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Hydrogen peroxide in deep waters from the Mediterranean Sea, South Atlantic and South Pacific Oceans

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Hydrogen peroxide (H_2O_2) is present ubiquitously in marine surface waters where it is a reactive intermediate in the cycling of many trace elements. Photochemical processes are considered the dominant natural H_2O_2 source, yet cannot explain nanomolar H_2O_2 concentrations below the photic zone. Here, we determined the concentration of H_2O_2 in full depth profiles across three ocean basins (Mediterranean Sea, South Atlantic and South Pacific Oceans). To determine the accuracy of H_2O_2 measurements in the deep ocean we also re-assessed the contribution of interfering species to 'apparent H_2O_2 ', as analysed by the luminol based chemiluminescence technique. Within the vicinity of coastal oxygen minimum zones, accurate measurement of H_2O_2 was not possible due to interference from Fe(II) . Offshore, in deep ($>1000\text{ m}$) waters H_2O_2 concentrations ranged from $0.25 \pm 0.27\text{ nM}$ (Mediterranean, Balearics-Algeria) to $2.9 \pm 2.2\text{ nM}$ (Mediterranean, Corsica-France). Our results indicate that a dark, pelagic H_2O_2 production mechanism must occur throughout the deep ocean. A bacterial source of H_2O_2 is the most likely origin and we show that this source is likely sufficient to account for all of the observed H_2O_2 in the deep ocean.

H_2O_2 is found ubiquitously in natural surface waters^{1–3}. Photochemistry^{4–6}, biological activity^{7–9}, and physical mixing^{1,10}, are considered to be the dominant processes controlling H_2O_2 concentration in the surface ocean. H_2O_2 concentrations from 20–800 nM are reported in surface marine waters with a diurnal oscillation typically observed^{11–13}. H_2O_2 is a reactive intermediate in the biogeochemical cycling of a range of elements in surface waters^{14,15}. It also has direct effects on surface dwelling microorganisms as ambient H_2O_2 concentrations are sufficient to trigger oxidative stress in many marine phytoplankton species^{16,17}.

Intriguingly, measurable concentrations of up to 6 nM H_2O_2 have been reported for the deep North Pacific¹⁸, far below the photic zone. The distribution of H_2O_2 throughout the deep ocean is however poorly constrained and thus the possible effects of H_2O_2 on biogeochemical cycles in these waters are presently difficult to assess. If H_2O_2 is found at low nM concentrations throughout the deep ocean this also raises questions about its origin. A photochemical source of H_2O_2 at these depths is not plausible so the foremost hypothesis is a dark biological source^{19,20} associated with bacterially mediated remineralisation of organic matter.

Here we report full depth profiles of H_2O_2 in three different ocean basins (Mediterranean Sea, South Atlantic and South Pacific Oceans) to determine whether measurable concentrations of H_2O_2 are found ubiquitously in the deep ocean. We also re-assess the robustness of the widely used flow-injection luminol based chemiluminescence method^{12,21,22} for the analysis of low ($<10\text{ nM}$) H_2O_2 concentrations in seawater.

Results

Verifying the accuracy of H_2O_2 measurements. As noted by Yuan and Shiller¹⁸, at low nM concentrations it is very difficult to establish conclusively that a measured, or 'apparent', concentration of H_2O_2 is real and not an artifact of sample handling or the result of compounds other than H_2O_2 causing a false positive signal. Potential systematic problems with light exposure, filtration and contamination from the atmosphere or de-ionized water residues exist. All of these potential H_2O_2 contamination sources were however eliminated. The use of opaque sample bottles and foil shields minimized background light. The ambient lighting used in our laboratory produced only a small increase in H_2O_2 concentrations even when seawater was deliberately exposed to light by removal of sample bottle lids and placement of samples directly below our 330 lumens light source ($<0.6\text{ nM min}^{-1}$, which is also inclusive of any contamination arising from atmospheric H_2O_2). Filtration was not

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Metal ion	Spike added	Measured H ₂ O ₂ concentration prior to spike/nM	% Recovery H ₂ O ₂
V(IV)	1.0 nM	42	113
	2.0 nM	42	149
	20 nM	42	303
Fe(III)	3.5 nM	42	104
Fe(II)	130 pM	42	113
	330 pM	42	130
	330 pM	1.3	153
	2.6 nM	1.3	202

Table 1. Apparent H₂O₂ signal produced in aged South Atlantic seawater when spiked with a selection of trace metal species.

used for normal sampling purposes and all sampling apparatus was pre-conditioned with seawater to ensure no contamination from de-ionized water derived H₂O₂.

With respect to the possible detection of species other than H₂O₂, the H₂O₂ luminol based FIA method is expected to be robust under most circumstances²². Organic peroxidases typically produce a low luminol response relative to the equivalent molar concentration of H₂O₂ and are only present at <1 nM in surface seawater²³. Yuan and Shiller¹⁸ for example found that the response of methyl hydroperoxide solution was 11% that of H₂O₂ (and this signal may have actually arisen from a H₂O₂ impurity). Some trace metal ions also cause a positive interference (Table 1)²². However, in oxygenated offshore seawaters the anticipated interferences should be minimal. Dissolved Fe (DFe) concentrations in the Atlantic for example rarely exceed 1 nM^{24,25} which is equivalent to an apparent H₂O₂ signal of <0.5 nM. The response of Fe(II) is however stronger than that of Fe(III) (Table 1). At low O₂ concentrations the oxidation rate of Fe(II) to Fe(III) is reduced such that high (nM) concentrations of Fe(II) can be found in the water column²⁶. For example, in the Southern Ocean Sarthou *et al.*²⁷ measured Fe(II) half-lives with respect to oxidation of 3–11 min in surface seawater (at pH 8.0–8.1 and O₂ 234–353 μM), whereas Scholz *et al.*²⁸ determined that the apparent Fe(II) half-life in bottom water of the Peruvian oxygen minimum zone (OMZ) was 16–18 h (at pH 7.6, O₂ <0.5 μM). Outside OMZs the half-life of Fe(II) in seawater is thus expected to be sufficiently short that the delay between sample collection and analysis (typically 10 min – 1 h) should be sufficient for Fe(II) to decay to undetectable (low pM) concentrations²⁹. Furthermore, as an apparent H₂O₂ signal arising from mainly non-H₂O₂ components is deducted from our measured H₂O₂ concentrations (see below), this should include any residual concentration of Fe(II). However, accounting for Fe(II) remains problematic in OMZs where nM Fe(II) concentrations are present during analysis.

It is also particularly difficult to assess the effect of dissolved V (DV) on apparent H₂O₂ concentrations as less data are presently available on the distribution of reduced V species in the marine environment. Vanadium exhibits a relatively uniform concentration profile in the ocean with typical dissolved concentrations of 30–40 nM and a dominant oxidation state of V(V)^{30,31}. Yet under reducing conditions a fraction of DV may exist as V(IV). Nanomolar concentrations of V(IV) have indeed been observed in sub-oxic and coastal waters³². As is the case for Fe(II) and Fe(III), any apparent H₂O₂ signal arising from V(IV) in oxic seawater (as per Table 1) should be accounted for by the deduction of an aged seawater blank from all measurements. But, as is the case for Fe(II), V(IV) interference may still be problematic in OMZs due to potentially enhanced concentrations which are subject to change as the sample is handled and analyzed. We present two depth profiles of DV in the S Pacific (Fig. 1) in order to assess the potential for this interference to occur within the vicinity of a coastal OMZ.

Trace metal samples were collected and analysed for Fe(II), DFe and DV at two stations (Stations 10 and 12, Fig. 1(a)) in the Peruvian OMZ. Oxygen depletion to <2 μmol kg⁻¹ was evident in the water column at both stations (Fig. 1(b)). Consistent with results reported elsewhere along the Peruvian coastline^{26,33,34} low O₂ waters below the surface mixed layer were enriched in DFe with the majority of DFe present as Fe(II). Up to 30 nM DFe was present at the shallow station (Station 10) and up to 2.4 nM at the offshore station (Station 12). Fe(II) concentrations were up to 12 nM and 0.77 nM at the shallow and offshore stations, respectively (Fig. 1(b)). The apparent H₂O₂ signal at these stations closely followed Fe(II) concentrations at all depths below 20 m, consistent with the interference demonstrated in aged seawater (Table 1). At both stations DV profiles exhibited a decline at intermediate depths associated with low O₂ concentrations (Fig. 1(b)). The solubility of V(V) in seawater exceeds that of V(IV), thus a decline in DV concentration is expected under reducing conditions when V(IV) is formed³⁵. Only a relatively small dissolved V(IV) concentration, ~1 nM, would be required to produce a nM apparent H₂O₂ signal (Table 1). Whilst we cannot determine the concentration of dissolved V(IV) present, it remains possible that this ion is a problematic interference with the luminol method for determination of H₂O₂ in coastal OMZs. It is not possible to correct for this interference as the concentration of reduced species (both Fe(II) and V(IV)) cannot be quantified with sufficient accuracy at the exact time H₂O₂ concentrations are measured. An aged (1–2 weeks old) seawater blank accounts for the presence of stable interfering species, but not unstable interfering species such as Fe(II) and V(IV) – the concentration of which must be known at the time H₂O₂ is measured if a correction is to be made. This difficulty arises, not only because of logistical constraints in coordinating the simultaneous measurement of multiple redox sensitive variables, but also because the measurement of Fe(II) via luminol itself is subject to a V(IV) interference³⁶ and the chemiluminescence response of luminol to some ions, including Fe(II), is non-linear³⁷. As an additional precaution we have therefore excluded all stations from our H₂O₂ dataset where

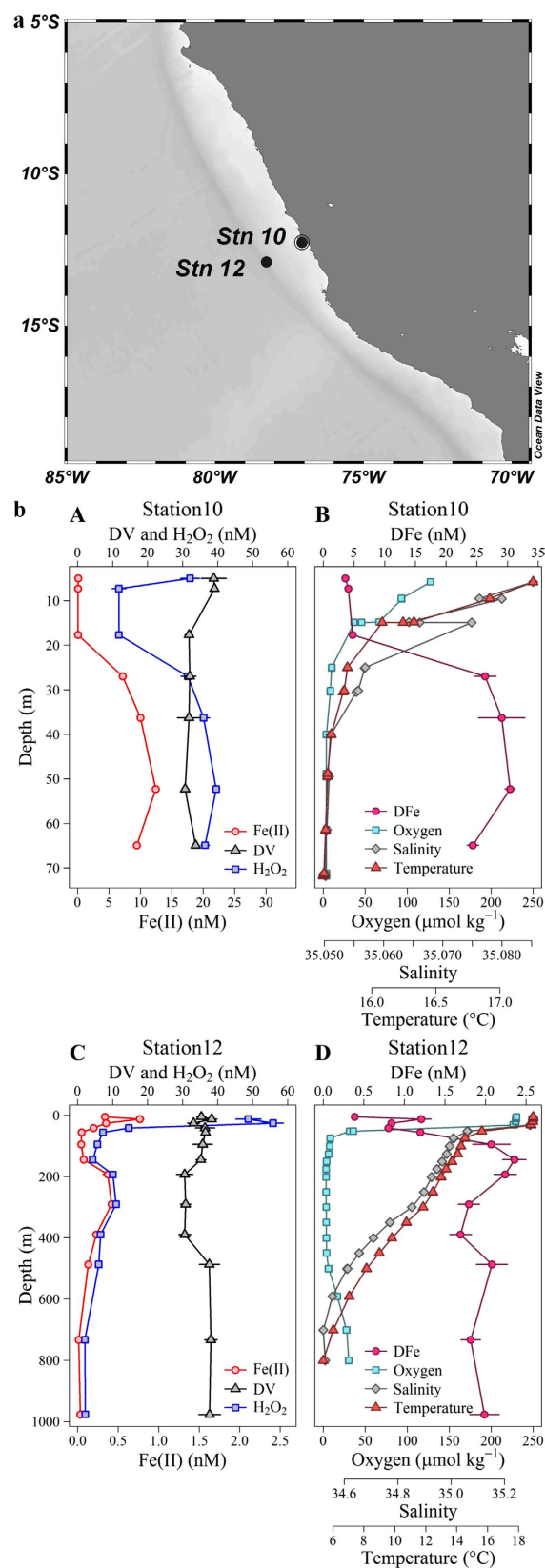


Figure 1. (a) Location of a shallow (Station 10) and offshore (Station 12) station within the Peruvian oxygen minimum zone (OMZ), plotted in Ocean Data View⁵⁴. (b) H₂O₂ concentrations (after deduction of an aged seawater blank) and concentrations of potentially interfering trace metals at two stations in the OMZ. A Station 10 H₂O₂, dissolved (<0.2 μm) V (DV), Fe(II). B Station 10 Salinity, temperature, dissolved Fe (DFe), dissolved O₂. C Station 12 H₂O₂, DV, Fe(II). D Station 12 Salinity, temperature, DFe, dissolved O₂. (b) made in R version 3.2.3 (2015), GWDG Göttingen, Germany, <http://ftp5.gwdg.de/pub/misc/cran/>.

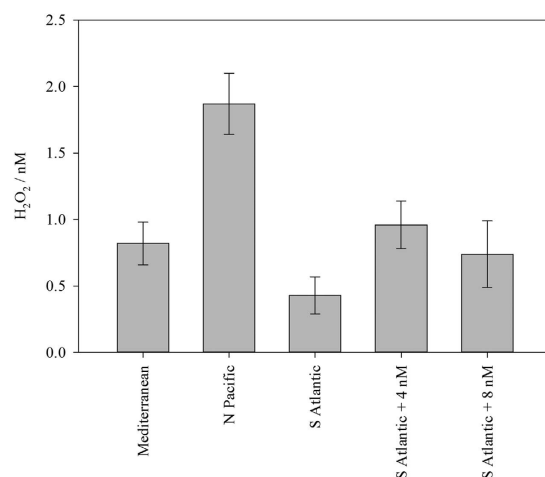


Figure 2. Apparent H_2O_2 measured after catalase treatment of different aged seawater samples. Mean ($n = 4$, $\pm$ standard deviation) H_2O_2 determined by flow injection analysis with no deduction made to account for positive interference from species other than H_2O_2 . South Atlantic seawater was spiked (+4 and 8 nM H_2O_2) and re-analyzed to verify the effectiveness of catalase addition.

Fe(II) exceeded 0.3 nM at multiple depths (Fe(II) was measured at all stations in the S Atlantic and S Pacific with a detection limit of ~ 0.2 nM, data not shown).

Some insight into what an apparent H_2O_2 signal is can be gained from use of the enzyme catalase, which removes H_2O_2 from solution. After surface seawater was aged in the dark at 6°C for >2 weeks a variable apparent H_2O_2 signal of >1 nM could still be observed in all surface seawater tested. An apparent H_2O_2 signal of 0.4–1.9 nM was still measured after treatment with catalase at 25°C for 1 h (Fig. 2). It is plausible that a residual, measurable concentration of H_2O_2 remained in seawater after catalase addition. Yet, if this residual H_2O_2 concentration depended primarily upon enzyme efficiency, we would expect to have observed the same residual H_2O_2 concentration in every catalase treated seawater sample. The residual signal however varied between water samples (Fig. 2). This indicated that the residual signal arose mainly from components other than H_2O_2 .

As a further test to determine if the apparent H_2O_2 signal could actually have arisen from H_2O_2 , we used the oxidation rate of Fe(II) in de-oxygenated seawater. Rate constants are available for the oxidation of Fe(II) via H_2O_2 in seawater (Equation (1)) at any environmentally relevant pH, temperature and salinity^{38,39}. Thus, by comparing the oxidation rate of Fe(II) spiked into catalase treated and non-catalase treated aged seawater, the concentration of H_2O_2 present in aged seawater was estimated. The apparent H_2O_2 signal determined for aged S Atlantic seawater ranged from 0.43–0.96 nM after the addition of catalase (Fig. 2). When a 5 nM Fe(II) spike was added to this seawater, after de-oxygenating and treatment with catalase, the Fe(II) signal was stable for in excess of 1 h. Whereas, without catalase a 1.8 nM decrease was observed (in 1 h). Using the rate constant $\log k\ 4.17\ \text{M}^{-1}\ \text{s}^{-1}$ (determined at 10°C and pH 8.17³⁹ matching our experimental conditions of 10.4°C and pH 8.2) and assuming a pseudo-first order reaction (with constant $[H_2O_2]$) according to Equation (1), we determined the theoretical decline in Fe(II) concentration.

$$d[\text{Fe(II)}]/dt = -k[\text{Fe(II)}][H_2O_2] \quad (1)$$

Assuming the decrease in Fe(II) concentration in non-catalase treated aged seawater could be attributed exclusively to oxidation by H_2O_2 , the H_2O_2 concentration in aged S Atlantic seawater prior to catalase treatment was 7 nM. No change was observed in Fe(II) concentration after catalase treatment. This suggested that catalase effectively lowered the initial 7 nM H_2O_2 to no more than a few hundred pM. If H_2O_2 were actually present in solution at the apparent concentrations measured (0.4–1.9 nM, Fig. 2), 0.4 nM of H_2O_2 should have resulted in a decline of 0.1 nM Fe(II) over 1 h, and 1.0 nM of residual H_2O_2 should have resulted in a decline of 0.25 nM Fe(II). The absence of Fe(II) decay was consistent with our interpretation of the residual post-catalase apparent H_2O_2 signal (Fig. 2) as being due to components other than H_2O_2 . For the purposes of calculating H_2O_2 concentration in seawater, the residual signal after aging and catalase treatment should therefore be treated as a blank. The detection limit of the method was therefore (3 standard deviations of the blank) 0.4–0.8 nM, similar to the typical blank of 0.6 nM reported elsewhere using similar apparatus^{5,40}. This was higher than the 0.1–0.3 nM detection limit if the signal arising only from the reagent and background light/electrical noise was considered.

H_2O_2 concentrations in the Mediterranean Sea and South Atlantic. Depth profiles of H_2O_2 were collected in the Mediterranean Sea (69 stations), S Atlantic (14 stations) and S Pacific Oceans. As outlined above, H_2O_2 measured at all 13 S Pacific stations and 30 S Atlantic stations was unreliable because of high (>0.3 nM) Fe(II) concentrations at multiple depths associated with coastal OMZs (Fig. 1). These stations are excluded and not discussed further.

The reproducibility of the apparent H_2O_2 signal when sampling triplicate samplers from the same depth (Fig. 3(a)) verified that the signal was not subject to random contamination. A comparison of H_2O_2 measurements

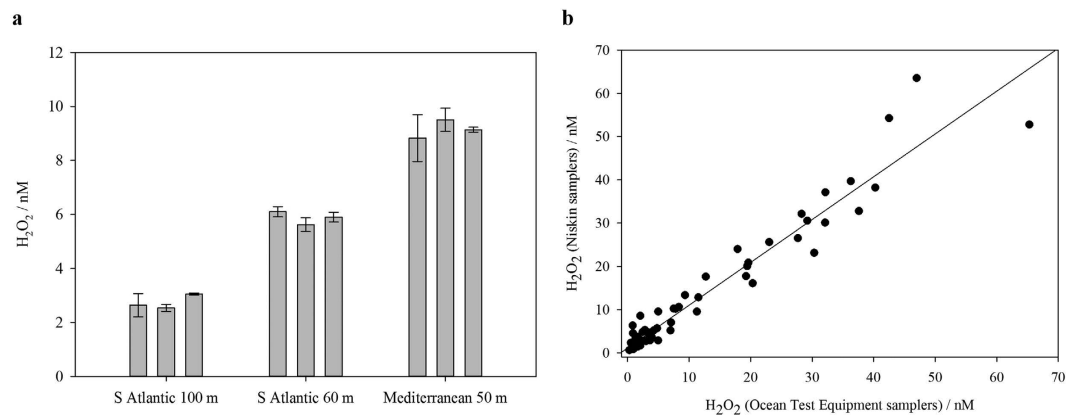


Figure 3. (a) Replicate apparent H_2O_2 measurements using 3 separate samplers deployed at the same depth. Mean ($n = 4$ injections) and standard deviation of apparent H_2O_2 shown. Apparent H_2O_2 included a signal arising from components other than H_2O_2 in solution. (b) Comparison between H_2O_2 samples from two sub-surface sampling methods. Ocean Test Equipment samplers mounted on a powder coated sampling rosette with a Kevlar cable, and Niskin samplers with internal metal components on a standard stainless steel rosette ($n = 67$ pairs) deployed at the same depths along the South Atlantic transect (December 2015, line of best fit plotted $R^2 = 0.94$).

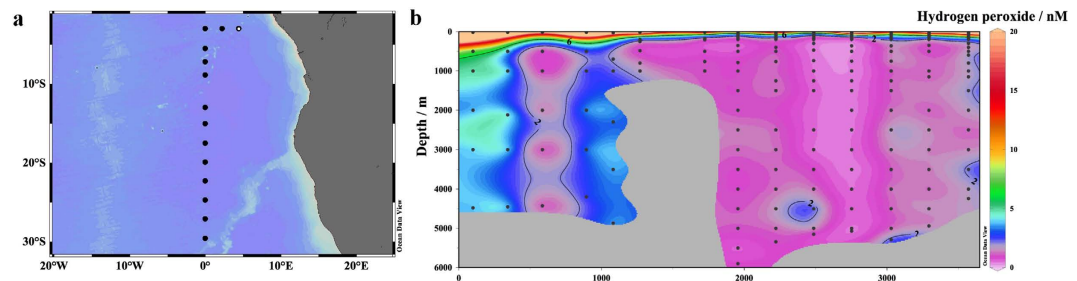


Figure 4. (a) GEOTRACES GA08 transect proceeding northeast-south in the South Atlantic, December 2015 (b) H_2O_2 concentrations in the South Atlantic plotted as distance along section shown (km) from northeast (left) to south (right). The first station (left) is marked on (a). Plotted in Ocean Data View⁵⁴.

using equipment that was, or was not, trace metal clean (OTE and Niskin samplers with internal stainless steel components), indicated a small (mean 1.2 nM based on linear intercept, $R^2 = 0.94$) overestimation of H_2O_2 concentration (Fig. 3(b)) when water was collected from Niskin samplers with metal components. The difference ($n = 67$ pairs) was statistically significant ($P < 0.001$, Wilcoxon Signed Rank Test – a paired test used here because it does not require a normally distributed population) and remained statistically significant both when the 3 highest concentrations were excluded ($n = 64$ pairs, $P < 0.001$) and when only concentrations < 5.0 nM were compared ($n = 40$ pairs, $P < 0.001$).

Proceeding from north-east to south-west along the S Atlantic transect, H_2O_2 was elevated at four of the first five stations throughout the water column, separated by a station with low H_2O_2 concentrations (Fig. 4) which were more typical of stations observed further south. These elevated concentrations may be associated with the OMZ, but this is difficult to confirm due to high (> 0.3 nM) Fe(II) concentrations within the OMZ along the shelf (at excluded stations, not shown) that interfered with the method for H_2O_2 analysis (Table 1). In the Mediterranean (Fig. 5) the vertical profile of H_2O_2 was very similar to that observed at the southern-most stations in the S Atlantic. High (> 4 nM) H_2O_2 was normally confined to approximately the uppermost 100 m. A notable exception was however found in shallower waters (< 500 m) for example off the coast of west Sicily (Fig. 5) where the gradient in H_2O_2 concentration declined more slowly with depth and elevated (at least 2–5 nM) H_2O_2 was found throughout the water column.

H_2O_2 concentrations in the surface ocean correlate strongly with irradiance⁵ and thus vary both diurnally and seasonally. Modest variation in temporally different reported concentrations in the same region is therefore expected. Our two most extensive datasets, for the S Atlantic (Fig. 6(a)) and the western Mediterranean (Fig. 6(b)), exhibited surface water concentrations of H_2O_2 with similar ranges to those reported by others^{5,12}. Price *et al.*¹² for example measured H_2O_2 concentrations ranging from 34–143 nM (mean 91 ± 29 nM) in surface waters from the Straits of Sicily, whereas here we report concentrations with a range 24–81 nM (mean 43 ± 22 nM).

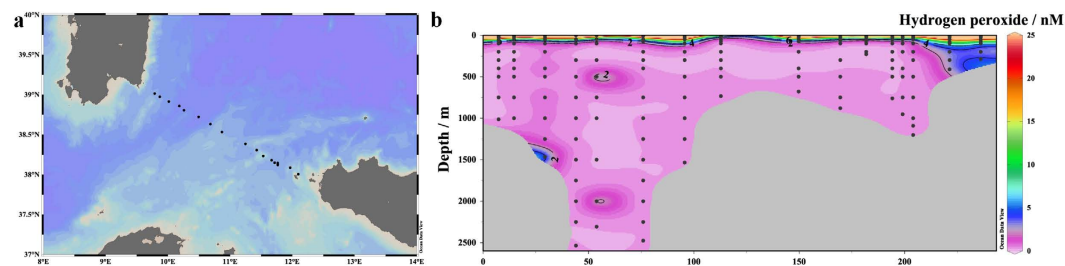


Figure 5. (a) Ocean Certain western Mediterranean transect between Sardinia and Sicily, August 2015 (b) H_2O_2 concentrations along the transect plotted as distance along section shown (km) from northwest (left) to southeast (right). Plotted in Ocean Data View⁵⁴.

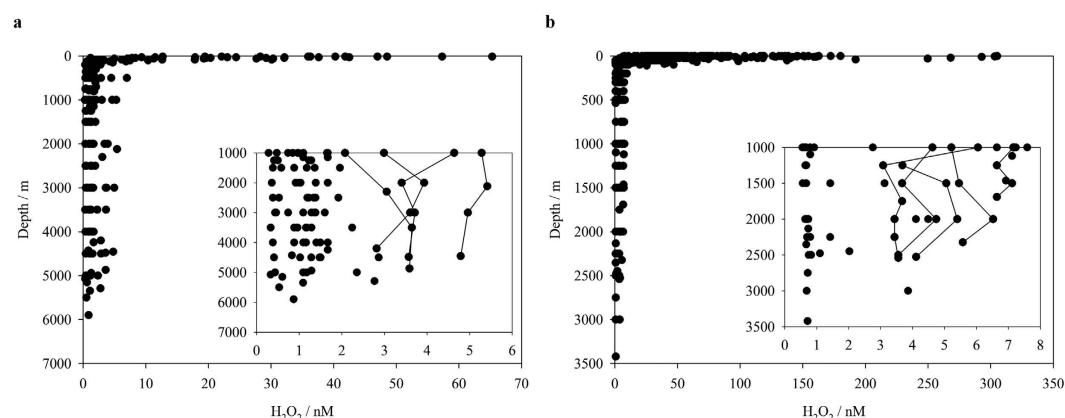


Figure 6. (a) H_2O_2 across all South Atlantic stations sampled. Only samples collected using trace metal clean Ocean Test Equipment samplers (181 data points, excluding 99 below detection limit) are shown (same data as Fig. 4b). Inset H_2O_2 at, or below, 1000 m (83 datapoints, excluding 80 below detection limit). For stations with multiple samples beneath 1000 m, profiles are connected if H_2O_2 exceeded 2.9 nM at any depth. (b) H_2O_2 across all Mediterranean stations. All sub-surface samples were collected from Niskin samplers free from internal metal components on a stainless steel cable (total 482 data points-including those shown in Fig. 5b, excluding 199 below detection limit). Inset H_2O_2 at, or below, 1000 m only (60 datapoints, excluding 64 below detection limit). For stations with multiple samples beneath 1000 m, profiles are connected if H_2O_2 exceeded 2.9 nM at any depth.

Discussion

A sharp decline in H_2O_2 concentration with depth was illustrated within the datasets as a whole (Fig. 6) consistent with the confinement of the dominant H_2O_2 source, direct photochemistry, to the surface ocean and H_2O_2 concentrations thus dropping rapidly below the thermocline¹. Our depth profiles of H_2O_2 suggested that the source of H_2O_2 in the deep ocean is pelagic with no obvious benthic sources evident at depth in the S Atlantic or Mediterranean (Figs 4(b) and 5(b)). A H_2O_2 source may be associated with the OMZ in the Angolan Basin (Fig. 4(b)), but this is difficult to confirm given our exclusion of shelf stations from the S Atlantic dataset and the uncertainty associated with false positive interferences from trace metals within the OMZ (Fig. 1).

The H_2O_2 concentrations observed in deep (>1000 m) waters at some stations in both the S Atlantic and Mediterranean were within the range 1–6 nM previously reported in the N Pacific¹⁸. However, the mean concentrations measured here are lower with the majority of measurements at >1000 m depth in both the Mediterranean and S Atlantic below detection (<0.4–0.8 nM). There is thereby moderate uncertainty associated with determining the mean deep (>1000 m) H_2O_2 concentration as this depends on how the datapoints below detection are treated. Using the most conservative approach, where we consider that datapoints below detection may take any value between 0 and the detection limit, the mean H_2O_2 concentration for the four transects where sufficient data points are available (Table 2) ranges from 0.25 ± 0.27 to 2.9 ± 2.2 nM. The mean H_2O_2 concentration >1000 m globally is therefore likely within the range 0.25–2.9 nM.

Unfortunately the rate of H_2O_2 decay has not been determined at *in situ* temperature and pressure for non-surface marine waters. Yet, when Yuan and Shiller¹⁸ incubated deep seawater (from 1500 and 5000 m in the N Pacific) at 21 °C (at which temperature most H_2O_2 decay experiments are reported) the observed decay constant, which is assumed to be first order, was 0.003 h^{-1} corresponding to a half-life of 230 h. This was slower than the decay measured in N Pacific surface waters where rate constants ranged from 0.004 – 0.02 h^{-1} , corresponding to half-lives of 33–170 h^{18,41,42}. As H_2O_2 decay is considered to be primarily microbially mediated⁴³

Transect	Depths*/m	H ₂ O ₂ Range/nM	Mean H ₂ O ₂ (±SD)/nM
Mediterranean, Sicily to Tunisia	100	0.61–3.1	1.5 ± 1.0
Mediterranean, Corsica to France	100	b/d–10	6.0 ± 4.2
	>1000	b/d–6.8	2.9 ± 2.2
Mediterranean, Balearics to Algeria	>1000	b/d–0.62	0.25 ± 0.27
Mediterranean, Sardinia to Sicily	100	b/d–9.9	1.7 ± 2.8
	>1000	b/d–6.1	0.55 ± 1.3
Mediterranean, Balearics to Spain	100	b/d–5.3	1.2 ± 2.1
S Atlantic, Angola Basin	100	0.46–9.6	3.6 ± 3.7
	>1000	b/d–4.6	0.90 ± 1.2
S Pacific, Peruvian upwelling**	100	2.8–9.0	5.0 ± 1.9
	>1000	b/d–2.1	1.1 ± 0.65

Table 2. Summary of deep ocean H₂O₂ data. b/d ‘below detection limit’ (0.4–0.8 nM), SD standard deviation of the mean H₂O₂ including the possible H₂O₂ concentration range for samples below detection (i.e. any value lower than the detection limit for each section), *100 m depth inclusive of samples 90–110 m, >1000 m depths inclusive of samples from 950 m. Mean/range not shown for groupings with <5 datapoints. **S Pacific H₂O₂ data is likely to be an over-estimate due to the presence of interfering species (eg Fe(II)) and is included for indicative purposes only.

decay constants in deep seawater would be expected to scale approximately with microbial activity¹⁸. Anticipated decay rates in the deep ocean would thereby be at least two orders of magnitude smaller than those in surface waters^{18,44,45}. The H₂O₂ decay rate constant measured at 1500 and 5000 m in the N Pacific is thereby quite high (14–72% of that measured in the same region for surface waters), which may imply that an inorganic mechanism also contributes to the observed decay rate¹⁸. There are several uncertainties here as it is unknown to what extent the mechanism for H₂O₂ decay in the deep ocean remains primarily enzymatic, as it is in surface waters, and it is also unclear whether a rate constant determined at 1 atm pressure and 21 °C can be extrapolated to *in situ* conditions at >1000 m depth.

None the less, if this rate constant is applicable to the deep ocean *in situ* and, as we suggest here, H₂O₂ is found throughout the deep ocean at a mean concentration of 0.25–2.9 nM, H₂O₂ must be produced at a rate of 0.8–9 pM h^{−1} to balance the measured decay. Reported rates of dark H₂O₂ production vary in the surface ocean, but two studies in different environments observed very similar ranges: peak surface dark H₂O₂ production rates for unfiltered coastal and Sargasso seawater are reported as 0.8–2.4 nM h^{−1}⁴⁶ and 1–3 nM h^{−1}¹⁹, respectively. The supposed dark production rate of H₂O₂ in the deep ocean would thereby have to be 0.03–1.1% of that found in surface waters. Measurements of leucine incorporation suggest that bacterial activity at 1000 m depth is approximately 0.8% of that at the surface⁴⁷. So, whilst not conclusive evidence, this calculation does at least suggest that a dark production mechanism, similar to that observed in surface waters^{19,46}, could feasibly account for all of the H₂O₂ present in the deep ocean.

Conclusions

Several trace metal species cause positive interferences with the widely used luminol-chemiluminescence method for H₂O₂ analysis in seawater. The majority of a residual 0.43–1.9 nM apparent H₂O₂ signal found in all aged, catalase treated seawater sampled cannot be attributed to H₂O₂ and should therefore be treated as a blank, which raises the detection limit of the method to 0.4–0.8 nM. Within the vicinity of OMZs, the concentration of Fe(II), and possibly also V(IV), is sufficiently high to dominate the apparent H₂O₂ signal in seawater below the thermocline. These interferences from transient species cannot presently be removed with great certainty.

After accounting for the apparent H₂O₂ signal arising from interfering ions, H₂O₂ appears to be present at very low nM concentrations in deep (below 1000 m) seawaters with the mean concentration varying across the different transects sampled: 2.9 ± 2.2 nM (Mediterranean, Corsica-France), 0.25 ± 0.27 nM (Mediterranean, Balearics-Algeria), 0.55 ± 1.3 nM (Mediterranean, Sardinia-Sicily) and 0.90 ± 1.2 nM (S Atlantic, Angola Basin). The source of this H₂O₂ remains uncertain, yet the observed distribution is consistent with a pelagic source. Estimates of deep ocean H₂O₂ decay rates and bacterial activity suggest that dark, bacterial production of H₂O₂ is sufficient to explain all H₂O₂ present at these depths.

Methods

Cruise work. Cruise work was conducted onboard RV Minerva Uno (Ocean Certain, August 2015, western Mediterranean Sea), FS Sonne (SFB 754, October 2015, South Pacific Ocean) and FS Meteor (GEOTRACES, November–December 2015, South Atlantic Ocean). All sample and reagent bottles for H₂O₂ analyses (opaque high density polyethylene (HDPE), Nalgene) were pre-cleaned by soaking sequentially in Mucosol detergent (Sigma-Aldrich) for 1 day and 1 M HCl for 1 week with 3 de-ionized water (18.2 MΩ cm^{−1}, Milli-Q, Millipore) rinses after each stage. Bottles were then dried in a laminar flow hood and stored in re-sealable plastic bags until required. Seawater for the analysis of H₂O₂ was collected using various methods to assess sampling artifacts. Seawater was either collected: from surface waters using a plastic bucket on a nylon line twice rinsed with surface seawater before sample collection, from a towfish which continuously pumped surface seawater through plastic tubing into a clean laboratory on deck, from trace metal clean Ocean Test Equipment (OTE) samplers mounted

on a powder coated sampling rosette with a Kevlar conducting cable complying with GEOTRACES specifications for trace metals, or from Niskin samplers (with, and without, internal metal components) mounted on a standard stainless steel sampling rosette with a stainless steel conducting cable. In all cases the HDPE sample bottles were rinsed 3 times with seawater, filled gently to overflowing, and sealed with no headspace. Samples were not filtered. For OTE and Niskin samplers, seawater was collected immediately after dissolved gas collection. Seawater was collected straight from the spigot of the samplers to minimize light exposure. All samples were analyzed within 2 h of sampling from the OTE or Niskin samplers. For surface samples, the time delay between sample collection and analysis for H_2O_2 was approximately 10 min.

Chemical analysis. H_2O_2 concentrations were determined via flow injection analysis (FIA) using the Co(II) catalyzed oxidation of luminol, which is the most commonly used analytical method in seawater^{12,21,22}. A FIA system was assembled using two 10-port valves (Valco, Vici), a photon multiplier tube (PMT, H9319-11, Hamamatsu), a glass flow cell with a mirrored base (Waterville Analytical Products) and a peristaltic pump (MiniPuls 3, Gilson). The flow cell, PMT and valve were arranged as per Yuan and Shiller²², but with two alternating reagent loops such that one loop was loading and one loop unloading, with the same flow rates (2.5 mL min^{-1} sample and 1.4 mL min^{-1} reagent) and target sample loop internal volume ($60 \mu\text{L}$) used²². It was verified that this configuration produced close to optimal chemiluminescence peak height responses by testing the effect of small changes to flow rate and tubing lengths (reagent loop and, separately, the length of tubing between the valve and detector) on the peak height produced for a 50 nM standard addition to aged seawater. H_2O_2 concentrations were derived from mean peak height and are reported as the mean ($\pm$ standard deviation) of 4 injections. The peak height response was always linear in the range 0–100 nM (R^2 typically > 0.99 and always > 0.98). The PMT was secured inside an electrical box to minimize background light, and all reagent and sample tubing was opaque (black PTFE, Global FIA, 0.8 mm internal diameter) except peristaltic pump tubing (PVC, Gradko).

The H_2O_2 reagent was prepared by adding the following reagents to aged de-ionized water (stored at room temperature for > 24 h in the dark) and making up to a total volume of 2 L: 0.54 g luminol (98%, ROTH), 44 g potassium carbonate (reagent grade, ROTH), 7.8 mL Co(II) solution (1000 ppm standard in 2% by volume HNO_3 , ROTH) and approximately 15 mL HCl (Trace Metal grade, Fisher Scientific) to adjust the pH of solution to 10.2²². Once mixed, H_2O_2 reagent was stored at approximately 6°C for in excess of 24 h (to maximize the luminol response²²) and then allowed to warm to room temperature for 6–12 h before use. Reagent remaining unused after 3 days was discarded. Between stations/experiments the FIA apparatus was rinsed with 0.1 M HCl followed by de-ionized water. To ensure the complete removal of acid and de-ionized water, an aged seawater blank was always run prior to re-commencing analysis. Calibrations were undertaken between stations, and with new reagent batches, using at least 6 standard additions of H_2O_2 (TraceSelect, Fluka) to aged (unfiltered) seawater obtained from > 1000 m depth (stored at room temperature in opaque HDPE bottles). H_2O_2 was sequentially diluted to create stock solutions of 100 mM and 100 μM using de-ionized water. The use of narrow-necked, opaque HDPE sample bottles, aluminium foil shields over the apparatus and reduced lighting in the laboratory minimized the potential for undesired photochemistry during sample analysis.

Experiments to verify the accuracy of H_2O_2 analysis. Mediterranean, Atlantic and Pacific seawaters were stored in the dark at 6°C for > 2 weeks for assessment of the baseline signal from a mixture of luminol reagent and seawater in the absence of high H_2O_2 concentrations. This aged seawater was then spiked with a freshly prepared solution of bovine catalase (Sigma) to a final enzyme concentration of 1–3 units mL^{-1} , and allowed to stand with gentle mixing at 25°C (to optimize enzyme performance) for 1 h. H_2O_2 spikes of 4 and 8 nM were also added to Atlantic seawater and treated identically to verify the effectiveness of the enzyme addition. The oxidation rate of a 5 nM Fe(II) spike in low O_2 seawater was used to estimate the concentration of residual H_2O_2 in aged, catalase treated seawater. Fe(II) was spiked into aged seawater with low O_2 in the presence and absence of catalase under a N_2 atmosphere. After seawater was aged in the dark at 6°C for 3 days, N_2 was bubbled through seawater for > 2 h. An Fe(II) spike was then added and the concentration of Fe(II) measured continuously for a period of > 1 h. Fe(II) concentration was measured via FIA using luminol^{48–50} with a dual Fe(II)/ H_2O_2 system⁵¹ assembled using the same components as the H_2O_2 system (see above). FIA was conducted in a laminar flow hood using the same procedures for handling reagent and seawater solutions as per the H_2O_2 analysis. Fe(II) reagent solution was made with 0.26 g luminol and 1.06 g potassium carbonate in 10 mL de-ionized water and stored overnight at 6°C to ensure complete dissolution. This was then added to a 2 L solution of de-ionized water containing 80 mL ammonium hydroxide (Trace Metal grade, Fisher Scientific), to which approximately 22 mL HCl (Trace Metal grade, Fisher Scientific) was added to adjust the final pH to 10.1. The mixed reagent was allowed to stand for > 24 h prior to use to maximize the luminol response³⁷.

The interference with the H_2O_2 luminol chemiluminescence method from Fe(II), Fe(III) and V(IV) was investigated by spiking solutions of these ions into S Atlantic seawater that had been aged in the dark. Fe(II) additions were made into both low (aged, no H_2O_2 spike) and high (aged, spiked to 42 nM) H_2O_2 Atlantic seawater as the luminol response to some interferences may be non-linear^{36,37}. Fe(II), V(IV) and Fe(III) stock solutions were made from ammonium Fe(II) hexahydrate (99%, Sigma Aldrich), vanadyl sulfate hydrate (99.99%, Sigma Aldrich) and Fe(III) chloride hexahydrate ($> 99.99\%$, Sigma Aldrich), respectively. Primary stocks were acidified using 1 mL HCl (Trace Metal grade, Fisher Scientific) per L solution and used on the day of preparation to minimize trace metal oxidation state changes.

To assess the potential for *in-situ* metal concentrations to interfere with H_2O_2 measurement in samples from OMZs, samples for trace metal analysis were collected from OTE samplers on a Kevlar wire adhering to GEOTRACES standards for trace metal sample collection (FS Sonne, October 2015, South Pacific). Sub-sampling was conducted under N_2 gas (purity $> 99.999\%$) in a metal free clean container where the air was filtered. Samples

for Fe(II) analysis were collected first and analysed immediately (analysis complete within 22 min of sub-sampling OTE samplers) without filtration as above. Dissolved seawater samples for trace metals (filtered with 0.2 µm, Acropack 500) were collected in 125 mL low density polyethylene (Nalgene) bottles (pre-cleaned according to GEOTRACES protocols: 1 day in Mucasil detergent, 1 week in 1.2 M HCl, 1 week in 1.2 M HNO₃ with 3 de-ionized water rinses after each stage) and stored double sealed in plastic bags. Samples were acidified in a laminar flow hood within 1 day of collection (140 µL Optima grade HCl, Fisher Scientific) and then stored acidified for >6 months. Samples were buffered to pH 6.4 using a 1.5 M ammonium acetate buffer before loading onto a WAKO resin⁵² for off-line pre-concentration (SeaFAST, Elemental Scientific Inc.). The buffer was prepared using ammonium hydroxide solution (Optima grade, Fisher Scientific) and acetic acid (Optima grade, Fisher Scientific) in de-ionized water adjusted to pH 8.5. Elution was performed in 1 M distilled HNO₃ (distilled using a sub-boiling PFA distillation system (DST-1000, Saville) from SPA grade HNO₃ (Romil)). Pre-concentrated samples were analysed by high resolution inductively coupled plasma-mass spectrometry (HR-ICP-MS, ELEMENT XR, ThermoFisherScientific) using isotope dilution for Fe and standard addition for V⁵³. Measurement of SAFe GEOTRACES reference water S produced concentrations of V 33.6 ± 1.1 nM (no consensus value available) and Fe 0.091 ± 0.004 nM (consensus value: 0.093 ± 0.008 nM).

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Author Contributions

M.H. and E.A. designed the study. M.H., C.S. and I.R. performed cruise and analytical work. M.H. and I.R. wrote the initial manuscript draft and then all authors contributed to its revision.

Additional Information

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