Title: Probing the photochemical reactivity of deep ocean refractory carbon (DORC): lessons from hydrogen peroxide and superoxide kinetics.

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24 Abstract

25 Most marine DOC is thought to be biologically-recalcitrant, especially that in the deep
26 ocean pool (> 1000 m). In particular, the deep waters of the North Pacific should contain the
27 most recalcitrant DOC because they do not form locally, with deep DOC having survived long
28 isolation from the surface during global-scale thermohaline circulation. One of the proposed
29 removal pathways involves photochemical reactions when refractory DOC circulates through
30 sunlit surface waters (Mopper et al., 1991). Here, we reevaluate the general photoreactivity of
31 refractory DOC by investigating the photochemical production of two reactive oxygen species
32 (ROS), hydrogen peroxide (H_2O_2) and superoxide (O_2^-), using controlled irradiations at sea and
33 in the laboratory. The photoproduction of these two ROS were compared between deep and
34 surface water samples collected in the Gulf of Alaska. For irradiated samples, initial superoxide
35 steady-state concentrations ($[\text{O}_2^-]_{\text{ss}}$) and first-order decay constants (k_{pseudo}) were similar between
36 surface and deep waters, ranging from $\sim 1 - 4$ nM and $4 - 12 \times 10^{-3} \text{ s}^{-1}$, respectively. Initial
37 photoproduction rates were comparable between surface and deep waters, ranging from $22 - 173$
38 nM h^{-1} for O_2^- and from $1 - 8.3 \text{ nM h}^{-1}$ for H_2O_2 , indicating that a large portion of photoproduced
39 O_2^- does not lead to H_2O_2 formation. In fact, the photoproduction ratio of O_2^- to H_2O_2 averaged
40 $\sim 4:1$ in paired experiments, instead of the $2:1$ stoichiometry expected for O_2^- dismutation to
41 form H_2O_2 . Continued irradiation for up to 48 hours showed photoproduced H_2O_2 to be much
42 lower in deep samples compared to surface samples with accumulation slowing or stopping in
43 deep samples despite both measurable $[\text{O}_2^-]_{\text{ss}}$ and photon absorbance by colored dissolved
44 organic matter (CDOM). These results are consistent with a loss of source material (i.e. CDOM)
45 for O_2^- photoproduction and a shift to a predominantly oxidative pathway for O_2^- decay. Low
46 photoproduction rates, loss of continued accumulation with extended radiation, and an apparent

47 loss of O_2^- source material argues that the deep refractory DOC pool is less photochemically
48 reactive than previously suggested.

60 **Keywords**

61 Hydrogen peroxide, superoxide, photochemistry, dissolved organic carbon, CDOM, Gulf of
62 Alaska, deep sea

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1. Introduction

The marine dissolved organic carbon (DOC) pool contains 662 ± 32 Pg C (Hansell et al., 2009) and is therefore one of Earth's largest active carbon reservoirs, similar in size to the carbon stored as CO₂ in the atmosphere. Because it remains largely uncharacterized, marine DOC has been classified based on qualitative assessments of its reactivity, ranging from labile DOC that is turned over on time scales of minutes to days to refractory DOC that survives from years to millennia (Carlson, 2002; Kirchman et al., 1993). Refractory DOC is largely biologically unavailable, with a mean radiocarbon age of 4000 – 6000 years (Bauer et al., 1992; Williams and Druffel, 1987), and comprises the majority of the oceanic carbon pool (~642 Pg C; Hansell, 2013). Because refractory DOC turns over on timescales of millennia, understanding changes in this pool may provide insight into its role in past and future climates (Sexton et al., 2011). For instance, ventilation and oxidation of DOC from the ocean's interior has been implicated in rapid hyperthermal warming events during the Palaeogene period (Sexton et al., 2011).

Refractory DOC is ubiquitous in the ocean, but in the deep ocean (>1000 m), the DOC pool is almost entirely refractory. Unfortunately, DOC dynamics in the deep sea remain largely unstudied due to the large spatial and temporal scales required to comprehensively sample deep ocean basins, even though deep ocean refractory carbon (DORC) represents ~70% of the entire DOC pool (Hansell et al., 2009). Recent analysis suggests that refractory DOC may be conserved during the majority of its circulation until reaching unknown local sinks that exist in both the deep far North Pacific and mid depth subtropical South Pacific (Hansell and Carlson 2013). On the other hand, one identified sink for refractory DOC appears to be via UV photooxidation as it circulates through the surface ocean (Mopper et al., 1991; Stubbins et al., 2012). Based on higher

photoproduction rates for select low molecular weight carbonyl compounds in short (< 5 h) irradiations of deep Atlantic water relative to surface samples, Mopper et al. (1991) estimated a residence time for photolabile bioreactory DOC of 500 – 2100 years, well below its measured average age. While this inconsistency may result from the assumptions made by Mopper et al. (1991) that the entire DOC pool is photoreactive and has a constant rate of photoproduction, there has been a lack of follow-up investigations to determine the actual strength of this photochemical sink for refractory DOC and further evaluation is warranted.

While direct links between free radical photoproducts and the carbon cycle have not been fully evaluated, the formation of reactive oxygen species (ROS) from CDOM is ubiquitous in irradiated marine waters and should be a useful proxy for DOC photoreactivity. Accordingly, Mopper and Zhou (1990) used higher photoproduction of hydroxyl radicals ($\cdot\text{OH}$) in deep Sargasso Sea samples relative to surface samples from the same location to argue for increased photoreactivity of deep ocean DOC. Hydrogen peroxide (H_2O_2) and its precursor, superoxide (O_2^-), are long-lived photoproducts relative to other ROS (e.g. $\cdot\text{OH}$ and $^1\text{O}_2$) and their study has the benefit of sensitive (subnanomolar) chemiluminescent detection methods (King et al., 2007; Rose et al., 2008a). Based on a detailed mechanistic investigation involving borohydride reduction and triplet state quenchers, Zhang et al. (2012) suggest that the formation of O_2^- arises via electron transfer from excited state CDOM to form oxygen reducing intermediates ($\text{CDOM}^{+/\cdot}$) that then react with O_2 to generate O_2^- . Traditionally, H_2O_2 formation has been described by O_2^- disproportionation, for which Zafiriou (1990) determined a pK_a of 4.60 ± 0.15 for O_2^-/HO_2 , and defined the overall second order rate law for O_2^- decay as

$$\frac{d[\text{O}_2^-]}{dt} = k_{\text{dis}}[\text{O}_2^-][\text{HO}_2] \approx 2k_{\text{D}}[\text{O}_2^-]^2 \quad (1)$$

where k_{D} for seawater = $(5 \pm 1) \times 10^{12} [\text{H}^+]$.

In addition to H₂O₂ formation via O₂⁻ dismutation, metals (e.g. Fe, Cu, and Mn) and CDOM also efficiently react with O₂⁻ in natural waters (Goldstone and Voelker, 2000; Heller and Croot, 2010a, 2010b; Wuttig et al., 2013). Because O₂⁻ can act as both an oxidant and a reductant, its decay pathways are far more complex than can be described by dismutation alone (Voelker and Sedlak, 1995; Zafiriou, 1990). Reactions of CDOM and/or metals (A) with O₂⁻ could produce either H₂O₂ or O₂ via the equations

where A_{ox} is an oxidant and A_{red} is a reductant. If the catalytic O₂⁻ decay described above is irreversible, then the amount of H₂O₂ generated per O₂⁻ should be between 0 and 1. On the other hand, if catalytic redox cycling is occurring between A_{ox} and A_{red}, then the same stoichiometry typical of dismutation (0.5 H₂O₂ per O₂⁻) should be maintained. These possible pathways create confusion in determining which pathway (or neither) is dominant in superoxide decay. Even though the fate of superoxide in seawater is complex and likely specific to season and oceanic region, examining open ocean O₂⁻ and H₂O₂ photochemical production together should be a better indicator of general photoreactivity as described by Zhang et al. (2012) than the photoproduction of CO and small organic carbon molecules (e.g. Mopper et al., 1991), which may require more specific reactions and starting material (Pos et al., 1998).

A number of studies using chemiluminescent methods have investigated the dominant superoxide decay pathways in seawater (Heller and Croot, 2010a, 2010b; Voelker et al., 2000; Wuttig et al., 2013). An interesting observation resulting from these recent superoxide studies is evidence for a widespread particle associated source of superoxide (Diaz et al., 2013; Hansard et al., 2010; Rose et al., 2008b). While some studies attribute O₂⁻ signals to a predominately

biological source, most of the open ocean $[O_2^-]_{ss}$ measurements have been made well after samples are removed from UV radiation and do not fully reflect the photochemical source (Hansard et al., 2010; Rose et al., 2008b). Despite the recognition that DOM photolysis in natural waters will produce O_2^- at significant rates (Micinski et al., 1993; Petasne and Zika, 1987; Zafiriou et al., 1990) direct O_2^- photoproduction rates in marine environments are rare. Additionally, early seawater superoxide studies by Micinski et al. (1993) and Zafiriou et al. (1990) using a NO probe may underestimate O_2^- photoproduction rates by 30 – 40% (Blough and Zepp, 1995). Therefore, further investigation of abiotic O_2^- production in seawater via photochemical reactions is also warranted.

To investigate whether DORC is photoreactive and that photochemistry is a significant global sink for DORC, we made direct measurements of both H_2O_2 and O_2^- concentrations during controlled laboratory and shipboard irradiations of deep ocean water (> 3000 m, containing essentially only DORC), mesopelagic water, and surface samples (DORC plus semi-labile fractions of DOC). Based on reports of higher LMW carbonyl compound photoproduction in deep relative to surface Atlantic samples (Mopper et al., 1991), we assumed that this same result would be found in our Gulf of Alaska samples. Accordingly, if our deep samples are more photoreactive than surface samples, this would indicate an increased susceptibility of DORC to photochemical oxidation and support the Mopper et al. (1991) suggestion that photochemistry is a significant removal mechanism for DORC.” Secondly, we further assess the differences between deep and surface samples using the dynamics and relationships between H_2O_2 and O_2^- in these experiments, thereby providing a better evaluation of direct O_2^- photoproduction rates and abiotic decay pathways in open ocean seawater.

2. Methods

2.1 Sample collection and handling

Surface seawater samples (~5m), abyssal seawater samples (deepest cast depth, ~3500-5000m), and mesopelagic samples (~1000m), were collected in the Gulf of Alaska onboard the R/V *Melville* in August 2013 (Fig. 1) and *in situ* hydrographic (depth, T, S) and chemical (nitrate, nitrite, ammonia, silicate, phosphate, oxygen) distribution data sets were collected for each cast and submitted to the U.S. Biological and Chemical Oceanography Data Management Office by the chief scientist (D. Hansell, University of Miami; <http://www.bco-dmo.org/dataset/527102>). Samples used for photochemical experiments were 0.2 μm gravity-filtered (Whatman; Polycap-36&75AS) directly from standard Niskin bottles into 2 L acid-cleaned (overnight in 2 M HCl), Milli-Q (Millipore; >18 M Ω) rinsed polycarbonate bottles (Nalgene). Filters were designated for use at a specific depth (surface, 1000 m, or deep), pre-cleaned with dilute HCl and Milli-Q before the cruise and, rinsed with Milli-Q and sample between each use at sea. Samples for shipboard irradiation experiments were typically partitioned directly into acid-cleaned, Milli-Q rinsed, 10 cm quartz spectrophotometric cells (Spectrocell; H₂O₂ measurements only) or into 0.6 or 1.0 L jacketed beakers (Ace Glass) with quartz lids, either within hours of collection or stored (typically 1 – 2 days) on the ship at 4 °C until use. All samples were maintained at ~20 °C with a recirculating water bath during irradiation. While all equipment in contact with seawater samples was acid cleaned as described above and nitrile gloves were used in handling samples, the R/V *Melville* was not equipped with special trace metal processing facilities and consequently, limited trace metal contamination could be present. Post cruise, remaining samples were shipped cold back to the laboratory and stored at 4 °C until use. CDOM absorption spectra exhibit no significant changes when stored in

this manner for up to 12 months (Johannessen and Miller, 2001; Swan et al., 2012) and photochemical properties are often assumed unchanged. Shipboard results are directly compared to post cruise laboratory irradiations to assess any potential effects from sample storage.

2.2 Determinations of Photoproducts

2.2.1 Hydrogen Peroxide Analysis

We measured H_2O_2 using a FeLume chemiluminescence (CL) system (Waterville Analytical) and modifications to the methods detailed in King et al. (2007). Briefly, samples mixed with an acridinium ester (2 μM 10-methyl-9-(*p*-formylphenyl)acridinium carboxylate trifluoromethanesulfonate, AE; provided by Dr. James J. Kiddle, Western Michigan University), which reacts with H_2O_2 to chemiluminesce at alkaline pH in this flow injection analysis system. The carrier solution (0.2 M HCl) and AE reagent were mixed and transported to a spiral flow cell located in front of a photomultiplier tube (PMT; Hamamatsu HC135 PMT, 900 V, 400 ms integration) using a peristaltic pump (Rainin). A sample syringe flushed and filled a 195 μL Teflon sample loop (VICI 10-port valve) to introduce a sample plug. Finally, the sample/carrier – AE solution mixed with 0.1 M carbonate buffer in the spiral flow cell to initiate CL (for system set up, see King et al. 2007). Decreased carbonate buffer pH (10.4 vs. 11.3 recommended in King et al., 2007) with 0.2 M HCl as the carrier prevented interference by precipitated MgOH_2 and helped decrease background CL. A ~ 2 mM H_2O_2 stock solution prepared from 30% H_2O_2 (J.T. Baker) was checked both prior and post cruise using its absorbance (molar absorptivity 38.1 $\text{M}^{-1} \text{cm}^{-1}$ at 240 nm; Miller and Kester, 1988) on a benchtop spectrophotometer (Perkin Elmer Lambda 40) with Milli-Q as the absorbance blank. Standards prepared in seawater samples matching those used in irradiations were checked for low or undetectable H_2O_2 starting values

against the same seawater with added catalase (20 μL of 100 units L^{-1} in 20 mL seawater; C100 Sigma-Aldrich). Under these conditions, the H_2O_2 detection limit, defined as three times the standard deviation of the blank, was 2.2 nM.

2.2.2 Superoxide Analysis

We also measured superoxide using the FeLume system employing the chemiluminescent reaction between O_2^- and a methyl *Cypridina* luciferin analogue (2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3(7*H*)-one; MCLA; TCI America), following the methods of Rose et al. (2008) and Garg et al. (2011). In this case, both the seawater sample and CL reagent pumped continuously to the flow cell where CL signal was measured (PMT 1200V; 800 ms integration time). The CL reagent contained 2.45 μM MCLA in 50 mM sodium acetate/50 μM diethylenetriaminepentaacetic acid (DTPA) solution, adjusted to pH 6 with concentrated HCl. We selected a pH of 6 because the chemiluminescent reaction of the conjugate base of MCLA (MCLA^- ; $\text{pK}_a = 7.64 \pm 0.03$) with molecular oxygen increases background signal at basic pH (Fujimori et al., 1993) and the rate of uncatalyzed superoxide dismutation increases at acidic pH (Equation 1). To prevent changes in signal due to changes in reaction temperature (Heller and Croot, 2010c), we equilibrated all solutions to room temperature prior to each experiment. Occasionally the enzyme superoxide dismutase (SOD; 200 U L^{-1} from manufacturer's assay; S5395-15KU Sigma) was added at the end of irradiation experiments to observe the subsequent signal reduction to near pre-irradiation background levels. A prepared stock solution of $\sim 1.5 \times 10^6 \text{ U L}^{-1}$ SOD in Milli-Q was stored at 4 $^\circ\text{C}$ in the dark when not in use. With SOD in solution, we observed no photochemical production when re-irradiating the sample, ensuring that changes in FeLume signal during non SOD irradiations were due to photochemically generated O_2^- . Because SOD can interfere with MCLA autooxidation (i.e. the

MCLA⁻ CL reaction with oxygen; Fujimori et al., 1993; Hansard et al. 2010), and lower the FeLume signal below the baseline, pre-irradiation baselines were always subtracted from O₂⁻ photoproduction data, never those containing SOD.

2.2.3 Superoxide System Calibration and Standards

The instability of superoxide in aqueous solutions creates analytical issues for all methods that produce superoxide standards in μ M concentrations (Heller and Croot, 2010c). To calibrate the MCLA-O₂⁻ reaction in the FeLume system for each freshly prepared MCLA reagent, we generated superoxide standards via the UV photolysis of acetone in an ethanol solution following modifications to the methods of McDowell et al. (1983) and the recommendations of Ong (2007). A solution of 41 mM acetone, 6 M ethanol, 30 μ M DTPA in 0.1 M borate buffer (pH = 12.5) was irradiated in a “superoxide generator” (Waterville Analytical) in a 1 cm quartz cell with a Hg lamp (Pen-Ray) for ≤ 30 seconds. We monitored superoxide concentration in real time using its absorbance at 240 nm, blanked with the absorbance of the solution at 400 nm (Ong 2007), and a O₂⁻ extinction coefficient (ϵ) corrected for the absorbance of H₂O₂ at 240 nm (Bielski 1978) as follows

$$\epsilon_{\text{O}_2^- \text{ CORRECTED}} = \epsilon_{\text{O}_2^-} - 0.5\epsilon_{\text{H}_2\text{O}_2} \text{ M}^{-1} \text{ cm}^{-1} = 2183 \text{ at pH 12.5} \quad (4)$$

Absorbance measurements were made using a USB2000-UV-VIS Miniature Fiber Optic Spectrometer (Ocean Optics) linked to a DT-MINI-2-GS light source (Ocean Optics) with Premium grade Si fiber optics. Un-irradiated acetone/ethanol solution was the absorbance blank. It has been noted that DTPA breaks down in UV light causing absorbance increases that can interfere with O₂⁻ determination (Heller and Croot, 2010c), but given the very short irradiation time required to generate O₂⁻ in our standard method, it is not likely that DTPA photolysis contributed significantly to the absorbance reading. For this reason, however, seawater

irradiations under the solar simulator and coincident superoxide data reported here never contain DTPA. When the superoxide reached about 100 μM , we sampled an aliquot and spiked it into filtered seawater samples equilibrated with 30 μM DTPA to eliminate metal catalyzed O_2^- decay (Heller and Croot, 2010a; Hansard et al., 2010). O_2^- concentrations after these spikes ranged from 5 - 50 nM ($[\text{O}_2^-]_0$) and were monitored by the FeLume for ~10 minutes. O_2^- decay was described with the following equation

$$\frac{-d[\text{O}_2^-]}{dt} = 2k_D[\text{O}_2^-]^2 + k_{\text{pseudo}}[\text{O}_2^-] \quad (5)$$

where k_D ($\text{M}^{-1} \text{s}^{-1}$) is the pH dependent dismutation rate constant and k_{pseudo} (s^{-1}) is the rate constant for the reaction of O_2^- with other sinks like CDOM and metals (Goldstone and Voelker, 2000). This equation is appropriate for modeling O_2^- in seawater when all pseudo-first order decay terms are summed (Equations 2 and 3), or metal sink terms are eliminated with the chelator DTPA, as is the case with our standards. We assumed that the FeLume signal (S , corrected for background chemiluminescence) is directly proportional to O_2^- concentration ($S = C [\text{O}_2^-]$) based on previous work (Heller and Croot 2011, 2010a, 2010b), and modeled S as a function of time (t) with the following equation (Garg et al., 2011; Heller and Croot, 2010a)

$$S = \frac{k_{\text{pseudo}} S_0}{k_{\text{pseudo}} e^{k_{\text{pseudo}} t} + k_D [\text{O}_2^-]_0 (e^{k_{\text{pseudo}} t} - 1)} \quad (6)$$

where S_0 is the FeLume signal at time zero and $[\text{O}_2^-]_0$ (M) is the spiked superoxide concentration. FeLume data was fit to Equation 6 with a non-linear regression of signal (S) over time (t) using *nlinfit* routine in MATLAB[®] and S_0 and k_{pseudo} as fitting parameters. The second order dismutation rate constant, k_D , was calculated for each seawater sample using Equation 1 (Zafiriou, 1990) and pH values determined with a Ross Ultra, glass combination pH electrode (Thermo Scientific) calibrated with NBS standard buffers. Although calibrating seawater pH

with Tris buffers of a composition and ionic strength similar to seawater is recommended (Marion et al., 2011), here we chose NBS buffers for direct compatibility with k_D values determined in seawater by Zafiriou (1990). For each set of standards, a linear regression of S_0 vs. $[O_2^-]_0$ gave signal to concentration ratio values (C) ranging from 1.53 to 3.62×10^4 counts nM^{-1} ($r^2 > 0.90$ for all standard curves, $n = 8$) which were used in subsequent O_2^- concentration determinations.

2.3 Photochemical Irradiations

We performed irradiations using two identical Suntest CPS solar simulators equipped with 1.5 kW xenon lamps (Atlas) fitted with a window glass to simulate sunlight reaching the earth's surface (~ 305 nm and above, Fig. 2). Two separate sample exposure configurations were used for photochemical experiments. The first was solely for photochemical H_2O_2 determinations to provide a highly reproducible and quantifiable light exposure. The second was for simultaneous determinations of photoproduct H_2O_2 and O_2^- in jacketed beakers. Both systems are described in detail below.

2.3.1 Experiments for Determination of H_2O_2 Photoproduction Rates

For these exposures, we distributed each seawater sample at room temperature into 15 matched 10 cm cylindrical quartz spectrophotometric cells, filled with no headspace and sealed using gas-tight caps fitted with Teflon faced, butyl rubber septa. Cells were placed vertically below the solar simulator into a black, water-cooled, aluminum irradiation block fitted with a half inch grey PVC lid with 1.6 cm apertures directly above the cell windows to eliminate residual off-axis photons. The block maintained the samples at $20^\circ C$ and allowed no transfer of light between cells during irradiation. We performed a few wavelength dependent experiments

on surface and deep seawater samples by aligning the block, and therefore each cell, under a variety of evenly spaced Schott long-band-pass cutoff filters (305, 320, 335, 345, 380, 425, and 480 nm). We measured no significant H₂O₂ photoproduction under the 425 and 480 nm cutoff filters. Similar studies suggest that less than 15% of total H₂O₂ photoproduction is caused by wavelengths above 400 nm (Kieber et al., 2014). Dark cells were kept in the water bath until the end of the irradiation. For broadband irradiations, the long band-pass cutoff filters were removed, allowing for the irradiation of 15 cells simultaneously (typically sampling in triplicate for each time point). We quantified spectral downwelling irradiance entering each cell, $E_0(\lambda)$ (average integrated $E_0(290 - 400 \text{ nm}) \sim 1.5 \text{ mol(photons) m}^{-2} \text{ s}^{-1} \text{ nm}^{-1}$), at 1 nm intervals with a UV-Vis portable spectroradiometer (OL756; Optronic Laboratories) fitted with a quartz fiber optic cable and 2 inch diameter integrating sphere. Solar irradiance was also measured throughout the cruise, replacing the 2 inch integrating sphere with a 6 inch diameter integrating sphere with a quartz dome window (OLIS-670; Optronic Laboratories). The OL756 was calibrated for both collection spheres with a NIST standard lamp (OL752-10 irradiance standard; Optronic Laboratories) pre and post cruise. Measured irradiance entering each cell very closely matches midday irradiance measured throughout the cruise (typical scans shown in Fig. 2).

We measured sample absorbance in each cell from 250 – 800 nm prior to, during, and post irradiation with a MAYA2000-Pro UV-VIS spectrometer (Ocean Optics) connected to a liquid waveguide capillary flow cell (1 m pathlength, LWCC-2100 Type II; World Precision Instruments). The LWCC was filled via a peristaltic pump positioned to pull the sample through Teflon tubing. Because Milli-Q water was used as the absorbance blank, a Perkin Elmer Lambda 40 dual beam spectrophotometer was used to check UV CDOM absorbance measurements in 10 cm spectrophotometric cells post cruise on the same samples made during the cruise using the

LWCC. Raw absorbance spectra were corrected for possible scattering, refractive index differences between salt water and the pure water blank, and instrument drift by fitting individual spectra to the equation:

$$A = F e^{-S\lambda} + O \quad (7)$$

using the MATLAB[®] nonlinear curve fitting routine *nlinfit*, where A (unitless) is absorbance, F is a fitting coefficient, S is the spectral slope coefficient, and O is the offset (Reader and Miller, 2011). Data were fit over the 680 – 800 nm range for 10 cm spectrophotometer cells and over the 660 – 700 nm range for the LWCC (D'Sa et al., 1999). The offset (O) was subtracted from the absorbance spectra, and any differences measured using the LWCC and the Lambda 40, while generally small below 350 nm, were attributed to differences in the baseline offset.

Because salt solutions have a higher refractive index than the freshwater on which both instruments are blanked, seawater should show an apparent negative absorbance at certain wavelengths, an effect that can be exacerbated in the long pathlength LWCC (D'Sa et al., 1999; Miller et al., 2002; Nelson et al., 2007). This refractive index effect can be corrected by preparing blanks with a similar refractive index to the samples (Miller et al., 2002) or by employing an empirical salinity correction (Nelson et al., 2007). We rarely observed this negative offset and absorbance spectra from both instruments were comparable in the UV (< 350 nm), indicating that CDOM absorption measurements made using pure water blanks in the LWCC are robust. Absorbance data was primarily used to correct H₂O₂ photoproduction data for CDOM fading and to normalize photoproduction rates for comparison among samples.

Corrected A spectra were converted to Napierian absorption coefficient ($a_g(\lambda)$; m⁻¹) with the equation:

$$a_g(\lambda) = [\ln(10) A]/L \quad (8)$$

over the 280 – 700 nm range, where L (m) is the pathlength. Sample irradiations in spectrophotometric cells typically lasted 3 to 12 hours but up to 24 or 48 hours for some samples. Percent CDOM loss was monitored at each wavelength from 290 – 350 nm and averaged to correct time series data.

2.3.2 Photochemical H_2O_2 and Superoxide Kinetic Experiments

For the photochemical superoxide experiments and paired O_2^-/H_2O_2 experiments, we irradiated samples under the solar simulator in acid-cleaned and Milli-Q rinsed 600 mL or 1 L jacketed beakers (Ace Glass) covered with quartz glass. Spectral irradiance entering the beakers was higher than that entering the spectrophotometric cells (Fig. 2) when measured as described in Section 2.3.1. O_2^- concentrations were monitored with MCLA chemiluminescence both during and post irradiation in 600 mL jacketed beakers to observe O_2^- steady-state concentrations ($[O_2^-]_{ss}$) and decay kinetics respectively. For determinations of initial steady-state values, samples were irradiated up to 15 minutes to establish a stable $[O_2^-]_{ss}$. Samples were well mixed with a Teflon stir bar for these short irradiations and maintained at 20 °C with a recirculating water bath. Post irradiation, the FeLume signal, and thus O_2^- decay, was again monitored and fit using Equations 5 and 6 for the first 60 seconds of decay, effectively combining metal sinks and all other first order sinks into the k_{pseudo} term. $[O_2^-]_0$ was replaced in equation 6 with $(C \times S_0)$ and again S_0 and k_{pseudo} were used as parameters for the nonlinear decay fit (Section 2.2.3). Assuming that at steady-state O_2^- production equals its decay, O_2^- photoproduction rates ($P_{O_2^-}$) were described using modeled $[O_2^-]_0$. This was determined by multiplying modeled S_0 together with C , to provide a $[O_2^-]_{ss}$ that accounts for the time (~10 sec) out of the light required for the sample to reach the detector, and k_{pseudo} using the following equation

$$P_{O_2^-} = 2k_D[O_2^-]_{ss}^2 + k_{pseudo}[O_2^-]_{ss} \quad (9)$$

During long term (> 20 hour) exposures, we monitored $[O_2^-]_{ss}$ in 600 mL beakers throughout the experiment to assess any changes in steady-state with continued irradiation. H_2O_2 concentrations were also periodically measured during these long term experiments to assess the stoichiometry between O_2^- and H_2O_2 production in these oligotrophic seawater samples. Additional long term irradiations were performed in 1 L beakers with H_2O_2 measurements at 24 and 48 hours. We assumed that H_2O_2 photoproduction is uniform in these optically thin (i.e. $a_g(\lambda) \times L \ll 1$) samples at the time points sampled and did not stir the 1 L beakers. Although photochemical degradation of H_2O_2 has been reported (Moffett and Zafiriou, 1993), it was estimated to be only 5% of the photoproduction rate and therefore was neglected when evaluating H_2O_2 results in our experiments.

3. Results and Discussion

3.1 General Trends in Initial H_2O_2 and O_2^- Photoproduction Rates

3.1.1 Initial H_2O_2 Photoproduction Rates and CDOM Normalized Rates

We determined initial photoproduction rates for H_2O_2 (P_{HO_2H}) over 6 hours during shipboard irradiations and over 7 – 9 hours during laboratory irradiations post cruise (Table 1) with a linear regression of photoproduced $[H_2O_2]$ (nM) verses irradiation time (h). Overall initial rates during the cruise and in the laboratory were also determined by pooling all production data for a given depth, regardless of station, and again fitting a linear regression to $[H_2O_2]$ vs. time (Table 1). Analysis of covariance (ANCOVA) was used to test for differences between depths in all rates determined in this manner. Photoproduction rates were slightly slower for deep samples ($2.36 \pm 0.66 \text{ nM h}^{-1}$) than for surface samples ($4.88 \pm 1.00 \text{ nM h}^{-1}$), but were not significantly

different ($p = 0.059$). These photoproduction rates were similar to those measured by Yuan and Shiller (2001) on a 0.4 μm filtered oligotrophic surface sample (5.4 nM h^{-1}) from the south central Atlantic, and to those measured by Yocis et al. (2000) on 0.2 μm filtered surface samples collected from the oligotrophic Antarctic (2.1 to 9.4 nM h^{-1}). Otherwise, most other published open ocean hydrogen peroxide photoproduction rate data are actually *net* production rates as they were measured either *in situ* (Avery et al., 2005; Obernosterer et al., 2001) or on unfiltered samples (Yuan and Shiller, 2005) and include biological sinks together with the photochemical source for H_2O_2 .

P_{HOOH} normalized to $a_g(325) \text{ (m}^{-1}\text{)}$, which removes variability due to CDOM and isolates differences from other sources, match within standard error (Table 1, $p = 0.5103$), indicating that slightly higher rates in surface samples are due to higher CDOM absorption coefficients and not a difference in their efficiency for H_2O_2 photochemical production. Previous studies have also indicated that the apparent quantum yield spectra for H_2O_2 , which describes the efficiency of H_2O_2 photoproduction, are similar in open ocean surface waters and that differences in H_2O_2 photoproduction may be primarily controlled by temperature (Kieber et al., 2014; Yocis et al., 2000) since superoxide dismutation (Equation 1) is a thermal reaction. Additional evidence for comparable initial rates between deep and surface waters were obtained post cruise for 7 – 9 hour laboratory irradiations in 600 mL jacketed beakers for paired $\text{H}_2\text{O}_2/\text{O}_2^-$ measurements. P_{HOOH} for deep water samples ($4.34 \pm 0.75 \text{ nM h}^{-1}$) and surface water samples ($4.99 \pm 1.70 \text{ nM h}^{-1}$) were essentially indistinguishable ($p = 0.784$). These rates determined in beakers are in the same range, but slightly higher than those measured in spectrophotometric cells, likely due to the 37.5% higher measured photon flux (integrated from 290 – 400 nm) entering the jacketed beakers (Fig. 2), and probably not related to storage effects. When normalized to $a_g(325)$, H_2O_2

photoproduction rates were somewhat higher for deep water (Table 1) but were not significantly different ($p = 0.2302$).

Other than having DOC concentrations $\sim 20 - 30 \mu\text{M}$ higher in surface water than in deep water (Table 1), a primary compositional difference between these samples is that surface water NO_3^- concentrations are typically low when compared to deep water concentrations (Table 1). Nitrate photolysis can lead to significant production of the highly reactive hydroxyl radical ($\cdot\text{OH}$) in seawater (Zafiriou, 1974; Zepp et al., 1987) and nitrate and nitrite were reported to account for 15 – 20% of $\cdot\text{OH}$ photoproduction rate in deep ($>700 \text{ m}$) waters collected in the Sargasso Sea (Mopper and Zhou, 1990). However, once in the surface, DORC will be exposed to sunlight without such high NO_3^- concentrations found at depth.

To evaluate the significance of higher NO_3^- on ROS rates in irradiated deep water, we performed additional irradiations with 50:50 deep:surface mixed samples while at sea and with 1000 m samples (containing the highest NO_3^- concentrations) back in the laboratory. For spectrophotometric cell irradiations containing mixed samples, initial H_2O_2 production data was pooled as above and a linear accumulation was calculated as $3.19 \pm 1.22 \text{ nM h}^{-1}$, in good agreement with both deep and surface water ($p = 0.1162$). Similarly, P_{HOOH} determined from irradiations of water collected at $\sim 1000 \text{ m}$ in jacketed beakers was $3.98 \pm 1.16 \text{ nM h}^{-1}$, the same as deep and surface water rates within error ($p = 0.8488$). $P_{\text{HOOH}}/a_g(325)$ rates shows no consistent trend to argue a significant role of nitrate in H_2O_2 photoproduction, with samples having much different nitrate concentrations showing similar $P_{\text{HOOH}}/a_g(325)$ rates (e.g. stations 20 & 27). Studies have shown that bleaching of CDOM and formation of DOM photoproducts by $\cdot\text{OH}$ is slow for solutions containing Suwannee River fluvic and humic acids (Goldstone et al., 2002; Grebel et al., 2009) and unimportant as pH was increased from 7 to 9 in a freshwater

stream (75 – 100% non $\cdot\text{OH}$ mechanisms for DOM loss, Molot et al., 2005). These results are consistent with limited influence of $\cdot\text{OH}$ production by nitrate photochemistry on observed initial H_2O_2 photoproduction rates in surface and deep water experiments. Therefore, the similar initial H_2O_2 photoproduction rates between deep and surface waters suggests that DORC has a similar photoreactivity to more biolabile fractions of DOM, but does not reflect an increased photoreactivity for DORC observed previously (Mopper et al., 1991).

3.1.2 Superoxide Steady-States and Decay Kinetics

Modeled $[\text{O}_2^-]_{\text{ss}}$ values were 1.2 – 16% higher than measured $[\text{O}_2^-]_{\text{ss}}$ at the flow cell for all but one sample. During these short irradiations under simulated sunlight, modeled $[\text{O}_2^-]_{\text{ss}}$ in deep samples (2.49 ± 1.84 nM) and in surface samples (2.94 ± 1.27 nM) were quite similar (Table 2). Repeat irradiations were used to check precision and measured steady-state concentrations varied slightly, between 2 and 16% of their original values. These steady-state concentrations measured during our irradiation experiments are ~ 4 to > 30 times higher than those measured in the field (Hansard et al., 2010; Rose et al., 2008b; Shaked et al., 2010). This likely reflects both the short transit time for the sample to reach our detector and the higher and constant photon flux under simulated sunlight relative to natural sunlight with variable cloud cover (Fig. 2). In an unfiltered sample under natural sunlight, Shaked et al. (2010) measured a $[\text{O}_2^-]_{\text{ss}}$ of ~ 140 pM in water from the Gulf of Aqaba. Otherwise, we are not aware of any $[\text{O}_2^-]_{\text{ss}}$ data in the literature that adequately reflects the photochemical source for superoxide. Hansard et al. (2010) measured superoxide concentrations in surface waters of the Gulf of Alaska ranging from 140 – 540 pM but, as the authors note, the 2 – 3 minute transit time required for the sample to reach the flow cell and react with MCLA greatly underestimates the contribution of photochemical O_2^- production. Based on these reports, and because the irradiance spectra

measured under our solar simulator is somewhat higher than natural sunlight in our study region (Fig. 2), the O_2^- steady-state measured during our experiments should be roughly representative of a maximum midday $[\text{O}_2^-]_{\text{ss}}$ in the open ocean near surface waters of the Gulf of Alaska.

Like $[\text{O}_2^-]_{\text{ss}}$, k_{pseudo} determined over the initial period of decay in our study was not significantly different in deep waters ($5.96 \pm 3.44 \times 10^{-3} \text{ s}^{-1}$) compared to surface waters ($4.60 \pm 1.07 \times 10^{-3} \text{ s}^{-1}$). Modeled pseudo-first order decay over the first 60 seconds (Table 2) generally matched that determined for the entire decay curve, but in some cases was faster during this initial phase (data not shown). Heller and Croot (2010b) also observed faster initial decay followed by slower decay for superoxide reacting with CDOM. This effect was explained by either a back reaction producing superoxide or the presence of two CDOM pools with high and low reactivity for superoxide. Their observations were made by monitoring the decay of O_2^- spikes and not from photogenerated O_2^- , which could potentially react with newly photogenerated CDOM transients (Zhang et al., 2012).

Pseudo first order decay constants determined here are in excellent agreement with k_{pseudo} values previously obtained in studies using additional precautions to collect trace metal clean samples in the Gulf of Alaska ($2 - 16 \times 10^{-3} \text{ s}^{-1}$; Hansard et al., 2010), providing support that metal contamination was not a significant issue in our experiments. Otherwise, k_{pseudo} values determined here are at the lower end of k_{pseudo} values determined from superoxide additions in the Eastern Pacific ($18.9 - 27.6 \times 10^{-3} \text{ s}^{-1}$; Rusak et al., 2011), the Southern Ocean ($4 - 74 \times 10^{-3} \text{ s}^{-1}$; Heller and Croot, 2010a), and DTPA amended seawater from the equatorial Atlantic, which solely reflects the CDOM sink ($9 - 34 \times 10^{-3} \text{ s}^{-1}$, Heller and Croot, 2010b). In these studies, spiked superoxide concentrations ranged from about 50 to 100 nM, ~100 – 1000 times higher than *in situ* concentrations discussed above. Interestingly, the k_{pseudo} values we determined for

decay of O_2^- standards starting at 7 – 42 nM, as required for FeLume calibration (Section 2.2.3), were about $15 - 35 \times 10^{-3} \text{ s}^{-1}$, in good agreement with the k_{pseudo} values determined from ~100 nM O_2^- spikes in DTPA amended seawater (Heller and Croot, 2010b) but higher than those determined post irradiation. While differences here may be due to the use of DTPA in these samples, we believe that this result provides evidence for a possible dependence of decay pathway distribution on initial O_2^- . Post-irradiation k_{pseudo} determinations typically started at $[\text{O}_2^-]_{\text{ss}}$ of ~ 1 – 4 nM (Table 2), so it may be difficult to compare pseudo-first order decay kinetics determined from superoxide spikes to those appropriate for naturally occurring conditions (Rusak et al., 2011).

3.1.3. Initial Superoxide Photoproduction Rates

We determined O_2^- photoproduction rates in two ways using the experiments discussed above. First we determined the “initial *net* O_2^- accumulation rate (*net* $P_{\text{O}_2^-}$)” using the first 15 seconds of irradiation and a linear regression of the FeLume signal, converted to $[\text{O}_2^-]$ with C , versus time. The benefit here is that actual measured $[\text{O}_2^-]$ data is used to determine these rates, removing errors associated with nonlinear fits and extrapolations. However, even when determined within 15 seconds in filtered samples, these initial $[\text{O}_2^-]$ accumulation rates reflect the *net* photoproduction since decay processes occur as $[\text{O}_2^-]$ increases to steady-state levels with continued exposure. Shaked et al. (2010) noted that *net* $P_{\text{O}_2^-}$ may depend heavily on the determination interval (here 15 s) and while they may be useful in assessing differences in O_2^- photoproduction rates between samples, they may not be informative of the true production rate occurring in a given experiment.

The second approach estimates O_2^- photoproduction by assuming that with constant irradiation, $[\text{O}_2^-]$ steady-state is maintained when production equals decay. To calculate O_2^-

photoproduction rates using this assumption, we use modeled $[O_2^-]_{ss}$ and k_{pseudo} with Equation 9. This approach, termed “modeled $P_{O_2^-}$ ”, accounts for the ~10 s transit time between $[O_2^-]$ measured in the FeLume flow cell and $[O_2^-]_{ss}$ in the jacketed beaker. This approach provides a better representation of the true O_2^- photoproduction rate by accounting for all loss mechanisms in the decay term that must be balanced by $P_{O_2^-}$ alone. The limitation is that errors for both modeled $[O_2^-]_{ss}$ and k_{pseudo} factor into the calculation.

3.1.3.1. Initial Net Superoxide Accumulation Rates (*net* $P_{O_2^-}$)

When averaged, *net* $P_{O_2^-}$ were similar between deep ($14.4 \pm 5.93 \text{ pM s}^{-1}$) and surface ($9.33 \pm 4.57 \text{ pM s}^{-1}$) waters, but a linear regression fit to all deep or all surface production data together found *net* $P_{O_2^-}$ to be higher for deep waters (18.3 versus 9.3 pM s^{-1} ; Table 2). These rates are comparable to initial O_2^- formation rates of $3 - 25 \text{ pM s}^{-1}$ measured over 4 to 10 seconds of sun exposure in surface samples from the Gulf of Aqaba (Shaked et al., 2010). To our knowledge, the only other O_2^- photoproduction rates reported for the open ocean using chemiluminescent methods were made over 20 minutes of irradiation under simulated sunlight showing O_2^- concentrations increasing $\sim 1.3 \text{ pM s}^{-1}$ for an unfiltered seawater sample and decreasing by about $\sim 0.006 \text{ pM s}^{-1}$ in a filtered sample (Rusak et al., 2011). Because the first time point was taken beyond 3 minutes, these are actually measurements of a series of $[O_2^-]$ steady-state values during the irradiation and their time course of change does not represent a photochemical production rate.

The observations that *net* $P_{O_2^-}$ in both deep and surface waters depend on our calculation method (average vs. linear regression) and that it shows a trend not observed for H_2O_2 photoproduction rates could be the result of measurements done over a much shorter time scale (secs vs. hrs for O_2^- and H_2O_2 , respectively). To check precision in these rates, repeat

irradiations were performed on several samples. $Net P_{O_2^-}$ was variable, with the second irradiation differing by 4 and ~ 35% and one sample showing a 90% change. These results argue that modeled $P_{O_2^-}$ may more effectively define depth differences in $P_{O_2^-}$.

3.1.3.2 Modeled Superoxide Photoproduction Rates

As expected when decay mechanisms are removed, modeled $P_{O_2^-}$ was generally higher than $net P_{O_2^-}$, and showed surprisingly large differences (–62 to 195%) from $net P_{O_2^-}$ in the same sample. Modeled $P_{O_2^-}$ ranged from 6.06 – 48.4 $pM s^{-1}$ in all samples (Table 2). For comparison, superoxide photoproduction rates inferred from H_2O_2 photoproduction rates determined during exposures to natural sunlight in higher CDOM coastal seawater with added SOD were on the order of 78 – 160 $pM s^{-1}$ (Petasne and Zika, 1987). Otherwise, direct measurements of O_2^- photoproduction rates in marine waters are surprisingly lacking, possibly due to the difficulty in quantifying low $[O_2^-]$ and its short lifetime in seawater.

When averaged, modeled $P_{O_2^-}$ for deep water was more variable ($15.0 \pm 13.3 pM s^{-1}$) but quite similar to that for surface water ($14.0 \pm 5.83 pM s^{-1}$), even when normalized to $a_g(325)$ (Table 2). Perhaps not surprisingly, the result that modeled $P_{O_2^-}$ does not change notably with sample depth, bears a striking resemblance to our initial photoproduction rates for H_2O_2 . Taken together with the similarity in $[O_2^-]_{ss}$ and k_{pseudo} values, our results do not support an increased photoreactivity for the DORC pool.

3.2 Trends in H_2O_2 Accumulation and O_2^- Steady-States with Continued Irradiation

3.2.1 Long Term H_2O_2 Photoproduction Rates

Because initial photochemical rates may not be sustained over time, we performed long term experiments to test this possibility. Using exposures up to 48 hours, we further examined

DORC photolability beyond its initial exposure to sunlight. Fig. 3. shows typical shipboard results for deep, surface and 50:50 mixed samples using water collected at Station 17 in both irradiation systems. Despite the similarity in initial rates between these three samples, H_2O_2 stops accumulating in the deep water sample with continued irradiation. This was not observed in the surface or mixed sample, perhaps due to differences in initial CDOM absorption coefficients for the different depths at this station ($a_g(325)$ listed in Table 1). These exposure experiments were repeated both shipboard and post cruise for water from several stations. H_2O_2 accumulation over 48 hr during shipboard irradiations was always lower in deep samples when compared to surface samples (Fig 5). By correcting H_2O_2 accumulation using the average % CDOM absorption fading over the 290 – 350 nm range, the nonlinear nature of these results could be partly explained (e.g. St. 24 and 29, Fig. 4). After 48 hours of irradiation the average % CDOM absorption loss was between 30 – 50% for deep water samples. In comparison, the average fading in surface water samples was only 5 – 30%, suggesting that surface waters may have been “prefaded” yet capable of producing much higher concentrations H_2O_2 photochemically. Even when corrected for fading, H_2O_2 either slows down (St. 24 and 29) or stops accumulating entirely in deep samples (St. 8 and 17; Fig. 3 and 4). In fact, for the longer irradiations shown in Fig. 3 and 4, photoproduced H_2O_2 in deep water did not exceed about ~ 40 nM and sometimes did not increase in concentration beyond 9 hours of irradiation.

As CDOM fades, the diminished photon absorption rates will lower the photoproduction of H_2O_2 , however we observed the accumulation of H_2O_2 stopping well before even 50% of the CDOM was lost in deep samples. Photodecomposition of H_2O_2 has been reported for samples collected from a “river to oligotrophic ocean” transect in the Eastern Caribbean (Moffet and Zafiriou, 1993), but photodecomposition rates were low at oligotrophic stations (less than 5% of

production rates). These results were from short (2 hour) irradiations and it is not known whether photodecomposition rates change with continued irradiation. *In situ* estimates suggest that H_2O_2 photodecomposition could be over 50% of the photoproduction rate when photoproduction rates are low (Yocis et al., 2000) so direct photodecomposition, or perhaps loss due to a photochemically generated product cannot be ruled out. We cannot find any published studies monitoring the photochemical production of H_2O_2 in deep seawater samples, but nonlinear H_2O_2 accumulation has been observed for surface seawater (Kieber et al., 2014) and for dilutions of Shark River Water (Andrews et al., 2000) with increasing photon dose even during short (< 2 h) exposures. Once corrected for CDOM loss, surface samples appear to maintain linearity up to at least 24 h (Fig. 4). It is also possible that H_2O_2 photoefficiency had already decreased in all irradiated samples since the earliest measured time points were after 6 h of irradiation. Even if this is the case, we can say with certainty that nonlinearity, even when corrected for loss of color, is most pronounced in deep samples, again providing compelling evidence from ROS dynamics that DORC is less photochemically reactive than previously suggested.

3.2.2 Paired H_2O_2 and O_2^- Dynamics with Continued Irradiation

To better interpret H_2O_2 results, we performed two shipboard experiments monitoring superoxide steady-state and hydrogen peroxide concentrations together in deep water samples throughout 20 – 30 h exposures under the solar simulator (Fig. 5). For these experiments, the sample was split and irradiated in two 600 mL jacketed beakers side by side. Throughout the experiment, one beaker was continually sampled and the other was left untouched until the end of the experiment to check for possible effects due to sampling. O_2^- and H_2O_2 values measured at the end of the experiment were the same, within error, between both beakers. Over the course of the irradiation, measured $[\text{O}_2^-]_{\text{ss}}$ continually decreased. For the irradiation of deep water

collected from Station 5 (Fig. 5A), we were unable to correct $[\text{O}_2^-]_{\text{ss}}$ for the time taken for the sample to travel to the FeLume because we did not monitor superoxide decay throughout the experiment. At the end of this irradiation experiment, however, we measured superoxide decay in the same way as was done for experiments measuring initial $[\text{O}_2^-]_{\text{ss}}$ and decay (Section 3.1.2). As before, we can assume that measured $[\text{O}_2^-]_{\text{ss}}$ throughout the experiment is slightly lower than that under the solar simulator due to a small loss of O_2^- during transfer to the FeLume. At the end of the first irradiation (Fig. 5A), k_{pseudo} , determined as described above (Section 2.2.2 Equation 6), was $13.4 \pm 2.16 \times 10^{-3} \text{ s}^{-1}$, slightly higher than k_{pseudo} values determined after short irradiations (Table 2).

To examine whether or not O_2^- decay kinetics changed throughout the long irradiation, we repeated the experiment with deep water from station 12, turning the solar simulator off after each $[\text{O}_2^-]_{\text{ss}}$ determination to measure O_2^- decay (Fig. 5B and C). During the first 7 h of irradiation, calculated k_{pseudo} values were $5.59 - 5.80 \times 10^{-3} \text{ s}^{-1}$, but after 9 hr of irradiation, k_{pseudo} values increased to $8.59 - 9.47 \times 10^{-3} \text{ s}^{-1}$ (decay after 4 – 11.5 hours shown in Fig. 5C). In addition to this apparent shift in decay kinetics, modeled $P_{\text{O}_2^-}$ also decreased with continued irradiation from $29.0 \pm 5.83 \text{ pM s}^{-1}$ during the first hour to $13.2 \pm 2.85 \text{ pM s}^{-1}$ at 18.7 hours.

Post cruise, we performed additional simultaneous irradiations on stored surface (top 5 m), 1000 m, and deep samples (Fig. 6) to determine if observed long term $[\text{O}_2^-]_{\text{ss}}$ decreases were unique to deep samples. As discussed previously, initial H_2O_2 photoproduction rates were similar for samples at all depths (Section 3.1.1) and similarly, H_2O_2 photoproduction between deep and 1000 m water were comparable over the course of the long irradiation (Fig. 6), never exceeding ~50 nM in deep water irradiations and ~80 nM in 1000 m water irradiations. At these two depths, in all but one 1000 m sample (Station 20), H_2O_2 concentrations did not increase beyond 12 hours

of irradiation. Surface water H_2O_2 photoproduction rates, however, were much more variable, with H_2O_2 concentrations ranging from ~60 nM (Station 07) to > 300 nM (Station 20) after 48 hours of irradiation.

The observation that $[\text{O}_2^-]_{\text{ss}}$ and modeled $P_{\text{O}_2^-}$ decrease with continued irradiation argues for a consumption of source, most simply explained by CDOM photobleaching. However, in these experiments, there was still a measurable superoxide steady-state even after H_2O_2 had stopped accumulating, meaning that CDOM was still capable of superoxide photoproduction. These results provide evidence that a large portion of photoproduced O_2^- decays through an oxidative pathway, and perhaps, in the DORC samples, decay mechanisms shift to an almost entirely oxidative pathway over long irradiations. Zhang et al. (2012) observed H_2O_2 photoproduction under anaerobic conditions, which they attributed to the introduction of O_2 during H_2O_2 analysis and long-lived reactive reducing intermediates formed during the irradiation. These photoproduced reductants will reduce O_2 to O_2^- as expected, but they are also likely capable of reducing O_2^- to H_2O_2 (Equation 3). Towards the end of our long irradiation experiments, O_2^- concentrations are low (~60 – 100 pM) and perhaps cannot compete for these reductants with the $> 10^6$ times higher concentrations of molecular oxygen present in seawater equilibrated with the atmosphere. Using standard aquatic fulvic acids and a soil humic acid, Sharpless et al. (2014) demonstrated that even after 59 hours of irradiation, photooxidation reduced DOM electron donating capacity while having little effect on electron accepting capacity, a result which does not support photochemical oxidation of electron donor groups to acceptor groups. Although the experimental source materials contained in our low CDOM Gulf of Alaska waters are most certainly different from those contained in commercially available fulvic and humic acids, the loss of electron donating capacity observed by Sharpless et al. (2014)

may help explain our observation that H_2O_2 accumulation slows tremendously or stops in deep ocean samples. Other than the results presented here, information is not available on the potential changes in the oxidative and reductive sinks for O_2^- and subsequent production stoichiometry for H_2O_2 in marine waters during long irradiations.

3.3 A comment on stoichiometry: How much O_2^- leads to H_2O_2 ?

While comparative studies in oligotrophic seawater are missing, the observation that H_2O_2 stops accumulating during irradiations in samples that have a measurable $[\text{O}_2^-]_{\text{ss}}$ and results reported elsewhere (Garg et al. 2011; Petasne and Zika, 1987) suggest that a portion of superoxide decays via an oxidative pathway (Equation 2), even after short irradiation experiments. For example, Petasne and Zika (1987) estimated that only 30% of superoxide photoproduction leads to H_2O_2 formation from irradiations of coastal seawater. The stoichiometry of dismutation (Equation 1) predicts 0.5 H_2O_2 produced per O_2^- , implying that the photoproduction rate of superoxide should be twice that for hydrogen peroxide. Because O_2^- photoformation data is limited and apparent quantum yield data for O_2^- is unavailable, currently the only option is to infer O_2^- photoefficiency from that for H_2O_2 . Therefore most studies assume this relationship holds when inferring O_2^- information from H_2O_2 observations (Heller and Croot, 2010a; Powers and Miller, 2014). However, most studies investigating O_2^- decay mechanisms have found that O_2^- decay typically follows first order kinetics, and not the second order fit required of dismutation (Heller and Croot, 2010a, 2010b; Rose et al., 2008b; Shaked et al., 2010). In fact, $[\text{O}_2^-]_{\text{ss}}$ models that use dismutation as the only sink for O_2^- in seawater overestimate steady-state concentrations by about an order of magnitude (Powers and Miller, 2014; Voelker and Sedlak, 1995; Zafiriou, 1990). Moreover, O_2^- readily reacts with CDOM, metals and other sinks, arguing for complex decay pathways in seawater and chemical kinetics

that approach a central limit of ~1.2 reaction order under these conditions (Bandstra and Tratnyek, 2005). At present, the relationship between H_2O_2 and O_2^- can only be constrained between 0 and 1, and can still be ~ 0.5 even if dismutation is not the dominant O_2^- sink depending on whether oxidative (Equation 2) or reductive (Equation 3) sinks are dominant.

To examine the portion of photoproduced O_2^- leading to H_2O_2 , we compared initial P_{HOOH} to modeled $P_{\text{O}_2^-}$ for the post cruise laboratory irradiations in jacketed beakers using the surface, deep, and ~1000m water samples discussed above (P_{HOOH} listed in Table 1). Ratios determined for surface and 1000 m water were generally lower than those for deep water. $P_{\text{HOOH}}:P_{\text{O}_2^-}$ ranged from 0.10 – 0.45 in surface water samples and from 0.13 – 0.46 in 1000 m water samples while ratios for deep water samples ranged from 0.37 – 0.67. In other words, photoproduction rates of O_2^- were generally more than a factor of two higher than those for H_2O_2 , averaging 4.11 times greater for these experiments (range = 1.5 – 9.8). The time scales of these measurements (less than 30 min experiments for O_2^- versus ~6 h for H_2O_2) may account for some of the deviation from dismutation stoichiometry because, as noted previously, a lack of reciprocity for H_2O_2 photoproduction has been observed during long irradiations here (Section 3.2) and for H_2O_2 photoproduction rates even during short irradiations (Kieber et al., 2014). However, these results do indicate that a ratio > 2 may be required when estimating O_2^- photochemistry from H_2O_2 photoproduction data, and previous estimates made using a 2:1 ratio (e.g. Heller and Croot, 2010a; Powers and Miller, 2014) are likely conservative.

While redox cycling of CDOM may be occurring here (e.g. Goldstone and Voelker, 2000), our results indicate that more O_2^- decays via an oxidative pathway (Equation 2) than a reductive pathway to produce H_2O_2 (Equation 3). Photobleaching plays an important role in loss of color but also in CDOM photooxidation. CDOM reductants produced by photochemical

charge separation have been proposed as the precursor for O_2^- (Zhang et al., 2012), thus suggesting that H_2O_2 is a convenient proxy for CDOM e^- loss (Sharpless and Blough, 2014). Therefore, it is not surprising that as CDOM is photobleached, it loses its capacity to generate these reduced species and to further reduce O_2^- to H_2O_2 . If this is the case, perhaps the oxidative sink (Equation 2) for O_2^- could be more important in our experiments, even during these short irradiations. More work is needed in marine systems to better understand redox cycling with CDOM from both autochthonous and allochthonous sources but at present, a stronger oxidative sink for O_2^- could explain why H_2O_2 photoproduction rates are comparatively low from deep ocean samples.

4. Conclusions

To probe the photochemical reactivity of deep ocean refractory DOC, we have provided the first simultaneously measured rates of both H_2O_2 and O_2^- photoproduction in the surface and deep waters of the subarctic North Pacific on samples analyzed at sea and in the laboratory, thus adding to the very limited number of direct measurements of O_2^- photochemical production in natural samples. Our initial production rates for both O_2^- (< 10 min; $\sim 23 - 173$ nM hr^{-1}) and H_2O_2 (< 6 hrs; $\sim 1 - 8$ nM hr^{-1}) were comparable between surface and deep waters. However, with continued irradiation up to 48 hours, photoproduced H_2O_2 was generally much lower in deep samples compared to surface samples and its accumulation in deep samples either stopped or slowed despite both measurable CDOM absorption coefficients and superoxide steady-state values. Because the accumulation of H_2O_2 appears to be exhausted in these deep samples, our data suggest that the photoreactive CDOM there is a very small component of the already low DOC pool (< 40 μM), in the most aged waters of the North Pacific. Swan et al. (2009) also found

that CDOM and DOC do not correlate in their large survey of Pacific waters, indicating that the processes controlling CDOM and DOC distributions are not related. A strong linear relationship between CDOM absorption coefficients and AOU has been reported in the interior of the Pacific Ocean (Swan et al., 2009; Yamashita and Tanoue, 2009), indicating that new CDOM accumulates via biological oxidation and this appears to be semilabile and photolabile (Helms et al., 2013).

In addition to the work monitoring LMW carbonyl photoproduction in deep and surface samples Mopper et al. (1991), Stubbins et al., 2012 found that dissolved polycyclic aromatics (i.e. dissolved black carbon) collected in the deep waters (3000 m) of the North Atlantic was very photoreactive, decreasing by 95% in a 28 day irradiation. The high molecular weight fraction of dissolved black carbon has a radiocarbon age of ~18,000 years (Ziolkowski and Druffel, 2010), and has led to its recent classification as an ultrarefractory component of marine DOC, containing about 12 Pg C (Hansell, 2013), a small part of the entire refractory DOC pool (~642 Pg C for refractory and ultra refractory together; Hansell, 2013). Therefore, its apparent age may not be controlled by its photo-inertness but rather by controls on its transport into sunlit waters (Stubbins et al., 2012). Additional evidence that dissolved black carbon should not be classified as refractory is that stable compounds in marine DOM tend to have higher H/C and O/C ratios and lower aromatic content than that of dissolved black carbon (Lechtenfeld et al., 2014). Therefore, the photochemical production rates of ROS observed here in deep water samples collected in the Gulf of Alaska are likely due to the photooxidation of new CDOM generated at depth and/or dissolved black carbon and probably not from bulk refractory DOC.

While one goal of this study was to probe the photoreactivity of deep ocean DOC, it also contributes significant new understanding of the processes controlling the photoproduction and

decay of superoxide in marine environments. We calculated O_2^- photoproduction rates for surface waters in the Gulf of Alaska ranging from 10.8 – 16.5 $pM\ s^{-1}$, well above those estimated for biological O_2^- production in this region ($< 0.3 - \sim 5.5\ pM\ s^{-1}$; Hansard et al., 2010) and in the Equatorial Pacific ($\sim 0.3 - 1.2\ pM\ s^{-1}$; Rose et al., 2008b). Because oceanic H_2O_2 distributions and photoproduction rates have been so widely studied, the photoproduction rates of O_2^- have often been inferred from those for H_2O_2 . However, of all the identified ROS formed from CDOM photochemistry in natural waters (i.e. O_2^- , H_2O_2 , $\cdot OH$ and 1O_2), O_2^- is the only one for which direct apparent quantum yield spectra have not been reported. Our results indicate that in the open ocean, a large fraction of photoproduced O_2^- does not lead to H_2O_2 , meaning that the relationship between these two ROS may not be a simple factor of two (Equation 1). Our shipboard experiments alone indicate that for surface waters, the photoproduction rate of O_2^- ranges between 1.76 and 4.53 times greater than that for H_2O_2 . Until O_2^- apparent yield spectra become available, applying this relationship to H_2O_2 apparent quantum yield spectra to obtain estimates of O_2^- photoproduction rates in marine waters will be poorly constrained and very likely inaccurate.

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Figure Captions

Fig. 1. Stations sampled between August 5, 2013 and August 22, 2013 in the Gulf of Alaska.

Fig. 2. Measured irradiance ($\text{W cm}^{-2} \text{ s}^{-1} \text{ nm}^{-1}$) under the solar simulator entering the various experimental setups used in this study (thin black lines) compared to measured irradiance on a sunny day (Station 6, 50.75N 141.73 W, August 8, 2013 at 2:45 PM, PST) and on a cloudy day (Station 8, 52.95 N 141.55 W, August 9, 2013, 2:30 PM, PST) collected on the ship (bold black lines). The inset graph shows the irradiance for all solar simulator setups from highest to lowest: 600 mL jacketed beakers on a stir plate, 1 L jacket beakers on the benchtop, and the average irradiance for spectrophotometric cells in the water cooled aluminum block).

Fig. 3. Photoproduced H_2O_2 (nM) for water collected at Station 17: surface (white symbols), deep (black symbols), and 50:50 mix of surface and deep water (gray symbols) for shorter irradiations in spectrophotometric cells (circles) and longer irradiations in jacketed beakers (squares). Circles are averages ± 1 standard deviation of 3 spectrophotometric cells, squares are single samples taken from the jacketed beaker, multiplied by a correction factor, 0.8727, for the difference in integrated irradiance (290 – 400 nm) reaching the surface of the cell vs. that reaching the surface of the 1 L jacketed beaker.

Fig. 4. Photoproduced H_2O_2 (± 1 standard deviation) in surface (white circles), deep (black circles), and 50:50 mix (gray circles) samples, measured in 1L jacketed beakers for long term irradiations (top) and that corrected for average % CDOM fading from 290 – 350 nm (bottom).

Fig. 5. Paired shipboard irradiations of deep seawater collected at Station 05 (A) and Station 12 (B) monitoring photoproduced H_2O_2 (nM; white circles) and $[\text{O}_2^-]_{ss}$ (nM; black circles), both ± 1 standard deviation. After each time point during the irradiation of Station 12 deep seawater, the irradiation was stopped to monitor O_2^- decay (C) where black lines are the O_2^- decay data and dashed lines are that modeled according to Equation 6.

Fig. 6. Irradiations for simultaneous monitoring of H_2O_2 photoproduction rates and O_2^- steady-states (bottom) for Station 07 (white circles), Station 8 (white triangles), Station 20 (gray squares) and Station 27 (black diamonds). All H_2O_2 and O_2^- data are reported ± 1 standard deviation.

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Table 1. Station information for samples used in determining initial H_2O_2 photoproduction rates (P_{HOOH} , nM h^{-1}) ± 1 standard deviation for 6 hr shipboard irradiations in spectrophotometric cells (Cells) and for 7 – 9 hour irradiations done in 600 mL jacketed beakers post cruise (Beaker). Pooled rates, determined by linear regression of all $[\text{H}_2\text{O}_2]$ versus time for a given depth, and r^2 values for both irradiation systems are listed at the bottom of the table.

Station	Lat N	Long W	Depth ^a m	$a_g(325)$ m^{-1}	[DOC] $\mu\text{mol kg}^{-1}$	[NO_3^-] $\mu\text{mol kg}^{-1}$	Irradiation system	P_{HOOH}^b nM h^{-1}	r^2	$P_{\text{HOOH}}/a_g(325)$ nM m h^{-1}
8	52.95	141.55	Bottom (3852.4)	0.185	36.96	37.09	Cells	4.90 ± 0.68 (3)	0.98	26.50 ± 3.66
			Surface (nd)	0.211	62.07	17.51	Cells	3.62 ± 1.37 (3)	0.87	17.16 ± 6.51
17	57.15	148.71	Bottom (5146.2)	0.143	37.52	35.89	Cells	2.01 ± 0.66 (7)	0.65	14.04 ± 4.64
			50:50 mix	0.240	53.98	20.50	Cells	4.09 ± 1.09 (6)	0.78	17.00 ± 4.54
			Surface (4.8)	0.338	70.43	5.10	Cells	7.13 ± 1.31 (3)	0.94	21.12 ± 3.87
24	53	152	Bottom (4541.1)	0.160	37.52	36.19	Cells	1.42 ± 0.71 (5)	0.58	8.85 ± 4.40
			50:50 mix	0.204	51.39	22.22	Cells	0.88 ± 0.26 (4)	0.86	4.33 ± 1.26
			Surface ^a (5.3)	0.248	65.27	8.25	Cells	4.00 ± 0.05 (3)	0.99	16.16 ± 0.18
29 (PAPA)	50	145	Bottom (4075.1)	0.147	38.3	36.39	Cells	1.19 ± 0.36 (5)	0.79	8.12 ± 2.43
			Surface (nd)	0.202	55.93	13.82	Cells	1.07 ± 0.68 (3)	0.71	5.32 ± 3.39
7	51.78	141.64	Bottom (3850.7)	0.162	37.82	37.89	Beaker	3.66 ± 1.58 (3)	0.84	22.57 ± 9.75
			Surface (5.3)	0.134	62.44	10.61	Beaker	1.66 ± 0.08 (3)	0.99	12.41 ± 0.56
8	52.95	141.55	1000 m (1005.4)	0.197	40.3	45.51	Beaker	2.68 ± 1.36 (3)	0.8	13.59 ± 6.89

20	55	152.66	Bottom (4274.1)	0.156	37.12	36.56	Beaker	4.98 ± 1.72 (3)	0.89	31.85 ± 11.01
			Surface (3.9)	0.393	67.31	38.88	Beaker	8.27 ± 0.50 (3)	0.99	21.05 ± 1.26
			1000 m (1011.9)	0.210	40.46	2.83	Beaker	4.41 ± 2.92 (3)	0.7	21.00 ± 13.88
27	51	152	Bottom (5078.9)	0.144	38.39	37.11	Beaker	4.62 ± 0.23 (3)	0.99	32.10 ± 1.61
			Surface (3.9)	0.242	63.33	45.47	Beaker	5.59 ± 2.21 (4)	0.76	23.10 ± 9.13
			1000 m (1011.1)	0.203	41.22	10.24	Beaker	4.71 ± 2.92 (4)	0.72	23.19 ± 10.13
					Depth	Irrad. system	$P_{\text{HOOH}}^{\text{b}}$ nM h ⁻¹	r ²	$P_{\text{HOOH}}/a_{\text{g}}(325)$ nM m h ⁻¹	r ²
				POOLED RATES	Bottom	Cells	2.36 ± 0.66 (20)	0.41	14.5 ± 3.76	0.45
					50:50 mix	Cells	3.19 ± 1.22 (10)	0.46	13.46 ± 4.88	0.49
					Surface	Cells	4.88 ± 1.00 (13)	0.68	17.5 ± 2.49	0.82
					Bottom	Beaker	4.34 ± 0.75 (9)	0.82	28.6 ± 5.05	0.82
					1000 m	Beaker	3.98 ± 1.16 (10)	0.60	19.60 ± 5.55	0.61
					Surface	Beaker	4.99 ± 1.70 (9)	0.55	19.7 ± 5.01	0.69

^aNumber in parenthesis is the is the sample depth recorded by the CTD.

^bNumber in parenthesis is the number of discrete samples used in determining initial P_{HOOH} .

Table 2. Initial $[O_2^-]$ net accumulation rate (Initial *net* $P_{O_2^-}$; pM s^{-1}) and $[O_2^-]_{ss}$ (nM) measured during short (< 30 min) *shipboard* irradiations in jacketed beakers and that modeled from Equation 6 (Mod. $[O_2^-]_{ss}$), correcting for the time required (~10 sec) for the sample to reach the flow cell. Initial *net* $P_{O_2^-}$ was determined with a linear regression of FeLume signal vs. time over the first 15 seconds of irradiation ($r^2 = 0.90 - 0.99$) and k_{pseudo} (s^{-1}) was determined *post irradiation* from a nonlinear regression of FeLume signal vs. time (Equation 6). Modeled O_2^- photoproduction rates (Mod. $P_{O_2^-}$; pM s^{-1}) were determined with Equation 5, by assuming at $[O_2^-]_{ss}$ photoproduction equals decay, and inputs of k_D (Zafiriou, 1990), modeled $[O_2^-]_{ss}$ and k_{pseudo} . Average (Avg.) modeled $[O_2^-]_{ss}$, k_{pseudo} and photoproduction rates (net and modeled) for bottom (> 3000 m) and surface (< 50 m) samples and pooled rates for Initial *net* $P_{O_2^-}$ (linear regression; Lin. Reg. and associated r^2) included. All values are ± 1 standard deviation and p -values compare surface and deep sample averages (ANOVA) and linear regressions (ANCOVA).

Station	Lat N	Long W	Depth m	$a_g(325)$ m^{-1}	[DOC] $\mu\text{mol kg}^{-1}$	$[NO_3^-]$ $\mu\text{mol kg}^{-1}$	Initial <i>net</i> $P_{O_2^-}$ pM s^{-1}	$[O_2^-]_{ss}$ nM	Mod. $[O_2^-]_{ss}$ nM	k_{pseudo} $\times 10^{-3} \text{ s}^{-1}$	Mod. $P_{O_2^-}$ pM s^{-1}
1	48.42	128.68	2539.8	0.185	37.15	39.64	5.19 ± 0.82	1.24 ± 0.19	1.44 ± 0.23	6.18 ± 0.86	9.10 ± 1.89
			50:50 mix	0.225	51.02	19.84	6.38 ± 0.98	1.91 ± 0.29	2.16 ± 0.33	7.72 ± 0.77	17.1 ± 3.07
3	49.28	134.67	3680.5	0.156	38.12	37.50	9.39 ± 1.43	1.04 ± 0.16	1.13 ± 0.19	8.86 ± 1.58	10.2 ± 2.43
			1010.4	0.181	40.62	44.99	n.d.	1.23 ± 0.19	1.34 ± 0.21	6.21 ± 0.78	8.51 ± 1.65
			4.2	0.123	64.49	6.85	9.75 ± 1.51	1.61 ± 0.24	1.84 ± 0.28	7.12 ± 0.51	13.4 ± 2.21
10	54.6	141.41	3720.2	0.211	38.63	40.51	15.9 ± 2.47	1.37 ± 0.21	1.45 ± 0.22	4.02 ± 0.62	6.06 ± 1.28
			50:50 mix	0.203	51.72	24.74	n.d.	4.21 ± 0.64	3.88 ± 0.72	12.1 ± 2.92	48.4 ± 14.3
			4.2	0.196	64.81	8.97	13.5 ± 2.08	3.73 ± 0.56	3.81 ± 0.59	3.97 ± 0.40	16.5 ± 2.78
12	56.47	141.24	3630.4	0.157	n.d.	37.96	23.24 ± 3.70	4.34 ± 0.67	4.66 ± 0.72	5.75 ± 0.87	29.0 ± 5.83
			2.9	0.391	n.d.	0.23	8.94 ± 1.37	3.25 ± 0.49	3.41 ± 0.52	2.84 ± 0.58	10.8 ± 2.48
29	50	145	4075.1	0.147	38.3	36.39	17.8 ± 2.77	2.49 ± 0.38	2.72 ± 0.47	5.01 ± 1.41	14.1 ± 5.90
			49.8	0.202	55.93	13.82	5.15 ± 0.82	2.66 ± 0.40	2.70 ± 0.42	5.37 ± 0.75	15.2 ± 3.01
Sample	Mod. $[O_2^-]_{ss}$ nM	k_{pseudo} $\times 10^{-3} \text{ s}^{-1}$	Avg. [DOC] $\mu\text{mol kg}^{-1}$	Avg. <i>net</i> $P_{O_2^-}$	Lin. Reg. <i>net</i> $P_{O_2^-}$	r^2	Avg. <i>net</i> $P_{O_2^-}/a_g(325)$	Lin. reg. <i>net</i> $P_{O_2^-}/a_g(325)$	r^2	Avg. mod. $P_{O_2^-}$	Avg. mod $P_{O_2^-}/a_g(325)$
Bottom ^a	2.49 ± 1.83	5.96 ± 3.44	38.09 ± 1.74	13.3 ± 7.88	18.3 ± 0.75	0.83	89.4 ± 42.5	144 ± 18.1	0.79	15.0 ± 13.3	81.1 ± 71.6
	(4)	(4)	(4)	(4)	(123)		(4)	(123)		(4)	(4)
Surface ^a	2.94 ± 1.27	4.60 ± 1.97	61.74 ± 5.04	9.33 ± 4.57	9.33 ± 1.88	0.31	50.3 ± 24.6	49.1 ± 10.6	0.26	14.0 ± 5.83	75.6 ± 31.4
	(4)	(4)	(3)	(4)	(123)		(4)	(123)		(4)	(4)
p^b	0.6421	0.3264	2.901×10^{-4}	0.0724	1.421×10^{-9}		0.0724	1.060×10^{-11}		0.8488	0.8488

^aNumber in parenthesis is the number of discrete measurements used in determining averages and linear regressions (pooled rates).

^bNumbers in bold are significantly different ($p < 0.01$).

Figure1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6