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# Reactive oxygen species in the world ocean and their impacts on marine ecosystems

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### ABSTRACT

Reactive oxygen species (ROS) are omnipresent in the ocean, originating from both biological (e.g., unbalanced metabolism or stress) and non-biological processes (e.g. photooxidation of colored dissolved organic matter). ROS can directly affect the growth of marine organisms, and can also influence marine biogeochemistry, thus indirectly impacting the availability of nutrients and food sources. Microbial communities and evolution are shaped by marine ROS, and in turn microorganisms influence steady-state ROS concentrations by acting as the predominant sink for marine ROS. Through their interactions with trace metals and organic matter, ROS can enhance microbial growth, but ROS can also attack biological macromolecules, causing extensive modifications with deleterious results. Several biogeochemically important taxa are vulnerable to very low ROS concentrations within the ranges measured in situ, including the globally distributed marine cyanobacterium *Prochlorococcus* and ammonia-oxidizing archaea of the phylum *Thaumarchaeota*. Finally, climate change may increase the amount of ROS in the ocean, especially in the most productive surface layers. In this review, we explore the sources of ROS and their roles in the oceans, how the dynamics of ROS might change in the future, and how this change might impact the ecology and chemistry of the future ocean.

### 1. Introduction

The distribution, abundance, and productivity of organisms in the World Ocean is intimately tied to biogeochemical cycles. Most marine ecosystems are dependent on primary production by aquatic photoautotrophs such as diatoms, cyanobacteria, seaweeds and seagrasses, and the growth of these organisms is in turn limited by the availability of nitrogen and/or phosphorus, or in some cases trace nutrients such as iron or vitamins. These molecules exist in a variety of chemical forms in the ocean, and because of their foundational importance in marine ecology, the enzymatic and abiotic reactions that shuttle them between their various states have been studied extensively.

Despite its ubiquity in both the ocean environment and in living biomass, the cycling of oxygen has received comparatively little attention from marine ecologists. The oxygen cycle is routinely imagined as a simple back-and-forth between the release of  $O_2$  as a by-product of photosynthetic water-splitting and the reduction of  $O_2$  to water by aerobic heterotrophic metabolism. But this formulation underestimates the complexity of environmental reactions involving oxygen, which in fact cycles continually through a variety of oxygen-containing

intermediates known as reactive oxygen species (ROS) (see Ref. [1] for an overview). Sometimes these ROS act as potent toxins; sometimes they are created by organisms as part of their natural growth processes; and in many cases they interact with other biogeochemical cycles in critical ways [1-3].

In this review, we will explore the roles played by ROS in the oceans. We will begin with a description of the chemistry of ROS and a survey of the biotic and abiotic mechanisms that generate them, follow up with an exploration of how ROS interact with marine biogeochemical cycles, and then consider some specific positive and negative interactions ROS have with marine organisms. Finally, we will close with a consideration of how the dynamics of ROS might change in the future due to human activity, and how this might impact the ecology and chemistry of the future ocean.

### 2. Aquatic chemistry of ROS

ROS are oxygen-containing molecules in which the redox state of oxygen is intermediate between that of  $\rm O_2$  and  $\rm H_2O$ , which form the dominant redox couple in most oxic natural waters. The co-existence of

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 $O_2$  and  $H_2O$ , despite their very different redox potentials, is a thermodynamically unfavorable situation that is enabled by two primary factors [4]:

- O<sub>2</sub> is relatively unreactive because its most energetically favorable state (the ground state) is a triplet state with two unpaired electrons; and
- 2. The continuous input of free energy into the global aquatic system, primarily due to sunlight-driven photo(bio)chemical reactions, prevents the system from reaching a thermodynamic endpoint in which  $O_2$  is fully consumed.

The triplet ground state of  $O_2$  (denoted  $^3O_2$ ) means that it is typically only able to react with other ground state molecules containing unpaired electrons, which forces the reduction of  $O_2$  to occur via a series of one-electron transfer steps [4]. Intracellularly, these one-electron transfer steps are often mediated by enzymes such that they occur in quick succession and the intermediate reaction products are not released [5]. In contrast, in the extracellular milieu of natural aquatic systems the sequence of one-electron transfer steps during the reduction of  $O_2$  can give rise to a range of ROS in free solution.

### 2.1. Thermodynamics

In oxic waters the overall thermodynamic drive is primarily for reduction of  $O_2$  (and ROS) to  $H_2O$ , coupled to oxidation of other chemical species [3]. In other words, ROS are predominantly oxidants. Nonetheless, there are circumstances in which some ROS can also function as reductants and drive redox cycling of various biogeochemically important elements [3]. While ROS may often be found in free solution in the aquatic milieu as noted above, there are circumstances in which they are bound to other species, either because they are not released as free species during formation, or due to additional reactions with other suitable substrates [4]. In this case, the resulting bound species may also function as oxidants or facilitate redox cycling.

This stepwise reduction of  $O_2$  to  $H_2O$  occurs via the following series of reactions [4]:

$$O_2 + e^- \rightarrow O_2^{\bullet -} \tag{1}$$

$$O_2^{\bullet -} + e^- + 2H^+ \rightarrow H_2O_2$$
 (2)

$$H_2O_2 + e^- \rightarrow HO^{\bullet} + OH^- \tag{3}$$

$$HO^{\bullet} + e^{-} + H^{+} \rightarrow H_{2}O \tag{4}$$

The thermodynamics of this sequence of redox reactions is illustrated in Fig. 1 under standard reaction conditions (unit activity and a temperature of  $298~\rm K$ ) at pH 7.

As illustrated in Fig. 1,  ${\rm O_2}^-$  is mildly reducing under standard reaction conditions at pH 7. In contrast,  ${\rm H_2O_2}$  is a relatively powerful two-electron oxidant from a thermodynamic perspective, and is not constrained to one-electron reactions in the same way as  $^3{\rm O_2}$ , but typically must overcome a large activation energy barrier [6]. Consequently,  ${\rm H_2O_2}$  reacts mostly as a one electron oxidant. One of the most well-known such reactions is the so-called Fenton reaction with Fe(II):

$$\mathbf{M}^{n+} + \mathbf{H}_2 \mathbf{O}_2 \rightarrow \mathbf{M}^{(n+1)+} + {}^{\bullet}\mathbf{O} \ \mathbf{H} + \mathbf{O}\mathbf{H}^-$$
 (5)

where  $\mathbf{M}^{n+}$  is Fe(II) in the case of the Fenton reaction. This reaction, and similar "Fenton-like" reactions with other trace metals and certain nonmetals, produce  ${}^{\bullet}\mathrm{OH}$  or high-valent metal ions [6], both of which are very strong and highly reactive oxidants.

In addition to these species formed during reduction of  $O_2$ , the electronically excited  $^1\Delta_g$  singlet state of dioxygen is typically also considered as one of the ROS. While two electronically excited states of dioxygen are possible, the highest energy  $^1\Sigma_g^{\ +}$  singlet state relaxes back

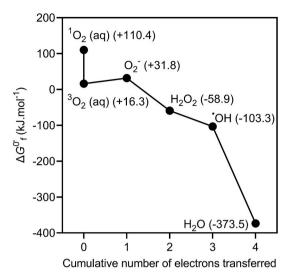


Fig. 1. Gibbs free energy of formation of ROS under standard conditions (1 M activity) in aqueous solution at pH 7.  $^{1}O_{2}$  refers to the  $^{1}\Delta_{g}$  excited state of dioxygen. Values were calculated from thermodynamic data reported in Sawyer (1991) [4].

to the  $^1\Delta_g$  state faster than it can undergo bimolecular reaction; hence the common notation  $^1O_2$  is used in this and other work in reference to the latter. While  $^1O_2$  is a relatively powerful oxidant, and not subject to the one-electron transfer constraints of  $^3O_2$ , in practice it is highly selective as an oxidant because it readily relaxes back to the  $^3O_2$  ground state through either energy transfer (particularly to water in aqueous environments), or by partial charge transfer in which formation of an intermediate charge-transfer complex results in relaxation of  $^1O_2$  back to  $^3O_2$  without completion of electron transfer [7].

When electron transfer is required to occur in single electron transfer steps, additional thermodynamic constraints are imposed on the system: not only must the overall reaction be thermodynamically favorable, but so too must each one-electron transfer step, else the reaction will be unable to proceed beyond the thermodynamic barrier imposed by a particular electron transfer step. Consequently, there are a limited set of redox reactions that are thermodynamically possible in oxic marine waters [3], as illustrated in Fig. 2. From a thermodynamic perspective, this explains why some intermediates in the redox reactions of various biogeochemically important species are only ever transient, and present at exceedingly low concentrations. While Fig. 2 does not provide an exhaustive examination of all possible redox couples that may be influenced by ROS, it does give an indication of some of the most important.

### 2.2. Reaction kinetics

Although thermodynamics is a critical control on the energetic favorability of potential reactions involving ROS, marine systems are dynamic and in many cases equilibrium is never reached. As such, the biogeochemistry of ROS is strongly influenced by reaction kinetics, which control their lifetimes and hence steady-state concentrations. The reactivity of the different ROS varies considerably. At the pH of seawater,  $O_2^{\bullet}$ —exists predominantly in the deprotonated form, which possesses a resonance-stabilized form with a "three-electron bond", and exhibits very little free radical character [5]. As such, it is relatively stable in marine waters and only reacts with other species possessing unpaired electrons in the valence shell, resulting in half-lives on the order of a few seconds to minutes in marine waters [1]. The reactivity of  $H_2O_2$  is usually kinetically limited due to the high activation energy barrier noted previously, with the overall reaction kinetics often controlled by rate of the formation of reaction intermediates that lower

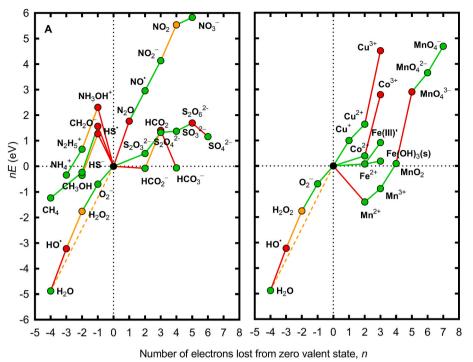


Fig. 2. Frost diagrams for some common redox active elements in natural waters at pH 8.1 accounting for typical activities of oxygen redox species in natural waters. (A) Representative species for the major elements C, N and S. (B) Trace metals Co, Cu, Fe and Mn. Red lines indicate redox couples that are absolutely unstable with respect to one-electron transfer processes involving the H<sub>2</sub>O/OH couple (lines with positive slope) or the H<sub>2</sub>/ H<sup>+</sup> couple (lines with negative slope); orange lines indicate redox couples that are conditionally unstable with respect to multi-electron transfer processes involving the H<sub>2</sub>O/O<sub>2</sub> couple; and green lines indicate redox couples that are thermodynamically permissible in aqueous solution at pH 8.1. The dashed orange line represents the redox potential associated with the four-electron transfer for complete reduction of O2 to H2O. Red symbols represent species that are thermodynamically unable to exist due to absolute instability with respect to one-electron transfer processes involving the H<sub>2</sub>O/OH couple (lines with positive slope) or the H<sub>2</sub>/H<sup>+</sup> couple; orange symbols represent species that are thermodynamically able to exist in the absence of processes to catalyze multielectron transfer processes involving the H2O/O2 couple; and green symbols represent species that are thermodynamically able to coexist with both the H2/ H<sup>+</sup> and H<sub>2</sub>O/O<sub>2</sub> couples. See Ref. [3] for details of concentrations and thermodynamic data used. Reproduced from Ref. [3] with permission.

the activation energy barrier [6]. For example, the Fenton reaction is thought to involve formation of an inner sphere complex between Fe(II) and  $\rm H_2O_2$  as the initial step, resulting in an overall reaction rate constant many orders of magnitude less than diffusion-controlled [6]. Consequently, typical half-lives of  $\rm H_2O_2$  in marine waters are considerably longer, on the order of hours to days [1]. In contrast,  $^{\bullet}\rm OH$  reacts at diffusion-controlled rates with many organic and inorganic compounds, and as such typically has a very short lifetime in natural waters (half-life  $\sim\!250$  ns, computed using data from Ref. [8]). Similarly,  $^1\rm O_2$  is highly reactive in aqueous environments with a half-life of around 4  $\mu s$  due to physical relaxation by solvent water [9].

Short half-lives and localized sources (as discussed further in Section III) may also result in spatially heterogenous distributions of ROS. This is particularly the case for  $^{\bullet}\text{OH}$  and  $^{1}\text{O}_{2}$ , but also to some extent for  $\text{O_2}^{\bullet}$  –, which may be formed through highly localized processes (e.g. in cells or at particle surfaces) and unable to diffuse far from the site of production due to their short half-life. This can result in significant spatial variability in steady-state concentrations, with the potential for concentrations near the site of ROS production that are many orders of magnitude greater than in the bulk solution, and associated differences in reaction kinetics.

### 3. Sources and sinks of ROS in the ocean

Naturally occurring  $\rm H_2O_2$  in the ocean was first reported in the 1960's [10], and even earlier than that, researchers were aware that  $\rm H_2O_2$  added to seawater was not stable, but disappeared with enzyme-like kinetics [11]. Nevertheless, the ability to study the distribution, sources, and sinks of ROS in the ocean awaited the development of sufficiently sensitive detection methods which would not occur until the 1980's. For instance, classical titration-based methods for quantifying  $\rm H_2O_2$  had limits of detection in the millimolar range, but the development of fluorescence methods in the 1980s [12] and chemiluminescence methods in the 2000s [13] made it possible to accurately measure the nanomolar  $\rm H_2O_2$  commonly found in natural waters (Fig. 4). In this section we first give an overview of the major ROS production and destruction pathways along with their impact on

steady-state ROS concentrations, and finally we describe some of the most common methods used for assessing seawater ROS concentrations in the field.

### 3.1. Sources of marine ROS

### 3.1.1. Photochemical production

Many studies in a wide variety of waters have supported the hypothesis that most ROS in bulk surface waters arise by photochemistry, especially via the photooxidation of dissolved organic matter (DOM) [14-17]. Colored DOM can absorb photons and enter an excited triplet state capable of reducing O2 to O2 -, which can then undergo spontaneous dismutation to H2O2 and O2. Because of its relatively long residence time, H<sub>2</sub>O<sub>2</sub> is the most easily measured ROS in the field and has been used as a proxy for the relative abundance of other ROS such as O2 - and OH. The rate of H2O2 accumulation is generally correlated with DOM concentration [18], and therefore steady-state H<sub>2</sub>O<sub>2</sub> concentrations are generally greater in terrestrially-impacted waters like coasts and estuaries, as opposed to the oligotrophic ocean gyres (Table 1 and references therein). ROS fluxes in bulk water and H<sub>2</sub>O<sub>2</sub> steady-state concentrations also decrease rapidly with depth and light level, dropping to near or below the limit of detection below the euphotic zone (Fig. 3a, Table 1). Because of the influence of sunlight, steady state H<sub>2</sub>O<sub>2</sub> concentrations in surface waters tend to exhibit a diel cycle with afternoon maxima and early morning minima (Fig. 3c) [19-21], and also a seasonal cycle peaking in the summer (Fig. 3b) [22,23]. Such trends have not been widely observed for other ROS, however.

H<sub>2</sub>O<sub>2</sub> production is stimulated by light in the visual range but is much faster under UV irradiation. While representing only a small fraction of the irradiance impacting natural seawater, photons in the UV-B range cause the majority of H<sub>2</sub>O<sub>2</sub> accumulation [24]. H<sub>2</sub>O<sub>2</sub> can also form in the atmosphere by the photolysis of water into hydrogen atoms and <sup>o</sup>OH [25]. These radicals can then join to form H<sub>2</sub>O<sub>2</sub> in the gas phase, which can subsequently be recruited into rain droplets, resulting in H<sub>2</sub>O<sub>2</sub> concentrations that are typically two orders of magnitude greater than that found in surface seawater [26]. Rain events have been shown to temporarily increase the in situ H<sub>2</sub>O<sub>2</sub> of seawater [26–32].

Table 1 H<sub>2</sub>O<sub>2</sub> concentrations in various waters.

| Type                  | Location  | [H2O2]             | Referen        |
|-----------------------|---|--------------------|----------------|
| Freshwater            | Jacks Lake, Ontario, surface                                      | 10-800             | [19]           |
|                       | Swedish Highland Lakes, surface                                   | nM<br>132–984      | [247]          |
|                       | 5wedish Highland Lakes, surface                                   | nM                 | [247]          |
|                       | Lake Erie, surface  | 50–175             | [248]          |
|                       |   | nM                 |                |
| Brackish              | Patuxent Estuary, surface   | 12-350             | [22]           |
|                       | Chesapeake Bay, surface   | nM<br>3–1700       | [15]           |
|                       | Chesapeake Bay, surface   | nM                 | [15]           |
|                       | Chesapeake Bay, surface   | 40–80 nM           | [248]          |
|                       | Coastal Germany, Intertidal salt flat<br>Southern Ocean, tidepool | 0.1–4.5 μM<br>2 μM | [23]<br>[249]  |
|                       | bounern beening nucepoor  | - ,                | [219]          |
| Coastal               | Southern Ocean  | 1.5 μM             | [249]          |
|                       | Antarctica<br>Peru  | 9–25 nM<br>80–500  | [250]<br>[251] |
|                       |   | nM                 |                |
|                       | Southern California   | 44–370<br>nM       | [252]          |
|                       | Baja California   | 50–125             | [248]          |
|                       | _   | nM                 | 54.03          |
|                       | Texas   | 14–170<br>nM       | [10]           |
|                       | Florida   | 80–210             | [15]           |
|                       | Code of Marian  | nM                 | F0=01          |
|                       | Gulf of Mexico  | 100–240<br>nM      | [253]          |
|                       | Bahamas   | 50-190             | [15]           |
|                       | Amazon River plume  | nM<br>25–165       | [21]           |
|                       | 7 mazon rever prume   | nM                 | [21]           |
| 0                     | Transical Parisis   | FO 1FO             | F0007          |
| Open ocean<br>surface | Tropical Pacific  | 50–150<br>nM       | [200]          |
|                       | Tropical Pacific, during rain                                     | 100-300            | [28]           |
|                       | North Pacific   | nM<br>25–120       | [254]          |
|                       | North Facility  | nM                 | [234]          |
|                       | South and Central Atlantic  | 16-68 nM           | [21]           |
|                       | Subtropical Atlantic  | 50–220<br>nM       | [18]           |
|                       | Equatorial Atlantic ITCZ  | 31-236             | [255]          |
|                       | Caribbean Sea   | nM<br>50–100       | [256]          |
|                       | Caribbean Sea   | nM                 | [230]          |
|                       | Gulf of Mexico  | 90–140             | [253]          |
|                       | Sargasso Sea  | nM<br>95–175       | [257]          |
|                       |   | nM                 |                |
|                       | Western Atlantic  | 40–175<br>nM       | [248]          |
|                       | Eastern Atlantic  | 30–80 nM           | [258]          |
|                       | North Atlantic  | 135–483            | [32]           |
|                       | Bermuda Atlantic Time Series                                      | nM<br>20–200       | [31]           |
|                       |   | nM                 | [0-]           |
|                       | Mediterranean Sea   | 100–140<br>nM      | [259]          |
|                       | Southern Ocean Indian Sector                                      | 5–20 nM            | [260]          |
| _                     |   |                    |                |
| Open ocean depths     | North Pacific, >1000 m  | <6 nM              | [261]          |
| асраю                 | Mediterranean, South Atlantic and                                 | <3 nM              | [262]          |
|                       | Pacific, >1000 m  | -134               | F013           |
|                       | South and Central Atlantic, below euphotic zone                   | <1 nM              | [21]           |
|                       | Bermuda Atlantic Time Series, 150                                 | $<\!2\;nM$         | [31]           |
|                       | m   |                    |                |
| Precipitation         | South and Central Atlantic, rain                                  | 3.5–71 μM          | [27]           |
|                       | Equatorial Atlantic ITCZ  | 1.5-22.8           | [255]          |
|                       |   | μM                 |                |

Table 1 (continued)

| Type | Location                           | [H2O2]    | Reference |
|------|------------------------------------|-----------|-----------|
|      | Gulf of Mexico, rain               | 40.2 μM   | [263]     |
|      | Florida Keys, rain                 | 28.4 μM   | [263]     |
|      | Miami/Bahamas, rain                | 30.7 μM   | [264]     |
|      | West Atlantic, rain                | 12.7 μM   | [263]     |
|      | Bermuda Atlantic Time Series, rain | 11–40 μΜ  | [31]      |
|      | Bermuda Atlantic Time Series, rain | 5–85 μM   | [26]      |
|      | New Zealand, rain                  | 10-30 μΜ  | [30]      |
|      | Equatorial Pacific, rain           | 8.5 μM    | [28]      |
|      | North Pacific ITCZ, rain           | 10.4 μM   | [28]      |
|      | Jacks Lake, Ontario, rain          | 1.3–34 μM | [19]      |
|      | Southern Ocean, snow               | 10–14 μM  | [249]     |

### 3.1.2. Stress production by organisms

In addition to direct photochemical ROS production, marine organisms can release H2O2 into the environment, often due to stress. Many enzymes capable of single-electron transfer to a substrate are also capable of reducing O2, and the ubiquity of O2 in oxic environments ensures that some H<sub>2</sub>O<sub>2</sub> will escape. Common metabolic pathways that produce H<sub>2</sub>O<sub>2</sub> as a by-product even under ideal conditions include the Mehler reaction (aka the water-water cycle) and photorespiration in photosynthetic organisms [33] as well as the electron transfer reactions of aerobic oxidative phosphorylation [34]. O2 reduction is enhanced when a primary electron transport carrier is not available in sufficient quantities due either to excess electron flow, nutrient deficiency, or other problems. For instance, in Fe-starved cyanobacteria, ROS buildup occurs due to a lack of ferredoxin, the Fe-containing primary cytoplasmic electron acceptor for photosystem I [35]. When more electrons flow through the photosynthetic electron transport chain than can be accepted by ferredoxin, O2 reduction becomes practically inevitable [33], and therefore excess ROS production and leakage of H<sub>2</sub>O<sub>2</sub> into the environment is likely to be common in low-Fe marine habitats, particularly near the ocean surface under conditions of high light [36]. Release of H2O2 has also been observed in Antarctic diatoms when suddenly exposed to light following the long periods of uninterrupted darkness characteristic of the polar winter [37]. Importantly, H<sub>2</sub>O<sub>2</sub> release by light-stressed photoautotrophs may contribute to the diel cycle in H<sub>2</sub>O<sub>2</sub> observed at the ocean's surface, leading to overestimates of the role of DOM photooxidation in ROS production. ROS buildup from unbalanced metabolism could also explain the toxicity of high-nutrient culture media to marine microorganisms adapted to oligotrophic lifestyles [38].

Low levels of ROS are continuously produced by many organisms for signaling purposes and as normal, short-lived metabolic intermediates. However, intracellular ROS accumulation - a condition generally referred to as oxidative stress – can occur as a general consequence of diverse stresses such as temperature, osmotic, and dehydration stress [39-44]. Exposure to anthropogenic pollutants can also induce oxidative stress in marine organisms. For example, silver nanoparticles and microplastics like polyacrylonitrile induced lethal oxidative stress in the photosynthetic microorganisms Prochlorococcus and Chlorella pyrenoidosa, respectively [45,46]. Exposure to heavy metals, elevated temperatures, acidification, organic pollutants, allelochemicals, and other stressors lead to ROS accumulation and other biomarkers of oxidative stress (e.g. DNA strand breaks) in a wide variety of marine animals, plants, and microbes [45-57]. However, it is not clear how much of this intracellular ROS is able to escape the organism and contribute to bulk ROS concentrations in the environment. O2 -, for instance, is incapable of crossing cellular membranes due to its charge, while highly reactive ROS such as \*OH have such short lifespans that they are unlikely to move far from their point of origin.

### 3.1.3. Extracellular ROS production by marine organisms

ROS are also released into the environment by healthy organisms as a normal part of their metabolism. For example, animals use  $H_2O_2$  at low

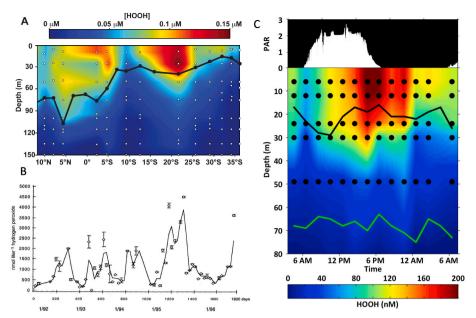


Fig. 3.  $H_2O_2$  profiles across time and space. A)  $H_2O_2$  depth profiles from samples across a Pacific transect in Jan–Feb 2007. B) Monthly  $H_2O_2$  measurements of a tide pool in Germany from 1991 to 1996. C)  $H_2O_2$  depth profiles and light intensity from a station in the South Pacific (Feb 2007) across a 24-h period; PAR, photosynthetically active radiation, with white bars indicating light intensity. In A and C dots represent sampled depths/times, with  $H_2O_2$  values interpolated between the points; black lines represent the depth of the surface mixed layer; and green line in C represents the deep chlorophyll maximum. Reprinted with permission from Ref. [200] (A) [273], (B), and [20] (C).

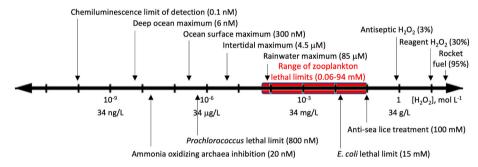


Fig. 4. H<sub>2</sub>O<sub>2</sub> concentrations in different environments and their biological impacts on various organisms.

concentrations as a signaling compound [58] and in high concentrations as an antimicrobial defense [59,60]. The rapid production of  $\rm H_2O_2$  bursts, often facilitated by NADPH-dependent oxidases, is an important component of the innate immune system of land plants and is also observed in aquatic phototrophs, where oxidative bursts have been observed after wounding or exposure to grazers or pathogens in a wide variety of macroalgae [61–66].

 $H_2O_2$  may also be produced as a weapon against prey or parasites. For instance, the coral  $Stylophora\ pistillata\ responded\ to\ physical\ stimulation\ (e.g.\ by\ pushing\ against\ its\ tissue)\ by\ releasing\ <math>H_2O_2$  from the site of stimulation, and the amount of  $H_2O_2$  released was proportional to the intensity of the stimulus [67]. Contact with prey organisms also stimulated  $H_2O_2$  release. The production of  $H_2O_2$  was unaffected by the presence or absence of zooxanthellae, indicating it was a native function of the cnidarian organism, possibly serving as a protection against pathogens or a way to stun and capture prey more effectively. Similarly,  $H_2O_2$  released as a response to wounding in the Antarctic seaweeds  $Ascoseira\ mirabilis\$ and  $Saccharina\ latissima\$ was able to inhibit grazing by amphipods, the primary herbivore feeding on these organisms in their native habitat [68,69].

Over the past decade, a variety of studies have also shown that extracellular production of  $O_2^{\bullet}$ —by marine microbes is nearly ubiquitous (e.g. Ref. [70]). This body of work has been summarized in detail in several excellent recent reviews [1,2,71] and will not be repeated here. However, it is worth emphasizing that many of these studies have suggested that this process may be "intentional", in the sense that the process may be exploited and even tightly regulated [72] to create or

maintain locally favorable biogeochemical conditions. For example, one of the most significant and potentially widespread uses of ROS in the marine environment is the facilitation of iron acquisition by extracellular  $O_2^{\bullet}$ –produced by microorganisms. The effectiveness of this process depends strongly on the concentration and speciation of iron but appears to be exploited by marine microbes under some conditions at least, as discussed in detail in Rose (2012) [73].

Algal blooms represent conspicuous hot spots for gross ROS production. Blooms are common in freshwater systems, but also occur in marine environments where they are often associated with the production of harmful toxins. H2O2 accumulation has been documented in the extracellular environment for a number of toxic species, albeit with substantial variation in production rates even between different strains of the same species [74-76]. It remains an open question how much of the ROS generation associated with blooms represents passive release or active production of ROS by the algae, and how much is passive photochemical generation caused by the high DOM conditions created by the bloom. For instance, production of  ${\rm O_2}^-$  and  ${\rm H_2O_2}$  was observed in filtered supernatants of cultures of several toxic algae [74], consistent with the same photochemical or other abiotic processes acting on DOM. On the other hand, the contribution of factors such as colony morphology [77], nutrient deficiency [78], CO<sub>2</sub> depletion [79], and lectin induction [76] to O<sub>2</sub>•-production all suggest active processes by the algae involved. Importantly however, some of these experiments were performed with axenic cultures whereas others also had undefined populations of associated bacteria; therefore it is not clear how much of the observed ROS production was caused by the algae and how much by

the bacterial component, or how the presence of different groups of bacteria may have altered the metabolism or ROS dynamics of the cultures during the experiments.

Regardless of the mechanisms underlying ROS production by algal blooms, they are capable of dramatically increasing ROS fluxes and steady-state H<sub>2</sub>O<sub>2</sub> concentrations compared to normal conditions. For example, the bloom-forming seaweed Ulva secretes sufficient H2O2 to more than double the ambient concentration of H<sub>2</sub>O<sub>2</sub> in coastal ecosystems where it dominates [80]. High concentrations of superoxide (~10 nM) were detected on the surface of coral Porites astreoides in the dark, independent of photosynthesis, and its symbionts also contributed extracellular superoxide in the dark and at low light levels [81]. ROS concentrations in a number of harmful algal blooms are so high that they have been considered a possible source of ichthyotoxicity [82-86] (although other studies such as [87] have argued against this conclusion). One possible factor that may exacerbate the impact of blooms on the prevailing H2O2 concentration is the fact that blooms have mechanisms for remaining at the sea surface, forming thin, very dense films at the air-water interface. One experiment using a recently developed microelectrode method for H<sub>2</sub>O<sub>2</sub> determination found H<sub>2</sub>O<sub>2</sub> concentrations 3–5 times greater in the upper few millimeters of the water column as compared to water a few centimeters deep during a freshwater Microcystis bloom that would have eluded older measurement protocols [88]; it is possible that such fine-scale measurements may reveal more high-ROS microenvironments in marine systems as well.

### 3.1.4. ROS production by non-photochemical abiotic processes

The final major source of ROS is via non-photochemical, abiotic redox processes, predominantly through the oxidation of reactive reduced species by O2. Such processes can potentially result in significant fluxes of ROS whenever there is a continuous supply of these reduced species to oxygenated waters, such as at the interface of reducing sediments with oxygenated waters. Murphy et al. demonstrated that, in the presence of hydrous ferric oxides, S<sup>2-</sup> undergoes net oxidation by O2 to produce O2 - and H2O2 [89]. More recently, Shaw et al. demonstrated a similar process for ROS production in hydrothermal vents [90] and calculated that the resulting flux of H<sub>2</sub>O<sub>2</sub> to the global ocean via this pathway is of a similar magnitude to that produced by photochemical processes in the surface ocean. Non-photochemical, abiotic ROS production could also occur at the interface of reducing microenvironments within the oxic water column, such as at the surfaces of abiotic particles (e.g. minerals). However, studies examining dark, particle-associated ROS production in the marine water column have primarily found this source to be biological (e.g. Refs. [81,91,92]).

### 3.2. Microorganisms: the predominant sink for marine H<sub>2</sub>O<sub>2</sub>

As has been previously mentioned, most ROS have very short residence times, and are rapidly eliminated by reaction with other molecules near their point of generation. Those reactions involving DOM or metals, or involved in toxic attacks on biomolecules, will be considered in later sections. Here we exclusively consider the role of microorganisms in the removal of H2O2 from their environment. Microbes are generally considered the primary agents of active H2O2 quenching in the ocean [1] and indeed in freshwater systems as well. Cooper and Zepp [93] found that H<sub>2</sub>O<sub>2</sub> decomposition in freshwater was arrested in chemically sterilized samples. Similarly, Petasne and Zika [94] found that 0.2 µm filtration of samples greatly reduced H<sub>2</sub>O<sub>2</sub> decay rates, and that most of the H<sub>2</sub>O<sub>2</sub> degradation ability was contained within the  $0.2\text{--}1~\mu\text{m}$  size fraction, implicating bacteria as the predominant players in H<sub>2</sub>O<sub>2</sub> removal. Both these studies found that re-seeding sterilized samples with cultured bacteria restored H2O2 degradation, confirming the important role played by microbes as the predominant sink for H<sub>2</sub>O<sub>2</sub> in natural waters.

Microorganisms that inhabit oxic waters have evolved many strategies for protecting themselves from ubiquitous  $H_2O_2$ . The most

conspicuous of these defenses are ROS-scavenging enzymes, including both catalases and peroxidases. These enzymes are widespread in both microbes and macroscopic life and have traditionally been thought to be essential for survival in the presence of atmospheric concentrations of O2, although recent evidence suggests that they are actually present in only a fraction of marine microbes [20,95]. Both catalase and peroxidase are phylogenetically diverse - there are multiple, independently evolved enzyme groups that catalyze these reactions - and many use redox-active metal cofactors containing Fe or Mn to facilitate the removal of H<sub>2</sub>O<sub>2</sub> [96]. Canonical catalase disproportionates H<sub>2</sub>O<sub>2</sub> directly into water and O2, but other catalases as well as metal-cofactor peroxidases are also capable of reducing H2O2 using electrons from other metabolites such as ascorbate. Peroxiredoxins, another group of peroxidases common in marine bacteria, do not have metal cofactors, but rather use redox-competent cysteine residues to reduce peroxides (e. g., H<sub>2</sub>O<sub>2</sub> as well as lipid and other organic peroxides) using electrons from small molecules (e.g., glutathione and thioredoxin) that are common intermediaries in cellular redox processes [97]. While some peroxiredoxins are clearly front-line defenses against H2O2 (e.g., AhpC in Escherichia coli [98] and the recently-discovered 2-Cys peroxiredoxin from Chattonella marina [99–101]), the function of others appears to be directed more toward scavenging of lipid or other organic peroxides

In the ocean, removal of  $\rm H_2O_2$  appears to occur primarily through the action of microbial catalases and peroxidases, although the relative importance of the two enzymes as well as the identities of the organisms expressing them remain incompletely understood. In a study of  $\rm H_2O_2$  decomposition using <sup>18</sup>O-labelled  $\rm H_2O_2$ , Moffett and Zafiriou [103] determined that the majority of  $\rm H_2O_2$  was converted to  $\rm O_2$ , indicating that most of the  $\rm H_2O_2$  was broken down using catalase. These results were recently revisited with improved methods, revealing that  $\rm H_2O_2$  removal processes proceed from catalase-like to peroxidase-like redox profiles with increasing depth [104]. Interestingly this same study also revealed that some intermediate depth samples exhibited unexpectedly high oxidation:reduction ratios, suggesting the existence of unknown powerful oxidants capable of scavenging  $\rm H_2O_2$  from the water column.

While catalase appears to be the predominant sink for H<sub>2</sub>O<sub>2</sub>, at least at the ocean's surface, only a minority of marine microorganisms produce it. In a re-examination of several diel metatranscriptomic studies from the surface ocean, the bacterial enzyme catalase-peroxidase (KatG) was much more abundant than the monofunctional catalase found in both bacteria and eukaryotes in all samples [20]. Algal ascorbate peroxidases were also widespread, and the expression of both enzymes was found to oscillate over the diel cycle, with higher expression during the day than at night, corresponding with increased rates of H2O2 removal during the day than at night. The taxonomic composition of the two enzymes varied between stations, but KatG sequences like those from heterotrophic bacterial clades Alteromonodales, SAR11, and SAR116 and green algal ascorbate peroxidase sequences were always abundant and over-represented relative to their share of the overall microbial population. Thus, a small subset of total microbial diversity in all these samples appeared to be responsible for the majority of the effort required to remove H<sub>2</sub>O<sub>2</sub> from the environment.

If only a fraction of the total microbial community is engaged in ROS removal and most of the removal is performed by intracellular enzymes, how do the other organisms survive? First, it is possible that some ROS scavenging enzymes are actually secreted into the environment; extracellular SODs are common in many bacteria and fungi [105], and a few organisms also express extracellular catalases [106–108]. Cellular contents may also be released into the environment as a result of sloppy feeding or viral lysis [109]. Residual  $\rm H_2O_2$  degradation has been detected in sterile-filtered seawater in a variety of studies as well [94, 110]. More likely, however, is that intracellular  $\rm H_2O_2$  degradation unavoidably also reduces the extracellular  $\rm H_2O_2$  concentration due to the fact that  $\rm H_2O_2$  is freely membrane permeable [111]. Thus, it is possible that the entire microbial community can be protected from

 $\rm H_2O_2$  by a minority of  $\rm H_2O_2$ -scavenging organisms. This phenomenon creates a feedback mechanism that may be responsible for the evolution of complex, market-like interdependencies in marine microbial communities wherein  $\rm H_2O_2$ -removing "helpers" protect their neighbors who in some cases "pay" them with other products, such as photosynthate or vitamins, a process described by the Black Queen Hypothesis [95,112].

### 4. The impact of ROS on marine biogeochemistry

As summarized previously, each of the various ROS exhibit quite different characteristics in terms of their reactivity. While all can act as oxidants, some are also able to act as reductants under different circumstances. Thus, ROS impact the biogeochemical cycling of a wide range of elements. Some of the major mechanisms by which ROS react with various elements, and the impact on their biogeochemical cycles, are discussed in further detail below.

### 4.1. ROS and organic carbon

Organic carbon (OC) molecules in their ground state are relatively stable against direct oxidation by 3O2, despite the thermodynamic favorability of the overall reaction with respect to the O<sub>2</sub>/H<sub>2</sub>O couple, due to the barrier imposed by the one-electron transfer requirement. This constraint is overcome when OC molecules are in an excited (typically singlet) state [4], as can occur by photochemical excitation of CDOM (although the majority of excited CDOM molecules transfer energy rather than electrons to  ${}^{3}O_{2}$ , yielding  ${}^{1}O_{2}$  [9]). While  ${}^{1}O_{2}$  may itself oxidize certain OC compounds, the effectiveness of this reaction is limited by its very short lifetime due to relaxation by solvent water [9]. Consequently, <sup>1</sup>O<sub>2</sub> is unable to diffuse far from the site of its formation, and the highest concentrations are typically found in hydrophobic microenvironments within large CDOM molecules (e.g. humic acids) [113]. In these localized environments, oxidation by <sup>1</sup>O<sub>2</sub> may represent a significant pathway for degradation of certain OC compounds, including organic pollutants [9].

OC is also readily oxidized by OH. Due to its high reactivity, OH is typically short-lived and thus this process is only significant where there is a continuous flux of \*OH production, usually from photochemical processes in marine systems. OH can also be formed though the reduction of H<sub>2</sub>O<sub>2</sub> via Fenton or Fenton-like reactions, but these are unlikely to occur at biogeochemically significant rates in most marine systems unless there is a continuous source of reduced trace metals. This can occur in the photic zone though photochemical reduction of some metals, such as Fe, giving rise to the so-called photo-Fenton reaction. The photo-Fenton reaction may be a major pathway for OC degradation under conditions like those found in marine waters [114], although evidence suggests that the primary oxidant may not necessarily be OH. This is consistent with the fact that the Fenton reaction does not necessarily produce free OH, but may instead yield other oxidants such as Fe(IV) (ferryl iron), depending on the complex speciation of iron [115]. While the reactivity of Fe(IV) towards OC may differ from that of free OH, it is still a powerful oxidant [116]. This may also be true for the Fenton-like system involving Cu(I) and H<sub>2</sub>O<sub>2</sub> [117].

The reaction between  ${}^{\bullet}$ OH and OC may be a significant sink for OC in marine waters [118]. This reaction initially produces carbon-centered radicals, which (unlike bulk OC) may subsequently react with  ${}^{3}$ O<sub>2</sub>. This process typically involves addition of O<sub>2</sub> to the carbon-centered radical to yield a peroxyl radical:

$$R^{\bullet} + {}^{3}O_{2} \rightarrow ROO^{\bullet}$$
 (6)

where R represents an organic group and ROO $^{\bullet}$  the peroxyl radical. Peroxyl radicals are typically far more stable than carbon-centered radicals under oxic conditions, since the latter typically react with  $^3O_2$  at near diffusion-controlled rates [119], but may undergo further reaction with  $O_2^-$  or other peroxyl radicals to yield organic peroxides:

$$ROO^{\bullet} + O_{2}^{\bullet-} + H^{+} \rightarrow ROOH + O_{2}$$
 (7)

$$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOR' + O_2$$
 (8)

where ROOH and ROOR' are organic peroxides. The fate of these organic peroxides is discussed further below.

In contrast to both  ${}^{1}O_{2}$  and  ${}^{\bullet}OH$ ,  $O_{2}^{\bullet}$  is highly selective in its reactions due to its unusual electronic structure, such that it readily reacts only with molecules possessing unpaired valence shell electrons, including a range of redox-active trace metals and organic radicals.  $O_2^{\bullet}$ is also unique among the ROS in that it is thermodynamically more favorable as a reductant than as an oxidant under typical conditions found in oxic marine waters (Fig. 1). As such,  $O_2^{\bullet}$  does not readily react with bulk OC, but may facilitate redox cycling of stable radical groups, such as semiquinones [120] and phenoxyl radicals [121,122], which are thought to be present in natural organic matter [122,123]. Goldstone and Voelker [124] first reported a non-metallic sink for  $O_2^{\bullet}$  in natural waters associated with the presence of humic and fulvic substances. Heller and Croot [125] showed that reactions with CDOM represented a significant sink of  $O_2^{\bullet}$  in tropical Atlantic surface waters, but that such a pathway was not a major sink for  $O_2^{\bullet}$  in the Southern Ocean [126]. King et al. [127] also showed that unidentified antioxidant compounds able to consume  $O_2^{\bullet}$  were present at concentrations of 100–400 pM in seawater samples from the South Atlantic Ocean, but only during daylight hours.

While  $\mathrm{H_2O_2}$  is a relatively powerful oxidant from a thermodynamic perspective [4], it does not readily oxidize OC under ambient conditions due to a high activation energy barrier for the reaction [6]. The oxidation of semiquinone-type radicals via a "metal-independent Fenton reaction" has been reported in aqueous solutions [128] and the formation of  $^{\bullet}\mathrm{OH}$  by such a pathway has been reported in solutions of irradiated humic acids [129]. However, this process has not been widely examined in marine waters.

Organic peroxides may reach concentrations comparable to  $H_2O_2$  of up to several hundred nM in surface nearshore marine waters [130], suggesting that they are similarly relatively unreactive. Nonetheless, relatively little is known about the fate of organic peroxides in the ocean, or their influence on biogeochemical cycles.

### 4.2. ROS and trace metals

ROS can potentially react with, and thus influence the redox speciation, of a range of redox-active trace metals in seawater. Among the biogeochemically relevant trace metals found in marine waters, Fe, Cu, Mn, Mo and V are particularly notable in terms of their ability to cycle between metastable redox states, at least in part because of the thermodynamic constraints imposed by the one electron transfer requirement in systems dominated by the O2/H2O redox couple [3]. This section will thus focus on these five trace metals as examples of trace metals with particular biological significance whose redox cycling in marine waters is well known to be influenced by ROS. This list is not intended to be exhaustive, however; there are a range of other redox active trace metals that are known to react with ROS under aqueous conditions (see, for example, compilations of reactions of  $O_2^{\bullet}$  [131] and OH [132] with various trace metals and other compounds), including in seawater (for example oxidation of Cr(III) by H2O2 [133] or the suggested involvement of ROS in Hg cycling [134]).

As stated previously,  $H_2O_2$  and \*OH are predominantly oxidizing in nature at the pH of typical marine waters, with the notable exception of the reduction of Cu(II) by  $H_2O_2$  (discussed further below). In contrast,  $O_2^{\bullet}$  is capable of functioning as both an oxidant and reductant and, as such, plays a somewhat special role in relation to the redox cycling of trace metals. The role of  $^1O_2$  in redox cycling of trace metals is likely to be limited due to its low concentrations and localization near the site of its predominantly photochemical generation, but due to their unpaired

valence shell electrons the trace metals discussed above may all react directly with  $^3\mathrm{O}_2$ , albeit at varying rates.

The general sequence of reactions between ROS and the trace metals may be described as follows:

$$M^{n+} + O_2 \leftrightarrow M^{(n+1)+} + O_2^-$$
 (9)

$$M^{n+} + O_2^- + 2H^+ \rightarrow M^{(n+1)+} + H_2O_2$$
 (10)

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + OH^{\bullet} + OH^{-}$$
 (11)

$$\mathbf{M}^{n+} + \mathbf{O}\mathbf{H}^{\bullet} \rightarrow \mathbf{M}^{(n+1)+} + \mathbf{O}\mathbf{H}^{-} \tag{12}$$

where M represents the metal and  $M^{n+}$  and  $M^{(n+1)+}$  are reduced and oxidized redox states, respectively. (The first reaction is shown as a two-way reaction due to the commonly reducing behavior of  $O_2^{\bullet}$ , in contrast to the other reactions). A critical control on the nature of ROS-induced redox cycling of trace metals is the relative rates of each of these reactions, leading to three general scenarios:

Scenario 1 – The forward reactions in eqs. (9)–(11) and/or 12 are fast relative to the backward reaction in eq. (9): the trace metal will exist primarily in its oxidized state and does not readily undergo ROS-induced redox cycling. The relative importance of  $^3O_2$  compared to other ROS as oxidants of the trace metals will depend on the relative rates of the forward reactions in eqs. (9)–(12).

Scenario 2 – The forward reactions in eqs. (9)–(12) are all slow compared to backward reaction in eq. (9): the trace metal will exist primarily in its reduced state and does not readily undergo ROS-induced redox cycling.

Scenario 3 – The forward and backward reactions in eq. (9) are both fast relative to the reactions in eqs. (10)–(12): the trace metal will be rapidly cycled between the reduced and oxidized states and reaches a steady-state redox equilibrium with the  $O_2/O_2^{\bullet}$  redox couple.

It is important to note that the relative reaction rates depend on both the rate constant, which is an intrinsic property of the reacting species, and the concentrations of  $^3\mathrm{O}_2$  and the various other ROS. Thus, the applicability of each of these scenarios will depend on both the chemistry of the trace metal involved and local conditions and concentrations.

### 4.2.1. Iron

The reactions of ROS with Fe are perhaps the most well-studied among these trace metals. In typical oxic marine waters, Fe falls into scenario 1 above. While Fe(III) is the thermodynamically stable redox state of free Fe in oxic marine waters, Fe(II) has been measured in a range of surface and deep ocean environments even under fully oxygenated conditions (e.g. Refs. [135,136]). The existence of measurable Fe(II) in these environments can result from photochemical and biological processes, in addition to physical transport from more reducing waters (e.g. in oxygen deficient zones [137], or potentially in reducing microenvironments in sinking particles [138]). The biogeochemical cycling of Fe in these oxic waters is tightly coupled to the cycling of  $\mathbf{O}_2$  and  $\mathbf{O}_2^-$  [73] via the two-way reaction:

$$Fe(II) + O_2 \leftrightarrow Fe(III) + O_2^{\bullet -}$$
(13)

The tight coupling between these redox pairs means that it is often difficult to resolve whether the redox cycling is initiated by the formation of Fe(II) or  $O_2^{\bullet}$ <sup>-</sup>[73], although arguably this may be unimportant in terms of the overall biogeochemistry. The dynamics of this system additionally depends on the chemical speciation of Fe, which is strongly influenced by the complexation of both Fe(II) and Fe(III) by organic matter [e.g. Refs. [139–141]]. This influence of iron speciation on reactivity with the  $O_2/O_2^{\bullet}$  couple has been examined in detail previously [73], and as such will not be explored further here.

Fe(II) may also be oxidized by  $H_2O_2$  and  ${}^{\bullet}OH$  according to the series of reactions shown in eqs. (10)–(12). Both the rates and mechanisms of these reactions depend strongly on the speciation of Fe(II), in addition to

the relative concentrations of the various ROS. For example [142], showed that humic-type organic matter can inhibit oxidation of Fe(II) by  $\rm H_2O_2$  [115]. additionally showed that the oxidation of Fe(II) by  $\rm H_2O_2$  yields  $^{\bullet}\rm OH$  when the Fe(II) is complexed by certain organic ligands, but in the absence of organic complexation at circumneutral pH appears to yield a different (but still highly oxidizing) species. In lake waters, the reduction of organically complexed Fe(III) by  $\rm O_2^{\bullet}{}^{-}$  was observed to generate an "autocatalytic Fenton reaction" [143], resulting in significant  $^{\bullet}\rm OH$  production due to redox cycling of Fe(II), and subsequent oxidation of OC by the generated  $^{\bullet}\rm OH$ . Such processes are also likely to occur in marine waters.

### 4.2.2. Copper

Many of the reactions of Cu with ROS are analogous to those of Fe with ROS, although with some important differences. Like Fe, Cu may exist in both reduced and oxidized states in marine waters, with the oxidized Cu(II) state the most thermodynamically stable. Unlike Fe(II), the reduced Cu(I) state reacts at near diffusion-limited rates with <sup>3</sup>O<sub>2</sub> except when complexed to certain stabilizing ligands, notably including Cl<sup>-</sup>, which is an important factor in why Cu(I) is able to persist at measurable concentrations in marine waters in some circumstances [144,145], However, Cu(I) and Cu(II), including their organic complexes, react at near-diffusion controlled rates with  $O_2^{\bullet}$  [146]. As such Cu also tends to fall into scenario 1 above, in which both Cu(I) and Cu(II) may coexist at biogeochemically significant concentrations particularly in surface waters [147,148]. Also similarly to Fe, both Cu(I) and Cu(II) may be complexed by organic matter in marine waters (e.g. Refs. [149, 150]), which influences the kinetics of the various reactions shown in eqs (1)-(4) and hence its overall redox cycling dynamics [151]. However, unlike Fe(III), Cu(II) can be reduced by H<sub>2</sub>O<sub>2</sub> at biogeochemically significant rates according to the reaction [152]:

$$Cu(II) + H_2O_2 \rightarrow Cu(I) + O_2^{\bullet -} + 2H^+$$
 (14)

This reaction introduces an additional mechanism by which ROS can induce redox cycling of Cu between the Cu(I) and Cu(II) states. Cu can also participate in Fenton-like processes under marine conditions, but as with Fe the formation of \*OH through this process depends on factors such as pH and complex speciation of Cu [117,153].

### 4.2.3. Manganese

Manganese has been measured to exist in three redox states in marine systems: Mn(II), Mn(III) and Mn(IV). The most oxidized Mn(IV) state is thermodynamically favored under oxic conditions with respect to the overall  $O_2/H_2O$  redox couple, but the first electron transfer step in the oxidation of Mn(II) by  $^3O_2$  is thermodynamically unfavorable (i.e. with respect to the  $O_2/O_2^{\bullet}$  redox couple) [154]. Hence, the oxidation of free, dissolved Mn(II) by  $^3O_2$  under conditions typical of marine waters is negligibly slow [155], but can be accelerated by microbes, organic complexation or adsorption to mineral surfaces (see Refs. [156, 157] for detailed reviews). Mn(II) is nonetheless often the most abundant redox state measured in oxic marine waters [157].

In contrast, the oxidation of Mn(II) to Mn(III) by  $O_2^{\bullet}$  is thermodynamically favorable under conditions found in marine waters [154], and has been shown to occur experimentally under similar conditions via either direct addition of  $O_2^{\bullet}$  [158,159] or by microbial  $O_2^{\bullet}$  production [160]. However, oxidation of Mn(II) via this pathway appears to be readily reversible, such that net oxidation of Mn(II) to Mn(IV) may not necessarily occur, due to the reverse reaction in which intermediate Mn (III) is reduced back to Mn(II) by  $H_2O_2$  [157,158,161]:

$$Mn(III) + H_2O_2 \rightarrow Mn(II) + O_2^{\bullet -} + 2H^+$$
 (15)

As such, for net oxidation of Mn(II) to Mn(III) to occur via this pathway,  $\rm H_2O_2$  produced by the reaction must be rapidly consumed via other processes.

Mn(III) may also be able to persist in marine waters when complexed

[157], and has been shown to represent a substantial proportion of the total dissolved Mn even under oxic conditions [162]; however there is currently only limited evidence for this. The major fate of Mn(III) is thought to be disproportionation:

$$Mn(III) + Mn(III) \rightarrow Mn(II) + Mn(IV)$$
 (16)

Mn(IV) is highly insoluble in marine waters, and predominantly exists as Mn(IV), or in mixed valence Mn(III)/Mn(IV), oxides. Reductive dissolution of these Mn oxides has been shown to be induced by reactions with both  $O_2^{\bullet}$  and  $H_2O_2$  [163]. The ranges of potential reactions of Mn(II), Mn(III) and Mn(IV) with  $O_2^{\bullet}$  and  $H_2O_2$  are consistent with observations of dynamic redox cycling of Mn in marine systems (e.g. Ref. [164]), and the suggestion that redox cycling of Mn may be a potential mechanism for the observed decay of  $H_2O_2$  in marine waters [104].

### 4.2.4. Vanadium

Vanadium can exist in the V(III), V(IV) or V(V) redox states in aqueous systems; however, the V(III) state is only thermodynamically favored under strongly reducing conditions [165]. In oxic marine systems, V(V) is the most thermodynamically stable state, but both the V (IV) or V(V) states have been measured at abundances of >10% of total dissolved V in oxic marine waters (e.g. Refs. [166,167]).

The rate constant for oxidation of VO(OH)<sup>+</sup>, the dominant V(IV) species at circumneutral pH, by  $^3O_2$  is around 1.1 M $^{-1}s^{-1}$  [168], which is only slightly lower than that for inorganic Fe(II) under similar conditions. However, as with many other trace metals discussed here, both V(IV) and V(V) form complexes with organic matter ([169] and references therein), which likely influences V redox dynamics. Given that oxidation by  $^3O_2$  proceeds via one-electron transfer steps, this reaction would be expected to yield  $O_2^-$ , but this has not been experimentally confirmed. V(IV) has been reported to react with HO<sub>2</sub> (the conjugate acid of  $O_2^{\bullet}$  –) under highly acidic conditions in aqueous solution [170]. It is also oxidized by  $H_2O_2$  under circumneutral conditions [171]. However, there remains a considerable knowledge gap around the dynamics of ROS-induced vanadium redox cycling in marine waters, including its reactions with  $O_2^-$  and  $H_2O_2$ , which have not been well studied under these conditions.

### 4.2.5. Molybdenum

Like vanadium, molybdenum can exist in three redox states in aqueous systems: Mo(IV), Mo(V) and Mo(VI) [172]. Of these, Mo(IV) is known to occur only under highly reducing conditions, with Mo(VI) being the most thermodynamically stable and dominant form under oxic conditions, and Mo(V) typically found in mildly reducing microenvironments [172]. Relatively few studies have examined Mo redox speciation in oxic waters. Wang et al. observed the coexistence of both Mo(V) and Mo(VI) in both fully oxic and low oxygen (<10  $\mu$ M dissolved O2) conditions in the Peconic River Estuary, with the reduced Mo(V) present as a greater proportion (up to 15%) of total dissolved Mo under the lower oxygen conditions but nonetheless present at measurable concentrations in some fully oxygenated surface waters. However, some doubts have been raised about the existence of Mo(V) in the oxygenated water column [173]. In any case, the diffusion of Mo(V) from reducing environments (e.g. sediments) into oxygenated waters would likely result in oxidation of Mo(V) to Mo(VI) by 3O2, given that reaction is thermodynamically favorable to proceed via one-electron steps [3]. This would suggest the involvement of ROS in redox cycling between Mo(V) and Mo(VI); however we are unaware of any experimental evidence for such processes in the literature at this time.

### 4.3. ROS and other inorganic species

ROS have been implicated in the biogeochemical cycling of a range of other elements in marine systems. In the case of iodine,  ${\bf I}^-$  reacts

exceedingly slowly with  ${}^3O_2$ , but reacts readily with  $O_2^{\bullet}$  [174],  $H_2O_2$  [175] and organic peroxides [176]. Consistent with these observations, Luther [177] demonstrated that the oxidation of halides under conditions typical of seawater is thermodynamically feasible by a range of ROS including  ${}^1O_2$ ,  $O_2^{\bullet}$ ,  $H_2O_2$  and  ${}^{\bullet}OH$ , with the effectiveness of each depending on the exact pathway.

ROS are additionally known to react with transient inorganic species that are found in seawater, including nitrogen radicals such as NO $^{\bullet}$  and sulfur radicals such as thiols [178]. While the reaction of  $O_2^{\bullet}$  with NO $^{\bullet}$  to yield peroxynitrite in alkaline solutions has been known for decades [179], recent work has shown this reaction is a major sink for photochemically generated NO $^{\bullet}$  in marine waters [180]. Where these radicals exist in oxygenated waters, often as transient species, reactions with ROS are likely through so-called 'cryptic cycles' in which the rapid turnover and low concentrations of the species involved renders them largely undetectable [181].

### 5. ROS impacts in marine ecosystems

In addition to the roles that ROS play in biogeochemical cycling, they also can create profound biological effects. ROS have predominantly been considered to have negative impacts on living organisms due to their ability to react, often indiscriminately, with biological macromolecules. However, increasing evidence indicates that ROS can also play beneficial roles for individual organisms and at the ecosystem level, including through potentially favorable moderation of the local redox environment (e.g. by increasing the bioavailability of micronutrients like iron [73]), but also through biological processes such as regulating growth and resisting pathogens. The beneficial effects of extracellular ROS have recently been reviewed by Hansel and Diaz [2], so will not be considered in further detail here. This section will therefore focus primarily on the potential negative impacts of ROS in marine ecosystems.

Steady-state concentrations and fluxes of ROS in the ocean are generally well below levels that cause toxicity in laboratory studies. For instance, the bacterium Escherichia coli was able to tolerate acute exposure to 15 mM H<sub>2</sub>O<sub>2</sub> with only a moderate loss of viable counts and a short lag period [182]; this value is almost five orders of magnitude higher than any in situ concentration measured in the surface ocean. Animals easily tolerate exposure to even higher concentrations of H<sub>2</sub>O<sub>2</sub>, with 3% (~100 mM) H<sub>2</sub>O<sub>2</sub> routinely used as a topical first-aid antiseptic in humans, for example. Environmental concentrations of free and OH are even lower, and orders of magnitude less than concentrations measured in healthy animal cells. Despite this, research over the past decade has revealed that some important marine microbial groups are vulnerable to even these low ROS concentrations. Moreover, some marine microenvironments, such as saltmarsh pools and certain kinds of algal blooms, may accumulate high concentrations of ROS capable of damaging even relatively well-protected organisms. In this section, we review the cellular targets of ROS attack and consider several key organisms that are particularly at risk.

### 5.1. Targets of ROS attack in marine organisms

ROS, especially OH, can attack all classes of biological macromolecules. OH may attack the backbone deoxyribose sugars in DNA, fragmenting them in a variety of ways leading to single- or double-strand breaks [105,183,184]. Also, the nucleotide bases themselves may be modified, either before or after incorporation into a DNA molecule. B-Hydroxyguanine, one result of OH attack on guanine, base pairs with thymine rather than cytosine, and if not repaired may lead to transversion mutations. RNA, including relatively long-lived rRNA and tRNA molecules, is even more vulnerable to oxidation than DNA [185], and oxidative mRNA damage predictably correlates with translational errors [186].

OH is also capable of many non-specific reactions on proteins,

oxidizing both side chains and polypeptide backbones. The typical result of such oxidation is the formation of carbonyl groups, and the detection of this "carbonylation" using antibodies is a common method for measuring oxidative protein damage in vitro [105]. Compared to  ${}^{\bullet}$ OH, other ROS have more well-defined protein targets.  $O_2^{\bullet}$  – degrades proteins that bind iron, such as ferritin, catalase, and Fe–S cluster enzymes [105,187,188], causing the release of free Fe into the cytoplasm, allowing destructive Fenton chemistry.  $H_2O_2$ , while more stable than either  $O_2^-$  or  ${}^{\bullet}$ OH, attacks certain proteins even in the absence of free Fe, including important, constitutively expressed proteins such as the  $\beta$ -subunit of ATPase and prokaryotic translation elongation factor G [189,190].

Lipids are also prone to attack by \*OH, and oxidized lipids are capable of dramatically magnifying the effects of ROS attack via radical propagation. In a typical scenario, \*OH attacks a double bond in an unsaturated lipid and abstracts a hydrogen atom, leaving a carbon radical and water. This new lipid radical may react with any other radical with no energy barrier. One possible reaction is with a second lipid radical, quenching the radical character of the system and forming a stable, cross-linked species that terminates radical propagation. This reaction is rare in oxic systems, however, compared to reaction with  $^3{\rm O}_2$  to form a lipid hydroperoxide and a new lipid radical. This propagation step may be carried out many times before the original lipid radical is quenched by a termination reaction. Lipid hydroperoxides are capable of attacking membrane proteins as well as facilitating the transition of  $^3{\rm O}_2$  to  $^1{\rm O}_2$  in the cytoplasm [191].

### 5.2. Major ROS-vulnerable marine populations

There are two ways that an organism living in the ocean may experience oxidative stress. First, the stress may arise intracellularly because of unbalanced metabolism, increased temperature or pH stress, or exposure to toxic substances in the environment such as xenobiotics and heavy metals. Some of these stressors were described above in section II. Second, the stress may arise through direct exposure to ROS in the environment. As described above, extracellular ROS fluxes in marine systems tend to be substantially lower than doses shown to negatively impact organisms in the laboratory. However, these observations may underestimate the direct risk organisms face from ROS in their native habitats. For instance, an E. coli population in natural river water exposed to light experienced  $\sim\!99\%$  mortality after 3 days unless catalase or sodium pyruvate (a \*OH trap) was added to the water prior to illumination [192]. Based on other studies, the likely H2O2 concentration of the river water was no greater than 10 µM (Table 1), more than 1000-fold less than the concentration necessary to kill laboratory populations of E. coli [182]. Laboratory culture media often contain hidden sources of ROS [193-196] which may select for disproportionately high levels of resistance over time, obscuring the sensitivity of native populations. More broadly, Xenopoulus and Bird [197] reported >50% reduction of bacterial productivity in a lake following exposure to 100 nM H<sub>2</sub>O<sub>2</sub> as compared to a catalase-treated control. Similar to these freshwater observations, a study in a coastal macrophyte-dominated ecosystem found that additions of as little as 20 nM H2O2 significantly reduced bacterial productivity, with 1 µM completely eliminating productivity over a 1 h timescale [80]; importantly however, other studies have shown no effect on bacterial production with  $H_2O_2$  addition [198].

Among the first marine organisms to be conclusively demonstrated to have high sensitivity to  $H_2O_2$  inhibition was *Prochlorococcus*, the numerically dominant phytoplankton genus throughout the temperate and tropical oligotrophic oceans. When grown in pure culture, *Prochlorococcus* is completely inhibited by 800 nM  $H_2O_2$ , but when grown in co-culture with a wide variety of heterotrophic bacteria, it can tolerate much higher exposures [199,200]. In the absence of bacteria, open ocean seawater from areas that are rich in *Prochlorococcus* rapidly accumulates  $H_2O_2$  concentrations at least this high when exposed to sunlight, suggesting that *Prochlorococcus* is obligately dependent on other

members of its community to survive. This sensitivity likely exists because Prochlorococcus has an extensively streamlined genome, which contains no genes for catalase or any heme peroxidases, essentially eliminating its capacity to defend itself from exogenous  $H_2O_2$  [201]. This lack of intrinsic  $H_2O_2$  resistance is reflected in conspicuous vulnerability of pure Prochlorococcus cultures to a wide variety of stresses, including dark exposure [202,203] and suboptimal temperatures [204]. While Prochlorococcus is more vulnerable than most, cyanobacteria in general appear to be vulnerable to lower doses of  $H_2O_2$  than most other studied organisms. Both unicellular cyanobacteria and filamentous forms experienced high mortality when exposed to  $\sim 10~\mu M$   $H_2O_2$ , a value that was consistent across numerous experiments in different laboratories [205–208]. On the other hand, eukaryotic microalgae (green algae, diatoms) survived doses as high as 1 mM [206, 207].

H<sub>2</sub>O<sub>2</sub> can also inhibit phytoplankton growth by altering the availability of limiting nutrients. The primary source of Fe in surface waters of the Atlantic ocean is atmospheric deposition, e.g. via dust from the Sahara Desert. Much of this Fe enters the ocean in the form of rainwater, which can also carry H<sub>2</sub>O<sub>2</sub> at concentrations hundreds of times higher than surface seawater (Table 1). Experiments in March 2000 at the Bermuda Atlantic Time Series stations showed that additions of either FeCl<sub>2</sub> or FeCl<sub>3</sub> to seawater increased chlorophyll-a concentrations over a three-day incubation, indicating Fe limitation of primary production in the absence of seasonal dust deposition [29]. However, when the Fe was added as a synthetic rainwater mixture also containing H2O2, the increase in phytoplankton growth compared to unamended controls was completely eliminated. The presence of H<sub>2</sub>O<sub>2</sub> effectively made the Fe unavailable, presumably through Fenton chemistry and precipitation of insoluble Fe(III) complexes. It is also possible that the increase in H<sub>2</sub>O<sub>2</sub> (to a final concentration of approximately 400 nM) may have directly damaged or killed phytoplankton in this experiment.

Another ecologically important group of organisms, the ammoniaoxidizing archaea (AOA) of the phylum Thaumarchaeota, are also catalase and peroxidase deficient and conspicuously vulnerable to H2O2 [209]. AOA are important regulators of the speciation of bioavailable nitrogen in the ocean, representing up to 40% of the total bacterioplankton in some parts of the World Ocean and directly or indirectly contributing significant fractions of the greenhouse gases N2O and CH4 to the atmosphere [210]. Some AOA strains are also among the most H<sub>2</sub>O<sub>2</sub>-vulnerable aerobic organisms known. For instance, the growth of one Southern Ocean strain was inhibited by 10 nM H<sub>2</sub>O<sub>2</sub>, nearly two orders of magnitude lower concentration than the lethal dose for Prochlorococcus, and the addition of only 6 nM H<sub>2</sub>O<sub>2</sub> to a field population completely eliminated nitrification while not affecting overall bacterial production at all [211]. Inhibition of AOA by nanomolar H<sub>2</sub>O<sub>2</sub> was also observed in isolates from coastal temperate seas and the deep ocean [212,213], and enhancement of growth was frequently seen after addition of H<sub>2</sub>O<sub>2</sub>-scavenging pyruvate or catalase, or by co-culture with helper bacteria [213-215]. AOA isolates have been observed to secrete H<sub>2</sub>O<sub>2</sub> into their growth media in proportion to the amount of ammonia oxidized, suggesting that their vulnerability may arise as a side effect of their primary metabolism [215]. If true, this may represent a difficult-to-overcome constraint on AOA in the ocean, and could account for the fact that they are primarily active in deep water, only thriving at the surface during polar winters when photochemically generated H<sub>2</sub>O<sub>2</sub> is at a minimum [211].

In general, macroscopic organisms, and even multicellular microscopic organisms such as fish and invertebrate larvae, are much less vulnerable to  $\rm H_2O_2$  in the nanomolar-micromolar range than these bacteria, although a number of them are inhibited by doses low enough to be of concern in the presence of some human activities (see section VI below). However, there are exceptions. For instance, one study demonstrated substantial variability in  $\rm H_2O_2$  tolerance in different clonal populations of the starlet sea anemone *Nematostella vectensis*, which inhabits coastal salt marshes where natural  $\rm H_2O_2$  concentrations

can reach the 100  $\mu$ M range. When anemones were sampled from South Carolina, New Jersey, and Nova Scotia, a clear north-south gradient in tolerance for acute  $H_2O_2$  exposure was observed, supporting the hypothesis that elevated temperatures correlate with greater exposure to  $H_2O_2$  [216]. Interestingly, when the same researchers sampled anemones from a variety of saltmarsh ponds in Massachusetts, they found substantial variation in  $H_2O_2$  tolerance between populations. Notably, these organisms were severely impacted by  $H_2O_2$  concentrations within the range observed in the estuarine waters they inhabit (Fig. 4), so this high variation over a short spatial scale suggests that  $H_2O_2$  in these ranges represents a sufficiently potent stress to affect local adaptation, at least in this species.

### 5.3. Impacts of ROS on marine biology experiments

Along with the discovery that some marine organisms are negatively affected by naturally occurring concentrations of ROS comes the possibility that cryptic ROS production caused by experimental procedures may impact results. For example, in a series of in situ mesocosm experiments in the Canary Islands in 2016 designed to test the effects of different degrees of ocean acidification on the structure and function of natural plankton assemblages, H<sub>2</sub>O<sub>2</sub> concentrations within the mesocosm enclosures were observed to increase significantly (>200 nM) in comparison to ambient conditions (~50 nM) after several days [198, 217]. The authors concluded that these increases were caused by the mesocosm enclosures inhibiting vertical mixture of surface and deep waters, causing H<sub>2</sub>O<sub>2</sub> formed near the surface to remain concentrated relative to conditions outside the enclosures. Elevated H2O2 correlated with decreased bacterial and phytoplankton abundances in these experiments, possibly indicating H2O2 toxicity that may have killed or otherwise inhibited these cells, but also confirming that reduced bacterial concentrations exerted a positive feedback on H<sub>2</sub>O<sub>2</sub> concentration by removing the primary H2O2 sink in the mesocosms. Further, the elevated H2O2 concentrations likely impacted the lability of DOM and trace metals and, as a consequence, the availability of important growth substrates.

Unexpected ROS production can also influence laboratory experiments with marine microorganisms. For example, HEPES and other zwitterionic pH buffers commonly used in culture media for algae and bacteria generate H<sub>2</sub>O<sub>2</sub> continuously when exposed to light, and can produce lethal concentrations for some of the more sensitive species (e. g. Prochlorococcus) in less than 24 h [194]. Collecting Thalassiosira weissflogii diatoms by syringe instead of peristaltic pump increased their release of H<sub>2</sub>O<sub>2</sub>, which could influence transcriptomic studies of these organisms by artifactually increasing oxidative stress gene expression, for example [75]. Based on these observations, researchers working in these systems need to consider H2O2 concentrations, and the effect of experimental procedures on them, in the same manner they measure light, temperature, and other physicochemical properties that may be important confounding variables, and should also consider the possibility that laboratory strains are substantially more H<sub>2</sub>O<sub>2</sub> resistant than their relatives in the ocean.

## 6. Impacts of human activity on marine ROS

There are several reasons to expect that the threat of oxidative stress will increase for marine organisms in the future as secondary effects of anthropogenic changes, including both the introduction of pollutants into marine systems as well as the broader impacts of CO<sub>2</sub> emissions on the physicochemical environment of the ocean. In this section we consider two vectors for increased oxidative stress, specifically the natural increase in ROS due to anthropogenic CO<sub>2</sub> increase, and secondarily human activities that may introduce or concentrate novel ROS sources in the environment.

### 6.1. Global CO2 increase and ROS

The  $\rm CO_2$  concentration of Earth's atmosphere is increasing at an unprecedented pace due to human consumption of fossil fuels. The two most profound sequelae of this change are i) an increase in global average temperature, both in the atmosphere and the ocean, and ii) an imbalance in the carbonate buffering system of aquatic ecosystems, leading to acidification. Both conditions could lead directly to increased ROS exposure for marine organisms, as well as creating secondary changes to marine environments that may exacerbate oxidative stresses.

First, higher temperatures are likely to lead to greater rates of biological  $\mathrm{H}_2\mathrm{O}_2$  production due to temperature stress on marine organisms, at least in the short term before local populations either adapt or are outcompeted by more temperature-tolerant competitors. When West Antarctic Peninsula seaweeds were exposed to 2-8 °C of warming they increased their rate of  $\mathrm{H}_2\mathrm{O}_2$  release into the surrounding seawater as well as exhibiting metabolic signs of oxidative stress [49]. Similarly, the microscopic gametophytes of three Arctic kelp species released greater amounts of  $\mathrm{O}_2^\bullet$  – into the surrounding medium when exposed to elevated temperatures [218]. In both cases, the seaweeds themselves appeared to be unharmed by the conditions, but the possibility of enhanced ROS release negatively impacting the surrounding community was not explored.

Increased  $H_2O_2$  release is also observed in sessile animals. For example, 16 different *Symbiodinium* coral symbionts exposed to heat stress showed substantial variability in signs of oxidative stress. Some strains exhibited severe electron transport chain damage resulting in production of large amounts of  $H_2O_2$ , whereas others avoided  $H_2O_2$  accumulation by downregulating PSII [219]. Like the anemones described earlier [216], this observation suggests a substantial amount of intraspecific variation in ROS tolerance that may increase the rate of evolution in a changing ROS environment, but perhaps at the cost of reduced photosynthesis or other unforeseen physiological trade-offs.

Increased temperature may also lead to higher ROS fluxes in future waters. Studies across time and space under present conditions reveal that shallower, hotter waters accumulate greater  $\rm H_2O_2$  concentrations (Table 1, Fig. 3). One predicted effect of global warming on the ocean is the shoaling of the mixed layer, where higher temperatures strengthen thermal stratification of the ocean and yield shallower thermoclines [220]. Fig. 3 shows clearly that  $\rm H_2O_2$  concentrations drop off rapidly below the thermocline, suggesting that most production occurs near the surface and only accumulates near the surface because  $\rm H_2O_2$  diffuses slowly across this barrier. Thus, shallower thermoclines may yield greater steady-state  $\rm H_2O_2$  concentrations at the ocean's surface, which may negatively impact ROS-sensitive organisms such as *Prochlorococcus*.

Ocean acidification may additionally enhance the susceptibility of marine organisms to environmental ROS. Most acidification research has focused on its interference with calcification in corals and other shelled organisms, but acidification also creates oxidative stress in a wide variety of both calcified and non-calcified species by imbalancing intracellular redox states, resulting in oxidative stress (e.g. Refs. [52,54, 221]) and enhanced vulnerability of some keystone species to a wide variety of both biotic and abiotic challenges [48,50,56]. In some cases, acidification may disrupt important symbiotic interactions as well. For instance, the synergistic "helper" interaction between Prochlorococcus and the bacterium Alteromonas appeared to shift from a commensal or mutualistic state under present-day conditions to an exploitative state under elevated CO<sub>2</sub>, with Alteromonas significantly downregulating its catalase genes and causing a dramatic increase in oxidative stress and cell death for Prochlorococcus [222]. Moreover, rates of hydroxyl radical formation by the Fenton reaction increase with H<sup>+</sup> concentration [223] as well as with temperature, suggesting that not just the concentrations, but also the lethality of ROS will be enhanced in future oceans, and that oxidative stress enhancement may underlie some of the synergistic effects of "multiple stressors" on marine life.

We must note, however, that much remains unknown about the

impact of  $\mathrm{CO}_2$  increase on the World Ocean in general, and other effects of acidification or temperature increase may reduce ROS stress on marine organisms. For instance, higher temperatures will increase the rates of all chemical, and most enzymatic, reactions, which will simultaneously increase both ROS production and removal processes and may also increase organismal maximum growth rates. All biogeochemical cycles, including both the biotic and abiotic cycling of DOM and other C compounds, will speed up, potentially increasing the bioavailability of many nutrients, which could reduce nutrient limitation stresses and/or improve the ability of organisms to protect themselves from ROS. Much more research into these processes will be necessary before we can draw any confident conclusions about ROS exposure will affect life in future oceans.

### 6.2. Pollution and marine ROS

In addition to the global impacts of  ${\rm CO_2}$  release, human activity is increasing the exposure of marine life to a wide variety of compounds that may increase oxidative stress. We have already discussed the effects of allelochemicals such as microplastics and heavy metals on oxidative stress in invertebrates, but in this section we will focus specifically on pollutants that may lead directly to increased ROS fluxes, particularly in coastal ecosystems.

### 6.2.1. Use of H<sub>2</sub>O<sub>2</sub> in aquaculture

Since 1993, H<sub>2</sub>O<sub>2</sub> has been used to control the growth of parasitic "sea lice" copepods and other ectoparasites in salmon aquaculture operations in Norway and elsewhere, with usage peaking in 2015 and remaining common today [224]. H<sub>2</sub>O<sub>2</sub> has been popular because of its relatively low toxicity profile compared to other effective pesticides and the fact that it is removed from water bodies after a few days by microbes as described above [225]. Relatively high H2O2 dosages are necessary for complete lice control, however, with approximately 100 mM necessary to achieve 98% reduction in parasite load (Fig. 4). Because water from salmon farms is released directly into coastal seas, there is concern that these concentrations, which are orders of magnitude higher than naturally occurring aquatic H<sub>2</sub>O<sub>2</sub> concentrations, may have toxic effects on off-target organisms. Indeed, studies have shown that several marine invertebrates and fish can be killed by lesser exposures, particularly in their immature forms (Table 2). For example, the LC50 for a 1-h H<sub>2</sub>O<sub>2</sub> exposure for European lobster Homarus gammarus larvae increased from 5 mM to 22 mM as the larvae progressed through stage I-IV molts [226], although one study showed that adult Calanus copepods were killed by significantly lower H2O2 doses than adults [227]. Notably, assays that utilized longer exposures typically reported lower LC50 values, indicating that chronic exposure to lower doses of H<sub>2</sub>O<sub>2</sub> can cumulatively increase damage even to complex multicellular organisms. For many of these species, lethal H<sub>2</sub>O<sub>2</sub> doses were substantially lower than the recommended dosing for delousing of salmon enclosures, but it is unclear whether concentrations of H2O2 released from these ponds into the ocean is sufficiently high to kill significant numbers of animals, or if instead dilution by the bulk seawater is sufficient to eliminate their negative impacts. Importantly, however, much lower sublethal doses of H2O2 contribute to other syndromes for these organisms, including impairing their ability to find refuge or otherwise avoid predation [226,227], reducing their feeding rate ([228], Japanese language paper cited in Ref. [216]), or slowing healing processes [216]. These impediments may indirectly contribute to increased mortality for species living in the vicinity of aquaculture operations or other environments where humans use H2O2. Combined with the facts that repeated H<sub>2</sub>O<sub>2</sub> treatment has negative impacts on salmon health [229] and appears to select for H2O2-resistant sea louse varieties [230], H2O2 has lost popularity in recent years in favor of mechanical and biocontrol delousing methods [225], but the practice remains widespread and worthy of further attention regarding its impacts on the surrounding natural environment.

Table 2 Lethal limits for  $H_2O_2$  exposure in various organisms.

|               | Species                                 | Type of assay          | Effective<br>dosage | Reference |
|---------------|---|------------------------|---------------------|-----------|
|               |   | assay                  | dosage              |           |
| Cnidarians    | Nematostella sp.                        | 14-d 100%<br>mortality | 163 μΜ              | [216]     |
| Crustaceans   | Mysis sp.                               | 1-h LC50               | 29 mM               | [265]     |
|               | Calanus<br>finmarchicus                 | 1-h LC50               | 180 μΜ              | [266]     |
|               | Calanus spp. Stage V<br>larvae          | 1-h LC50               | 6.3 mM              | [227]     |
|               | Calanus spp. Stage V<br>larvae          | 25-h LC50              | 2.3 mM              | [227]     |
|               | Calanus spp. Adult                      | 1-h LC50               | 1.4 mM              | [227]     |
|               | Calanus spp. Adult                      | 25-h LC50              | 900 μΜ              | [227]     |
|               | Crangon<br>septemspinosa                | 1-h LC50               | 94 mM               | [265]     |
|               | Atemia salina                           | 24-h LC50              | 24 mM               | [267]     |
|               | Crophium volutator                      | 96-h LC50              | 1.4 mM              | [268]     |
|               | Homarus                                 | 1-h LC50               | 48 mM               | [265]     |
|               | americanus, stage I<br>larva            |                        |                     |           |
|               | Homarus gammarus,<br>larvae stages I-IV | 1-h LC50               | 5.2–22 mM           | [226]     |
| Molluscs      | Dreissena<br>polymorpha                 | 72-h LC50              | 890 μΜ              | [55]      |
| Fish          | Siganus fuscescens                      | 24-h LC50              | 6.6 mM              | [269]     |
| 1 1011        | Trachurus japonicus                     | 24-h LC50              | 2.6 mM              | [269]     |
|               | Oncorhynchus<br>mykiss juveniles        | 3-h 90%<br>mortality   | $\sim 9 \mu M^a$    | [270]     |
| Phytoplankton | Microcystis<br>aeruginosa               | 3-h LC50               | 7.9–195 μΜ          | [207]     |
|               | Selenastrum<br>capricornutum            | 3-h LC50               | 119–625.3<br>μΜ     | [207]     |
|               | Raphidocelis<br>subcapitata             | 3-h LC50               | 359–2088<br>μM      | [207]     |
|               | Prochlorococcus                         | 24-h 100%<br>mortality | 800 nM              | [200]     |
| Zooplankton   | Daphnia sp.                             | 48-h LC50              | 164 μΜ              | [271]     |
| - F           | Moina sp                                | 48-h LC50              | 58.8 μM             | [271]     |
| Bacteria      | Escherichia coli                        | 3 h-LC50               | 7.8 mM              | [272]     |
| Ducteria      | Escherichia coli                        | 6 h 100%<br>mortality  | 15 mM               | [182]     |
|               | Vibrio coralliilyticus                  | 0.5 h- LC50            | ${\sim}15~\mu M$    | [67]      |

<sup>&</sup>lt;sup>a</sup> Measured in the presence of harmful alga *Heterosigma carterae*; algal toxins likely enhanced ROS lethality.

### 6.2.2. Use of $H_2O_2$ to combat harmful algal blooms

Harmful cyanobacterial blooms are increasing globally, and this trend may continue in the next decades [231]. One study showed that in 100 lakes investigated in North America and Europe, 60% have observed a substantial increase in cyanobacteria since the industrial revolution [232], and recently, the model of Chapra et al. [233] predicted that the average days per year with cyanobacterial blooms in USA will increase from  $\sim$ 7 d to 18–39 d by 2090. Rising temperatures and atmospheric CO<sub>2</sub> as well as the increasing frequency of extreme hydrologic events increase the growth rates of algae and alter critical nutrient thresholds for the development of algal blooms [234,235]. The expansion of agriculture in developing countries also accelerates the input of nitrogen and phosphorus into coast area, which also promotes the occurrence of algal blooms.

A large body of work from Russia and the former Soviet Union, summarized in English by Skurlatov and Ernestova [236], suggested that environmental  $\rm H_2O_2$  has the positive effect of reducing the frequency and severity of toxic algal blooms. Consequently, toxin-producing freshwater cyanobacteria were rare in waters where the concentration of  $\rm H_2O_2$  exceeded 0.3  $\mu$ M. When waters became loaded with ROS "traps" from wastewater effluents, however, toxic blooms frequently formed. As  $\rm H_2O_2$  is generally toxic at lower concentrations to microorganisms than macroorganisms, it has been proposed as a very promising algicide in the control of cyanobacterial blooms [231,234,235]. Researchers in 1986 discovered that as little as 50  $\mu$ M  $\rm H_2O_2$  strongly suppressed

photosynthesis and growth in the cyanobacterium *Oscillatoria rubescens* [237]. Afterwards, laboratory and field tests [206,238,239] proved the effectiveness of  $\rm H_2O_2$  in selectively eliminating cyanobacterial blooms and preventing bloom-forming species from surviving during winter [214]. Both high light and blue light were found to enhance the inhibition of  $\rm H_2O_2$  to *Microcystis aeruginosa PCC 7806* [240]. A range of authors observed a dose-effect response between  $\rm H_2O_2$  and their effectiveness in the inhibition of different cyanobacteria. Considering the cost and ecological risk, a maximum dose of 5 mg  $\rm L^{-1}$  (~150  $\mu M$ ) was recommended for use in freshwater systems [241].

One major concern of using  $H_2O_2$  to control *Microcystis* blooms was the release of the toxin microcystin from the lysing cells. However, a field experiment showed that the particulate and water soluble microcystin degraded 90% in less than 3 days. The decomposition of microcystin may be related to microbial degradation [242] but also to  $^{\circ}$ OH catalyzation due to Fenton chemistry involving the added  $H_2O_2$  [243]. Though  $H_2O_2$  could not completely eliminate the harmful cyanobacterial bloom, it created a possibility for the rapid succession of phytoplankton populations from HAB species to non-harmful species, which could be readily consumed in classic microbial food chains [244].

Most applications of H<sub>2</sub>O<sub>2</sub> to control algal blooms have been in freshwater systems, but recent efforts have also shown promise in marine systems. In the Norfolk Broads, a series of shallow saltwater lakes in Great Britain, the eukaryotic chrysophyte Prymnesium parvum is a toxic eukaryotic alga that has been responsible for massive fish kills. Administration of approximately 1.5 mM H<sub>2</sub>O<sub>2</sub> to the Broads during a period between blooms reduced the ambient *P. parvum* levels by >90%, with effects starting only 2 h after exposure [245]. The H<sub>2</sub>O<sub>2</sub> treatment also led to sizable changes in the bacterial community, with especially conspicuous losses of unicellular cyanobacterial taxa. Interestingly, the community rebounded within 4 d after treatment, suggesting that H<sub>2</sub>O<sub>2</sub> did not cause a permanent shift in community structure although it could possibly help to reduce the magnitude of toxic taxa during a bloom. Similar concentrations were applied to a bloom of the toxic dinoflagellate Alexandrium in a brackish creek in the Netherlands, resulting in 99.8% reduction of algal biomass as well as elimination of the algal toxin from the water column [246]. The dosage necessary to destroy Alexandrium also caused severe reduction in zooplankton as well as altering the macroinvertebrate and even fish communities, however, reflecting the concerns raised in the previous section on the trade-offs in using H<sub>2</sub>O<sub>2</sub> as a biocontrol agent.

### 7. Conclusions

The oxygen cycle in the ocean is considerably more complex than the simple shuttling of O between water and organic matter, with reactive intermediates engaged in a wide variety of reactions that have critical importance for the biogeochemistry of the sea. Improvements in our technological capability to measure ROS fluxes have led to an improved understanding of the cycling of DOM and trace metals in the ocean and have revealed previously unsuspected mortality pathways for microorganisms as well as potential confounding factors for oceanographic experiments. However, we still know comparably little about the mechanistic reasons for why some organisms are so much more susceptible to ROS damage than others, or how changing environmental conditions impact ROS fluxes and their effect on marine life. Critically, we also do not understand fully how human activity will affect ROS fluxes, nor how our use of ROS such as H2O2 for biocontrol may affect ecosystems. Because many stress pathways involve ROS, future efforts to mitigate harm to the environment from human activity must involve a deeper understanding of the pathways of formation and degradation of these enigmatic molecules.

### **Declaration of interests**

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Redox Biology 52 (2022) 102285

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