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1 *R E S E A R C H P A P E R*

2

3 **Modelling ROS formation in boreal lakes from interactions**  
4 **between dissolved organic matter and absorbed solar photon flux**

5

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12 A B S T R A C T

13 Concentrations of dissolved organic matter (DOM) are increasing in a large number of lakes  
14 across the Northern hemisphere. This browning serves a dual role for biota by protecting against  
15 harmful ultraviolet radiation, while also absorbing photosynthetically active radiation. The  
16 photochemical activation of DOM and subsequent formation of reactive oxygen species (ROS)  
17 is a potentially harmful side effect, but can be difficult to measure directly *in situ*. In this study,  
18 we combine a data set of physico-chemical properties from 71 Nordic lakes with *in vitro* ROS  
19 formation quantum yields to predict ROS formations across a representative boreal ecosystem  
20 gradient. For the upper centimeter of the water column, we calculate ROS formations in the  
21 range of 7.93–12.56  $\mu\text{mol L}^{-1} \text{h}^{-1}$ . In the first meter, they range between 1.69–6.69  $\mu\text{mol L}^{-1}$   
22  $\text{h}^{-1}$  and in the remaining depth the range is 0.01–0.46  $\mu\text{mol L}^{-1} \text{h}^{-1}$ . These ROS formations are  
23 comparable with previously field-measured hydrogen peroxide formation rates and likely affect  
24 both phyto- and zooplankton, as well as lake chemistry. Interestingly, wavelengths of the visible  
25 spectrum ( $> 400 \text{ nm}$ ) contribute more than half of the overall ROS formation in surface-near  
26 water layers. The association between DOM and ROS formation was found to be two-fold.  
27 While DOM promotes ROS formation in the first centimeters of the water column, the shading  
28 effect of light attenuation overpowers this with increasing depth. In the context of water  
29 browning, our results indicate the emergence of an underestimated oxidative stress environment  
30 for lake biota in the upper centimeters of the water column.

31

32 **Keywords:** Dissolved organic matter, environmental modelling, lake ecology, ROS formation,  
33 ultraviolet radiation

34 **1. Introduction**

35 The increase of dissolved organic matter (DOM) in lakes and rivers of the Northern  
36 hemisphere has been well documented over the past decades (Solomon et al., 2015). Likely  
37 causes for this water browning include decreased deposition of sulfur (Monteith et al., 2007),  
38 changes in vegetation (Larsen et al., 2011; Finstad et al., 2016), transformation of land use  
39 (Evans et al., 2012), shifts in hydrology (de Wit et al., 2016), and climate change in general  
40 (Erlandsson et al., 2008). The major fraction of DOM are highly aromatic organic molecules  
41 (often measured as total organic carbon; TOC), which attenuate light over a broad spectrum of  
42 wavebands. The attenuation is strongest for harmful ultraviolet radiation (UVR; Williamson et  
43 al., 2015) and decreases in exponential fashion with increasing photon wavelength; thus,  
44 photosynthetically active radiation (PAR; Thrane et al., 2014) is also absorbed. This can have  
45 both direct positive and negative effects on biota, since there is a tradeoff between  
46 photoprotection from harmful radiation on the one hand, and strong attenuation of PAR on the  
47 other hand, which reduces photosynthesis in aquatic ecosystems (Thrane et al., 2014; Kelly et  
48 al., 2014; Seekell et al., 2015). An indirect effect of light attenuation is the photoactivation of  
49 DOM and subsequent release of free radicals and reactive oxygen species (ROS; Cooper and  
50 Zika, 1983; Scully et al., 1996; Richard et al., 2007). ROS are known to have an overall negative  
51 impact on biota, e.g., via oxidative stress-mediated membrane damage, lipid oxidation, and  
52 genetic damage (Cooke et al., 2003; Vehmaa et al., 2013). The latter has also been demonstrated  
53 for ROS derived from photoactivated DOM (Wolf et al., 2017).

54 The underlying photochemical mechanisms of ROS formation from photoactivated DOM  
55 are well-studied and described and discussed in detail elsewhere (see, e.g., Richard and  
56 Canonica, 2005). Briefly, the absorbance of photons by DOM molecules results in an increased  
57 electron excitation status (the triplet state of DOM; itself highly reactive), which subsequently  
58 causes spontaneous reactions with surrounding molecular oxygen, cascadingly forming

59 peroxides and oxygen-based radicals, e.g., superoxides, singlet oxygen, and the hydroxyl  
60 radical (Cooper et al., 1988; Dalrymple et al., 2010; Zhang et al., 2014; Krumova and Cosa,  
61 2016).

62 Past efforts to quantify *in situ* ROS formation from photoactivated DOM have mostly  
63 focused on the measurement of hydrogen peroxide formation in lakes (H<sub>2</sub>O<sub>2</sub>; Cooper and Zika,  
64 1983; Cooper et al., 1988; Abele-Oeschger et al., 1997; Croot et al., 2004; Yuan and Shiller,  
65 2005). This is primarily owing to the fact that other ROS have very fast decay rates and may  
66 last for microseconds only; while H<sub>2</sub>O<sub>2</sub>, in comparison, is comparably long-lived, with half-  
67 lifes of up to several days in surface waters (Croot et al., 2004). H<sub>2</sub>O<sub>2</sub> is mainly degraded  
68 enzymatically (Petasne and Zika, 1997), but also via photo-degradation and the Fenton pathway  
69 (Mostofa et al., 2013; Halliwell and Gutteridge, 2015). Rates of photolysis typically range  
70 between 5–50 % of the H<sub>2</sub>O<sub>2</sub> formation rate (Moffett and Zafiriou, 1993; Yocis et al., 2000)  
71 and the consumption of H<sub>2</sub>O<sub>2</sub> in the Fenton pathway is usually multiple orders of magnitude  
72 lower than its production (Moffett and Zika, 1987). *In-situ* measurements of total ROS  
73 formation from photoactivated DOM in lakes are scarce, and often H<sub>2</sub>O<sub>2</sub> concentrations or  
74 formation rates are reported (Kieber et al., 2014; Cory et al., 2016). While measurements of  
75 individual ROS *in situ* are intriguing and deserve strong attention, an approximation of the  
76 overall oxidative stress burden in the aquatic environment can be a starting point for more  
77 detailed future research, especially in the context of water browning.

78 Most laboratory *in vitro* studies achieve photoactivation of DOM by “blitzing” with strong  
79 UVR ( $\lambda \leq 400$  nm) to investigate the total ROS formation capacity. This neglects possible ROS  
80 formation from wavelengths of the visible light spectrum ( $\lambda > 400$  nm; Kieber et al., 2014).  
81 Despite their relative low energy, the total absorbed flux of photons of these wavelengths far  
82 surpasses that of UVR (Gueymard, 2004). In natural systems, the absorption of UVR will be  
83 strongest in the upper layers of the water column, whereas for visible light, the absorption is

84 stronger in deeper waters (Kirk, 2011). Despite a predictably low ROS formation quantum yield  
85 in the visible spectrum, this indicates a potential contribution of visible light to the overall ROS  
86 formation.

87 In the present study, we aim to provide a realistic assessment of the environmental oxidative  
88 stress burden by modelling ROS formation of 71 Nordic lakes along an ecosystem gradient of  
89 varying DOM levels. ROS formation quantum yields for four different wavebands (ultraviolet,  
90 blue, green, and red) were experimentally determined to define the ROS formation quantum  
91 yield as a function of the photon wavelength. This information was combined with multiple  
92 absorption spectra from the lakes. Together with a standardized global solar photon flux, we  
93 modeled total *in situ* ROS formations at different depth layers and throughout the water column.  
94 We further investigated, if, despite a low ROS formation quantum yield, visible light provides  
95 a relevant share of the total amount of ROS formation.

96 **2. Material and methods**

97 *2.1. In-vitro determination of ROS formation quantum yields*

98 Wavelength-dependent ROS formation quantum yields were determined in *in vitro* assays,  
99 based on the assumption that photoactivated DOM will produce ROS. The source of DOM was  
100 the Nordic Aquatic Humic Acid Reference (International Humic Substances Society, St. Paul,  
101 MN, USA), which was isolated using reverse osmosis (Gjessing et al., 1999). It was dissolved  
102 in double distilled H<sub>2</sub>O (0, 5, 10, and 20 mg C L<sup>-1</sup>) and concentrations were verified on a TOC-  
103 V<sub>CPH</sub> Total Organic Carbon Analyzer (Shimadzu, Kyoto, Japan).

104 UVA- and red-green-blue (RGB) -radiation exposures (390, 450, 510, and 630 nm) were  
105 conducted using two fully programmable 96-LED boards (UVA: Microwell 96 LED Controller,  
106 Version 3.2, 13.06.2014; RGB: Microwell 96 RGB Controller, Version 1.0, 19.08.2014;  
107 [https://tindie.com/stores/Dead\\_Bug\\_Prototypes/](https://tindie.com/stores/Dead_Bug_Prototypes/); Dead\_Bug\_Prototypes, Sandnes, Norway) at  
108 20 μmol m<sup>-2</sup> s<sup>-1</sup>. The photon flux irradiance was scripted in Arduino (version 1.8.1;  
109 <https://www.arduino.cc/en/Main/Software/>) and calibrated using a SpectraPen LM 500-UVIS  
110 spectroradiometer (Photon Systems Instruments, Drásov, Czech Republic).

111 The determination of individual ROS formations for the four different wavelengths followed  
112 the method described in Wolf et al. (2017), which is based on the principles explained by  
113 Marchesi et al. (1999) and Gomes et al. (2005). In the first step, the non-fluorescent 2',7'-  
114 dichlorofluorescin diacetate (DCFH-DA; Sigma-Aldrich, St. Luis, MO, USA) was  
115 enzymatically deacetylated to (non-fluorescent) 2',7'-dichlorofluorescin (DCFH) with 20 U  
116 esterase (from porcine liver; CAS 9016-18-6; Sigma-Aldrich). DCFH reacts with ROS to form  
117 the fluorescent 2',7'-dichlorofluorescein (DCF). DCFH is a suitable probe as proxy for "total"  
118 ROS, as it is relatively unspecific (Chen et al., 2010). It detects – directly or indirectly –  
119 peroxides (Zmijewski et al., 2010), hydroxyl radicals (Cohn et al., 2008), superoxide anions

120 (Marchesi et al., 1999), and singlet oxygen (Douillard et al., 2011). While it does not provide  
 121 information on the different ROS, which may also differ in their reactivity, it is an elegant probe  
 122 for an approximation of the overall ROS formation levels.

123 Five replicates of each DOM × irradiance combination were prepared in black 96-well plates  
 124 (Nunc 96F Nontreated MicroWell; Thermo Fisher Scientific, Roskilde, Denmark) and 25 μmol  
 125 DCFH L<sup>-1</sup> added to each well (100 μL volume in total). A hydrogen peroxide standard (0.03–  
 126 72.1 nmol L<sup>-1</sup>; in triplicates) was used as reference. The plates were incubated at 20 ± 0.3 °C  
 127 for an hour, before measuring fluorescence on a BioTek Synergy Mx plate reader (BioTek  
 128 Instruments; Winooski, VT, USA). As recommended by the manufacturer, the excitation  
 129 wavelength was 504 nm and fluorescence was measured at 529 nm. The experiment was run  
 130 three times, resulting in three independent values per wavelength. ROS formations ( $C_{\text{ROS}}$ ; mol  
 131 m<sup>-3</sup>) were calculated from the fluorescence intensities in relation to the hydrogen peroxide  
 132 standard curve.

133 The amount of absorbed photons ( $E_{\text{abs,ROS}}$ ; mol) was calculated as:

$$E_{\text{abs,ROS}} = \frac{q_{\text{p},\lambda} \times (1 - 10^{-a(\lambda)})}{V} \times t \quad (1)$$

134 Here,  $q_{\text{p},\lambda}$  is photon flux (mol m<sup>-2</sup> s<sup>-1</sup>) at the given wavelength (390, 450, 510, or 630 nm),  
 135  $a(\lambda)$  (m<sup>-1</sup>) the *in vitro* absorbance of DOM at the given wavelength,  $V$  (m<sup>3</sup>) the exposure  
 136 volume, and  $t$  (s) the experimental time (IUPAC, 2006). Individual ROS formation quantum  
 137 yields ( $\Phi_{\text{ROS}}$ ; dimensionless) were determined using the amount of absorbed photons ( $E_{\text{abs,ROS}}$ ):

$$\Phi_{\text{ROS}} = \frac{C_{\text{ROS}}}{E_{\text{abs,ROS}}} \quad (2)$$

138 The ROS formation quantum yield was then modelled as a function of the wavelength  
 139 ( $\Phi_{\text{ROS}}(\lambda)$ ), assuming an exponential hyperbolic relationship, which has been proven to be  
 140 suitable for other quantum yields of photo-activated DOM (Zhang et al., 2006; Xie et al., 2009):



$$\Phi_{\text{ROS}}(\lambda) = m_1 \times e^{\frac{m_2}{\lambda - m_3}}. \quad (3)$$

141 Here,  $m_1$  (dimensionless),  $m_2$  (nm), and  $m_3$  (nm) are fitting parameters, and  $\lambda$  (nm) is the photon  
 142 wavelength. To obtain the standard deviation for the ROS formation quantum yield function,  
 143 1'000'000 Monte Carlo simulations were run on the posterior residual distribution and average  
 144 values for the standard deviation were calculated from these simulations. A visualization of the  
 145 ROS formation quantum yield is shown in SI-Figure 1 of the Supporting information.

146

## 147 2.2. Predictive modelling of ROS formation in lakes

148 Seventy-seven Nordic lakes were sampled in July and August 2011. The details of the  
 149 monitoring and sampling regime are given by Thrane et al. (2014). As some spectral endpoints  
 150 could not be obtained for all lakes, only 71 were part of the modelling approach in this  
 151 communication.

152 While Thrane et al. (2014) used individual, lake- and date-specific irradiances, the data set  
 153 only contained data for 400–700 nm. To accommodate for other wavelengths, especially the  
 154 UVR waveband < 400 nm, we used the current standard model for solar irradiance ASTM  
 155 G137-03, with an air mass of 1.5 atmospheres at a 37 ° global tilt (downloaded from  
 156 <http://ampsmodeling.org/spectralData.html>; ASTM, 2012). This spectrum represents a global  
 157 annual average of the solar photon flux that arrives on the earth's surface. A visual excerpt is  
 158 given in SI-Figure 2 in the Supporting information. The spectral photon flux ( $E_p(\lambda)$ ; mol m<sup>-2</sup>  
 159 s<sup>-1</sup> nm<sup>-1</sup>) from 280–4000 nm was used for all subsequent calculations.

160 Absorption spectra for DOM from lake samples ( $a_{\text{DOM}}(\lambda)$ ; m<sup>-1</sup>; Twardowski et al., 2014),  
 161 non-algal particles from lake samples ( $a_{\text{NAP}}(\lambda)$ ; m<sup>-1</sup>; Shen et al., 2012), phytoplankton from  
 162 lake samples ( $a_{\text{PP}}(\lambda)$ ; m<sup>-1</sup>; Küpper et al., 2007; Thrane et al., 2015), and standardized water  
 163 ( $a_{\text{Water}}(\lambda)$ ; m<sup>-1</sup>; Wozniak and Dera, 2007) were modelled. Procedures and equations are detailed

164 in the Supporting information (SI-Figures 3–6). All absorption coefficients were used for  
 165 extrapolation outside their initially measured range, i.e., to cover both parts of the light  
 166 spectrum below 400 nm and above 700 nm. The total absorption coefficient spectrum ( $a_{\text{Total}}(\lambda)$ ;  
 167  $\text{m}^{-1}$ ) was calculated as the sum of  $a_{\text{DOM}}(\lambda)$ ,  $a_{\text{PP}}(\lambda)$ ,  $a_{\text{NAP}}(\lambda)$ , and  $a_{\text{Water}}(\lambda)$  (Kirk, 2011). The  
 168 relative contribution of DOM to the total absorption ( $k_{\text{DOM}}(\lambda)$ ) was calculated as the quotient of  
 169  $a_{\text{DOM}}(\lambda)$  and  $a_{\text{Total}}(\lambda)$ ; see SI-Figure 7 for a visualization.

170 The wavelength-specific photon flux absorption for each lake was modelled. The amount of  
 171 DOM-absorbed photons per depth unit ( $E_{\text{abs,p}}(\lambda)$ ;  $\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$ ) for each wavelength was  
 172 calculated as follows ( $z$  is the depth; m):

$$E_{\text{abs,p}}(\lambda) = E_{\text{p}}(\lambda) \times e^{-a_{\text{Total}}(\lambda) \times z} \times k_{\text{DOM}}(\lambda). \quad (4)$$

173 Wavelength specific ROS formation quantum yields ( $\Phi_{\text{ROS}}(\lambda)$ ) were calculated using  
 174 equation (3) from the *in vitro* studies. The ROS formation ( $C_{\text{ROS}}(\lambda)$ ;  $\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$ ) was  
 175 calculated by multiplying the amounts of absorbed photons at a given wavelength with the  
 176 wavelength-specific ROS formation quantum yield:

$$C_{\text{ROS}}(\lambda) = E_{\text{abs,p}}(\lambda) \times \Phi_{\text{ROS}}(\lambda). \quad (5)$$

177 To eliminate wavelength dependency of the ROS formation, the integral of  $C_{\text{ROS}}(\lambda)$ , was  
 178 calculated using the trapezoid method from 280–4000 nm ( $C_{\text{ROS}}$ ;  $\text{mol m}^{-2} \text{s}^{-1}$ ). Integrating  
 179 equation (5) over depth intervals allows for the calculation of ROS formation in volumetric  
 180 layers ( $C_{\text{ROS}}(z)$ ;  $\text{mol m}^{-3} \text{s}^{-1}$ ):

$$C_{\text{ROS}}(z) = \int_{z_0}^{z_1} C_{\text{ROS}} dz. \quad (6)$$

181 where  $z_0$  is the starting depth (m) and  $z_1$  the ending depth (m) of the integration. With this, it is  
 182 possible to calculate the ROS formation for any desirable volumetric layer of the water column.  
 183 In this study, absorbed photons for layers of the first centimeter (0–0.01 m), meter (0.01–1 m),  
 184 and maximum lake depth (1 m–lake depth; m) were calculated.

185 To identify a threshold between positive and negative influence of DOM on ROS formation,  
 186 the relationship between TOC ( $c_{\text{TOC}}$ ;  $\text{mg L}^{-1}$ ) in lakes and areal ROS formation ( $C_{\text{ROS}}$ ;  $\text{mol m}^{-2}$   
 187  $\text{s}^{-1}$ ) was analyzed for depth dependency assuming an exponential relationship:

$$C_{\text{ROS,A}} = A_{\text{TOC}} \times e^{-s_{\text{TOC}} \times c_{\text{TOC}}}. \quad (7)$$

188 The curve parameters for shape  $A_{\text{TOC}}$  ( $\text{mol m}^{-2} \text{s}^{-1}$ ) and slope  $s_{\text{TOC}}$  ( $\text{L mg C}^{-1}$ ) were fit in the  
 189 modelling procedure. The areal ROS formation from the wavelength integral of equation (5)  
 190 was determined for a 100-step sequence between 0–0.1 m. For all 100 ROS formations,  
 191 individual models of equation (7) were fit and the slope parameter  $s_{\text{TOC}}$  was extracted. The slope  
 192 parameter was used as proxy for the correlation between DOM and ROS production, i.e., a  
 193 positive slope indicates that DOM has a positive influence on ROS formation, whereas a  
 194 negative slope means DOM has a negative influence. The value of the slope shows the intensity  
 195 of this relationship.

196

### 197 2.3. Statistical analyses

198 All data was analyzed using open-source statistical software R (version 3.4.1; R Core Team,  
 199 2017) and its add-on packages MASS (version 7.3-47; Venables and Ripley, 2002), nlme  
 200 (version 3.1-131; Pinheiro et al., 2017) and MuMIn (version 1.15.6; Bartoń, 2016).

201 To investigate the correlations between ROS formations and abiotic parameters, linear  
 202 mixed-effects (LME) models were applied. For three discrete volumetric ROS formations,  
 203 namely in the first centimeter (0–0.01 m), the first meter (0.01–1 m), and in the remaining water  
 204 column (1 m–maximum lake depth; m), these LME models were applied. The explanatory  
 205 variables were lake chemical parameters total iron (Fe;  $\mu\text{g L}^{-1}$ ), total nitrogen (TN;  $\text{mg L}^{-1}$ ),  
 206 total organic carbon (TOC;  $\text{mg L}^{-1}$ ), and total phosphorus (TP;  $\mu\text{g L}^{-1}$ ). None of these four  
 207 parameters have been used in the modelling of the three volumetric ROS formations.

208 Before analyses, the respective response variables were power-transformed to maximize log-  
209 likelihood (Box and Cox, 1964). To identify a suitable set of combinations of the explanatory  
210 variables, the selection of parametrization was based on Akaike's corrected information  
211 criterion (AICc; Akaike, 1974; Burnham and Anderson, 2004). For this, the AICc of all possible  
212 model combinations was determined using maximum likelihood. All models within a  $\Delta$  of 2 of  
213 the lowest AICc were updated using restricted maximum likelihood. Model averaging of the  
214 full model was then used to summarize the information contained in the competing "best"  
215 models, i.e., to find the ideal parametrization (Burnham and Anderson, 2002).

216 After determination of the final set of parameter combinations for each model, the influence  
217 of fixed-effect variables of the averaged full models was analyzed in Wald  $F$ -tests with  
218 marginal (type III) sum-of-squares (Pinheiro and Bates, 2000; Li and Redden, 2015), with a  
219 significance threshold of  $P < 0.05$ . Unless stated otherwise, all results are given as mean  $\pm$   
220 standard deviation.

## 221 3. Results

### 222 3.1. In-vitro assays and modelling

223 The *in vitro* part of this study produced a ROS formation quantum yield model. It is  
 224 dependent on the photon wavelength and decreasing exponential hyperbolically (SI-Figure 1).  
 225 Individual *in vitro* ROS quantum yields for the four different wavelengths were  $8.00 \pm 2.70 \times$   
 226  $10^{-3}$  for 390 nm,  $3.00 \pm 1.17 \times 10^{-3}$  for 450 nm,  $1.12 \pm 0.52 \times 10^{-3}$  for 510 nm, and  $0.16 \pm 0.09$   
 227  $\times 10^{-3}$  for 630 nm. This is in line with previously published quantum yields for the formation  
 228 of triplet state DOM, singlet oxygen, and the hydroxyl radical from photo-activated DOM under  
 229 irradiance from UV-B, UV-A, and blue wavebands (Marchisio et al., 2015). The model  
 230 parameters for equation (3) were estimated as follows (mean  $\pm$  standard error):  $m_1 = 5.03 \pm 24.3$   
 231  $\times 10^{-5}$ ,  $m_2 = 1134 \pm 2605$  nm, and  $m_3 = -166.8 \pm 302.7$  nm. The modelling of DOM absorption  
 232 spectra was possible for 71 lakes. A graphical summary of the DOM absorption spectra is  
 233 shown in SI-Figure 3. The fraction of DOM-absorbed photons decreases monotonically from  
 234  $92.1 \pm 7.7$  % at 280 nm to  $5.0 \pm 3.6$  % at 720 nm (SI-Figure 7).

235

### 236 3.2. Areal ROS formation

237 Areal ROS formation throughout the water column was strongly dependent on the  
 238 wavelength of the absorbed photons (Figure 1). In the upper layers of the water column, the  
 239 contribution of the UVR waveband to the ROS formation was highest, with a maximum of  $44.1$   
 240  $\pm 2.4$  % at the surface. The notable increase in ROS formation around 400 nm is a result of the  
 241 increasing solar photon flux (SI-Figure 2). With increasing depth, this contribution diminishes  
 242 and can be considered negligible for depths below one meter (Table 1). ROS formation  
 243 throughout the water column shows a characteristic exponential decline (Figure 2). The  
 244 calculated average ROS formation at the surface was  $3.20 \pm 0.28 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . At depths of

245 one and ten meters, calculated ROS formations were  $0.39 \pm 0.21 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $0.03 \pm 0.06$   
246  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. The turning point, where the correlation between DOM and ROS  
247 formation switches from positive to negative, was calculated at a depth of 1.23 cm (95%  
248 confidence interval: 0.71–1.82 cm; Figure 3). Below this turning point, DOM has a negative  
249 effect on ROS production, owing to the attenuation and the shift in the wavelength composition  
250 towards longer wavelengths, which are less effectively absorbed by DOM (cf. SI-Figure 7).  
251 While DOM is a determinant for this threshold, it is surprisingly shallow in all lakes.

252

### 253 3.3. Volumetric ROS formation

254 Volumetric ROS formation followed a similar pattern as its areal equivalent above, as it  
255 decreased with depth (Figure 4). In the first centimeter, ROS formation was highest with  $11.11$   
256  $\pm 0.88 \mu\text{mol L}^{-1} \text{h}^{-1}$ . Over the first meter of the water column, this value decreased to  $3.37 \pm$   
257  $1.07 \mu\text{mol L}^{-1} \text{h}^{-1}$ , and further decreased when integrating over the remaining lake depth ( $0.13$   
258  $\pm 0.11 \mu\text{mol L}^{-1} \text{h}^{-1}$ ). In the first centimeter of the water column, ROS formation was positively  
259 associated with TOC, but negatively with TP; TN and Fe were not significantly correlated to  
260 ROS formation. In the first meter of the water column, ROS formation was negatively  
261 correlated with Fe, TOC, and TP; the correlation with TN was again not significant. For ROS  
262 formation in lake depths below one meter, only Fe and TP had a significant negative correlation;  
263 TOC and TN had no significant association with ROS formation. Details on model parameters  
264 are given in SI-Table 1.

**265 4. Discussion**

266 This study provides a mathematical modelling framework to study ROS formation in natural  
267 systems along an environmentally relevant ecosystem and wavelength gradient. As Andrews et  
268 al. (2000) pointed out, wavelength composition of the absorbed photons has a significant impact  
269 on apparent quantum yields in nature, e.g., for photo-bleaching, O<sub>2</sub> uptake, and H<sub>2</sub>O<sub>2</sub>  
270 production. Here, we extend the UVR-based approach of previous studies (Scully et al., 1996;  
271 Scully et al., 1997) to lake systems and low, but realistic UVR exposures.

272 Interestingly, our results indicate that the UVR waveband contributes, at most, half of the  
273 total ROS formation. The majority of the photochemically formed ROS stem from wavebands  
274 of the lower visible light spectrum (Figure 1; Table 1). This can be directly attributed to our  
275 modeling approach, i.e., using a standardized irradiance spectrum as starting point for  
276 modelling (SI-Figure 2). While previous studies already pointed towards UV-A being more  
277 important than UV-B for photochemical ROS formation in natural systems (Abele-Oeschger et  
278 al., 1997), our calculations take into account the overall contribution of UVR and visible light  
279 to the total ROS formation, demonstrating that also shortwave visible light (blue and green)  
280 contributes substantially to ROS formation. This can be interpreted as a direct consequence of  
281 using the solar irradiance, which significantly increases around a wavelength of 400 nm (SI-  
282 Figure 2). Furthermore, despite using wavelengths of up to 4000 nm, there is no noteworthy  
283 impact of photons with wavelengths > 700 nm to the overall ROS formation (Figure 1). This is  
284 due to our modelling approach, combining the solar photon flux with the sharply declining ROS  
285 formation quantum yield; and proves the robustness of our modelling methodology.

286 Unsurprisingly, we find a clear depth dependency of both areal and volumetric ROS  
287 formation. Despite the contribution from shortwave visible light, almost the entire ROS  
288 production takes place in the upper surface layer, with a sharp decline along the first meter.  
289 Even at modest concentrations of DOM, ROS formation becomes virtually negligible at depths

290 below one meter. This mirrors the previously published depth profiles of peroxide formation in  
291 lakes by Scully et al. (1997).

292 Our calculated ROS formations in the first meter of the water column are within the range  
293 of previously reported *in situ* H<sub>2</sub>O<sub>2</sub> formations in lakes (Scully et al., 1996; Abele-Oeschger et  
294 al., 1997; Scully et al., 1997; Cory et al., 2016), albeit being up to ten times higher in their  
295 maximum values. This is not unexpected, as we modelled total ROS instead of H<sub>2</sub>O<sub>2</sub>, and  
296 former studies did not necessarily specify the sampling depth, which has a great influence on  
297 overall ROS formation (Figures 1 and 2). Furthermore, the photo-activated triplet state of DOM  
298 is believed to contribute significantly to the overall reactivity (Timko et al., 2014; Marchisio et  
299 al., 2015).

300 While our study thus provides insight into an approximation of the overall oxidative stress  
301 burden and total ROS formation in these lakes, the biological consequences on ecosystem scale  
302 are hard to decipher, and will depend largely on the individual ROS. Their unlike  
303 characteristics, e.g., concerning half-life, reactivity, and steady-state concentration, will  
304 eventually determine the consequences for biota in natural systems.

305

#### 306 4.1. Drivers of ROS formation *in situ*

307 TOC offers two contrasting “modes of action” for ROS formation in aquatic systems: the  
308 formation of ROS from photoactivated DOM in the upper layers *versus* the strong light  
309 attenuation with increasing depth. At the very surface, TOC is strongly associated with ROS  
310 formation, while in depths below one meter, TOC was no longer correlated to ROS formation.  
311 Similar to TOC, TP changed its correlation to ROS formation with increasing depth; this is not  
312 surprising, as TOC and TP are closely correlated in natural systems (Chen et al., 2015).  
313 Interestingly, the correlation between Fe and ROS formation increases with depth. This could  
314 be due to the possible influence of Fe on the absorption spectra of DOM. As Fe and TOC are



315 strongly associated in boreal lakes (Maranger and Pullin, 2003; Weyhenmeyer et al., 2014), this  
316 could indicate a qualitative switch between the two factors in the context of ROS formation.  
317 While TOC is a good predictor for ROS formation in surface-near regions, Fe-based predictions  
318 fare better in deeper waters.

319 Besides from photoactivated DOM, ROS formation could also be induced via photoactivated  
320 nitrate and nitrite (Vaughan and Neil, 1998). But even the eutrophic boreal lakes under  
321 investigation in this study were a lot richer in organic carbon (Thrane et al., 2014), and thus the  
322 contribution of inorganic nitrogen to the overall ROS formation is likely not strong. Similarly,  
323 Fe is known to both influence DOM absorption spectra (Thrane et al., 2014; Molot et al., 2015)  
324 and induce ROS formation via the Fenton pathway (Mostofa et al., 2013), but was only  
325 measured in low concentrations.

326 Another key driver of photochemical ROS formation in natural systems is the solar photon  
327 flux. We used a standardized spectrum to even out effects of seasonality and other confounding  
328 factors, e.g., cloud cover or variability in the stratospheric ozone layer (ASTM, 2012). In nature,  
329 seasonality and confounding factors influence the solar photon flux massively (Nann and  
330 Riordan, 1991; Sikorski and Zika, 1993a; Sikorski and Zika, 1993b; Dye, 2004). Subsequently,  
331 ROS formation in natural systems is also tied to the same fluctuations (Cooper and Lean, 1989).  
332 Nevertheless, our calculations even these effects out in long-term scenarios by using a  
333 standardized solar photon flux.

334

#### 335 *4.2. Implications for lake biota*

336 ROS formation negatively affects bacterial communities, while having beneficial effects on  
337 algal growth (Xenopoulus and Bird, 1997; Drábková et al., 2007; Baltar et al., 2013). However,  
338 because of the photon absorbance-related breakdown of DOM, smaller organic molecules result  
339 in more readily available carbon sources for protists (Lindell et al., 1996; Moran and Zepp,

340 1997; Tranvik and Bertilsson, 2001; Kissman et al., 2017). A recent study has also  
341 demonstrated detrimental effects of natural ROS formation on DNA integrity of zooplankton  
342 (Wolf et al., 2017), and the ROS formations calculated in this study are in proximity of those  
343 applied by Wolf et al. (2017). Most ROS, with the exception of H<sub>2</sub>O<sub>2</sub>, immediately decay and  
344 exist at steady-state concentrations in natural systems, thus only affecting biota when being  
345 formed in the immediate vicinity of organism. H<sub>2</sub>O<sub>2</sub> concentrations could potentially build up  
346 during the day, possibly affecting small biota, e.g., microbes (Moffett and Zafiriou, 1990;  
347 Mostofa and Sakugawa, 2009).

348 Planktonic biota in the upper layers of lakes could – at the very least – be sensitized by  
349 increased ROS formation. This could increase vulnerability, e.g., to anthropogenic pollutants  
350 (Lushchak, 2011; Bundschuh and McKie, 2016). It is also likely that a prolonged state of  
351 sensitization affects plankton life history in the long term (Yurista and O'Brien, 2001).  
352 Upregulations of defense mechanisms (e.g., anti-oxidant enzymes, DNA-repair enzymes, and  
353 pigmentation; MacFayden et al., 2004; Rautio and Tartarotti, 2010) would withdraw energy  
354 reserves from other metabolic cravings, such as growth and reproduction.

355 In the context of an increased water browning, i.e., increasing levels of DOM, the results of  
356 our study show a dual impact for biota. An increase in DOM will increase the ROS formation  
357 in the upper centimeters (Figure 3). This can affect phytoplankton, microbes, and pelagic  
358 zooplankton residing close to the surface. On the other hand, increased water browning will  
359 also increase the attenuation of light (Thrane et al., 2014). Thus, the ROS formation in the water  
360 column below the first few centimeters will experience a decrease in ROS formation, as less  
361 photons reach the DOM molecules in deeper waters.

362 **5. Conclusions**

363 Several studies have demonstrated a current water browning (i.e., increased levels of DOM)  
364 of surface waters in boreal areas (Williamson et al., 2015; de Wit et al., 2016; Finstad et al.,  
365 2016). With more DOM in freshwater ecosystems, the photochemical formation of ROS is  
366 affected. In this study, we have combined an *in vitro* ROS formation quantum yield model with  
367 monitoring data to assess how ROS formation in lakes is related to DOM, depth, and  
368 wavelength. This allowed for determination of both depth- and wavelength-dependency of the  
369 modeled ROS formation.

370 Interestingly, visible light is responsible for a significant share of ROS formation, with UVR  
371 having its strongest influence at the surface. ROS formations have a sharp vertical decline, and  
372 our results indicate that it is only important in the first meter of the water column. The influence  
373 of DOM on ROS formation changes with depth. While promoting ROS formation in the first  
374 centimeters, the light-attenuating properties of DOM remove photons from the water column,  
375 effectively shielding organisms.

376 Whether ROS formation in natural systems will be detrimental for biota depends, amongst  
377 other factors, on duration, degradation rates, and the mixing regime. Additionally, there may  
378 be more complex interactions, e.g., if certain clades of biota are more susceptible than others  
379 (Lindholm et al., 2016). Still, the net effect on ecosystem productivity will likely be negative,  
380 owing to the dominating role of light attenuation and thus reduced primary production. As water  
381 browning continues in freshwater systems of the Northern hemisphere, this showcases the  
382 multifaceted role of DOM in natural systems.

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386 **Appendix A. Supporting information**

387 Supporting information on the Material and methods and detailed statistical results can be  
388 found at the online version of this article.

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632 **Tables**

633 **Table 1**

634 Relative contributions of UVR ( $\lambda < 400$  nm) and visible light ( $\lambda \geq 400$  nm) to the overall ROS  
 635 formation in six discrete depth layers. Data is presented as mean  $\pm$  standard deviation. For the  
 636 ROS formation at a depth of ten meters, lakes shallower than ten meters were excluded from  
 637 the calculations; hence, the lower  $n$ . Note the steep decrease of UVR's relative contribution  
 638 within the first meter of the water column.

Two-dimensional layer	Depth (m)	Relative contribution to ROS formation		$n$
		$\lambda < 400$ nm	$\lambda \geq 400$ nm	
Surface	0	44.1 $\pm$ 2.4 %	55.9 $\pm$ 2.4 %	71
One millimeter	0.001	43.8 $\pm$ 2.5 %	56.2 $\pm$ 2.5 %	71
One centimeter	0.01	41.4 $\pm$ 3.6 %	58.6 $\pm$ 3.6 %	71
One decimeter	0.1	24.1 $\pm$ 10.1 %	75.9 $\pm$ 10.1 %	71
One meter	1	1.8 $\pm$ 4.5 %	98.2 $\pm$ 4.5 %	71
Ten meters	10	0.0 $\pm$ 0.0 %	100.0 $\pm$ 0.0 %	54

639

640 **Figures captions**

641 **Fig. 1.** Wavelength-dependent ROS formation ( $\text{nmol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$ ) for three different two-  
 642 dimensional depth layers (surface, one meter, and ten meters; indicated on the right side of each  
 643 graph). The notable increase in ROS formation around 400 nm is a result of the increasing solar  
 644 photon flux (SI-Figure 2). Note the changing range of the y-axis and the decreasing contribution  
 645 of UVR radiation to ROS formation with increasing depth (see also Table 1). Solid lines are  
 646 mean values and shaded areas represent the standard deviation.  $n = 71$  for the surface and one  
 647 meter depth, and  $n = 54$  for ten meters depth.

648

649 **Fig. 2.** Depth profile of ROS formation ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in 74 Nordic lakes. Note that the y-axis  
 650 has been logarithmized for better visualization and to accommodate the steep decrease of ROS  
 651 formation within the first meter of the water column. Solid line is the mean and the shaded area  
 652 represents the standard deviation.

653

654 **Fig. 3.** Depth profile of the slope parameter ( $s_{\text{TOC}}$ ) for the correlation between DOM ( $\text{mg TOC}$   
 655  $\text{L}^{-1}$ ) and areal ROS formation ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), as expressed in equation (7).  $s_{\text{TOC}}$  is used as proxy  
 656 for the quality and quantity of this correlation (see Material and methods). The solid line shows  
 657 the depth profile of the  $s_{\text{TOC}}$  values and the shaded area is the 95 % confidence interval of  $s_{\text{TOC}}$ .  
 658 Positive  $s_{\text{TOC}}$  values can be interpreted as DOM having a positive correlation with ROS  
 659 formation; whereas negative values indicate a negative correlation of DOM on ROS formation.  
 660 The dotted line represents the “turning point” of the correlation, i.e., the depth where the quality  
 661 of the correlation between DOM and ROS formation changes from positive to negative.

662



663 **Fig. 4.** Overview of the influence of DOM (mg TOC L<sup>-1</sup>) on ROS formation ( $\mu\text{mol L}^{-1} \text{h}^{-1}$ ) for  
664 three different volumetric depth layers (first centimeter, first meter, and remaining lake depth;  
665 indicated on the right side of each graph). The solid line is the prediction of the AICc-based  
666 fully averaged LME model (see Material and methods) and the shaded area is the 95 %  
667 confidence interval. Notice the changing ranges of the y-axes and the qualitative change in  
668 correlation between the first centimeter (positive) and the first meter (negative) and remaining  
669 lake depth (nonexistent). Orange color indicates significant correlations of ROS formation and  
670 DOM (see Material and methods).