleaned 1 L amber glass bottles using pre-combusted GC 50, Advantech ($0.45 \mu\text{m}$) glass fiber filter. To effectively minimize any chemical transformation, the samples were stored in a mini-refrigerating system with operating condition set at 4°C and transported to the laboratory, where analysis was carried out on arrival.

Instrumentation and photoirradiation experiments

The High-Performance Liquid Chromatography system used for analysis of the ROS and the solar simulators used for the photo-irradiation experiments are described in Supplementary Materials. The data obtained from the irradiated samples using the solar simulators were normalized to 2-nitrobenzaldehyde, which has a degradation rate ($J_{2\text{NB}}$) of 0.00929 s^{-1} . This degradation rate is equivalent to the sunlight intensity determined at noon under a clear sky in Higashihiroshima city ($34^\circ 25' \text{N}$) on May 1, 1997 (0.908 kW m^{-2}) (Arakaki *et al.*, 1998).

Analytical methods

Measurements of the physicochemical properties of the rivers Details for the measurements of temperature, pH, electrical conductivity (EC), inorganic anion and cation, dissolved organic carbon (DOC), and H₂O₂ concentrations are given in Supplementary Materials. The absorbance of each filtered river water sample was measured in a quartz cell at 190 to 600 nm on a UV-Visible recording spectrophotometer (UV-2400PC, Shimadzu, Kyoto, Japan) using Milli-Q water as a reference. The absorption coefficients a_λ were calculated using the Napierian equation as follows:

$$a_\lambda = \frac{2.303A_\lambda}{l}, \quad (17)$$

where A_λ is the sample absorbance at the reference wavelength, and l is the path length.

Photochemical measurement of •OH and NO• A benzene probe was used to trap •OH formed in irradiated river water samples for •OH measurements (Takeda *et al.*, 2004; Nakatani *et al.*, 2007b). Benzene reacts rapidly with •OH and lacks reactivity in direct photolysis (Al Housari *et al.*, 2010). The river water samples (7 mL) were spiked with benzene (1.2 mM final concentration) and irradiated for 40 min in a solar simulator. Aliquots taken at 10 min intervals were analysed by HPLC at excitation and emission wavelengths of 270 and 298 nm, respectively. The calibration curve of a standard phenol solution was used to determine the photoformation rate of the radical ($R_{\bullet OH}$).

Photochemically generated NO• in river water samples was measured by a standard method using 4,5-diaminofluorescence-2 (DAF-2) as a probe that produces a highly fluorescent product (DAF-2T) (Olasehinde *et al.*, 2009). The river water samples (5 mL) were mixed with DAF-2 (5 μM final concentration) and irradiated for 30 min. Aliquots were taken at set intervals and analysed by HPLC. The calibration curve of standard DAF-2T in phosphate buffer was used to determine the photoformation rate of the radical ($R_{NO\bullet}$).

$R_{\bullet OH}$ and $R_{NO\bullet}$ were calculated using the following equations:

$$R_{\bullet OH} = \frac{R_{\text{phenol}}}{F_{\text{benzene-OH}} Y_{\text{phenol}}}, \quad (18)$$

$$R_{NO\bullet} = \frac{R_{\text{DAF-2T}}}{F_{(\text{DAF-2})-NO\bullet} Y_{\text{DAF-2T}}}, \quad (19)$$

where R_{phenol} and $R_{\text{DAF-2T}}$ are the experimentally obtained photoformation rates of phenol and DAF-2T, respectively; $F_{\text{benzene-}\bullet OH}$ and $F_{(\text{DAF-2})-NO\bullet}$ are the fractions of •OH and

NO• that react with the probes rather than other natural scavengers in the river water, respectively. They were calculated using Eq. (20), and ranged from 0.92 to 0.99 for •OH, while 0.9 to 0.96 for NO•. Y_{phenol} and $Y_{\text{DAF-2T}}$ are the yields of phenol and DAF-2T from the reactions with the radicals, respectively. According to previous reports, Y_{phenol} is 0.75 ± 0.07 (Arakaki and Faust, 1998) and $Y_{\text{DAF-2T}}$ is 0.042 ± 0.003 (Olasehinde *et al.*, 2009).

$$F = \frac{K_D [\text{optimum probe concentration}]}{K_D [\text{optimum probe concentration}] + \sum (k_s [S])}. \quad (20)$$

From the above equation, K_D represents the second order reaction rate constant of benzene with •OH equivalent to the value: $7.8 \times 10^9 \text{ s}^{-1}$ (Buxton *et al.*, 1988; Nakatani *et al.*, 2007b) or DAF-2 with NO• equivalent to the value: $6.28 \times 10^6 \text{ s}^{-1}$ for NO• (Olasehinde *et al.*, 2009) in the air saturated solution, and $\sum (k_s [S])$ represents the scavenging rate constant. The optimum probe concentrations were 1.2 mM and 5 μM for •OH and NO•, respectively.

$\sum (k_s [S])$ was determined using competition kinetics from the slopes and intercepts of the plots of $1/R_{\text{phenol}}$ versus $1/[\text{benzene}]$ for •OH and $1/R_{\text{DAF-2T}}$ versus $1/[\text{DAF-2}]$ for NO•.

$$\begin{aligned} \frac{1}{R_{\text{phenol}}} &= \left[\left(\frac{\sum (k_s [S])}{R_{\bullet OH} Y_{\text{phenol}} k_{\text{benzene}}} \right) \times \frac{1}{[\text{benzene}]} \right] + \left[\frac{1}{R_{\bullet OH} Y_{\text{phenol}}} \right], \quad (21) \\ \frac{1}{R_{\text{DAF-2T}}} &= \left[\left(\frac{\sum (k_s [S])}{R_{NO\bullet} Y_{\text{DAF-2T}} k_{\text{DAF-2}}} \right) \times \frac{1}{[\text{DAF-2}]} \right] + \left[\frac{1}{R_{NO\bullet} Y_{\text{DAF-2T}}} \right], \quad (22) \end{aligned}$$

Equations (21) and (22) are summarized in Eq. (23) as follows:

$$\sum (k_s [S]) = \frac{(\text{slope} \times K_D)}{\text{Intercept}}. \quad (23)$$

The steady-state concentrations, $[\bullet OH]_{ss}$ and $[NO\bullet]_{ss}$, and lifetimes, $\tau_{\bullet OH}$, $\tau_{NO\bullet}$ were determined using the following equations:

Table 1. Water quality data, H_2O_2 concentrations, and a_{300} values in river water samples

Rivers	WT (°C)	pH	EC (mS cm ⁻¹)	Cations		
				Na ⁺ (μM)	NH ₄ ⁺ (μM)	K ⁺ (μM)
Mikasa	24.7 (23.9–25)	7.8 (7.6–7.9)	0.13 (0.09–0.17)	632 (631–632)	3.93 (3.4–4.63)	30.6 (25.9–34.9)
Ohta	23.4 (19.9–27.1)	7.5 (7.1–7.9)	—	210 (146–259)	1.95 (1.26–2.86)	21.2 (13–29.2)
Kurose	20.6 (9.2–27.2)	7.3 (6.7–8.0)	0.29 (0.04–0.9)	1190 (174–4600)	95 (0.4–550)	92.4 (17.5–162)
Ashida	23.9 (23.6–24.8)	7.4 (7.1–7.6)	0.09 (0.06–0.12)	226 (132–317)	5.37 (3.68–10.6)	61.7 (48.7–83.2)
Yodo	12.7 (11.4–13.5)	7.3 (7.2–7.3)	0.16 (0.15–0.17)	542 (505–565)	—	66.3 (64.4–68.1)
Yamato	23.3 (17.1–29.1)	7.6 (7.2–7.8)	0.19 (0.07–0.29)	803 (272–1290)	8.73 (BDL–14.5)	119 (71.3–164)
Shonai-Yada	18.4 (15.8–20.7)	7.2 (6.9–7.4)	0.13 (0.05–0.18)	351 (223–469)	9.85 (0.5–21.9)	67.7 (52–85.9)
Urui	18.1 (17.4–18.7)	7.5 (7.3–7.7)	0.12 (0.08–0.16)	304 (206–384)	2.56 (1.8–3.58)	49.4 (41.8–55.3)
Kokubu	22.4 (16.1–29.7)	7.6 (7.4–7.7)	0.46 (0.4–0.52)	1340 (1030–1580)	172 (119–234)	151 (116–194)
Average	21.2 (9.2–29.7)	7.4 (6.7–8.0)	0.21 (0.04–0.9)	679 (132–4600)	46.1 (BDL–550)	77.1 (13–194)

Rivers	Anions					DOC (mg C L ⁻¹)	H ₂ O ₂ (nM)	a_{300} (m ⁻¹)
	Cl ⁻ (μM)	NO ₃ ⁻ (μM)	NO ₃ ⁻ (μM)	SO ₄ ²⁻ (μM)	HCO ₃ ⁻ (μM)	CO ₃ ²⁻ (μM)		
Mikasa	194 (136–279)	2.1 (1.69–2.38)	27.9 (19.5–41.2)	85.3 (66.4–116)	359 (245–489)	1.03 (0.46–1.81)	374 (312–413)	7.93 (7.14–9.26)
Ohta	162 (124–181)	0.7 (0.25–1.61)	21.3 (13.8–33.5)	38.4 (23.5–59.2)	222 (83–474)	0.95 (0.51–1.26)	271 (169–402)	4.92 (2.44–6.36)
Kurose	769 (78.6–3660)	11.3 (BDL–35.8)	134 (BDL–306)	482 (31.7–2100)	179 (30.1–628)	0.43 (0.01–3.01)	196 (71–369)	14.6 (2.81–36.2)
Ashida	126 (82.8–165)	1.06 (0.43–1.52)	37.6 (17.4–53.6)	75.7 (41.4–118)	166 (78.8–245)	0.22 (0.05–0.46)	188 (174–228)	12.2 (10.4–15.1)
Yodo	378 (356–393)	0.59 (0.49–0.67)	60.4 (55.3–64.4)	134 (124–140)	137 (117–144)	0.09 (0.06–0.1)	106 (78–131)	4.84 (4.59–5.07)
Yamato	585 (166–958)	2.99 (1.92–4.7)	107 (48.8–160)	141 (100–168)	269 (95.8–427)	0.57 (0.07–1.08)	427 (276–566)	10.1 (8.89–11.2)
Shonai-Yada	237 (179–327)	4.51 (2.52–7.06)	63.9 (43–91.3)	201 (132–291)	101 (59.2–158)	0.07 (0.02–0.14)	341 (264–428)	11.1 (9.26–12.4)
Urui	142 (105–187)	0.84 (BDL–1.5)	105 (70.3–182)	98.7 (67.2–141)	228 (135–336)	0.35 (0.11–0.69)	238 (181–304)	6.34 (2.49–12.6)
Kokubu	1070 (705–1370)	38.1 (25.2–45.9)	250 (121–371)	353 (292–421)	251 (151–297)	0.46 (0.18–0.74)	1240 (250–2930)	10.1 (8.13–12.2)
Average	462 (78.6–3660)	8.0 (BDL–45.9)	95.6 (BDL–371)	204 (23.4–2100)	210 (30.1–628)	0.42 (0.01–3)	391 (71–2930)	9.52 (2.44–36.2)

Note: data are means (minimum–maximum). Abbreviations: WT, water temperature; BDL, below detection limit. A - indicates “not measured”. HCO_3^- and CO_3^{2-} concentrations were not measured directly. Instead, these values were estimated using the method described by Mook (2001). Details are given under Supplementary Materials.

$$[X]_{ss} = R_X / (\sum (k_s [S])), \quad (24)$$

$$\tau_x = 1 / \sum (k_s [S]), \quad (25)$$

where X can be either •OH or NO•.

Photochemical measurement of ¹O₂ Furfuryl alcohol was used as a probe for ¹O₂ photodegradation studies as described by Haag *et al.* (1984). Methanol (1%) was added to each river water sample to effectively scavenge •OH, after which the samples were spiked with FFA (100 μM) and irradiated under the solar simulator for 1–2 h. Aliquots were obtained at set intervals and analysed by HPLC. The [¹O₂]_{ss} was monitored using degradation of FFA and calculated with Eq. (26).

$$[{}^1\text{O}_2]_{ss} = \frac{K}{K_{\text{FFA}, {}^1\text{O}_2}}, \quad (26)$$

where $K_{\text{FFA}, {}^1\text{O}_2}$ is the reaction rate constant of FFA with ¹O₂ and has a value of $1.09 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ (Haag *et al.*, 1984); and K is the first order degradation rate constant of FFA during irradiation of the FFA-spiked water sample. The photoformation rate ($R_{{}^1\text{O}_2}$) was calculated using Eq. (27).

$$[{}^1\text{O}_2]_{ss} = \frac{R_{{}^1\text{O}_2}}{k_d}, \quad (27)$$

where k_d ($2.5 \times 10^5 \text{ s}^{-1}$) (Rodgers and Snowden, 1982) is the dissociation rate constant of ¹O₂ in water because water is its main scavenger (Peterson *et al.*, 2012).

Other studies Details about the percentage contributions (% C) of known sources to the •OH formation rates and statistical analysis are given in Supplementary Materials.

RESULTS AND DISCUSSION

Physicochemical properties of the river water samples

The water quality parameters of the rivers are presented in Table 1. The water temperature ranged from 9.2°C to 29.7°C (average, 21.2°C) and the pH ranged from 6.7 to 8.0. The EC values of the river water samples ranged from 0.04 to 0.9 mS cm⁻¹. Cations and anions that are produced from dissolved salts and inorganic compounds are a measure of the level of pollution in a river. In this study, their concentrations ranged from below the detection limit (bdl) for NH₄⁺, NO₂⁻, and NO₃⁻, to as high as 4600 μM for Na⁺. The lowest NO₂⁻ concentration (bdl) was observed in both the Urui and Kurose rivers, and the highest in the Kokubu River with a value (45.9 μM),

which was about an order of magnitude higher than NO₃⁻ (371 μM) in the same Kokubu river. The DOC values in this study ranged from 0.51 mg C L⁻¹ in the Ohta River to 7.98 mg C L⁻¹ in the Kurose River and was consistent with the values (2.03–7.67 mg C L⁻¹) reported for some rivers and lakes in the middle to lower Yangtze region in China (Xu *et al.*, 2020). In the present study, the H₂O₂ concentrations ranged from a low of 71 μM in the Kurose River to a high of 2930 nM in the Kokubu River. The absorption coefficients at 300 nm (a_{300}), which is an indicator of the CDOM abundance, ranged from 2.44 m⁻¹ in the Ohta River to 36.2 m⁻¹ in the Kurose River and were consistent with earlier reports. Peterson *et al.* (2012) found that the a_{300} values of river samples and river-impacted samples were $\geq 10 \text{ m}^{-1}$ and $2 \text{ m}^{-1} \leq a_{300} < 10 \text{ m}^{-1}$ respectively. Table 1 shows the distribution of CDOM across the nine rivers.

Photochemical generation of ROS

Photoformation rates of •OH, NO•, and ¹O₂ The distributions of ROS photoformation across the rivers are shown in Figs. 2a–c.

Generally, there was greater variation of ROS photoformation in the Kokubu and Kurose Rivers while the other rivers had lower and more consistent values. From Table 1, the higher concentrations of organic and inorganic dissolved substances in both rivers reveal their low water quality, which could be a reason behind their higher ROS photoformation rates (Sunday *et al.*, 2020). Supplementary Figs. S1a–c show that there were inverse relationships between the rivers annual average flow rates and ROS photogeneration rates, thus indicating higher ROS photogeneration rates in polluted rivers compared to the clean ones. For example, the Kokubu River has the shortest length and the lowest flow rate in this study (Table S1), this limits the dilution effect on pollutants within the river system, as compared with bigger rivers like the Ohta and Yodo.

Supplementary Table S3 shows the average (mean), minimum, and maximum values of the photoformation rates of the ROS. $R_{\bullet\text{OH}}$ ranged from $13.9 \times 10^{-12} \text{ M s}^{-1}$ in the Kurose River to $944 \times 10^{-12} \text{ M s}^{-1}$ in the Kokubu River because of the high NO₂⁻ concentration. The lowest ($2.76 \times 10^{-12} \text{ M s}^{-1}$) and highest ($2610 \times 10^{-12} \text{ M s}^{-1}$) $R_{\text{NO}\bullet}$ values were both observed in the Kurose River, and were recorded in the upstream and downstream regions, respectively. The large difference in $R_{\text{NO}\bullet}$ within the same river could point to anthropogenic inputs in the downstream section of the river (Anifowose *et al.*, 2015b). The lowest $R_{{}^1\text{O}_2}$ value ($9.48 \times 10^{-9} \text{ M s}^{-1}$) was observed in the Ohta River, which also had the lowest CDOM value (2.44 m⁻¹), and the maximum $R_{{}^1\text{O}_2}$ value ($133 \times 10^{-9} \text{ M s}^{-1}$) was measured in the Kurose River, which had the

Fig. 2. Box plots for distributions of (a) the $\bullet\text{OH}$ photoformation rate (R_{OH}), (b) the $\text{NO}\bullet$ photoformation rate ($R_{\text{NO}\bullet}$), (c) the $^1\text{O}_2$ photoformation rate ($R_{^1\text{O}_2}$), (d) the $\bullet\text{OH}$ steady-state concentration ($[\bullet\text{OH}]_{\text{ss}}$), (e) the $\text{NO}\bullet$ steady-state concentration ($[\text{NO}\bullet]_{\text{ss}}$), and (f) the $^1\text{O}_2$ steady-state concentration ($[^1\text{O}_2]_{\text{ss}}$) across the rivers. Letters indicate the following rivers: A = Mikasa, B = Ohta, C = Kurose, D = Ashida, E = Yodo, F = Yamato, G = Shonai-Yada, H = Urui, and I = Kokubu.

highest CDOM value (36.2 m^{-1}).

Among the three radicals, $^1\text{O}_2$ had the highest average photoformation rate ($3.64 \times 10^{-8} \text{ M s}^{-1}$), followed by $\text{NO}\bullet$ ($3.77 \times 10^{-10} \text{ M s}^{-1}$), and finally $\bullet\text{OH}$ ($1.91 \times 10^{-10} \text{ M s}^{-1}$). The ROS photoformation rates were in the order $^1\text{O}_2 > \text{NO}\bullet > \bullet\text{OH}$. The observation in this study of $^1\text{O}_2$ having a higher photoformation rate than other ROS is consistent with earlier observations by Kaur and Anastasio (2017) in fog waters.

Steady-state concentrations of $\bullet\text{OH}$ ($[\bullet\text{OH}]_{\text{ss}}$), $\text{NO}\bullet$ ($[\text{NO}\bullet]_{\text{ss}}$), and $^1\text{O}_2$ ($[^1\text{O}_2]_{\text{ss}}$) The distributions of $[\bullet\text{OH}]_{\text{ss}}$, $[\text{NO}\bullet]_{\text{ss}}$, and $[^1\text{O}_2]_{\text{ss}}$ are shown in Figs. 2d–f, respectively. From the figures, both $[\text{NO}\bullet]_{\text{ss}}$ and $[^1\text{O}_2]_{\text{ss}}$ were still observed to be more dominant in the Kurose and Kokubu, rivers, whereas, $[\bullet\text{OH}]_{\text{ss}}$ behaved differently. The average (i.e., mean), minimum, and maximum values of the steady-state concentrations of the ROS are listed in Table S3. The minimum $[\bullet\text{OH}]_{\text{ss}}$ was $1.53 \times 10^{-16} \text{ M}$ in the Kurose River and the maximum $[\bullet\text{OH}]_{\text{ss}}$ was $16 \times 10^{-16} \text{ M}$ in the Shonai-Yada river. According to Takeda *et al.* (2004), $[\bullet\text{OH}]_{\text{ss}}$ are generally low because of the high re-

Fig. 3. Box plots for the distributions of (a) the scavenging rates of $\bullet\text{OH}$, (b) the scavenging rates of $\text{NO}\bullet$, (c) the lifetimes of $\bullet\text{OH}$, and (d) the lifetimes of $\text{NO}\bullet$ across the rivers. Letters indicate the following rivers: A = Mikasa, B = Ohta, C = Kurose, D = Ashida, E = Yodo, F = Yamato, G = Shonai-Yada, H = Urui, and I = Kokubu.

activity and short lifetime of the radical. The $[\text{NO}\bullet]_{\text{ss}}$ ranged from $(10.2\text{--}1520) \times 10^{-12} \text{ M}$ and was lowest in the Ashida River and highest in the Kokubu River. The lowest $[^1\text{O}_2]_{\text{ss}}$ ($3.79 \times 10^{-14} \text{ M}$) was observed in the Ohta River and the highest ($53.4 \times 10^{-14} \text{ M}$) was in the Kurose River. The average $[\text{NO}\bullet]_{\text{ss}}$ ($1.55 \times 10^{-10} \text{ M}$) was about three orders of magnitude higher than that of $[^1\text{O}_2]_{\text{ss}}$ ($1.45 \times 10^{-13} \text{ M}$), which is over three-orders of magnitude higher than that of $[\bullet\text{OH}]_{\text{ss}}$ ($7.25 \times 10^{-16} \text{ M}$). The steady-state concentrations were in the order $[\text{NO}\bullet]_{\text{ss}} > [^1\text{O}_2]_{\text{ss}} > [\bullet\text{OH}]_{\text{ss}}$.

Scavenging rate constants and lifetimes of $\bullet\text{OH}$, $\text{NO}\bullet$, and $^1\text{O}_2$ The distribution of the scavenging rate constants (Σk_s [S]) and lifetimes (τ) of both $\bullet\text{OH}$ and $\text{NO}\bullet$ across the rivers are shown in Figs. 3a–d, while their averages (mean), minimum, and maximum values are reported in Supplementary Table S4. The lowest $\Sigma k_{s,\text{OH}}[\bullet\text{OH}]$ was $3.3 \times 10^4 \text{ s}^{-1}$ in the Mikasa River and the highest was $9.0 \times 10^5 \text{ s}^{-1}$ in the Kokubu River. The τ_{OH} across the rivers ranged from $1.11 \mu\text{s}$ in the Kokubu River to $30 \mu\text{s}$ in the Mikasa River. The Urui River had the lowest value for $\text{NO}\bullet$ (0.41 s^{-1}) and the Yamato River had the highest (7.41 s^{-1}). The $\tau_{\text{NO}\bullet}$ ranged from 0.135 s in the Yamato River to 2.44 s in the Urui River. $^1\text{O}_2$ is mainly scavenged by water, therefore, the scavenging abilities of other potential

Table 2. Comparison of the photoformation rates and steady-state concentrations of $\bullet\text{OH}$, $\text{NO}\bullet$, and $^1\text{O}_2$ in various natural water samples

Reactive species	Photoformation rate	Steady-state concentration	References
$R_{\bullet\text{OH}}$	($\times 10^{-12} \text{ M s}^{-1}$)	($\times 10^{-16} \text{ M}$)	
Japanese rivers	13.9–944	1.53–16	This study
Kurose river, Japan	1–1340	0.04–119	Anifowose <i>et al.</i> (2015b)
Rivers along Middle to Lower Yangtze region, China	51–117	0.176–0.311	Xu <i>et al.</i> (2020)
Lakes along Middle to Lower Yangtze region, China	110–182	0.25–1.03	Xu <i>et al.</i> (2020)
Canal Fumemorte (fresh water), Southern France	15.2 $\pm$ 0.1	0.94 $\pm$ 0.01	Al Housari <i>et al.</i> (2010)
Vaccaries (estuarine water), Southern France	19.3 $\pm$ 0.1	1.72 $\pm$ 0.01	Al Housari <i>et al.</i> (2010)
Seto Inland Sea, Japan	7.2–424	0.03–1.89	Olasehinde <i>et al.</i> (2012)
$R_{\text{NO}\bullet}$	($\times 10^{-12} \text{ M s}^{-1}$)	($\times 10^{-12} \text{ M}$)	
Japanese rivers	2.76–2610	10.2–1520	This study
Kurose river, Japan	1–3540	0.2–685	Anifowose <i>et al.</i> (2015b)
Coastal water of Yellow sea, China	up to 2.52	bd1–500	Liu <i>et al.</i> (2017)
Seto Inland Sea, Japan	1.4–9.17	3–41	Anifowose and Sakugawa (2017)
$R_{^1\text{O}_2}$	($\times 10^{-9} \text{ M s}^{-1}$)	($\times 10^{-14} \text{ M}$)	
Japanese rivers	9.48–133	3.79–53.4	This study
Lake Superior, North America	—	0.59–71	Peterson <i>et al.</i> (2012)
Canal Fumemorte (fresh water) Southern France	13.6 $\pm$ 0.1	5.44 $\pm$ 0.04	Al Housari <i>et al.</i> (2010)
Vaccaries (estuarine water), Southern France	51.4 $\pm$ 5.4	20.6 $\pm$ 2.2	Al Housari <i>et al.</i> (2010)
Seto Inland Sea, Japan	3.32–22.7	1.2–8.2	Sunday <i>et al.</i> (2020)

quenchers in the river system can be ignored (Peterson *et al.*, 2012). The dissociation rate constant of $^1\text{O}_2$ in water is $2.5 \times 10^5 \text{ s}^{-1}$ (Rodgers and Snowden, 1982), and is equivalent to its scavenging rate constant with an inverse of $4 \mu\text{s}$ as its lifetime. The average $\sum k_{s,\bullet\text{OH}}[\bullet\text{OH}]$ was $2.07 \times 10^5 \text{ s}^{-1}$ and that of $\text{NO}\bullet$ was 2.65 s^{-1} . Their lifetimes were $9.03 \mu\text{s}$ and 0.62 s , respectively. The order for the scavenging rate constants was $^1\text{O}_2 > \bullet\text{OH} > \text{NO}\bullet$ and that for the lifetimes was the reverse ($\text{NO}\bullet > \bullet\text{OH} > ^1\text{O}_2$).

Comparison with previous results A comparison of our ROS values with previous results showed that they were in the same order (Table 2). We found a $R_{\bullet\text{OH}}$ range of $(13.9\text{--}944) \times 10^{-12} \text{ M s}^{-1}$ and a $[\bullet\text{OH}]_{ss}$ range of $(1.53\text{--}16) \times 10^{-16} \text{ M}$ which were consistent with the reported values of Anifowose *et al.* (2015b). Corresponding values reported for fresh and estuarine waters of Southern France and some water samples collected in the middle to lower Yangtze region in China, were lower than those found in our study. The main precursor to $\bullet\text{OH}$ photoformation for those samples was DOM (Al Housari *et al.*, 2010; Xu *et al.*, 2020). Although CDOM has lower quantum yield for $\bullet\text{OH}$ photogeneration [$\phi_{\bullet\text{OH}}^{\text{CDOM}} = 10^{-5}\text{--}10^{-4}$] than nitrite [$\phi_{\bullet\text{OH}}^{\text{NO}_2} = (2.5\text{--}6.5) \times 10^{-2}$], the higher ability of the former to absorb sunlight can make it high enough to be the main source of $\bullet\text{OH}$ (Vione *et al.*, 2014). Our corresponding $\sum k_{s,\bullet\text{OH}}[\bullet\text{OH}]$ ($(0.33\text{--}9) \times 10^5 \text{ s}^{-1}$) and $\tau_{\bullet\text{OH}}$ ($1.11\text{--}30 \mu\text{s}$) values are consistent with those reported across lakes and rivers $[(0.97\text{--}4.5) \times 10^5 \text{ s}^{-1}$ and $(0.3\text{--}22.5 \mu\text{s})]$ having different levels of anthropogenic impacts (Minero *et al.*, 2007; Anifowose *et al.*, 2015b).

The $R_{\text{NO}\bullet}$ range in our study was $(2.76\text{--}2610) \times 10^{-12} \text{ M s}^{-1}$, which was within the range reported by Anifowose *et al.* (2015b) for samples from the Kurose River $[(1\text{--}3540) \times 10^{-12} \text{ M s}^{-1}]$. Lower values of $R_{\text{NO}\bullet}$ were reported for coastal water samples from the Yellow Sea and the Seto Inland Sea by Liu *et al.* (2017) and Anifowose and Sakugawa (2017), respectively. The $\text{NO}\bullet$ concentrations were higher in rivers and in coastal areas than in the sea (Table 2). In our study, which covered different rivers, the $\text{NO}\bullet$ concentration range $[(10.2\text{--}1520) \times 10^{-12} \text{ M}]$ was higher than that measured in the Kurose river by Anifowose *et al.* (2015b) $[(0.2\text{--}685) \times 10^{-12} \text{ M}]$. Our results were also much higher than the concentrations measured in coastal water samples from the Yellow Sea ($\text{bd1--}500 \times 10^{-12} \text{ M}$) (Liu *et al.*, 2017) and Seto Inland Sea $[(3\text{--}41) \times 10^{-12} \text{ M}]$ (Anifowose and Sakugawa, 2017). These differences could be attributed to higher NO_2^- surface concentrations in rivers and coastal areas than in the sea (Liu *et al.*, 2017; Tian *et al.*, 2019). The $\sum k_{s,\text{NO}\bullet}[\text{NO}\bullet]$ range of $0.41\text{--}7.41 \text{ s}^{-1}$ and $\tau_{\text{NO}\bullet}$ range of $0.135\text{--}2.44 \text{ s}$ in our study were comparable to those obtained by Anifowose *et al.* (2015b) in the Kurose River ($0.77\text{--}19.66 \text{ s}^{-1}$ and $0.05\text{--}1.3 \text{ s}$). A $\tau_{\text{NO}\bullet}$ range of $3\text{--}20 \text{ s}$ has been reported for seawater samples from the central equatorial Pacific (Zafiriou *et al.*, 1980) and of $10\text{--}100 \text{ s}$ for samples from the Seto Inland Sea (Olasehinde *et al.*, 2010). These values show that $\text{NO}\bullet$ is more stable in seawater than in river water, probably because of the higher quantities of natural scavengers in river water.

The $R_{^1\text{O}_2}$ and $[^1\text{O}_2]_{ss}$ ranges across the rivers were

Fig. 4. Relationships between (a) R_{OH} and NO_2^- concentrations, (b) R_{NO} and NO_2^- concentrations, and (c) singlet oxygen and a_{300} .

$(9.48\text{--}133) \times 10^{-14} \text{ M s}^{-1}$ and $(3.79\text{--}53.4) \times 10^{-14} \text{ M}$, respectively, and were consistent with the values obtained by Al Housari *et al.* (2010) for samples from the Southern France Canal Fumemorte [$(5.44 \pm 0.04) \times 10^{-9} \text{ M s}^{-1}$ and $(13.6 \pm 0.1) \times 10^{-14} \text{ M}$] and estuarine water samples [$(20.6 \pm 2.2) \times 10^{-9} \text{ M s}^{-1}$ and $(51.4 \pm 5.4) \times 10^{-14} \text{ M}$]. Peterson *et al.* (2012) reported a $[^1O_2]_{ss}$ range across Lake Superior in North America $(0.59\text{--}71) \times 10^{-14} \text{ M}$, which also agreed with the results from our study. The 1O_2 values reported for the Seto Inland Sea were lower than those for the river water samples (Sunday *et al.*, 2020) (Table 2).

Sources and sinks of the ROS

Sources of $\bullet OH$ The calculated average percentage contributions of NO_2^- , NO_3^- , and H_2O_2 to $\bullet OH$ photoformation across the rivers were 63.9%, 13.1%, and 1.5%, respectively (Supplementary Table S5). This indicates that NO_2^- is a major source of $\bullet OH$ in Japanese river waters, with a regression equation of $y = 19.2x + 36.9$ ($r^2 = 0.96$, $p < 0.01$) (Fig. 4a). NO_3^- was also correlated with $\bullet OH$ as shown in Table 3 ($r^2 = 0.65$, $p < 0.01$). NO_2^- photolysis is believed to dominantly produce $\bullet OH$ (Zafiriou and True, 1979b) when river water has a relatively high

NO_2^- concentration. The contribution of NO_2^- to $\bullet OH$ generation was comparable to that of NO_3^- because of its higher photochemical reactivity—the photolysis rate constant of $\bullet OH$ with NO_2^- is about hundred times higher than that of NO_3^- —which compensates for its lower concentration compared with NO_3^- in natural water (Vione *et al.*, 2006). UV irradiation of natural waters is believed to produce H_2O_2 , which further yields $\bullet OH$ (Wang *et al.*, 2001) as shown in Eqs. (5)–(7). The H_2O_2 concentration was significantly correlated with $\bullet OH$ ($r^2 = 0.37$, $p < 0.01$), which suggested that direct photolysis of H_2O_2 and the photo-Fenton reaction may have contributed to $\bullet OH$ photoformation.

NO_2^- has a relatively unstable oxidation state and it can easily be oxidized to the more stable NO_3^- , which is more abundant. While both are ubiquitous in nature, they may enter the natural water environment via natural sources i.e., by atmospheric deposition through the nitrogen cycle (which results into formation of nitrate and ammonium ions) and through point and non-point anthropogenic sources via artificial fertilizers, direct discharge, and nitrogenous compounds generated by residential, industrial, and agricultural sources (He *et al.*, 2011; Sugimoto *et al.*, 2019). The organic form of nitrogen may undergo ammonification and nitrification processes to produce ammonia and ammonium ions which are converted into nitrites and are in turn oxidized to stable nitrate forms in natural waters. Tap (drinking) water in Japan mostly comes from river water (after cleaning and sterilization). Thus, keeping low nitrite and nitrate concentrations in river water is necessary in attaining a good quality of tap water. According to the Ministry of Health, Labour and Welfare Japan (2020), nitrite ion concentration in tap water must be less than 0.04 mg-N L^{-1} (ca. $2.86 \mu\text{M}$). Also, the sum of nitrite and nitrate must be less than 10 mg/L in tap water. In Japan, agricultural input is significant to the total nitrogen deposit in surface waters. Residential and industrial wastewater also contain a lot of nitrogen compounds, which further result to nitrite and nitrate pollution in river waters.

Sources of $NO\bullet$ Our results showed that as the NO_2^- concentration increased across the rivers; corresponding increases were observed in their $NO\bullet$ photoformation rates with a slope of 4.14×10^{-5} (Fig. 4b). The photoformation rate constant of $NO\bullet$ from NO_2^- (in Milli-Q water) had a slope of 4.13×10^{-5} (Supplementary Materials) which was consistent with that obtained in our study (Fig. 4b). The correlation coefficient of the NO_2^- concentration with R_{NO} ($r^2 = 0.72$, $p < 0.01$) showed that $NO\bullet$ was sourced from NO_2^- . Several reports point to NO_2^- as a major photochemical source of $NO\bullet$ in natural water (Smith and Yoshinari, 2008; Olasehinde *et al.*, 2010; Anifowose *et al.*, 2015b). In their study on $NO\bullet$ photoformation across the Seto Inland Sea, Olasehinde *et al.* (2010) observed a

Table 3. Pearson's multiple correlation matrix of the reactive oxygen species and other river water parameters

Temp.	pH	EC	Na ⁺	NH ₄ ⁺	K ⁺	Cl ⁻	NO ₂ ⁻	NO ₃ ⁻	SO ₄ ²⁻	DO	H ₂ O ₂	a ₃₀₀	R _{OH}	R _{NO•}	R _{O₂}
Temp.	1														
pH	0.358**	1													
EC	0.038	-0.035	1												
Na ⁺	0.019	-0.12	0.912**	1											
NH ₄ ⁺	0.031	-0.184	0.923**	0.888**	1										
K ⁺	0.027	0.139	0.782**	0.688**	0.619**	1									
Cl ⁻	0.039	-0.117	0.925**	0.969**	0.719**	0.539**	1								
NO ₂ ⁻	0.102	0.142	0.725**	0.639**	0.680**	0.587**	0.732**	1							
NO ₃ ⁻	-0.304*	0.052	0.726**	0.579**	0.705**	0.856**	0.390**	0.409**	1						
SO ₄ ²⁻	0.033	-0.320*	0.802**	0.912**	0.452**	0.856**	0.367**	0.252*	0.137	1					
DOC	0.362**	0.123	0.540**	0.364**	0.318*	0.666**	0.482**	0.201	0.079	0.329**	1				
H ₂ O ₂	0.398**	0.264*	0.502**	0.314*	0.400**	0.550**	0.605**	0.203	0.067	0.843**	0.201	1			
a ₃₀₀	0.340*	0.173	0.077	0.115	0.049	0.392**	0.255*	0.804**	0.432**	0.590**	0.605**	0.432**	1		
R _{OH}	0.01	0.136	0.761**	0.539**	0.736**	0.572**	0.978**	0.848**	0.294*	0.881**	0.625**	0.898**	0.225	1	
R _{NO•}	0.22	0.108	0.664**	0.424**	0.506**	0.440**	0.848**	0.162	0.142	0.881**	0.625**	0.898**	0.225	0.520**	1
R _{O₂}	0.456**	0.232	0.179	0.108	0.416**	0.181	0.303*	0.162	0.142	0.881**	0.625**	0.898**	0.225	0.520**	1

*Correlation is significant at the 0.05 level (two-tailed).

**Correlation is significant at the 0.01 level (two-tailed).

linear relationship with NO₂⁻ concentration ($r^2 = 0.98$). In the same vein, Anifowose *et al.* (2015b) observed the same trend ($r^2 = 0.95$) in samples from the Kurose River. Compared with the r^2 value (0.95) of Anifowose *et al.* (2015b), the r^2 value (0.72) obtained in our study, is indicative that some rivers with higher NO₂⁻ concentration had lower photoformed NO• and vice versa. These imbalances could arise from various nitrogen processes (nitrification, denitrification, annamox) which could produce or consume NO• (Schreiber *et al.*, 2012). Apart from the contribution of inorganic form of NO₂⁻ to NO•, the photochemical transformation of organic nitrogen present in dissolved organic matter may produce NO₂⁻ (Kieber *et al.*, 1999) which could in turn generate NO•.

Sources of ¹O₂ A positive and statistically significant relationship ($r^2 = 0.81$, $p < 0.01$) was observed between a_{300} and ¹O₂ in the river water samples (Fig. 4c). DOC also, had a statistically significant correlation ($r^2 = 0.78$, $p < 0.01$) with ¹O₂ (Table 3). Based on the coefficient of determination obtained between the a_{300} and the DOC ($r^2 = 0.71$, $p < 0.01$), one could infer that CDOM is a major component of DOM across the Japanese rivers (Supplementary Fig. S2). Peterson *et al.* (2012), in a multiyear field study on Lake Superior, observed a linear relationship between [¹O₂]_{ss} and a_{300} ($r^2 = 0.88$) and DOC ($r^2 = 0.95$). They concluded that DOC and a_{300} were the best parameters for predicting the production rates of ¹O₂ and the results of our study agree with this. The ¹O₂ producing efficiencies of the rivers we studied were compared by normalizing the results to DOC. The DOC normalized values were in the range of $(2.87\text{--}15.7) \times 10^{-14}$ M mg DOC⁻¹ L with the highest value obtained in the Ohta River, which has the lowest concentration of DOC among the rivers in this study. A study of surface and ocean waters in the Southeastern USA by Zepp *et al.* (1977) gave a DOC-normalized value range of $(1.4\text{--}5.8) \times 10^{-14}$ M mg DOC⁻¹ L. Haag and Hoigne (1986) reported a range of $(0.3\text{--}3) \times 10^{-14}$ M mg DOC⁻¹ L for lake, river, and waste-water samples. They established that large macromolecular structures do not necessarily induce ¹O₂ formation.

Sinks of •OH The •OH sinks with the inorganic anions (Cl⁻, NO₂⁻, NO₃⁻, SO₄²⁻, CO₃²⁻ and HCO₃⁻) were calculated using the following equation:

$$\% \text{ Sink} = k_{i,\bullet\text{OH}} \times [i] / \sum(k_{s,\bullet\text{OH}} [\bullet\text{OH}]) \quad (28)$$

where $k_{i,\bullet\text{OH}}$ is the rate constant for the reaction of the ion with •OH (listed in Supplementary Table S6), $[i]$ is the concentration of the ion, and $\sum(k_{s,\bullet\text{OH}} [\bullet\text{OH}])$ is the scavenging rate constant of •OH across the rivers. The result obtained showed that about 35% of •OH was consumed by these ions (Fig. S3). This suggests that 65% of the photoformed •OH reacts directly with river DOM, and

we can infer that $\bullet\text{OH}$ loss caused by dissolved anions is not an important process. On the basis of the high percentage consumption by DOM, the $\bullet\text{OH}$ sink across the Japanese rivers could be estimated by multiplying the DOC concentration by a general scavenging rate constant of $k_{\text{C},\bullet\text{OH}} = [(7.5 \pm 6.8) \times 10^8 \text{ L (mol C)}^{-1} \text{ s}^{-1}]$ obtained by subtracting the contribution of inorganic species from the overall scavenging rate constant. This is of the same order as the result obtained by Arakaki *et al.* (2013) for terrestrial water samples $[(5.4 \pm 3.6) \times 10^8 \text{ L (mol C)}^{-1} \text{ s}^{-1}]$ from lakes, rivers, reservoirs, and ground-water in countries other than Japan. According to Mopper and Zhou (1990), $k_{\text{C},\bullet\text{OH}}$ may vary according to the source of the DOM and the natural water history. Therefore, we could infer that our estimated value may be a better representation of the major $\bullet\text{OH}$ sinks across Japan. This model was employed in prediction of $\sum(k_{\text{s},\bullet\text{OH}} [\bullet\text{OH}])$ from DOC values reported by Anifowose *et al.* (2015b) and were found to lie within 95% confidence interval of the experimental values of $\sum(k_{\text{s},\bullet\text{OH}} [\bullet\text{OH}])$ of the same study as shown in Supplementary Fig. S4.

Sinks of $\text{NO}\bullet$: *A case study of the Kurose River* Unlike other rivers that were a few times studied, the Kurose River has been well studied for its $\text{NO}\bullet$ activity during the last decade (Anifowose *et al.*, 2015b). It has a record of spatial variation of $\text{NO}\bullet$ ranging from low concentration in the upstream section to high concentrations in the anthropogenically-impacted downstream as also observed in our study. Because of this, calculation of the $\text{NO}\bullet$ flux was carried out for the Kurose River.

From the data obtained from the Japan Meteorological Agency (2020), a $P_{\text{NO}\bullet}$ range of 1–10 ppbv and average wind speed of 2.5 m s^{-1} were recorded at Higashihiroshima within the study period. Using Eqs. (S9) and (S10), these values together with the Kurose River average $\text{NO}\bullet$ concentrations were employed in calculating the $\text{NO}\bullet$ flux ($F_{\text{NO}\bullet}$ in $\text{mol m}^{-2} \text{ s}^{-1}$) between the river and the air boundary layer. From Henry's law constant for $\text{NO}\bullet$ ($k_{\text{H}} \approx 1.9 \times 10^{-3} \text{ M atm}^{-1}$), the average $\text{NO}\bullet$ concentration measured across the river surface ($209 \times 10^{-12} \text{ mol L}^{-1}$), corresponded to an atmospheric $\text{NO}\bullet$ concentration ($P_{\text{NO}\bullet}$ (river)) of $1.1 \times 10^{-7} \text{ atm}$. This value is up to 110 times greater than the actual atmospheric $\text{NO}\bullet$ concentration (1–10 ppbv). Thus, $\text{NO}\bullet$ flux was calculated to range from $1.44\text{--}1.57 \text{ pmol m}^{-2} \text{ s}^{-1}$ and this translates to an annual flux of $(4.65\text{--}5.05) \times 10^2 \text{ g (NO}\bullet) \text{ y}^{-1}$, corresponding to $(2.17\text{--}2.36) \times 10^2 \text{ g N y}^{-1}$ from the Kurose River with an estimated average surface area of 1.02 km^2 (Supplementary Materials). From this estimated value, the atmosphere could be regarded as a sink for river $\text{NO}\bullet$. Supplementary Table S7 shows that the values obtained in our study for the Kurose River were consistent with previous reports by different investigators for natural water samples.

Although $\text{NO}\bullet$ is stable, the presence of its unpaired electron makes it reactive towards other radicals. Equation (13) suggests there is a reaction between $\bullet\text{OH}$ and $\text{NO}\bullet$. From our study, the percentage contribution of $\bullet\text{OH}$ towards $\text{NO}\bullet$ consumption was negligible ($\sim 0.0003\%$), as observed by Anifowose *et al.* (2015b) (Supplementary Materials). The superoxide anion ($\text{O}_2^{\bullet-}$), though not determined in this study, reacts in a diffusion-controlled manner with $\text{NO}\bullet$ to produce peroxyxynitrite (ONOO^-) (Eq. (14)). This reaction is a sink for $\text{NO}\bullet$ (Blough and Zafiriou, 1985; Zielonka *et al.*, 2010; Adesina *et al.*, 2018).

Sinks of $^1\text{O}_2$ $^1\text{O}_2$ can undergo both physical quenching and chemical quenching (DeRosa and Crutchley, 2002). According to Eq. (15), physical quenching with water molecules returns $^1\text{O}_2$ to its ground state without product formation or O_2 consumption, and it is the main mechanism by which it is scavenged (Peterson *et al.*, 2012). There are other dissolved quenchers in natural water, though their combined contributions are far lower than the dissociation rate constant of $^1\text{O}_2$ with water. In chemical quenching, $^1\text{O}_2$ reacts with organic compounds to produce new products (Eq. (16)). Because of the high concentration affinity of $^1\text{O}_2$ to hydrophobic components of DOM (Latch and McNeill, 2006), it can partake in the partial photooxidation of DOM (Cory and Kling, 2018) to produce carboxylic-rich alicyclic molecules (CRAM) which are recalcitrant to biodegradation (Cory *et al.*, 2010). Upon photo stimulation, these compounds could be respired by bacteria to CO_2 , thereby contributing to the aquatic carbon cycle (Cory *et al.*, 2014). Thus, as much as $^1\text{O}_2$, produced by CDOM can also be involved in its transformation, CDOM can be seen as major source and minor quencher of $^1\text{O}_2$ (Latch and McNeill, 2006; Loiselle *et al.*, 2012).

Modelling of the ROS

It is possible to generate a model for the ROS by considering their precursors. Regression analysis using SPSS 22.0 software gave a comprehensive model for the three ROS using their source compounds as independent variables. An average model was obtained for predicting each of the ROS. The main source of $\bullet\text{OH}$ and $\text{NO}\bullet$ was NO_2^- . Hence, the model for $\bullet\text{OH}$ was $R_{\bullet\text{OH}} (10^{-12} \text{ M s}^{-1}) = 19.2 [\text{NO}_2^-] - \mu\text{M} + 36.9$ while that of $\text{NO}\bullet$ was $R_{\text{NO}\bullet} (10^{-12} \text{ M s}^{-1}) = 41.4 [\text{NO}_2^-] - \mu\text{M} + 44$. In the same vein, we modelled $^1\text{O}_2$ from a_{300} and obtained: $R_{^1\text{O}_2} (10^{-9} \text{ M s}^{-1}) = 3.52 (a_{300}) - \text{m}^{-1} + 1.61$. These models were fitted to previous data generated in our laboratory for $\bullet\text{OH}$ and $\text{NO}\bullet$ ($n = 72$) as reported by Anifowose *et al.* (2015b), and $^1\text{O}_2$ ($n = 42$) as presented in Supplementary Table S8. Almost all of the data were found to lay within 95% confidence interval of the experimental values (Supplementary Figs. S5a–c). This is an indication that the mod-

els are viable and could be useful for ROS predictions in Japanese rivers on a large scale.

CONCLUSIONS

This study of different rivers along the west-east axis of Japan revealed possible variations in the photochemical generation rates, steady-state concentrations, scavenging rates, and lifetimes of $\bullet\text{OH}$, $\text{NO}\bullet$, and $^1\text{O}_2$. The major source of photogenerated $\bullet\text{OH}$ and $\text{NO}\bullet$ was NO_2^- while $^1\text{O}_2$ originated from river CDOM. Because of higher concentrations of source compounds in the Kurose and Kokubu rivers, they had higher ROS photoformation rates than the other rivers. The models generated from these data could be used to predict the photoformation rates of the three ROS from known sources in rivers across Japan. The general scavenging rate constant of $\bullet\text{OH}$ as a function of the DOC concentration was [$k_{\text{C},\bullet\text{OH}} = [(7.5 \pm 6.8) \times 10^8 \text{ L (mol C)}^{-1} \text{ s}^{-1}]$]. This equation could be used to estimate the scavenging rate constant of $\bullet\text{OH}$ in river water in Japan from DOC concentrations.

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SUPPLEMENTARY MATERIALS

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 Figures S1 to S5
 Tables S1 to S8