Sources and Sinks of Hydrogen Peroxide at Station ALOHA

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Abstract

Hydrogen peroxide (H_2O_2) dynamics were studied in the water column at Sta. ALOHA (22°45'N 158°W). Samples were taken twice a day during 34 cruises from 1994 to 1998 to assess temporal and depth variability. Measurements were made using the dimerization of (p-hydroxyphenyl) acetic acid by H_2O_2 in the presence of peroxidase and detected by fluorometry. Beginning in 2001, a similar measurement protocol was used in conjunction with a continuous flow surface pumping and detection system. The "typical" climatological vertical distribution of total peroxide (H_2O_2 plus organic peroxide) had relatively high concentrations (40 to 70 nM) from the surface to about 50 m followed by a decrease to about 10 nM at 150 m. Under conditions of enhanced stratification, as typically occurs in summer, near-surface waters had even higher peroxide concentrations (>70 nM). At depths >150 m, concentrations of <10 nM were observed and dominated by organic peroxides. Although this pattern represents the mean condition of peroxides distribution at Sta. ALOHA, seasonal, daily and even higher frequency variations in time and space were observed reflecting the complex spectrum of H_2O_2 sources and sinks.

The seasonality is mainly due to the high irradiance occurring in summer which sustains a photoproduction of H_2O_2 and, perhaps, higher summertime DOM. The impact of wet deposition during episodic rainfall events and a variety of biogenic sources and sinks of H_2O_2 were also documented. Extremely low concentrations (<10 nM) of H_2O_2 were observed in surface waters on rare occasions, especially during large blooms of the cyano-bacterium *Trichodesmium*. This H_2O_2 scavenging is probably related to high catalase activity in this N_2-fixing microorganism and this may be a large sink for H_2O_2 during selected periods. The continuous pumping/detection system documented complex dynamics in H_2O_2 concentrations in the surface waters. Large variations (>50 nM in amplitude) were observed in time and/or space when peroxide concentrations were high (60-100 nM). By contrast, during *Trichodesmium* blooms we observed only small variations (mean 26 ± 7.5 nM), probably due to the much lower H_2O_2 concentrations.

Introduction

Hydrogen peroxide (H_2O_2) is ubiquitous in surface ocean waters and may play a fundamental role in biogeochemistry including trace metal bioavailability and the selection for, or against, selected groups of microorganisms (Johnson *et al.*, 1989; Moffet and Zafiriou, 1990, 1993; Palenik and Morel, 1988). Our interest in studying H_2O_2 in the oligotrophic North Pacific Ocean was inspired by three potential ecological applications: (1) the use of H_2O_2 as a tracer for vertical advection of surface ocean waters (Johnson *et al.*, 1989; Miller and Kester, 1994), (2) the role of H_2O_2 as an agent for coupled photolytic/chemical alteration of dissolved organic matter, thereby contributing to the global carbon cycle (Mopper and Zhou, 1990; Miller and Kester, 1994; Miller and Moran 1997) and (3) the influence of H_2O_2 on microbial metabolism including gene regulation, and even the survival of selected microbial groups based on the ability to metabolize or otherwise detoxify H_2O_2.

Worldwide concentrations of H_2O_2 range from 10^{-9} to 10^{-6} mol l^{-1} depending on light intensity, temperature, season, DOM loading and geographical location (Moffet and Zika, 1987; Johnson *et al.*, 1989; Cooper *et al.*, 1987; Karl *et al.*, 1993). Generally, concentration vs. water depth profiles display highest H_2O_2 concentrations in near surface waters and relatively steep gradients below the mixed-layer; below approximately 200 m H_2O_2 concentration approaches the analytical limit of detection (1-5 nM; Zika *et al.*, 1985; Moffet and Zafiriou, 1993; Weller and Schrems, 1993). There are multiple potential sources and sinks for H_2O_2 in the sea (**Table 1**). Several of these processes were investigated at Sta. ALOHA (A Long term Oligotrophic Habitat Assessment), located 100 km north of Oahu, Hawaii (22°45' N, 158° W).

H_2O_2: SOURCES AND SINKS

SOURCES	CONTROLS
1. DOM + light → H_2O_2 (probably via superoxide anion disproportionation)	• DOM concentrations and fluxes • DOM composition • light intensity and wavelength
2. cyanobacteria and eucaryotic algae (dark production, presumably reduction of O_2)	• distribution and abundance of putative microbes
3. marine phytoplankton (amino acid oxidase)	• distribution and abundance of putative microbes • amino acid concentrations and fluxes
4. atmosphere → ocean flux (esp. wet deposition)	• rainfall
SINKS	**CONTROLS**
1. biological degradation	• microbial biomass and activity • catalase activity
2. quenching	• DOM concentrations and fluxes • DOM composition • non-organic quenching agents
3. adsorption	• particulate matter concentration and composition
4. hydrolysis (enzymatic and non-enzymatic)	?

Table 1

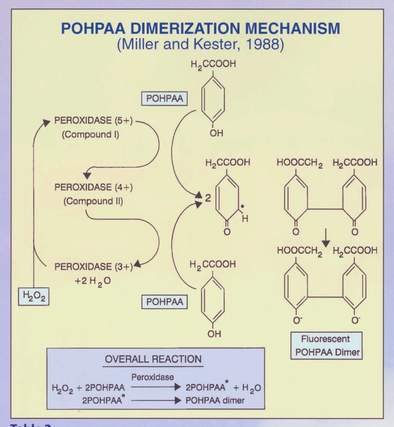


Table 2

Materials and Methods

Vertical profiles of H_2O_2 concentrations were measured using discrete water samples collected at approximately 0300-0600 hr and at 1500-1800 hr to investigate diurnal variability. Measurements began in January 1994 (HOT-51) and ended in June 1998 (HOT-94). We recently re-established the time-series in July 2001 (HOT-128), and more recently added a continuous flow underway H_2O_2 detection system (**Figure 1**).

For the discrete measurements, seawater was drawn from the CTD rosette into amber Nalgene HDPE 30 ml bottles. Total peroxide was determined by the para-hydroxyphenyl acetic acid (POHPAA) dimerization mechanism (Miller and Kester, 1988; **Table 2**). For each cast, a second set of samples was also taken to measure catalase-insensitive, "organic" peroxide, by the addition of catalase to destroy H_2O_2. Organic peroxide was estimated as the difference between the total peroxide (measurement #1) and the organic peroxide (measurement #2). No further characterization was made of the organic peroxide fraction.

Sample analysis was made by fluorescence using a Perkin-Elmer LS-5 fluorescence spectrometer (Ex = 313 nm, Em = 400 nm) relative to reagent H_2O_2 made in seawater taken at 1000 m where concentrations of total peroxide are very low (average < 5 nM). For the underway system (**Figure 1**), a peristaltic pump was used to collect samples from the ship's underway seawater system. The fluorometric reagent was then added to the sample prior to being introduced into a fluorescence spectrometer (Ex = 313 nm, Em = 400 nm). A relative baseline was made by adding catalase to surface seawater and a standard curve of reagent H_2O_2 made in the deep seawater was used to calibrate the method.

Figure 1: Photo of the shipboard laboratory components of the underway continuous H_2O_2 measurement system. Shown are the pump, reagent reservoir, reaction coils and fluorescence detector unit and data recorder/computer.

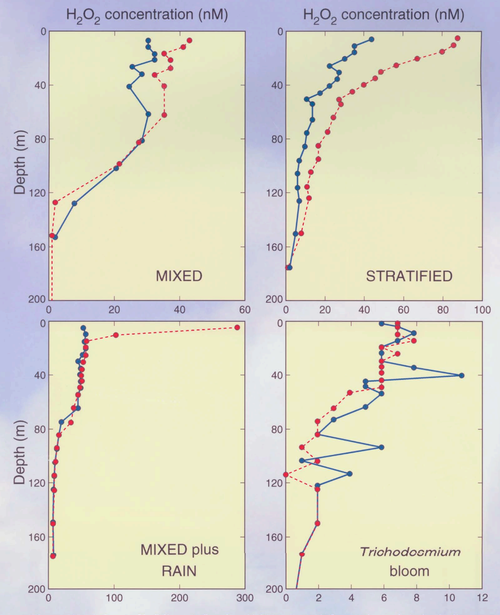


Figure 2: H_2O_2 profile "diversity" at Sta. ALOHA. In each frame the red circles indicate sunset and blue circles sunrise profiles; the difference is net photo-production of H_2O_2. [Left, top] typical mixed water column profiles resulting from vigorous wind mixing and lower average light; [Right, top] typical stratified water column profiles resulting from seasonal stratification and higher average light and, perhaps, higher DOM; [Left, bottom] rare, H_2O_2 wet deposition event (i.e., heavy rainstorm) on HOT-58; [Right, bottom] anomalously low surface H_2O_2 concentrations observed during a near surface *Trichodesmium* "bloom" event (note scale change). We currently believe that *Trichodesmium* stimulates H_2O_2 removal (via catalase) rather than repressing H_2O_2 production, although direct experiments have not been performed.

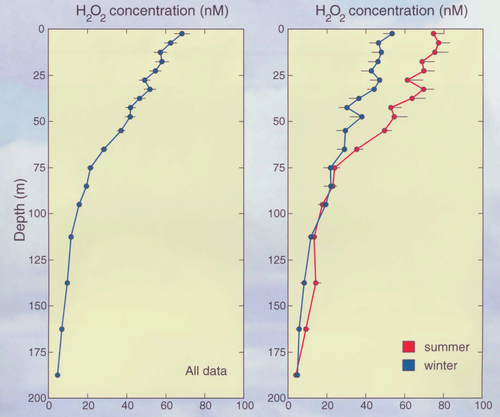


Figure 3: H_2O_2 at Sta. ALOHA. Shown on the left is the climatological H_2O_2 distribution as a function of depth for all samples collected over a 4-year period. Data are shown as mean ± 1 se. Shown on the right are data collected in winter only (Dec-Feb) contrasted with data collected in summer only (June-Aug), again as mean +(summer) or –(winter) 1 se.

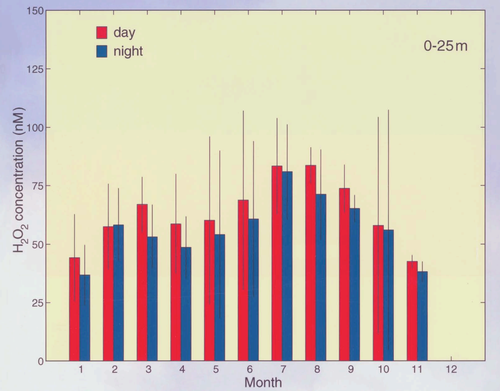


Figure 4: Monthly H_2O_2 concentrations (mean ± SD) for samples collected in the upper portion of the water column (0-25 m) showing variable inventories with maxima in summer, and variable day-to-night net photoproduction.

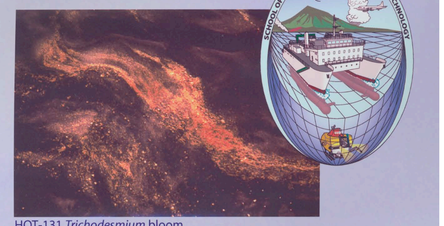
Results and Discussion

The marine "H_2O_2 cycle" is a complex interaction of numerous potential sources and sinks (**see Table 1**). At Sta. ALOHA the main source appears to be photochemical production in the near surface waters. Paired samples collected on separate hydrocasts before and after the daylight period can be used to assess the extent of daily net H_2O_2 photoproduction. For selected cruises, the results obtained are consistent with this proposed production mechanism, especially when the water column is highly stratified (**Figure 2**). However, on many cruises we have failed to observe any day/night differences in H_2O_2 inventories. For the 34 cruise observation period, 68% of the 0-25 m H_2O_2 concentrations measured during the day displayed approximately >20 nM higher concentrations than the night time profiles, 20% show the reverse pattern and 12% of the profiles show no significant difference between day and night profiles. Deep mixed layers would reduce the total effective light flux, and result in more uniform and lower near-surface H_2O_2 concentrations (**Figure 2**). Generally the magnitude of the change is positively correlated with absolute concentration; the higher the H_2O_2 concentration, the greater the daily variation thereof.

Surface water H_2O_2 concentrations at Sta. ALOHA ranged from >10 nM to 290 nM (**Figures 2 and 3**). Aperiodic wet deposition of H_2O_2 from the atmosphere accounted for the highest values that were observed in this study (**Figure 2**). Below the surface mixed-layers, H_2O_2 decreased exponentially with depth to values <10 nM below approximately 200 m. H_2O_2 concentrations show the predicted summer enhancement (due to combined water column stability and light); near-surface H_2O_2 concentrations in summer were approximately 20 nM higher than in winter (**Figures 3 and 4**), although there is large cruise-to-cruise variation due to the relatively rapid dynamics of H_2O_2.

Beginning in July 2001, an underway system was used simultaneously with the manual method described above. Continuous measurements of surface H_2O_2 concentrations at Sta. ALOHA in December 2001 (**Figure 5, bottom**) show large temporal/spatial variations in H_2O_2 that appear to be independent of photoproduction. Without depth-resolved profiles it is difficult to determine the primary controls on these patterns, but they are likely related variations in mixing or local rainfall. A dynamic range of 100 nM was observed in surface H_2O_2 during the December 2001 cruise. During the HOT-131 cruise, a *Trichodesmium* bloom in the area resulted in lower overall peroxide concentrations and a smaller dynamic range, both as expected (**Figure 5, top**). Apparently *Trichodesmium* blooms either scavenge H_2O_2 or prevent it from being formed in the first place – most likely the former.

The underway system for measuring surface H_2O_2 concentrations in real time is still in an experimental phase but initial results are very promising. This system could lead to an operational underway detection system for *Trichodesmium* (and perhaps other N_2-fixing microorganisms) once the cause-and-effect mechanisms are better established.



HOT-131 *Trichodesmium* bloom

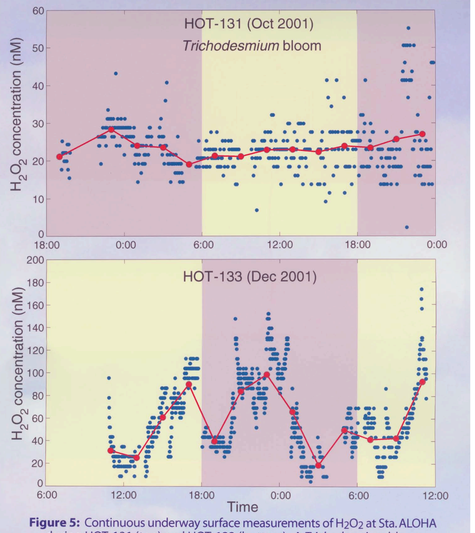


Figure 5: Continuous underway surface measurements of H_2O_2 at Sta. ALOHA during HOT-131 (top) and HOT-133 (bottom). A *Trichodesmium* bloom was encountered during HOT-131 which resulted in lower than expected mean H_2O_2 concentrations and limited time/space variation. In contrast, the HOT-133 cruise had higher concentrations with evidence of both net photoproduction during daylight hours and spatial variations (caused by local wet deposition?) at night. The red data points and solid red line show the hourly mean H_2O_2 concentrations.

Conclusions and Perspectives

Concentrations of H_2O_2 in the oligotrophic waters of Sta. ALOHA provide useful information regarding biological, chemical and physical (mixing) processes. Distributions observed are consistent with a light-dependent photochemical reaction as the major source. Although more detailed information on local H_2O_2 sources and sinks is necessary before meaningful models can be developed, the results available to date provide ample incentive to continue these investigations. The development of an underway H_2O_2 measurement system provides explicit evidence for temporal/spatial heterogeneity in H_2O_2 dynamics in near-surface waters. More research needs to be conducted on the H_2O_2 sink mechanisms, especially the apparent active H_2O_2 scavenging by blooms of *Trichodesmium*. This is a work in progress.

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Acknowledgments

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PUBLIC DATA ACCESS
http://hahana.soest.hawaii.edu/HOT/HOT_JGOFS