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Production of Extracellular Reactive Oxygen Species by Marine Biota

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Abstract

Reactive oxygen species (ROS) are produced ubiquitously across the tree of life. Far from being synonymous with toxicity and harm, biological ROS production is increasingly recognized for its essential functions in signaling, growth, biological interactions, and physiochemical defense systems in a diversity of organisms, spanning microbes to mammals. Part of this shift in thinking can be attributed to the wide phylogenetic distribution of specialized mechanisms for ROS production, such as NADPH oxidases, which decouple intracellular and extracellular ROS pools by directly catalyzing the reduction of oxygen in the surrounding aqueous environment. Furthermore, biological ROS production contributes substantially to natural fluxes of ROS in the ocean, thereby influencing the fate of carbon, metals, oxygen, and climate-relevant gases. Here, we review the taxonomic diversity, mechanisms, and roles of extracellular ROS production in marine bacteria, phytoplankton, seaweeds, and corals, highlighting the ecological and biogeochemical influences of this fundamental and remarkably widespread process.

1. INTRODUCTION

In the words of Paracelsus, who is credited with being the father of the field of toxicology, "All things are poison, and nothing is without poison; the dosage alone makes it so a thing is not a poison." In a similar vein, reactive oxygen species (ROS) are paradoxically both essential and detrimental to life, and the tipping point is primarily a function of concentration.

All aerobic organisms form ROS inside their cells (intracellular production) as a by-product of respiration or photosynthesis. Within eukaryotic cells, for instance, mitochondria account for 90% of the O_2 consumed and thus are a pivotal source of intracellular ROS (Hopkins 2016). Oxidative stress involves the accumulation of these ROS to toxic levels within the cell, where the ROS alter the redox state of critical enzymes or destroy essential biomolecules, such as membranes and proteins. Yet within higher organisms (e.g., animals, fungi, and plants), it is well appreciated that low levels of intracellular ROS are also essential for basic life functions, such as cell signaling. Since ROS other than hydrogen peroxide have a limited ability to passively diffuse or be actively transported across biological membranes (Korshunov & Imlay 2002), organisms maintain healthy internal levels of ROS through the regulation of antioxidant molecules and enzymes (Fridovich 1998), such as the superoxide scavenger superoxide dismutase (SOD).

The ROS superoxide and hydrogen peroxide are also actively produced outside the cells (extracellular production) of a broad range of organisms, spanning bacteria to animals. Extracellular ROS production has long been acknowledged in higher eukaryotes (animals, fungi, and plants). Specific enzymes, including those belonging to the NADPH oxidase (NOX) family of transmembrane oxidoreductases, regulate eukaryotic extracellular ROS production (Lara-Ortiz et al. 2003). These enzymes are not limited solely to phagocytic cell types and instead are phylogenetically widespread, including in plants (Lamb & Dixon 1997), animals (Griendling et al. 2000), and phytoplankton (Anderson et al. 2016, Kim et al. 2000), as well as bacteria (Hajjar et al. 2017). In fact, similar to intracellular ROS production, extracellular ROS production is known to play essential roles in organismal physiology, including cell differentiation and growth, signaling, wound repair, defense against pathogens, and the innate immune response (Aguirre et al. 2005).

With the exception of macroalgae, the ubiquity and importance of extracellular ROS production within marine organisms have only recently come to light. Yet ROS in the oceans often remain vilified as toxic compounds, recognized primarily for their negative impacts on organismal health and function. In fact, the mere presence of the ROS hydrogen peroxide and superoxide, or their inferred presence due to the upregulation of antioxidants, is frequently equated to stress, disease, and death in macro- and microorganisms, without the establishment of direct causal links or empirical evidence. However, an increasing number of studies are attributing biogenic ROS to beneficial physiological processes within marine systems, such as micronutrient acquisition in phytoplankton (Rose et al. 2005), microbial growth (Hansel et al. 2019), photosynthetic health (Diaz et al. 2019), pathogen resistance (Armoza-Zvuloni et al. 2016a), feeding in corals (Armoza-Zvuloni et al. 2016b), and the evolution of life over Earth history (Taverne et al. 2018). We therefore find ourselves at the precipice of a new view of the complex and multifaceted role of ROS in the health and physiology of marine organisms.

Here, we provide a brief synopsis of the current knowledge regarding extracellular ROS in marine biota, with an emphasis on the ecological and physiological basis for its production. This review is necessarily brief, due in large part to the need for more research on extracellular ROS production by marine biota. We refer readers to the many references throughout, as well as previous seminal and informative reviews on ROS within other organismal and environmental systems (Aguirre & Lambeth 2010, Rose 2012, Weinberger 2007). In this review, we expand upon and update recent reviews on extracellular ROS production by phytoplankton (Diaz & Plummer 2018) and marine microbes (Zinser 2018a).

Reactive oxygen species (ROS): chemically reactive oxygen-containing molecules, some of which form during sequential reduction of molecular oxygen to water

Oxidative stress: disturbance in the balance between intracellular ROS and antioxidants that has potential to damage biomolecules and initiate cell death

Hydrogen peroxide: ROS formed via divalent reduction of oxygen or often via dismutation of superoxide, with a half-life of minutes to days

Antioxidant: enzyme or small molecule that degrades reactive chemicals and radicals, including ROS, thereby inhibiting them from oxidizing essential biomolecules

Superoxide: ROS formed via monovalent reduction of oxygen, with a half-life of seconds to minutes and a limited capacity to cross membranes

Superoxide dismutase (SOD): redox-active metalloenzyme found throughout aerobic life that catalyzes dismutation of superoxide to molecular oxygen and hydrogen peroxide

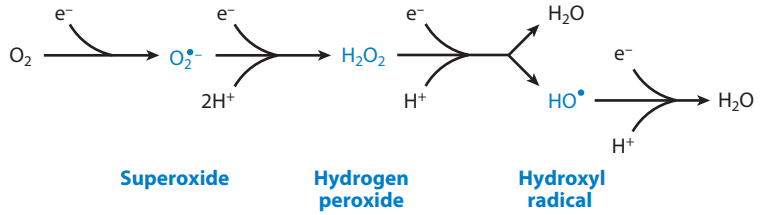

Figure 1

Reactive oxygen species (*blue text*) formed via the sequential monovalent reduction of molecular oxygen to water.

2. WHAT ARE REACTIVE OXYGEN SPECIES?

ROS are short-lived oxygen-bearing molecules with half-lives that range from fractions of seconds to days. They include hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}/HO_2$), hydroxyl radicals ($HO^{\bullet}$), singlet oxygen ($^1O_2$), and carbonate radicals ($CO_3^{\bullet-}$). The primary ROS within marine and biotic systems form via the sequential monovalent reduction of molecular oxygen to water (Fridovich 1998) (**Figure 1**).

In this review, we focus on superoxide and hydrogen peroxide, the most abundant ROS within marine systems. Superoxide is the single-electron reduced form of O_2 and the conjugate base of the hydroperoxyl radical ($HOO^{\bullet}$) (Bielski 1978). With a pK_a of 4.8, the reactive anion superoxide ($O_2^{\bullet-}$) dominates over the hydroperoxyl radical within marine waters. Superoxide may undergo catalyzed or uncatalyzed dismutation to form hydrogen peroxide and O_2 (Moffett & Zafiriou 1990, Petasne & Zika 1987). Degradation of superoxide and hydrogen peroxide occurs via multiple pathways, including reactions with metals, with organic matter, and via enzymatic activity (see Section 4). Within marine waters, the typical half-lives for superoxide are on the order of seconds to minutes, while those of hydrogen peroxide are frequently hours to days (Hansard et al. 2010; Heller & Croot 2010a; Rose et al. 2008, 2010).

Within biological systems, ROS may be formed both inside (intracellular) and outside (extracellular) the cell. While hydrogen peroxide may be actively or passively transported across biological membranes, the superoxide anion, which predominates under typical physiological pH conditions, is not membrane permeable (Korshunov & Imlay 2002). Thus, intracellular superoxide production typically makes a negligible contribution to extracellular pools of superoxide.

NADPH oxidase: family of membrane-bound enzymes that couple oxidation of intracellular NADPH to reduction of extracellular oxygen, forming primarily superoxide

Dismutation: molecule of intermediate oxidation state self-reacting to form one molecule with a higher and one with a lower oxidation state

3. EXTRACELLULAR REACTIVE OXYGEN SPECIES PRODUCTION

Production of extracellular ROS has been observed, or inferred based on the presence of NOX enzymes, in a wide variety of marine organisms, including prokaryotes; protists; invertebrates such as corals, echinoderms, and tunicates; and fish (Aguirre & Lambeth 2010). In many of these organisms, our understanding of the biogeochemical controls, biochemical pathways, and physiological reasons for this production is still in its infancy, but the last decade has brought new insight. In this short time frame of inquiry, it has already become apparent that extracellular ROS production is a closely regulated process that is an essential function for a broad diversity of marine organisms.

3.1. Macro- and Microbiological Extracellular Reactive Oxygen Species Production

This review focuses on extracellular ROS production by heterotrophic bacteria, phytoplankton, macroalgae, and corals, based on the widespread biogeochemical and ecological importance of these groups.

3.1.1. Heterotrophic bacteria. The first heterotrophic marine bacterium to demonstrate the ability to produce superoxide extracellularly was a bacterium in the *Roseobacter* clade (Learman et al. 2011) isolated from an estuary (Hansel & Francis 2006). Subsequent surveys of an ecologically and taxonomically diverse range of bacterial heterotrophs revealed the widespread presence of this process in marine bacteria, with sustained steady-state concentrations and gross rates spanning orders of magnitude, from 2 to 32 nM (at 10^5–10^8 cells mL^{-1}) and ~0.02 to 110 amol $cell^{-1}$ h^{-1}, respectively (Diaz et al. 2013, Hansel et al. 2019, Sutherland et al. 2019). These measurements include two strains within the widespread and numerically abundant SAR11 clade (Sutherland et al. 2019), the ubiquitous coral symbiont *Endozoicomonas* (Diaz et al. 2016, Zhang et al. 2016a), and several bacteria within the ecologically relevant *Roseobacter* clade (Hansel et al. 2019). Bacterial extracellular superoxide production varies with microbial species, life stage, and cell density but is agnostic to light and time of day. While the production rates given above are lower by an order of magnitude or more than those by most phytoplankton (see Section 3.1.2), this disparity in rates is considerably less in most cases when rates are normalized to surface area (Diaz et al. 2013).

Far less is known about extracellular hydrogen peroxide production by marine bacteria. A recent survey of a dozen bacterial species revealed consistent hydrogen peroxide degradation rates but high variability in production rates (Bond et al. 2020). Production rates spanned several orders of magnitude as a function of species ($<10^{-3}$ to $>10^2$ amol $cell^{-1}$ h^{-1}). These rates varied considerably depending on experimental flow conditions, pointing to the potential contribution of intracellular hydrogen peroxide to extracellular pools. As several of these strains had previously been measured for extracellular superoxide production (Diaz et al. 2013), comparison of extracellular hydrogen peroxide and superoxide production rates suggested that hydrogen peroxide arose from sources beyond just superoxide dismutation.

3.1.2. Phytoplankton. Extracellular ROS production is common among freshwater and marine phytoplankton, including many that produce harmful algal blooms (HABs). It has been recognized among marine phytoplankton since the early report of hydrogen peroxide production by *Pleurochrysis carterae* (Palenik et al. 1987); since then, it has been identified in representative cyanobacteria such as *Prochlorococcus*, *Synechococcus*, and *Trichodesmium* (Hansel et al. 2016, Rose et al. 2008, Sutherland et al. 2019), as well as many lineages of eukaryotic phytoplankton, including raphidophytes, dinoflagellates, prymnesiophytes, diatoms, the cryptophyte *Geminigera cryophila* (Sutherland et al. 2019), and the pelagophyte *Aureococcus anophagefferens* (Diaz et al. 2018). In culture, phytoplankton (~10^3–10^6 cells mL^{-1}) are typically capable of sustaining steady-state concentrations of extracellular hydrogen peroxide between ~0.01 and several micromolar and superoxide concentrations between ~0.1 and tens of nanomolar (Diaz & Plummer 2018; Diaz et al. 2018, 2019; Hansel et al. 2016; Plummer et al. 2019; Schneider et al. 2016; Sutherland et al. 2019). Net rates of extracellular hydrogen peroxide production (10^{-2}–10^5 amol $cell^{-1}$ h^{-1}) and superoxide production (10^{-5}–10^4 amol $cell^{-1}$ h^{-1}) vary by multiple orders of magnitude within and among phytoplankton species. This variability is controlled by several physiological and environmental factors, including cell size, cell density, growth phase, and irradiance. HAB-forming raphidophytes of the genus *Chattonella* are capable of the highest rates of extracellular ROS production, although other species are able to reach comparable levels, such as *Prorocentrum minimum* (Park et al. 2009) and *Karenia mikimotoi* (Kim et al. 2019). The lowest reported rates of phytoplankton-derived extracellular superoxide production were detected in representative strains of the numerically dominant marine picocyanobacterium *Prochlorococcus* (Sutherland et al. 2019). Several other phytoplankton taxa were shown to generate extracellular superoxide and/or hydrogen peroxide at intermediate rates, including the HAB-forming *Pseudo-nitzschia* sp. and *Karenia brevis* (Diaz et al. 2018), a noncalcifying strain of the prominent bloom-forming marine coccolithophorid *Emiliania*

huxleyi (Plummer et al. 2019), and the important polar phytoplankton species *Phaeocystis antarctica* (Sutherland et al. 2019).

3.1.3. Seaweeds. Extracellular ROS production is widespread among green, red, and brown macroalgae. Much of the research in this field has focused on the inducible oxidative burst, or the ability of seaweeds to rapidly release high concentrations of ROS in response to environmental stimuli. Yet the constitutive production of extracellular ROS in the absence of any external cue is also common, as seen in the Fucales (Küpper et al. 2002). The first report of extracellular ROS production among macroalgae was the hydrogen peroxide burst of the rhodophyte *Eucheuma platycladum* (Collén et al. 1994). ROS bursts have also been observed in other rhodophytes, such as the Gracilariaceae (Weinberger et al. 2010), *Chondrus crispus* (Collén & Davison 1999), and *Pyropia* spp. (Hou et al. 2015, Luo et al. 2015); the chlorophytes *Ulva* spp. (Collén & Pedersén 1996), *Dasycladus vermicularis* (Ross et al. 2005), and *Cladophora glomerata* (Choo et al. 2004); and phaeophytes such as *Sargassum muticum*, the Desmarestiales, and the Laminariales, including *Laminaria digitata* (Küpper et al. 2001) and the giant kelp *Macrocystis pyrifera* (Küpper et al. 2002). Hydrogen peroxide is the most commonly identified ROS in the macroalgal oxidative burst, with rates normalized to algal fresh weight ranging from ∼0.2 to 60 $\mu mol\ g^{-1}\ h^{-1}$ (Küpper et al. 2002; Ross et al. 2005; van Hees & Van Alstyne 2013; Weinberger & Friedlander 2000; Weinberger et al. 2002, 2010) and expected concentrations as high as several millimolar at the site of production, which become diluted to micromolar levels in bulk seawater (Bouarab et al. 1999, Küpper et al. 2001, Weinberger et al. 2002). Superoxide has also been detected in oxidative bursts by *Pyropia haitanensis* (Luo et al. 2015), *L. digitata* sporophytes (Küpper et al. 2001), and the gametophytes of *L. digitata*, *Alaria esculenta*, and *Saccharina latissima* (Müller et al. 2012). As is evident in *L. digitata*, extracellular ROS production transcends reproductive life stages in other seaweeds as well, including *Fucus serratus* (Coelho et al. 2002, 2008; Küpper et al. 2002), *Saccharina* (McDowell et al. 2015, Mizuta & Yasui 2010, Müller et al. 2012), and *P. haitanensis* (Luo et al. 2014).

3.1.4. Corals. The first investigations of extracellular ROS production in corals showed that superoxide and hydrogen peroxide are present at the surfaces of both healthy and stressed colonies of the coral *Stylophora pistillata* within aquarium incubations (Saragosti et al. 2010, Shaked & Armoza-Zvuloni 2013). Subsequent field measurements showed substantial external superoxide levels at the surfaces of pigmented and bleached corals during a bleaching event within Kaneohe Bay, Hawaii. Coral-derived superoxide concentrations ranged from levels below that of bulk seawater (e.g., *Fungia scutaria* and *Montipora capitata*) up to 250 nM (e.g., *Porites lobata*), the highest superoxide concentrations measured in marine systems (Diaz et al. 2016). Consistent with biological production, superoxide concentrations at coral surfaces rapidly declined over short distances (centimeters) away from the coral, as expected based on the short lifetime of superoxide in those waters (half-life <40 s). Through the development of a submersible chemiluminescent sensor, recent direct in situ measurements of superoxide at the surfaces of corals within several reefs in Cuba similarly revealed species-level variability in external superoxide levels (Grabb et al. 2019) and the consistently high levels of superoxide associated with corals of the genus *Porites*, regardless of species or geographic region (Diaz et al. 2016, Zhang et al. 2016a).

Similar trends in superoxide and hydrogen peroxide levels were observed as a function of coral species from various reef localities, where, for instance, the steady-state ROS levels were high for *Porites* sp. (H_2O_2 = ∼500 nM; $O_2^{\bullet-}$ = ∼120 nM), intermediate for *Pocillopora* sp. (H_2O_2 = ∼250 nM; $O_2^{\bullet-}$ = ∼55 nM), and near zero for *Fungia* sp. (Diaz et al. 2016, Shaked & Armoza-Zvuloni 2013). This species-specific variability in ROS levels at coral surfaces is a consequence of differences in both production and degradation of ROS. In fact, corals also release antioxidants

into their surroundings, with antioxidant release varying widely across coral species (Armoza-Zvuloni & Shaked 2014, Saragosti et al. 2010, Shaked & Armoza-Zvuloni 2013). Given this ability to control both hydrogen peroxide and superoxide production and decay, the stable nonzero levels of these ROS maintained at the surfaces of corals suggest some biological function that is tightly regulated (Diaz et al. 2016, Shaked & Armoza-Zvuloni 2013).

3.2. Mechanisms of Extracellular Reactive Oxygen Species Production

NOX enzymes, which are the only enzymes known to generate ROS as their sole function, have a demonstrated or suspected role in extracellular ROS production by bacteria, phytoplankton, seaweeds, and corals. However, a diversity of additional mechanisms for extracellular ROS production also exist in these organisms.

3.2.1. Heterotrophic bacteria. At present, the mechanism or mechanisms of extracellular superoxide production have been identified only in one heterotrophic marine bacterium—the coastal isolate *Roseobacter* sp. AzwK-3b (Hansel & Francis 2006). In this bacterium, superoxide is produced by a soluble heme-containing peroxidase (Andeer et al. 2015) within the peroxidase–cyclooxygenase superfamily (Zamocky et al. 2015). The enzyme is loosely anchored on the outer membrane (**Figure 2**) and constitutes the predominant fraction of the exoproteome (Learman & Hansel 2014). Sequences homologous to these heme-containing peroxidases are widely distributed among taxonomically and ecologically diverse bacteria (Andeer et al. 2015, Zamocky et al. 2015), suggesting that this mechanism of extracellular superoxide production may be prevalent. NOX-like protein sequences have also been identified within a taxonomically diverse range of bacteria (Hajjar et al. 2017), but the distribution and relevance of this enzyme and its putative role in extracellular superoxide production in marine bacteria have not yet been evaluated.

3.2.2. Phytoplankton. Mechanisms of extracellular superoxide production by phytoplankton include the NOX family of NADPH oxidases (**Figure 2**). The ability of the broad-spectrum flavoenzyme inhibitor diphenylene iodonium (DPI) to abolish extracellular superoxide production has been interpreted to indicate a role for NOX enzymes in a wide range of phytoplankton species, including *Prorocentrum minimum*, *Symbiodinium* spp., *Thalassiosira weissflogii*, *Thalassiosira pseudonana*, *Phaeodactylum tricornutum*, *Dunaliella tertiolecta*, and *Chlamydomonas reinhardtii* (Anderson et al. 2016, Kustka et al. 2005, Laohavisit et al. 2015, Park et al. 2009, Saragosti et al. 2010). Yet DPI is not specific to the NOX family and thus does not necessarily rule out the involvement of other oxidoreductases and peroxidases. Nevertheless, the freshwater alga *C. reinhardtii* possesses two putative NOX enzymes within the respiratory burst oxidase homolog (Rboh) family, referred to as Rbo1 and Rbo2. Approximately 60% of extracellular superoxide production is eliminated in mutant *C. reinhardtii* lacking Rbo1 (Anderson et al. 2016), confirming the essential involvement of NOX enzymes but also suggesting the potential presence of other superoxide-generating mechanisms. In *Chattonella marina* and the closely related *Chattonella ovata*, extracellular superoxide production has been linked to a putative NOX homolog recognized by antibodies raised against mammalian NOX (Kim et al. 2000). Furthermore, the presence of multiple putative *Nox* sequences has recently been confirmed in the transcriptomes of *Chattonella antiqua*, *Chattonella subsalsa*, and several other red tide flagellates (Shikata et al. 2019). Bioinformatics analysis has also revealed putative *Nox* homologs in the genomes of other phytoplankton, including the diatoms *P. tricornutum*, *T. pseudonana*, *Thalassiosira oceanica*, and *Fragilariopsis cylindrus* (Diaz et al. 2019, Hervé et al. 2006); the marine picoeukaryotes *Micromonas pusilla* and *Ostreococcus tauri*; *E. huxleyi*; and the dinoflagellate *Symbiodinium microadriaticum* (Diaz et al. 2019). NOX enzymes have also been identified

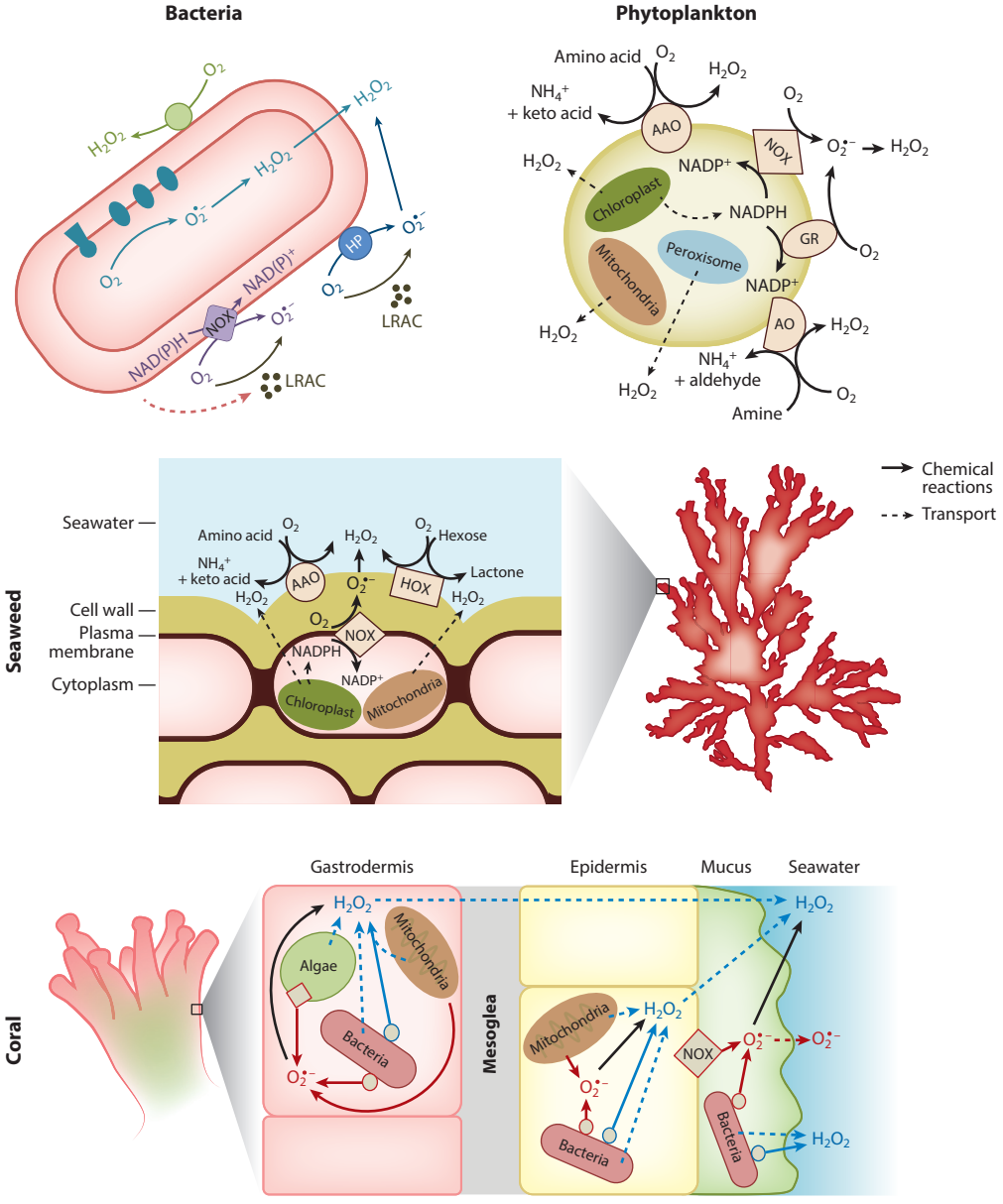

Figure 2

Simplified schematics illustrating sites of superoxide and hydrogen peroxide formation within bacteria, phytoplankton, seaweed, and coral. Extracellular superoxide and hydrogen peroxide formation is mediated via transmembrane and outer-membrane enzymes. Hydrogen peroxide also forms via dismutation of superoxide (*solid arrows*) and is transported across biological membranes (*dashed arrows*). Microbe-derived labile redox-active compounds (LRACs) are also suspected to play a role in bacterial superoxide production, but their identity remains unknown. For corals, algal and bacterial symbionts contribute to reactive oxygen species fluxes, with the enzymatic pathways presumed to be similar to those depicted for bacteria and phytoplankton in the top panels. Solid arrows reflect chemical reactions; dashed arrows indicate transport. Additional abbreviations: AAO, amino acid oxidase; AO, amine oxidase; GR, glutathione reductase; HOX, hexose oxidase; HP, heme peroxidase; NOX, NADPH oxidase.

in some cyanobacteria (Magnani et al. 2017), including one marine strain that probably acquired it via gene transfer from a eukaryote (Zhang et al. 2013). However, the in vivo ROS-generating potential of these NOX enzymes remains largely unexplored.

Phytoplankton generate extracellular ROS using several other enzymes besides NOX (**Figure 2**). For example, some phytoplankton, including *Prymnesium parvum*, *Pleurochrysis*, and *Amphidinium*, generate extracellular hydrogen peroxide through cell surface deaminases such as amine oxidase and amino acid oxidase (Palenik et al. 1988). In addition, DPI-inhibitable, NADPH-dependent extracellular superoxide production by *T. oceanica* was recently attributed to the activity of a putative cell surface flavoenzyme with high sequence similarity to glutathione reductase (GR) (Diaz et al. 2019). Putative homologs of *T. oceanica* GR were found in model marine phytoplankton genomes and ocean metagenomes, suggesting that GR-mediated extracellular superoxide production by phytoplankton may be more phylogenetically widespread. In the case of GR and NOX, it is believed that NADPH is supplied via photosynthesis, based on the ability of light to stimulate extracellular ROS production in a wide range of phytoplankton species (Diaz & Plummer 2018, Diaz et al. 2019, Hansel et al. 2016, Plummer et al. 2019). However, phytoplankton still generate substantial extracellular ROS in the dark, indicating that additional mechanisms are at play (Diaz et al. 2019, Hansel et al. 2016, Schneider et al. 2016, Zhang et al. 2016b) and remain to be discovered. For example, unknown cell-free superoxide-generating enzymes were recently implicated in cultures of several freshwater phytoplankton (Chaput et al. 2019) and marine HAB-forming species (Diaz et al. 2018). These water-soluble proteins are unlikely to include NOX enzymes, which are highly hydrophobic transmembrane enzymes, and therefore represent promising targets for further investigation.

3.2.3. Seaweeds. Proposed mechanisms of extracellular ROS production among macrophytes include at least three ROS-generating enzymes (**Figure 2**): NOX homologs (Rboh), oligosaccharide oxidase, and amino acid oxidase. In red (Weinberger et al. 2010), green (Ross et al. 2005), and brown (Küpper et al. 2001, 2002) macroalgae, the potential involvement of NOX enzymes in the oxidative burst has been widely inferred from inhibition assays involving DPI. *Rboh* gene sequences have been discovered in the genomes of several seaweeds, including the red algae *Chondrus crispus* (Hervé et al. 2006), *Pyropia* spp. (Hervé et al. 2006, Luo et al. 2015), and *Gracilariopsis lemaneiformis* (Wang et al. 2018). Furthermore, positive relationships between ROS production and *Rboh* gene copy number (Luo et al. 2014) and expression (Chen et al. 2016; Luo et al. 2014, 2015) support a role for NOX homologs in the production of extracellular ROS in seaweeds such as *P. haitanensis*. McDowell et al. (2015) observed that extracellular hydrogen peroxide production by the kelp *S. latissima* is dependent on light via photosynthetic electron transport. This observation is consistent with the light-dependent production of photosynthetic NADPH as a substrate for NOX, but it could also reflect the passive release of hydrogen peroxide produced during light reactions of photosynthesis. Similarly, neither of these mechanisms can be ruled out in the green alga *Ulva rigida*, which upregulates extracellular hydrogen peroxide production as a function of increasing irradiance (Collén & Pedersén 1996).

Oligosaccharides representing the degradation products of macroalgal cell walls are well-established chemical elicitors of the ROS burst in seaweeds (Chen et al. 2016; Hou et al. 2015; Küpper et al. 2001, 2002; Luo et al. 2015; Weinberger & Friedlander 2000; Weinberger et al. 2005, 2010). These oligosaccharides directly stimulate the DPI-insensitive production of hydrogen peroxide by cell surface oligosaccharide oxidase (Hou et al. 2015; Weinberger et al. 2005, 2010). Cell-surface localized hexose oxidase (HOX) belonging to the broad family of oligosaccharide oxidases has been purified from the red algae *C. crispus* and *Ptilophora subcostata*, in which HOX is believed to play a role in the ROS burst (Ogasawara et al. 2016). An additional mechanism of

extracellular hydrogen peroxide production implicated in the red alga *C. crispus* involves the enzyme amino acid oxidase, which is elicited by the release of L-asparagine by the green algal parasite *Acrochaete operculata* (Weinberger et al. 2002). Multiple elicitors of extracellular ROS production besides oligosaccharides and amino acids have been reported in macroalgae, including bacterial flagellar peptides (Luo et al. 2015), as well as free fatty acids (Küpper et al. 2009) and hormones such as abscisic acid (Wang et al. 2018). These observations may lead to the discovery of additional enzymatic and signaling mechanisms involved in extracellular ROS production by seaweeds.

3.2.4. Corals. The coral holobiont contains a number of potential ROS sources, including endosymbiotic bacteria and algae (Symbiodiniaceae), ectosymbiotic (surface-associated) microbes within the coral mucus, and the coral animal itself (**Figure 2**). Yet direct evidence of the sources and mechanisms of extracellular ROS production in corals is currently lacking.

Due to superoxide's short lifetime and limited ability to cross biological membranes, it is unlikely that any superoxide derived by tissue-hosted symbionts could traverse the several biological membranes required to contribute to external pools, even under stressful conditions. Instead, superoxide measured at the coral surface could derive from the coral host and/or microbes within the mucus layer. Several lines of evidence have pointed to the coral animal as the primary source of extracellular superoxide. First, Symbiodiniaceae were ruled out as dominant contributors based on the observation that extracellular superoxide concentrations were independent of algal symbiont abundance and bleaching status (pigmented versus bleached) in both field and aquarium-based investigations for a broad diversity of coral species (Diaz et al. 2016, Saragosti et al. 2010). Second, equivalent superoxide levels at the surfaces of aquarium-hosted *Porites astreoides* colonies before and after mucus removal showed that mucus-hosted microbes play a negligible role in extracellular superoxide production (Zhang et al. 2016a). Lastly, symbiont-deficient coral larvae were found to produce significant extracellular superoxide in a density-dependent manner in the absence of light (Diaz et al. 2016), pointing to the coral animal as the source of superoxide. In fact, in situ superoxide levels at the surface of *Porites compressa* colonies did not change significantly as a function of light over a diel cycle, further pointing to the decoupling of superoxide from algal activity and instead implicating the coral itself as the source of superoxide (Diaz et al. 2016).

In contrast to superoxide, hydrogen peroxide has a relatively long half-life and is able to freely cross cell membranes through aquaporins. Thus, hydrogen peroxide measurements at the surfaces of corals include contributions from epithelial and gastrodermal coral cells, as well as microbiome members residing within the tissues of the coral (**Figure 2**). The evidence to date suggests that both the coral and endosymbiotic algae are involved in hydrogen peroxide production. In aquarium incubations containing *S. pistillata* where hydrogen peroxide production was stimulated by water flow, pigmented colonies produced external hydrogen peroxide, yet bleached colonies did not, implicating Symbiodiniaceae as the primary source of hydrogen peroxide (Armoza-Zvuloni & Shaked 2014). Yet a follow-up study found hydrogen peroxide production in both bleached and pigmented corals that occurred in response to physical stimuli at specific polyps, providing evidence that the coral animal was responsible for the ROS produced (Armoza-Zvuloni et al. 2016b). Hydrogen peroxide was also produced by the azooxanthellate red branching gorgonian *Lophogorgia chilensis* in response to heat and physical stress (Mydlarz & Jacobs 2006), ruling out symbiotic algae as the ROS source.

While the enzymatic pathways for ROS production in corals have not been defined, NOX enzymes (NOX2 and, more recently, NOX4) have been identified in the related cnidarian sea anemone *Nematostella vectensis* (Gandara et al. 2017, Sumimoto 2008, Zhang et al. 2013). Furthermore, a recent transcriptomic study of the branching, stony coral *Acropora cervicornis* found expression of an NADPH oxidase homologous to a mouse NOX3 (Libro et al. 2013). Indeed, DPI

strongly inhibited superoxide production by both pigmented and bleached *S. pistillata* (Saragosti et al. 2010) and hydrogen peroxide production by physically and thermally stressed gorgonian corals in aquarium incubations (Mydlarz & Jacobs 2006). Thus, similar to other eukaryotes, NOX-like enzymes are likely responsible, at least in part, for superoxide and hydrogen peroxide production within corals.

3.3. The Physiological and/or Functional Basis for Extracellular Reactive Oxygen Species Production

Extracellular ROS play a multitude of beneficial roles across bacteria, phytoplankton, seaweeds, and corals. These functions include, but are not necessarily limited to, protection against physiochemical threats, biological defense, and innate physiology.

3.3.1. Heterotrophic bacteria. Extracellular superoxide and hydrogen peroxide production has been measured from a wide diversity of healthy, active bacteria during exponential growth (Bond et al. 2020, Diaz et al. 2013, Hansel et al. 2019, Learman et al. 2011, Sutherland et al. 2019, Zhang et al. 2016a). Steady-state concentrations and production rates were often higher for cells during the exponential phase than during the stationary phase (Diaz et al. 2013, Hansel et al. 2019), indicating that ROS production is decoupled from stress and cell lysis. In fact, extracellular superoxide levels in a bacterium within the *Roseobacter* clade (*Ruegeria pomeroyi* DSS-3) were tightly regulated over a life cycle in batch culture via a balance of both production and decay processes (Hansel et al. 2019). This regulation allowed for nearly constant superoxide levels outside the cells during active growth, which declined upon entering the stationary phase. Similarly, SOD-deficient mutants of the bacteria *Escherichia coli* and *Salmonella typhimurium* grew normally during exponential phase but died on entering the stationary phase (Carlioz & Toutati 1986, Storz et al. 1987). In wild-type *E. coli* strains, superoxide levels declined by more than 80% upon entering the stationary phase via expression of a periplasmic SOD (Benov & Fridovich 1994). It is therefore apparent that bacteria regulate levels of extracellular superoxide outside the cell as a function of growth stage through coordinated production and degradation processes.

A strong inverse relationship between extracellular superoxide production and cell density has also been demonstrated for a wide diversity of heterotrophic bacteria (Diaz et al. 2013, Hansel et al. 2019, Sutherland et al. 2019). In fact, removal of extracellular superoxide through the addition of exogenous SOD resulted in significantly diminished growth by two *Roseobacter* bacteria, pointing to superoxide or the dismutation product hydrogen peroxide in growth regulation (Hansel et al. 2019). These results are consistent with previous findings for *E. coli* and are reminiscent of cell signaling behavior in other model systems (Albert 2005, Jeong et al. 2000).

While it is clear that bacteria regulate the levels of superoxide outside the cell as a function of cell density and growth stage, the fate of superoxide and the exact mechanism of superoxide-enhanced growth remain unknown. Based on more recent findings in the animal and plant sciences, superoxide levels at the cell surface may modulate membrane biophysics and induce lipid signaling cascades involved in cell growth and proliferation (Saran 2003). While hydrogen peroxide may similarly play a key role in marine bacterial physiology, there are limited data on the dynamics of hydrogen peroxide production by bacteria. Additionally, the limited data that are available are difficult to interpret due to the ability of intracellular hydrogen peroxide to contribute to extracellular pools (Bond et al. 2020). Given the ample evidence for the important role of extracellular hydrogen peroxide as a cell signal in other cell types (D'Autreaux & Toledano

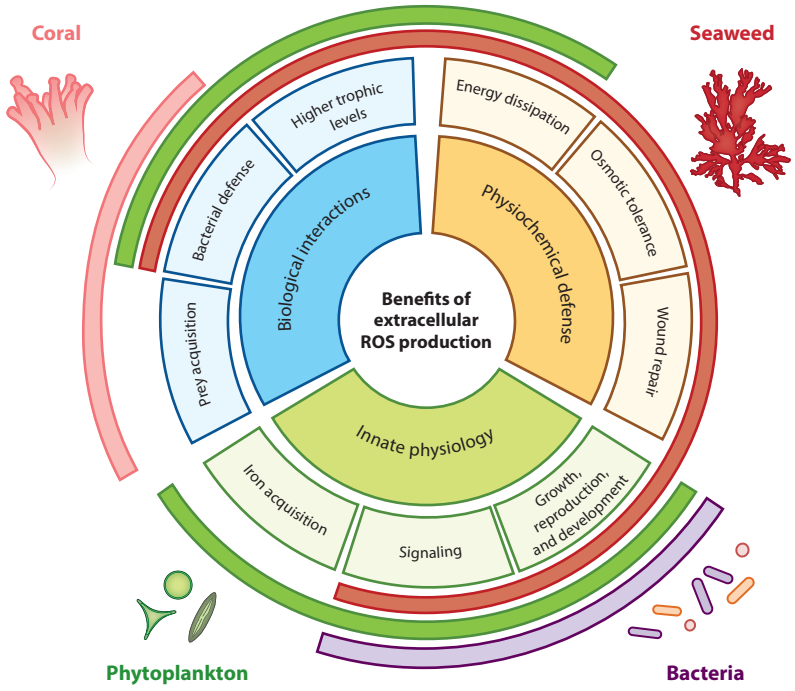

Figure 3

The physiological benefits of extracellular superoxide and hydrogen peroxide production in bacteria, phytoplankton, seaweeds, and corals. The benefits for each organismal group may extend beyond the ones given here; only benefits with current scientific evidence are shown. Abbreviation: ROS, reactive oxygen species.

2007), it is likely that hydrogen peroxide, like superoxide, is a key infochemical involved in bacterial growth and proliferation (**Figure 3**).

3.3.2. Phytoplankton. Extracellular ROS production may promote the welfare of phytoplankton in a variety of biological interactions (**Figure 3**). For example, the ability of HAB-forming phytoplankton to generate abundant extracellular ROS has been implicated in the toxicity and therefore the success of these blooms, although the evidence is inconsistent and controversial (Astuya et al. 2018, Diaz & Plummer 2018, Kim et al. 2019, Li et al. 2018). Microbial production of extracellular ROS consumes substantial oxygen (Sutherland et al. 2020) and should therefore be considered in HABs that involve suffocation as a fish-killing mechanism (Kim & Oda 2010). Furthermore, extracellular ROS production has been hypothesized as a phytoplankton defense mechanism against grazing (Martel 2009) because lectins, which are involved in microzooplankton prey recognition, stimulate extracellular ROS production in phytoplankton prey (Diaz & Plummer 2018, Kim et al. 2019). Indeed, the toxic dinoflagellates *Alexandrium* spp. induce ROS-dependent mortality in their microzooplankton predators (Flores et al. 2012). Conversely, microzooplankton could potentially produce extracellular ROS to facilitate predation, but this hypothesis remains largely unexplored.

Phytoplankton-derived extracellular ROS production may also shape interactions with bacteria. For instance, extracellular superoxide production by *C. marina* has antimicrobial effects (Kim et al. 1999; see also Diaz & Plummer 2018 and references therein). In *Prochlorococcus*, which has

adaptively lost the hydrogen peroxide–degrading enzyme catalase, catalase-positive helper heterotrophic bacteria are responsible for degrading extracellular hydrogen peroxide to keep it at nontoxic levels (Morris et al. 2008, 2011). This relationship is thought to play a major role in the success of phytoplankton species and overall plankton community structure (Zinser 2018b). In other ways, phytoplankton ROS production can be a vulnerability. For example, viruses hijack ROS metabolism (Sheyn et al. 2016, Vardi et al. 2012) by stimulating extracellular hydrogen peroxide production (Evans et al. 2006) and initiating ROS-activated apoptosis (Bidle et al. 2007, Vardi et al. 2012) in *E. huxleyi*, which is involved in bloom demise. However, *Micromonas polaris* does not accumulate ROS during viral infection (Piedade et al. 2018), indicating that the role of ROS in virus-induced phytoplankton mortality is variable.

Catalase: heme-based metalloenzyme found throughout aerobic life that catalyzes the decomposition of hydrogen peroxide to water and oxygen

Besides modulating biological interactions, extracellular ROS production may play vital roles in the innate physiology of phytoplankton (**Figure 3**). Consistent with an abundance of evidence from other phytoplankton (Diaz & Plummer 2018), *E. huxleyi* (Plummer et al. 2019) and *T. oceanica* (Diaz et al. 2019) upregulate extracellular superoxide production during active growth and as a function of decreasing population density, suggesting a community-dependent signaling process in a wide range of species, including *Synechococcus* and *P. antarctica* (Sutherland et al. 2019). SOD and catalase additions inhibit the growth of *C. marina* (Oda et al. 1995); however, the negative growth response to SOD additions could not be replicated in *E. huxleyi* (Plummer et al. 2019), *T. oceanica* (Diaz et al. 2019), or a mutant strain of *C. marina* exhibiting approximately half the wild-type rate of extracellular superoxide production (Kim et al. 1999). In fact, SOD additions enhanced growth in *E. huxleyi* (Plummer et al. 2019) and *T. oceanica* (Diaz et al. 2019), which was hypothesized to involve a hydrogen peroxide–dependent proliferation cue, similar to fungal and animal signaling systems (Bauer 2014, Oshikawa et al. 2010, Rossi et al. 2017).

Another aspect of basal phytoplankton physiology potentially involving extracellular ROS production is iron acquisition (Rose 2012). Superoxide can both oxidize and reduce iron, thereby influencing the oxidation state and, potentially, the bioavailability of this often limiting nutrient in marine phytoplankton communities. The role of extracellular superoxide production in iron acquisition has been demonstrated in some phytoplankton species but appears to be absent in others (see, for instance, Kustka et al. 2005, Roe & Barbeau 2014, Rose et al. 2008).

Finally, extracellular ROS production may be involved in the acclimation of phytoplankton to physiochemical stimuli, such as light stress (**Figure 3**). Enzymatic NADPH oxidation coupled to the production of extracellular superoxide has been proposed as a photoprotective mechanism in the marine model diatom *T. oceanica* (Diaz et al. 2019). This function is likely present in *C. marina* (Yuasa et al. 2020) and other species, as suggested by light-enhanced extracellular superoxide production in additional phytoplankton taxa (see Diaz & Plummer 2018 and references therein). In *C. marina*, NOX expression is significantly upregulated during the day (Shikata et al. 2019), which is further suggestive of a photoprotective role for extracellular ROS production.

3.3.3. Seaweeds. Extracellular ROS are involved in the macroalgal defense response to a variety of physical challenges (**Figure 3**). Several physical stimuli commonly induce the seaweed oxidative burst, including heat stress (Luo et al. 2014, 2015; Wang et al. 2018), osmotic stress (Ross & Van Alstyne 2007, Wang et al. 2018), desiccation (Ross & Van Alstyne 2007), and wounding (Collén et al. 1994; Luo et al. 2015; McDowell et al. 2014a, 2015, 2016). During wounding, the giant unicellular green alga *Dasycladus vermicularis* relies on an instantaneous sealing mechanism involving an initial gelling process, followed by the release of micromolar hydrogen peroxide, which oxidatively cross-links, browns, and hardens the wound plug in a peroxidase-mediated reaction with phenylpropanoids (Ross et al. 2005). Similarly, the oxidative burst increases cell wall strength in developing embryos of the intertidal kelp *F. serratus*, improving their resilience to

osmotic stress (Coelho et al. 2002). Additionally, extracellular hydrogen peroxide production has been proposed as a mechanism of photosynthetic energy dissipation in species such as *U. rigida* (Collén & Pedersén 1996).

In addition to protecting against physical stressors, the seaweed ROS burst is involved in biological defense (**Figure 3**). Biological attack often involves degradation of the macroalgal cell wall, leading to the production of oligosaccharide degradation products that trigger the ROS burst as a primary immune response (Küpper et al. 2002, Weinberger 2007, Weinberger & Friedlander 2000, Weinberger et al. 2010). In the red alga *Gracilaria conferta*, the oligoagar-stimulated ROS burst eliminates up to 60% of resident bacterial epiflora (Weinberger & Friedlander 2000). This oligosaccharide-induced ROS production is associated with lower susceptibility to parasitism in *L. digitata* (Küpper et al. 2002), increased resistance to rotting in *Pyropia yezoensis* (Hou et al. 2015), and improved growth and product yield in the aquaculture of *P. haitanensis* (Chen et al. 2016). Antimicrobial effects are lost, however, when ROS production is inhibited via application of DPI (Bouarab et al. 1999, Küpper et al. 2002). ROS production may also control interactions between seaweeds and macrofaunal grazers. For instance, during its wound-activated ROS burst, the kelp *Ascoseira mirabilis* inhibits grazing by amphipods, preventing the loss of 30% of kelp biomass (McDowell et al. 2014b). Furthermore, *A. mirabilis* also protects the red alga *Palmaria decipiens* from being grazed, but this effect disappears in the dark when the ROS burst is also suppressed (McDowell et al. 2016), illustrating the complex interactions among macroalgal taxa and abiotic conditions such as light availability.

ROS-mediated defenses in macroalgae likely arise through multiple mechanisms. Some seaweeds produce ROS concentrations (micromolar to millimolar) that are directly toxic or noxious to pathogens (Bouarab et al. 1999, Weinberger et al. 2002), epiphytes (Küpper et al. 2001), and grazers (McDowell et al. 2014b). ROS may also participate in a network of chemical reactions that amplify macroalgal defenses. For example, the ROS burst can coincide with an increase in halogenating activity (Cosse et al. 2009; Weinberger et al. 1999, 2007), involving haloperoxidases that utilize hydrogen peroxide to form hypohalous acids and volatile organohalogens. In addition to having impacts on climate and ozone destruction, these halogenated species inhibit microbial quorum sensing and biofilm formation (Punitha et al. 2018). ROS may also drive signaling pathways that are involved in the expression of haloperoxidases in *L. digitata* (Cosse et al. 2009) and the upregulation of secondary defense metabolites such as phlorotannins in the same species (Küpper et al. 2002).

Beyond macroalgal defenses, extracellular ROS production plays a basal role in the growth, differentiation, and reproduction of some macroalgae (**Figure 3**). In the kelp *Saccharina japonica*, the formation of reproductive organs is dependent on ROS production in the plasma membrane and cell wall, which is thought to be involved in the loosening of cell walls, as in higher plants (Mizuta & Yasui 2010). The germination of *S. japonica* zoospores (Küpper et al. 2002) and the growth and development of *F. serratus* embryos (Coelho et al. 2008) are similarly dependent on ROS production. Furthermore, embryonic patterning in *F. serratus* requires an interdependent extracellular ROS–calcium signaling cascade (Coelho et al. 2008).

3.3.4. Corals. ROS production in corals has long been associated with stress, disease, bleaching, and apoptosis, based largely on the expectation that UV- and heat-induced damage to the photosynthetic machinery of Symbiodiniaceae and coral mitochondrial membranes (Lesser 2011, Weiss 2008) leads to elevation of intracellular ROS to toxic levels that induce oxidative stress (Lesser 2006) and trigger apoptosis signaling pathways (Cai & Jones 1998). Nevertheless, it is certainly reasonable to assume that corals behave similarly to other eukaryotic systems, in which ambient intracellular ROS are also important for maintaining redox homeostasis, health, and physiological function (D'Autreaux & Toledano 2007).

Extracellular ROS production has also been observed in healthy and stressed corals, where enhanced production may not simply be related to oxidative stress, but instead may provide beneficial functions to the coral host (**Figure 3**). For instance, there is indirect evidence that superoxide plays a role in coral thermotolerance, resistance to pathogenic disease, and protection against pathogenic bleaching. More specifically, coral-derived NAD(P)H oxidoreductases, which are putatively involved in extracellular superoxide production, are associated with increased thermotolerance of the coral *Acropora millepora* (Dixon et al. 2015) and resistance to pathogenic white band disease in *Acropora cervicornis* (Libro et al. 2013). Furthermore, *Vibrio shiloi*, a coral-bleaching pathogen of *Oculina patagonica*, produces an extracellular SOD during infection (Banin et al. 2003). By contrast, SOD-deficient mutants of *V. shiloi* are avirulent and do not induce bleaching. These findings may point to superoxide production by corals as a strategy for protection, including pathogen deterrence, similar to other eukaryotic systems such as seaweeds, plants, fungi, and animals. In fact, the high levels of superoxide associated with the typically stress-tolerant *Porites* spp. in comparison with the stress-prone *Montipora* and *Montastraea* spp. are consistent with a protective or beneficial role of superoxide in the coral host (Diaz et al. 2016, Grabb et al. 2019, Zhang et al. 2016a).

More recently, hydrogen peroxide production in the stony coral *S. pistillata* was also linked to defense against pathogens as well as prey acquisition. In incubation experiments with fragments of *S. pistillata*, hydrogen peroxide was rapidly released in response to the introduction of *Artemia salina* nauplii to the surrounding water (Armoza-Zvuloni et al. 2016b). Similar behavior between bleached and pigmented fragments indicated that the source of hydrogen peroxide was primarily the coral animal and not the algal symbionts in response to the prey. Additionally, hydrogen peroxide production occurred at the site of stimulus, rather than as a whole-colony response. Similarly, *S. pistillata* fragments rapidly produced high levels of hydrogen peroxide upon contact with several species within the virulent genus *Vibrio* (Armoza-Zvuloni et al. 2016a), suggesting that production may be a means of pathogenic defense. In fact, estimates of hydrogen peroxide concentrations within the coral diffusive boundary layer are well within concentration ranges (10–20 μM) that induce mortality of the coral pathogen *Vibrio coralliilyticus*.

Rapid hydrogen peroxide production has also been documented in response to physical and chemical stimuli and distress in both stony and gorgonian corals (Armoza-Zvuloni et al. 2016b, Mydlarz & Jacobs 2006). Similar to plant and animal systems, this coral oxidative burst may play a protective role, including serving as a defense against pathogen invasion and/or acting as a chemical signal to induce immune and wound-healing responses during times of stress. Given the likely role of NADPH oxidases and presumably peroxidases in ROS production in corals, ROS production undoubtedly plays a multifaceted role in coral health and function, possibly including immune response, nutrient acquisition, feeding, wound response, cell signaling, cell growth, and other functions, as is widely appreciated in other eukaryotic systems.

4. BIOGENIC REACTIVE OXYGEN SPECIES IN MARINE WATERS

Oceanic ROS levels are a function of several abiotic and biotic production and degradation processes, and each group of organisms discussed in this review has the potential to contribute substantially to these fluxes. While limited in number, hydrogen peroxide and superoxide measurements have been made in a variety of marine systems, ranging from oligotrophic to productive ocean waters in the presence and absence of light. Due to a half-life on the order of seconds to minutes (Heller & Croot 2010b, Rose et al. 2008, Wuttig et al. 2013) and lack of an in situ sensor, oceanic superoxide measurements to date have been based on shipboard measurements of either dark, steady-state concentrations or projections of in situ concentrations modeled from

decay kinetics (Hansard et al. 2010; Roe et al. 2016; Rose et al. 2008, 2010; Rusak et al. 2011). According to the limited superoxide field measurements using these approaches, nonphotochemical marine superoxide levels typically range from low picomolar levels in oligotrophic regions to low nanomolar levels in coastal waters (Diaz et al. 2016; Hansard et al. 2010; Roe et al. 2016; Rose et al. 2008, 2010; Rusak et al. 2011). Higher superoxide concentrations (~10–100 nM) have also been recorded in some shallow reef systems where water could be directly pumped into boat-based instruments (Diaz et al. 2016) or using a recently developed handheld submersible chemiluminescent sensor (Grabb et al. 2019), both of which allowed for at least partial contributions of light-mediated (photochemical or phototrophic) pathways. A greater number of hydrogen peroxide measurements have been made in the ocean in comparison with superoxide, which is certainly a consequence of its longer half-life and thus relatively greater ease in measurement. Within marine waters, hydrogen peroxide concentrations are typically two to three orders of magnitude higher than superoxide concentrations. Hydrogen peroxide concentrations typically range from tens to hundreds of nanomolar, spanning the oligotrophic open ocean to more productive coastal waters (Heller & Croot 2010a, Shaked & Armoza-Zvuloni 2013, Yuan & Shiller 2005). Elevated hydrogen peroxide values (e.g., 150–300 nM) have been observed as a result of rainwater inputs (Avery et al. 2005, Hanson et al. 2001, Yuan & Shiller 2001) and within coral reef ecosystems (Shaked & Armoza-Zvuloni 2013).

The imprint of biological processes is evident in the measurements of ROS concentrations and production rates from a variety of marine ecosystems. While biological activity appears to be involved in oceanic ROS production, correlations between superoxide and hydrogen peroxide production and chlorophyll *a* distributions within field data have varied widely (Rose et al. 2008, 2010; Rusak et al. 2011; Vermilyea et al. 2010). The role of biological activity was initially inferred based on ROS production being associated with particles—in other words, production was removed by filtering. Indeed, while photochemical ROS production has long been appreciated in sunlit waters (Cooper & Zika 1983, Cooper et al. 1989, Kieber et al. 2002), several recent studies showed that rapid, particle-associated formation of superoxide and hydrogen peroxide also occurred in the absence of light (Hansard et al. 2010, Moffett & Zafiriou 1990, Palenik & Morel 1988, Rose et al. 2008, Rusak et al. 2011). More recently, use of nonselective and selective biocides has more specifically linked ROS production to biological activity. Addition of microbial poisons and enzyme or metabolic inhibitors stunts or completely inhibits ROS production in natural samples (Moffett & Zafiriou 1990, Rose et al. 2010, Zhang et al. 2016b), even though some enzymatic ROS production can persist after cell death (Diaz et al. 2019, Schneider et al. 2016). Indeed, extrapolation of superoxide production rates by heterotrophic bacteria (Diaz et al. 2013) and cyanobacteria (Rose et al. 2008) can account for measured rates of superoxide within a variety of marine environments, further pointing to microbes as a dominant source.

5. ENVIRONMENTAL IMPLICATIONS

ROS production by marine biota, including heterotrophic bacteria, phytoplankton, seaweeds, and corals, has the potential to contribute profoundly to marine community ecology and biogeochemistry, regardless of whether such impacts are physiologically directed or an adventitious side reaction (**Figure 4**). The new knowledge of abundant biological and dark sources of ROS in the ocean indicates that superoxide and hydrogen peroxide likely play an underrecognized role in global marine biogeochemistry, including the dark ocean—a region that constitutes approximately 95% of our global habitat (**Figure 4**). In particular, ROS profoundly impact many nutrient metals, carbon, O_2, and climate-relevant gases. For instance, while the concentration and lifetime of superoxide in natural seawater are low (Burns et al. 2012, Hansard et al. 2011, Rose et al. 2008),

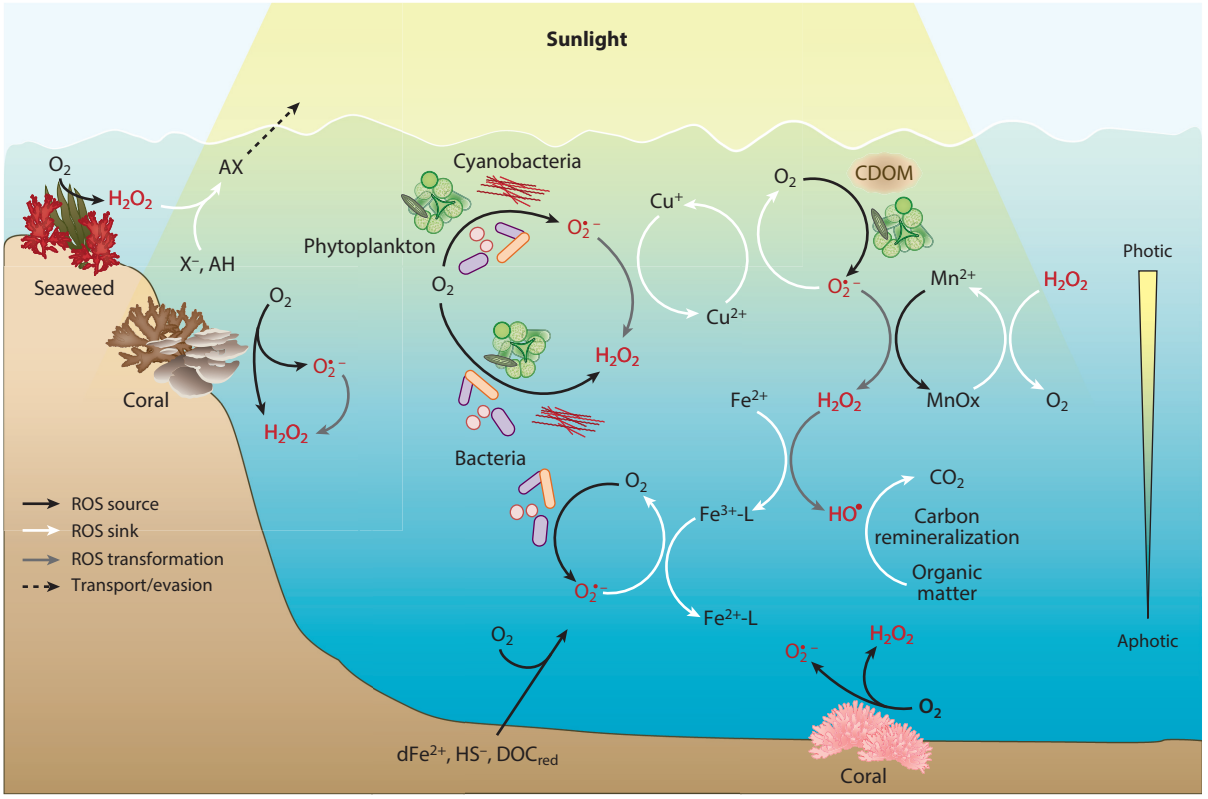

Figure 4

Simplified schematic depicting the major sources (*black arrows*) and a suite of sinks (*white arrows*) of superoxide and hydrogen peroxide within the ocean. Transformation of one reactive oxygen species (ROS) to another is depicted by a gray arrow. Abiotic sources include photochemical excitation of colored dissolved organic matter (CDOM) and abiotic oxidation of reduced metals (e.g., ferrous Fe, Fe^{2+}), hydrogen sulfide (HS^-), and dissolved organic carbon (DOC_{red}). ROS transformation and decay pathways include reactions with various metals, including copper (Cu), iron (Fe), and manganese (Mn), and carbon, which can lead to carbon remineralization to CO_2. Haloperoxidase-mediated oxidation of halide ions (X^-), such as Cl^-, Br^-, and I^-, by hydrogen peroxide forms organic and inorganic halogenated species that can undergo volatization. For simplicity, this reaction network schematic is not exhaustive and omits other important metal- and dissolved organic matter–coupled reactions with superoxide and hydrogen peroxide (and other ROS more broadly). Additional abbreviations: AH, organic substrate; AX, organohalogen compounds; dFe^{2+}, dissolved Fe^{2+}; Fe^{2+}/Fe^{3+}-L, ligand-bound iron; MnOx, manganese oxide.

superoxide has the ability to rapidly reduce [e.g., Cu(II) and Fe(III)] or oxidize [e.g., Mn(II), Cu(I), and Fe(II)] aqueous metal ions (Hansard et al. 2011, Rose 2012, Voelker & Sedlak 1995, Voelker et al. 2000). Superoxide also reacts with and modifies organic matter (Heller & Croot 2010a, Wuttig et al. 2013), with a reactivity toward specific chemical moieties (Heller et al. 2016). In fact, in productive sunlit waters, dissolved organic matter may serve as both a sink and source of superoxide (Heller et al. 2016). Furthermore, the extracellular production of superoxide may also be a key process in the global oxygen cycle by serving as a net sink of up to one-fifth of the marine oxygen budget, which has strong implications for our understanding of marine respiration rates and carbon budgets, in addition to mechanisms of ocean deoxygenation (Sutherland et al. 2020).

Hydrogen peroxide also plays a direct and indirect role in a suite of elemental cycles. For instance, it is an important reductant of manganese oxides (Sunda & Huntsman 1994), which ultimately impacts the scavenging of several essential micronutrient trace elements (e.g., cobalt and

nickel) in the surface ocean. On the flip side, hydrogen peroxide is an oxidant of Fe(II) (Millero & Sotolongo 1989, Moffett & Zika 1987), which generates hydroxyl radicals ($^{\bullet}OH$), a particularly reactive oxidant of organic matter (Pullin et al. 2004) involved in the remineralization of recalcitrant forms of carbon. As illustrated in macroalgae, hydrogen peroxide also participates in the enzymatic formation of halogenated compounds, including volatile species that can lead to the destruction of ozone and the formation of cloud condensation nuclei in the atmosphere, which have important impacts on climate.

SUMMARY POINTS

1. Despite the widely held perception that reactive oxygen species (ROS) are associated solely with toxicity and cellular damage, superoxide and hydrogen peroxide are not unequivocal indicators of poor health; they are also essential secondary metabolites produced by healthy, actively growing marine organisms.
2. The last decade has revealed that extracellular ROS production is a widespread trait among common marine prokaryotic and eukaryotic organisms spanning the tree of life.
3. An understanding of the biochemical pathways for extracellular ROS production in many marine species remains in its infancy but includes the phylogenetically ubiquitous transmembrane NOX family of NADPH oxidases as well as soluble oxidoreductases and peroxidases.
4. There is mounting evidence that superoxide and hydrogen peroxide serve a suite of essential physiological functions for marine organisms, akin to terrestrial animal and plant systems, including cell signaling, defense, and wound repair, to name a few.
5. Superoxide and hydrogen peroxide are key redox reactants, influencing the cycling of carbon, nutrients, metals, O_2, and climate-relevant gases in an often cryptic manner due to their rapid fluxes and reaction kinetics.
6. ROS play a multifaceted role in the ecology of marine systems by serving as growth promoters and defense molecules.

FUTURE ISSUES

1. Due to the cryptic nature of ROS, there is a critical need to improve in situ sensing technologies deployable at various temporal and spatial scales to quantify ROS and to distinguish between abiotic and biological ROS sources within marine systems.
2. Key organisms that remain underexplored in terms of ROS production, such as marine fungi, archaea, and microzooplankton, need to be investigated, which will undoubtedly reveal new insights on marine ecology and biogeochemistry.
3. Recent evidence that microbial ROS production is driven by a variety of enzymes, including NOX enzymes, highlights a vital need for exploration of the biochemical and regulatory pathways involved in ROS production within a diversity of marine organisms.
4. While parallels to known animal and plant systems can be drawn, there is a need to directly identify the physiological benefits provided by ROS to many marine organisms,

along with a need to understand how this ROS production is regulated to promote organismal health.

5. Given the diversity of roles of ROS in ecology and biogeochemistry, there is a need to better understand the fate of ROS within the ocean and to incorporate these reaction networks into ecological and biogeochemical models.
6. Important advances may be achieved within ocean sciences by embracing a holistic view of ROS in organismal health and function, consistent with the known beneficial and detrimental roles of ROS in a range of living organisms.

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Contents

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