REVIEW

Production of extracellular reactive oxygen species by phytoplankton: past and future directions

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In aquatic environments, phytoplankton represent a major source of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide. Many phytoplankton taxa also produce extracellular ROS under optimal growth conditions in culture. However, the physiological purpose of extracellular ROS production by phytoplankton and its wider significance to ecosystem-scale trophic interactions and biogeochemistry remain unclear. Here, we review the rates, taxonomic diversity, subcellular mechanisms and functions of extracellular superoxide and hydrogen peroxide production by phytoplankton with a view towards future research directions. Model eukaryotic phytoplankton and cyanobacteria produce extracellular superoxide and hydrogen peroxide at cell-normalized rates that span several orders of magnitude, both within and between taxa. The potential ecophysiological roles of extracellular ROS production are versatile and appear to be shared among diverse phytoplankton species, including ichthyotoxicity, allelopathy, growth promotion, and iron acquisition. Whereas extracellular hydrogen peroxide likely arises from a combination of intracellular and cell surface production mechanisms, extracellular superoxide is predominantly generated by specialized systems for transplasma membrane electron transport. Future insights into the molecular-level basis of extracellular ROS production, combined with existing high-sensitivity geochemical techniques for the direct quantification of ROS dynamics, will help unveil the ecophysiological and biogeochemical significance of phytoplankton-derived ROS in natural aquatic systems.

KEYWORDS: harmful algae; phytoplankton bloom; oxidative stress; redox homeostasis; biological interactions; monitoring; cryptic biogeochemistry
INTRODUCTION

Reactive oxygen species (ROS) include intermediates in the four-electron reduction of oxygen to water: superoxide, hydrogen peroxide and hydroxyl radical. Biological ROS production has been the subject of scientific inquiry since the discovery of the ubiquitous antioxidant enzyme, superoxide dismutase (SOD) (McCord and Fridovich, 1969). Since then, it has been well established that all oxygen-metabolizing organisms generate ROS and that this ROS production has potentially self-harmful effects. Yet more recently, awareness has been growing that biological ROS production can promote growth and survival. Extracellular ROS production regulates cell differentiation by fungi (Aguirre et al., 2005), innate immunity in seaweeds (Weinberger, 2007) and white blood cells (Babior, 1999), heterotrophic feeding by corals (Armoza-Zvuloni et al., 2016) and reproduction by sea urchins (Shapiro, 1991). Extracellular ROS production by the harmful bloom-forming phytoplankton species Chattonella marina has been implicated in its toxicity (Kim and Oda, 2010), growth (Oda et al., 1995) and iron acquisition (Garg et al., 2007; Liu et al., 2007), while many other phytoplankton generate extracellular ROS under non-stressful conditions for reasons that remain mysterious.

ROS occur naturally in the environment, as the products of both abiotic and biologically driven chemical reactions. In natural waters, ROS are present at low concentrations ($10^{-18}$–$10^{-6}$ mol L$^{-1}$) and are short-lived (μsec–days), yet ubiquitous (Table I). Aquatic ROS profoundly shape the biogeochemical cycling of carbon, as well as toxic and nutrient metals (Siciliano et al., 2002; Pullin et al., 2004; Barbeau, 2006; Learman et al., 2011; Rose, 2012; Zinser, 2018). In oxygenated surface waters, biological production of ROS can be a substantial and dominant ROS source, especially in areas of elevated biological productivity, such as phytoplankton blooms (Rose et al., 2008b; 2010; Hansard et al., 2010; Vermilyea et al., 2010; Rusak et al., 2011; Dixon et al., 2013; Marsico et al., 2015; Cory et al., 2016). Despite prodigious extracellular ROS production by many cultivated phytoplankton species and the quantitative contribution of phytoplankton communities to aquatic ROS fluxes, the physiological significance of phytoplankton-derived ROS and the wider implications for food web dynamics and biogeochemistry are poorly understood. Here, we review the rates, mechanisms and ecophysiological roles of extracellular ROS production by phytoplankton, with a focus on marine taxa and the ROS superoxide and hydrogen peroxide. We also propose future research directions to clarify the ecosystem-scale significance of phytoplankton-derived ROS.

SURVEY OF EXTRACELLULAR ROS PRODUCTION BY PHYTOPLANKTON

A broad diversity of phytoplankton produce extracellular ROS under optimal growth conditions in culture, including eukaryotic phytoplankton and cyanobacteria (Fig. 1; Supplementary Table S1). The major ROS superoxide (Oda et al., 1997; Marshall et al., 2005a; Fortune et al., 2010; Mooney et al., 2011; Dorantes-Aranda et al., 2015; Schneider et al., 2016; Cho et al., 2017), hydrogen peroxide (Oda et al., 1997; Kim et al., 1999a; Fortune et al., 2010; Schneider et al., 2016; Cho et al., 2017) and hydroxyl radical (Oda et al., 1992a; Yang et al., 1995; Cho et al., 2016) have all been examined in phytoplankton. Compared to previous studies on hydroxyl radical production, however, the literature on superoxide and hydrogen peroxide generation by phytoplankton is much more expansive. Thus, the focus here is primarily on superoxide and hydrogen peroxide.

Extracellular ROS production has been quantified on a per-cell basis in at least 21 eukaryotic phytoplankton species, and the majority of these are capable of forming harmful algal blooms (HABs) (Yang et al., 1995; Kim et al., 1999a; Marshall et al., 2005a; Mooney et al., 2011; Cho et al., 2017). Raphidophytes are the most thoroughly studied HAB group in terms of the production and potential functions of extracellular ROS, especially the Chattonella species C. marina and C. antiqua (Oda et al., 1997; Marshall et al., 2003, 2005a, 2005b), as well as Heterosigma akashiwo, Olisthodiscus luteus and Fibrocapsa japonica (Oda et al., 1992b; Yang et al., 1995; Oda et al., 1997; Marshall et al., 2005a; Fortune et al., 2010). Extracellular ROS production has been examined in harmful bloom-forming dinoflagellates, including Alexandrium spp. (Marshall et al., 2005a; Mooney et al., 2011; Dorantes-Aranda et al., 2015; Mardones et al., 2015; Cho et al., 2017), M. polykrikoides (Kim et al., 1999a, 2002; Tang and Gobler, 2009b; Griffith and Gobler, 2016) and Karenia mikimotoi (Yamasaki et al., 2004; Dorantes-Aranda et al., 2015; Cho et al., 2017).

Among non-HAB forming eukaryotic phytoplankton, extracellular ROS are produced by the symbiotic dinoflagellates Symbiodinium spp. (Saragosti et al., 2010;
Zhang et al., 2016a), the coccolithophorid Pleurochrysis carterae (Palenik et al., 1987) and diatoms, including the genus Thalassiosira (Kustka et al., 2005; Rose et al., 2006b; Milne et al., 2009; Waring et al., 2010; Schneider et al., 2016). Extracellular superoxide production has also been quantified in at least four species of cyanobacteria (Rose et al., 2005, 2008b; Godrant et al., 2009; Fujii et al., 2011; Hansel et al., 2016).

Cell-normalized rates of extracellular ROS production vary widely among phytoplankton species ($10^{-4}$–$10^{3}$ fmol cell$^{-1}$ h$^{-1}$; Fig. 1; Supplementary Table S1). The ichthyotoxic raphidophyte C. marina is capable of the highest extracellular ROS production rates, yet other HAB species can reach similar rates of ROS production, including Alexandrium catenella (Mardones et al., 2015), M. polykrikoides (Kim et al., 1999a, 2002), K. mikimotoi (Yamasaki et al., 2004; Dorantes-Aranda et al., 2015; Cho et al., 2017) and F. japonica (Dorantes-Aranda et al., 2015). Overall, HAB-forming eukaryotes generate much more extracellular ROS than other phytoplankton taxa, including the harmful freshwater cyanobacterium Microcystis aeruginosa (Fujii et al., 2011), as well as non-HAB species such as Thalassiosira spp. Cell size is a major control on the interspecific variability in extracellular ROS production (Oda et al., 1997; Marshall et al., 2003a; Diaz et al., 2013), yet when accounting for cell size, some harmful algae still generate substantially more ROS than non-harmful species (Kustka et al., 2005). Thus, to some degree, extracellular ROS production may be related to the tendency of some phytoplankton species to form HABs.

In addition to large interspecific variability, extracellular ROS production rates also vary considerably within phytoplankton species (<10 to $10^{3}$-fold; Fig. 1; Supplementary Table S1). Major factors underlying this variability include growth phase (Kawano et al., 1996; Kim et al., 1999a, 2004; Skeen et al., 2004; Garg et al., 2007; Portune et al., 2010), cell density (Yang et al., 1995; Twiner and Trick, 2000; Kim et al., 2002; Marshall et al., 2005b; Dorantes-Aranda et al., 2015), cell lysis (Dorantes-Aranda et al., 2013; Mardones et al., 2015), inter-strain differences (Ishimatsu et al., 1996; Oda et al., 1997; Portune et al., 2010).
Examining the effect of these parameters on extracellular ROS production by phytoplankton has helped to illuminate potential ecophysiological functions (see section Ecophysiological roles of phytoplankton-derived extracellular ROS).

**SUBCELLULAR PATHWAYS OF EXTRACELLULAR ROS PRODUCTION**

In phytoplankton, ROS production occurs at several major sites: the chloroplasts and mitochondria (or thylakoid membranes in cyanobacteria), peroxisome (eukaryotes only), cell surface and the cell-free environment (Fig. 2). The principal ROS-generating reaction at these sites is the formation of superoxide by the single-electron reduction of oxygen. In turn, rapid dismutation of superoxide by SOD is a primary mechanism for the production of hydrogen peroxide. Hydrogen peroxide can also be produced independently from superoxide through the two-electron reduction or oxidation of oxygen and water, respectively (Fig. 2).

Intracellular ROS arise through several key processes. For example, in chloroplasts, ROS production within the stromal fluid occurs via the Mehler reaction, which is mediated by photosystem I (PSI; probably via iron-sulfur clusters) and/or with the involvement of a stromal factor (SF) such as monodehydroascorbate reductase (MDAR) (Asada, 1999, 2006). ROS production also occurs in the chloroplast lumen at several sites within photosystem II (PSII) such as the oxygen-evolving complex (OEC) and cytochrome b559 (cytb559), although ROS production in PSII is thought to be minor compared to PSI (Pospíšil, 2014). In mitochondria, intracellular production of ROS in the matrix and intermembrane space is primarily mediated by complex I (CI), complex III (CIII) and membrane-bound NAD(P)H dehydrogenases (DH) (Møller, 2001; Murphy, 2009). ROS production within the peroxisome matrix occurs via several oxidoreductases such as glycolate oxidase (GO) and xanthine oxidase (XO). On the peroxisome membrane, ROS are produced via NAD(P)H-dependent reactions mediated by MDAR, cytochrome b (cytb), and/or peroxisome

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**Fig. 2.** Mechanisms of superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) production in phytoplankton. Chloroplast: PSI—photosystem I, PSII—photosystem II, SF—stromal factor, OEC—oxygen-evolving complex, cytb$_{559}$—cytochrome b559; Mitochondria: CI—complex I, CIII—complex III, DH—NAD(P)H dehydrogenase; Peroxoxomes: GO—glycolate oxidase, XO—xanthine oxidase, MDAR—monodehydroascorbate reductase, cytb—cytochrome b, PMP29—peroxisome membrane polypeptide 29; Cell surface and cell-free environment: OR—oxidoreductase. Intracellular hydrogen peroxide can diffuse within and outside of cells (white arrows), but intracellular superoxide is unlikely to escape the cell.
membrane polypeptide 29 (PMP29) (Noctor et al., 2002; Foyer et al., 2009; del Rio and Lopez-Huertas, 2016).

The movement of superoxide and hydrogen peroxide within the intracellular space differs strongly, such that fundamentally different processes are likely involved in the biogenic fluxes of these ROS into the environment. For example, intracellular hydrogen peroxide readily diffuses across membranes, which may be an important route for the release of biogenic hydrogen peroxide into seawater, as seen for 

C. marina (Kim et al., 2000a, 2007). However, as a much shorter-lived (~µs) anion at physiological pH with a limited diffusive distance (~100 s of nm), superoxide does not readily cross biological membranes (Korshunov and Inlay, 2002; Lesser, 2006). Even the complete lysis of cells under severe oxidative stress cannot release enough superoxide to account for the steady-state concentrations that have been measured in natural waters (Rose, 2012). Intracellular processes such as photosynthesis are, therefore, unlikely to be a direct source of biologically derived extracellular superoxide. Indeed, 

Symbiodinium spp. (Saragosti et al., 2010; Zhang et al., 2016a) and 

Thalassiosira spp. (Schneider et al., 2016) produce extracellular superoxide in the dark, indicating the presence of non-photosynthetic mechanisms for superoxide production. Furthermore, the photosynthetic inhibitor dichlorophenyldimethylurea (DCMU) does not alter extracellular superoxide production by 

C. marina and H. akashiwo (Oda et al., 1998).

Rather than originating from intracellular sources, most extracellular superoxide is likely produced directly at the cell surface. Cell surface NADPH oxidoreductases catalyze the production of extracellular superoxide in many organisms, including protozoa, seaweeds, fungi, plants, animals (Saran, 2003; Aguirre et al., 2005; Hervé et al., 2005; Bedard et al., 2007; Weinberger, 2007; Anderson et al., 2011) and the freshwater alga 

Chlamydomonas reinhardtii (Anderson et al., 2016). In fact, extracellular superoxide production by 

C. marina occurs on the cell surface through an NADPH oxidoreductase that is homologous to human neutrophil NADPH oxidase (Shimada et al., 1993; Kim et al., 2007). Light exposure stimulates extracellular superoxide production by 

C. marina (Dorantes-Aranda et al., 2013; Li et al., 2015), 

Thalassiosira spp. (Schneider et al., 2016) and 

Symbiodinium spp. (Saragosti et al., 2010), which suggests that photosynthesis (Kim et al., 1999a; Marshall et al., 2002) may play an indirect role in extracellular superoxide production by supplying NADPH to the cell surface NADPH oxidoreductase, as suggested previously (Saragosti et al., 2010). In addition to superoxide, extracellular hydrogen peroxide may also be directly generated at the cell surface. For example, extracellular hydrogen peroxide production by 

Pyrnesium parvum is mediated by amino acid oxidases during metabolism of exogenous organic nitrogen sources (Palenik et al., 1988).

Extracellular ROS production can also occur in the cell-free environment. For instance, the 

C. marina NADPH oxidoreductase can become dislodged from the cell surface and actively generate superoxide in cell-free spent media (Kim et al., 2000a, 2007). Cell-free hydrogen peroxide production has also been documented for the model diatom 

Phaeodactylum tricornutum (Schneider et al., 2016), although the mechanism remains unresolved.

**ECOPHYSIOLOGICAL ROLES OF PHYTOPLANKTON-DERIVED EXTRACELLULAR ROS**

ROS commonly arise as metabolic byproducts, whose damaging effects on vital biomolecules such as DNA, lipids and proteins are well known (Lesser, 2006). However, ROS production can be directed through specialized pathways (Fig. 2) to participate in a variety of regulatory and signaling processes that aid in the growth and survival of the organism making the ROS (Foyer and Noctor, 2005; Lesser, 2006; Mittler et al., 2011; Scheibe and Dietz, 2012; Schmitt et al., 2014). As discussed below, extracellular ROS production by phytoplankton may modulate biological interactions such as HAB toxicity, allelopathy, grazing and viral infection, while also aiding in growth and iron acquisition. ROS may serve many of these functions in the same phytoplankton species while also sharing similar purposes across different phytoplankton taxa. For instance, even though 

Chattonella is the most prolific producer of extracellular ROS among phytoplankton, the role of extracellular ROS in this genus may not be unique, as outlined in the following sections.

**Ichthyotoxicity of HABs**

ROS-forming HABs have caused immense financial losses to aquaculture industries in Australia (Hallegraeff et al., 1998), Japan (Okaichi, 1997) and Chile (Fuentes et al., 2006; Mardones et al., 2010). ROS are involved in the noxious or toxic effects of several HAB-forming species, such as raphidophytes (Yang et al., 1995; Oda et al., 1997; Kim et al., 1999b), and the dinoflagellates 

M. polykrikoides (Kim et al., 1999a; Tang and Gobler, 2009b, 2010) and 

Alexandrium spp. (Flores et al., 2012; Mardones et al., 2015). For example, antioxidants alleviate the toxic effect of multiple HAB species on various marine organisms (Yang et al., 1995; Oda et al., 1992b, 1997; Kim et al., 1999a, 1999b; Tang and Gobler, 2009a, 2009b, 2010; Flores et al., 2012). Furthermore, fish mucus and/or surface
receptor-binding lectins stimulate ROS production by several raphidophytes, suggesting a role for extracellular ROS in modulating these interactions (Tanaka et al., 1994; Nakamura et al., 1998; Oda et al., 1998; Jenkinson and Arzul, 2001). Recently, several raphidophytes, suggesting a role for extracellular ROS in modulating these interactions (Tanaka et al., 1994; Nakamura et al., 1998; Oda et al., 1998; Jenkinson and Arzul, 2001; Marshall et al., 2003, 2005b; Mardones et al., 2015). This mode of ROS toxicity helps to explain how transient ROS molecules can exert potentially harmful effects at concentrations that are not directly cytotoxic and over spatio-temporal scales that may exceed ROS lifetimes and diffusive distances. In some cases, cell lysis and the concomitant stimulation of extracellular ROS production are thought to be an important aspect of ichthyotoxicity in fish-killing phytoplankton species (Dorantes-Aranda et al., 2013, 2015; Mardones et al., 2015).

Despite the evidence suggesting that HAB-derived ROS are harmful, chemical additions of ROS that represent concentrations expected during harmful blooms of H. akashiwo, C. marina and M. polykrikoides have been insufficient to completely account for toxic effects on fish and invertebrates (Twiner et al., 2001; Marshall et al., 2003; Woo et al., 2006; Tang and Gobler, 2009a). Such lines of evidence have been used as an argument against the potentially harmful effects of ROS during HABs.

Other biological interactions
Phytoplankton-derived extracellular ROS may shape other biological interactions, such as grazing, allelopathy, and viral infection. For example, similar lectin-receptor-binding processes have been implicated in the production of extracellular superoxide by phytoplankton (Oda et al., 1998) and the recognition and capture of phytoplankton prey by the microzooplankton species Oxyrrhis marina (Wootton et al., 2007). Thus, lectin-stimulated extracellular ROS production by phytoplankton has been proposed to play a role in grazing interactions (Martel, 2009). In fact, extracellular ROS production by Alexandrium spp. has been linked to the mortality of microzooplankton grazers (Flores et al., 2012). Extracellular ROS production by C. marina and other raphidophytes also modulates interactions with non-predatory organisms, such as the bacterium Vibrio alginolyticus, by inhibiting its growth in an antioxidant-dependent manner (Oda et al., 1992b, 1997; Kim et al., 1999b). Furthermore, viral infection of the cosmopolitan phytoplankton species Emiliania huxleyi is associated with elevated levels of intracellular ROS and extracellular hydrogen peroxide, although the mechanisms and role(s) of this ROS production are not well understood (Evans et al., 2006).

Growth
The production of extracellular ROS by a broad diversity of phytoplankton under optimal growth conditions (Fig. 1) suggests that ROS may serve a role in the baseline physiology of these microorganisms. In particular, ROS production may have important consequences for cellular physiology, viability and growth. For example, the removal of superoxide and hydrogen peroxide through the addition of exogenous SOD and catalase, respectively, inhibits the growth of C. marina and changes its cell morphology from spindle to round-shaped (Oda et al., 1995). This morphological shift is also observed in C. antiqua when superoxide is removed via oxidation by an electrode (Tanaka et al., 1992). These results suggest that extracellular ROS play an essential role in the vitality and survival of Chattonella spp. Hansel et al. (2016) recently summarized several lines of evidence suggesting a role for extracellular superoxide production in growth regulation by a number of different microbial species. For example, extracellular superoxide is an autocrine growth promoter in other microorganisms such as Saccharomyces cerevisiae, Escherichia coli and Salmonella typhimurium. In these microorganisms, the transition to stationary phase requires a decrease in superoxide concentrations, which is accomplished by cell surface SODs (Saran, 2003; Buetler et al., 2004). Observations demonstrating that biomass-normalized extracellular superoxide production by C. marina is highest in exponential phase and lower in stationary phase (Oda et al., 1995; Kawano et al., 1996; Garg et al., 2007) are consistent with the positive relationship between superoxide and growth. Similar growth phase-dependent trends in superoxide production have been observed for other raphidophyte species such as C. antiqua and H. akashiwo (Skeen et al., 2004; Portune et al., 2010), as well as the dinoflagellate M. polykrikoides (Kim et al., 1999a).

Many HAB species modulate cell-normalized ROS production rates in an inverse relationship with cell density (Yang et al., 1995; Twiner and Trick, 2000; Kim et al., 2002; Marshall et al., 2005b), consistent with a potential signaling role for ROS, as recently proposed for the
Iron acquisition

Besides a potential role as an autocrine growth signal, extracellular ROS production may promote the growth of phytoplankton by more indirect, alternative means via metal nutrient acquisition. For example, superoxide is a potent oxidant and reductant of iron. Under some environmental conditions, extracellular superoxide can increase the bioavailability of iron, especially when this micronutrient is growth-limiting (Rose, 2012). In fact, extracellular superoxide production has been proposed as a strategy for iron acquisition by Lyngbya majuscula (Rose et al., 2005), T. erythraeum (Roe and Barbeau, 2014), M. aeruginosa (Fujii et al., 2011) and C. marina (Garg et al., 2007; Liu et al., 2007), although superoxide had no effect on iron uptake by Thalassiosira spp. (Kustka et al., 2005) or Chlorella kessleri (Middlemiss et al., 2001). Ultimately, the ability of superoxide to facilitate iron acquisition depends on prevailing biogeochemical conditions, which dictate the effect of this ROS on the steady-state concentrations of biologically labile mononuclear inorganic complexes of iron (II) and iron (III) (Rose, 2012). The reader is referred to Rose (2012) for a detailed review on the potential role of extracellular superoxide in microbial iron acquisition.

FUTURE RESEARCH DIRECTIONS

In aquatic environments, ROS concentrations can be low or undetectable due to rapid reactions with carbon and metals. ROS therefore “invisibly” drive major transformations of key elemental cycles via cryptic biogeochemistry (Hansel et al., 2015). Similarly, we suggest that ROS may play a cryptic role in biological interactions. For example, previous work has revealed that antioxidants can alleviate the toxic effects of ROS-producing HABs (Oda et al., 1992b, Yang et al., 1995; Oda et al., 1997; Kim et al., 1999a, 1999b; Tang and Gohler, 2009a, 2009b, 2010; Flores et al., 2012), yet representative HAB-derived ROS concentrations are insufficient to induce toxicity (Twiner et al., 2001; Marshall et al., 2003; Woo et al., 2006; Tang and Gohler, 2009a). However, organisms may experience higher doses of ROS than suggested by steady-state ROS concentrations, depending on the underlying kinetics and pathways of ROS production and degradation. For example, ROS concentrations represent the balance of ROS production and decay. Low concentrations of ROS may, therefore, disguise rapid production rates, if decay rates are also high. Depending on the identity and efficiency of ROS-degrading constituents (e.g. PUFAs), high ROS production rates by natural HABs could potentially be toxic without necessarily leading to elevated concentrations of ROS in the surrounding environment.

In order to test this “cryptic interactions” hypothesis, ROS fluxes and concentrations should be assessed together, particularly in natural systems. For example, the majority of phytoplankton-ROS research has been conducted using controlled laboratory experiments with model cultures. Yet much remains to be discovered about phytoplankton-driven ROS dynamics in natural aquatic environments. In fact, the scarcity of ROS measurements during natural HABs makes it difficult to assess whether ROS levels reach toxicity thresholds during these events. In addition to cryptic toxicity, the potential (cryptic) role of phytoplankton-derived extracellular ROS in other biological interactions such as grazing, allelopathy and viral infection should be considered. By potentially mediating biological interactions within and across trophic levels in these ways, ROS may modify food web dynamics and shape aquatic ecosystem health and large-scale biogeochemical cycling via pathways that remain to be discovered.

The inverse dependence of extracellular ROS production rates on phytoplankton cell density and the potential role of extracellular ROS in phytoplankton growth suggest that elevated ROS concentrations and production rates could be expected in aquatic systems leading up to phytoplankton blooms. For instance, in Lake Erie, total hydrogen peroxide concentrations (attributed to biological production) peaked approximately 2 weeks before the appearance of Microcystis spp. blooms and the occurrence of maximum microcystin levels during summer 2014 and 2015 (Cory et al., 2016). We suggest that a possible link between phytoplankton bloom formation and elevated biological ROS production could be taxonomically
widespread, which has implications for the evaluation and development of ROS-based strategies for predicting algal blooms. In contrast to cryptic cycling, this bloom prediction hypothesis suggests that biological ROS production may peak before a phytoplankton bloom, leading to ROS concentrations and production rates, far from being hidden or invisible, which can be used as a bloom-forecasting index. Given the short lifetimes of ROS (Table I), such a prediction tool could be responsive to ecosystem variables over relatively short timescales, and thus provide a high temporal-resolution indicator (~days to weeks) of potential interest to natural resource managers.

ROS measurements can be technically complex, especially in systems with high organic matter and metal loading. However, future research on phytoplankton-derived ROS is tractable in a range of environments, given currently available geochemical tools for the high sensitivity detection of ROS concentrations and dynamics in complex natural systems (Rose et al., 2008a; Godrant et al., 2009; Heller and Croot, 2010; Yamaguchi et al., 2010; Miller et al., 2011; Burns et al., 2012; Marisco et al., 2015; King et al., 2016; Roe et al., 2016; Zhang et al., 2016b). For a review of ROS quantification methods and technological advancements, the reader is referred to papers by Bartosz (2006), Soh (2006) and Burns et al. (2012). In addition to direct ROS measurements, future advances in the understanding of subcellular ROS production mechanisms will lead to new insights on phytoplankton-derived ROS. A variety of methods have been utilized to characterize the molecular basis of ROS production in microorganisms, including gene knockouts (Anderson et al., 2016), as well as chemical activity assays combined with peptide fingerprinting (Andeer et al., 2015) and immunoblotting and immunofluorescence (Kim et al., 2000a). Tracking molecular targets for ROS production in the field could provide critical information on natural ROS dynamics, especially if ROS cycling is rapid and difficult to detect by direct geochemical means.

SUPPLEMENTARY DATA
Supplementary data can be found online at Journal of Plankton Research online.

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