

**Hydrogen peroxide impacts on marine phytoplankton
community structure:
A modeling story**

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Doctor of Philosophy

Degree

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Dedication

To the past iterations of myself for whom this dissertation seemed unattainable;
thank you for persevering.

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Abstract

Microbial community structure in the oligotrophic ocean has evolved so that organisms unable to survive in monoculture can flourish in this harsh, nutrient depleted environment. The oligotrophic ocean contains high concentrations of hydrogen peroxide (H_2O_2), a type of harmful reactive oxygen species, which is produced when light hits pigmented detrital material.

Prochlorococcus is the most abundant photosynthetic organism in the oligotrophic ocean and is estimated to perform half of the carbon fixation there, yet it cannot live in monoculture at the level of H_2O_2 produced in one day of its natural environment. The less streamlined sister of *Prochlorococcus*, *Synechococcus*, as well as co-isolated heterotrophic bacteria, are able to survive the H_2O_2 of this environment but cannot outcompete *Prochlorococcus* for low nutrient concentrations here. *Prochlorococcus* has evolved dependence on other microorganisms, requiring them to constantly detoxify ambient H_2O_2 . Thus, allowing *Prochlorococcus* to survive in mixed-culture communities but not to competitively exclude the community members without facing H_2O_2 damage. Mechanisms that control the oceanic carbon cycle are an essential area of current and future climate change research, yet they are complex and poorly understood. Mathematical models accounting for various biological and physical processes allow for the assessment of factors that exert control on carbon and nutrient cycling.

Current phytoplankton global models attribute 25% of marine primary production to *Prochlorococcus* but exclude H_2O_2 as a source of microbial death in those calculations. Beginning with a simple Monod-based representation of H_2O_2 dynamics, this thesis provides a basal quantitative groundwork to assess H_2O_2 detoxification and damage in globally important plankton genera. The tradeoffs between growth advantage (*Prochlorococcus*) and H_2O_2 detoxification (*Synechococcus*) were found to control the toggle between competitive and coexistent behavior. The leakiness of H_2O_2 detoxification was found to be a key driver of coexistence both in dimensionless and spatially/ temporally resolved frameworks. The stepwise build of this model keeps resource competition and coexistence theories rooted in the (micro)biology while allowing for quantitative ecological evaluation of H_2O_2 impacts on globally relevant microbial dynamics.

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Chapter 1 HYDROGEN PEROXIDE AND PHYTOPLANKTON
INTERACTIONS OF THE OPEN OCEAN: A BASE
UNDERSTANDING

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INTRODUCTION

Oceans cover approximately 70% of the earth (Raven and Falkowski 1999) and are largely composed of ‘nutrient deserts.’ The oligotrophic ocean by definition is nutrient poor, with nitrogen, along with iron and phosphorus shown, to be colimiting to microbial growth (Moore *et al.* 2013). Nitrogen (N) is considered to be limiting in 75% of ocean productivity (Bristow *et al.* 2017). Microorganisms that dominate these oligotrophic waters tend to be small, physically streamlined cells (Azam *et al.* 1983; Beaulieu *et al.* 2008; Dortch and Packard 1989; Moya *et al.* 2009; Thompson *et al.* 2019) that can uptake sufficient amounts of rare nitrogen due to their increased surface area to volume ratio (Lee and Fuhrman 1987; Pianka 1970; Ben A. Ward *et al.* 2017). Small microbes, mainly picophytoplankton (Buitenhuis *et al.* 2012; Falkowski, Woodhead, and Vivirito 1992), serve as the base of the microbial loop (Field 1998; Bristow *et al.* 2017; Follows and Dutkiewicz 2011), and thus the marine ecosystem (Falkowski, Woodhead, and Vivirito 1992), by fixing gaseous carbon to bioavailable components (Gruber *et al.* 2009; Raven and Falkowski 1999). Primary production and carbon fixation in marine oligotrophic environments is intimately tied to the growth of phytoplankton communities (Hartmann *et al.* 2014; Williams *et al.* 2013).

Two picocyanobacterial genera (*Prochlorococcus* and *Synechococcus*) are responsible for roughly 25% of global carbon fixation (Field 1998; Follows *et al.* 2007; Partensky, Hess, and Vaulot 1999). Once carbon is initially fixed via phytoplankton, such as cyanobacteria and algae, heterotrophic microbes, like *Alteromonas*, viruses (Jover *et al.* 2014; Weitz and Wilhelm 2012), and eukaryotic grazers (Banse 1995) determine the secondary fate of carbon by recycling it, funneling it to higher trophic levels, or storing it (Arístegui 2005; Boyd 2015; Buchan *et al.* 2014; Dortch and Packard 1989; Duarte *et al.* 2013). The carbon cycle is thus tied to the other nutrient cycles, such as phosphorus, nitrogen, and iron (Follows and Dutkiewicz 2011; Gruber and Galloway 2008; Redfield 1958; Voss *et al.* 2013) through the metabolisms, geographical ranges, and life histories of marine microorganisms (Falkowski 1997; Fuhrman 2009, 1999; Passarge *et al.* 2006; Fuhrman 2002).

The carbon cycle plays an integral role in climate change science (Le Quéré *et al.* 2009; Friedlingstein *et al.* 2006). It is predicted that as global carbon emissions continue to rise (Sokolov *et al.* 2009; Plattner *et al.* 2008) marine systems that canonically act as carbon sinks (Tagliabue, n.d.; Boyd 2015; Gruber *et al.* 2009; Raven and Falkowski 1999; Siegel *et al.* 2014) will be significantly affected (Friedlingstein *et al.* 2006; Gruber *et al.* 2009; Hennon *et al.* 2018). Nitrogen deposition is expected to increase in the future (Voss *et al.* 2013) yet increased stratification is set to favor streamlined, smaller-celled competitors (Henson *et al.* 2021; Knight and Morris 2020; Sallée *et al.* 2021; Wang *et al.* 2023). CO₂ concentration itself is expected to exhibit contrasting controls on community members (Dutkiewicz *et al.* 2015; Knight and Morris 2020). Despite various studies addressing this topic (Beman *et al.* 2011; Hennon *et al.* 2018; Hutchins and Fu 2017; Liao *et al.* 2019; Plattner *et al.* 2008; Sokolov *et al.* 2009; Wang *et al.* 2019), it is still unclear how climate change will affect oligotrophic waters and their microbial inhabitants. Therefore, understanding of the drivers that control the microbial biomass of the primary producers and their community members are applicable to current theories about marine carbon and nutrient cycle (Martin *et al.* 1987) and also inform predictions about future oceanic conditions.

Genera like *Prochlorococcus*, *Synechococcus*, and *Alteromonas spp.* all grow together in the oligotrophic ocean, yet differ in their diel (daily) and seasonal spatial patterns (Biller, Berube, *et al.* 2015; Berube *et al.* 2015; Marie *et al.* 1997; Partensky, Hess, and Vaulot 1999; Sohm *et al.* 2016; Liu and Campbell 1995) as well as geographic range (Barton *et al.* 2010; Buitenhuis *et al.* 2012; Flombaum *et al.* 2013; Follows *et al.* 2007; Rippka *et al.* 2001; Sohm *et al.* 2016; Thompson *et al.* 2013; Thomson *et al.* 2010). *Prochlorococcus* generally outgrows *Synechococcus* by ten-fold (Morris *et al.* 2011; Thompson *et al.* 2013) in oligotrophic waters. Presumably this is due to its streamlined size, and associated benefits, as compared to *Synechococcus* (Flombaum *et al.* 2013; Partensky, Hess, and Vaulot 1999). In laboratory culture however, it was not possible to mirror these microbial dynamics until hydrogen peroxide (H₂O₂) and heterotrophic bacteria were introduced (Calfee 2021; Morris *et al.* 2008). It was shown that *Prochlorococcus*'s streamlined form lacks the ability to survive alone in the concentrations of (H₂O₂) abiotically produced in this environment (Morris 2011; Zinser 2018a) likely due to its lack of H₂O₂ detoxifying catalase (Harada *et al.* 2021; Bernroither *et al.* 2009). Thus,

Prochlorococcus has evolved a dependence on catalase-positive microbes that share its environment, like *Synechococcus spp.* and *Alteromonas spp.*, as shown in Figure 1.1, to allow it to survive in mixed-culture communities and dominate production there (Biller, Berube, *et al.* 2015; Berube *et al.* 2015; Morris *et al.* 2011; Morris, Lenski, and Zinser 2012).

This dissertation investigates how these differing controls and the underlying mechanisms impact plankton community structures in the open ocean through contemporary computation modeling approaches. Thus, we first review current knowledge on H_2O_2 in the ocean and how phytoplankton are currently known to differ in reaction with exogenous H_2O_2 . Then we cover known models that simulate phytoplankton dynamics implicitly, via correlative representations, or explicitly, via trait parameterization, and known environmental toggles.

After reviewing the current knowledge of oceanic production of reactive oxygen species (ROS) like H_2O_2 and global marine phytoplankton modeling efforts in relation to nutrient supplies, this dissertation will concentrate on the intersection of H_2O_2 and nutrient stresses on plankton dynamics. It will explore H_2O_2 influences on singular plankton (Chapter 2) before investigating how coculture interactions are augmented by differing nitrogen (N) and H_2O_2 assumptions (Chapter 3). Trait space explaining the steady state concentration predictions of phytoplankton and H_2O_2 is further explored as a function of H_2O_2 parameters. Trait differences are visualized in separate different biogeographical predictions for prominent phytoplankton via utilization of microbial trait model projections on top of a global circulation model (Chapter 4).

REVIEW

Reactive oxygen species, and specifically H_2O_2 , are constant threats to microbial cells in the aerobic environment (Morris, Rose, and Lu 2022; Zinser 2018a; 2018a). Despite both being the primary H_2O_2 sink in marine systems (Morris, Rose, and Lu 2022; Zinser 2018a) and having known differential affects plankton that grow in the surface ocean (Morris *et al.* 2011; Zinser 2018a; Morris, Rose, and Lu 2022) currently no ocean microbial model accounts for the differential influence of H_2O_2 dynamics on oceanic microbial abundances. Gyres where *Prochlorococcus* is found to dominate numerically are defined by harsh abiotic conditions like high incident light (up to $2000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at the surface (Morris *et al.* 2011; Zinser *et al.* 2007; Cooper *et al.* 1988)), yearly maximum temperatures above 30°C (Martiny *et al.* 2009),

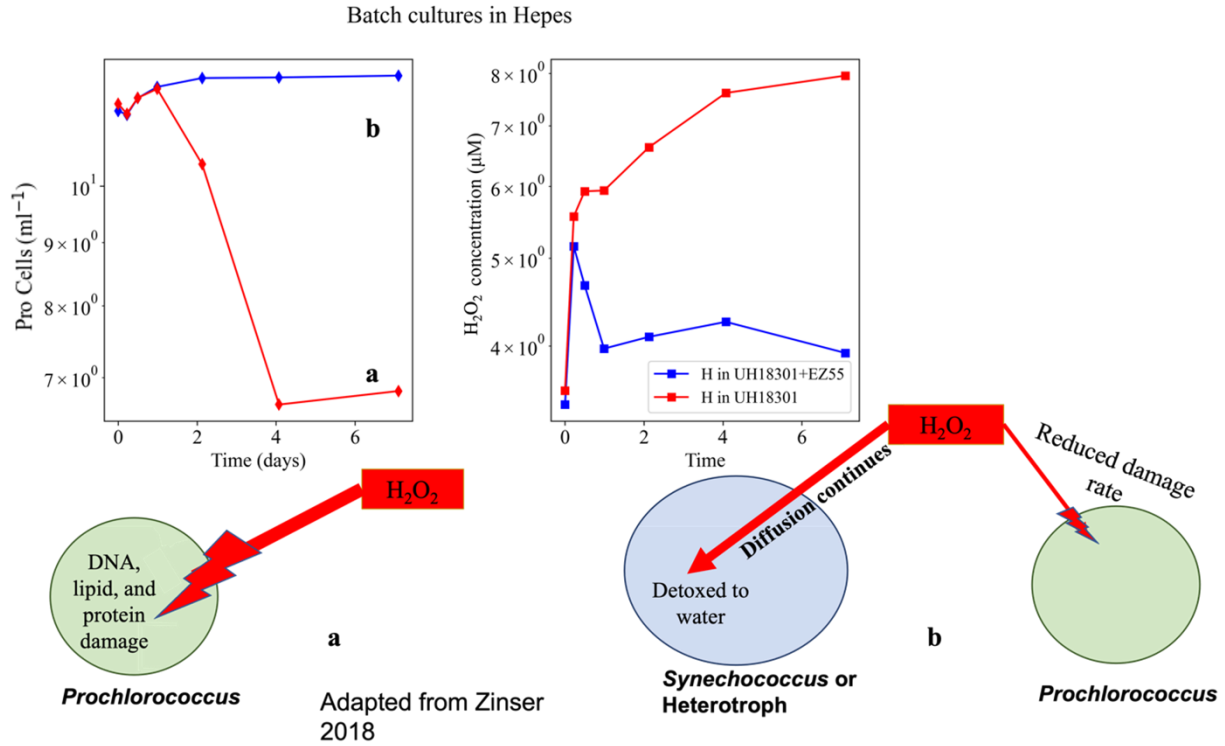


Figure 1.1 Concurrent monoculture or coculture dynamics for *Prochlorococcus* and H₂O₂

Prochlorococcus abundance (left) and H₂O₂ concentrations (right) in batch cultures spiked with photochemical- H₂O₂ production via HEPES buffer either in axenic (monoculture) assays (a, red) or coculture with *Alteromonas*, a heterotrophic bacterial helper, assays (b, blue). (HEPES buffer H₂O₂ production is shown in Appendix A, section A3.) The monoculture (a) and coculture (b) dynamics shown in representative bubble diagrams of a and b are below data plots. The bubble diagrams are assumed to represent the mechanisms on which the corresponding dynamics (a, red or b, blue) depend. Computational modeling concerning *Prochlorococcus* without (a, red) or with (b, blue) a helper microbe (like *Alteromonas*) to act as a H₂O₂ sink, is based upon the H₂O₂ diffusional interactions shown within this figure. These assumptions are continued through this dissertation. (Adapted from Zinser (2018b) with data from Morris 2011).

and daily reactive oxygen species production from biotic and abiotic sources (Blot *et al.* 2011; Cooper *et al.* 1988; Imlay 2003; Nishiyama, Allakhverdiev, and Murata 2006). Temperature has been studied in relation to *Synechococcus* and *Prochlorococcus* community structure, with *Synechococcus* shown to dominate *Prochlorococcus* in the colder (Sohm *et al.* 2016), more eutrophic waters (Flombaum *et al.* 2013). Light has been shown to coincide with temperature as the main determinant between *Prochlorococcus* ecotype niche differentiation (Martiny *et al.* 2009; Rippka *et al.* 2001) and as a regulator of *Synechococcus* growth, as *Synechococcus* is resigned to the shallower depths than *Prochlorococcus* (Biller, Berube, *et al.* 2015; Berube *et al.* 2015; Zinser *et al.* 2007).

Concepts from resource competition theory (Grover 1997; Tilman 1982; 1977; Hardin 1960) are often used to explain *Prochlorococcus*' dominance in nutrient-deplete water. Its streamlined size allows *Prochlorococcus* to have a higher surface area to volume ratio than *Synechococcus* indicating that *Prochlorococcus* has both a lower requirement for membrane-constructing molecules (like phospholipids) and an increased ability to uptake essential nutrients from the environment (Beaulieu *et al.* 2008; Ward *et al.* 2017; 2012; Moore and Chisholm 1999; Edwards *et al.* 2012). Competition theory categorizes organisms into either '*r*' or '*k*' strategists, based on the carrying capacity, *k*, or net growth rate, *r* (Pianka 1970). Leading to the organization of groups defined to compete with others by either maximizing the ability to grow quickly (by maximizing their maximum growth rate value, and *k*) like diatoms (Kirchman 2016), or maximizing the ability to take up nutrients (by minimizing their nutrient affinity value, and *r*); *Prochlorococcus* is a classic example of the latter (Armstrong and McGehee 1980; Pianka 1970; Treusch *et al.* 2009). While it is clear that *Prochlorococcus* is streamlined for ammonium use (Garcia-Fernandez, de Marsac, and Diez 2004), nitrogen is a constant constraint on growth (Biller, Berube, *et al.* 2015; Berube *et al.* 2015; Bristow *et al.* 2017) as nitrogen composes 7% of microbial cell mass in proteins and nucleic material, while phosphorus generally composes 1% (Krumhardt *et al.* 2013). This means that for a microbe that is streamlined for growth, further streamlining or the evolutionary cutting a protein from one's genetic repertoire, is arguably more advantageous than cutting a single gene, as nitrogenous molecular construction and maintenance costs for proteins are considered alongside the molecular and spatial costs of gene storage and replication as well (Lynch and Marinov 2015; Read *et al.* 2017). *Prochlorococcus* has been

noted to be especially streamlined genetically and physically (Garcia-Fernandez, de Marsac, and Diez 2004; Avrani and Lindell 2015; Chisholm 2017; Zinser and Martiny 2023; Partensky and Garczarek 2010), meaning that it likely strategically cut genes, and associated proteins and functions, from various areas of cellular life (Kettler *et al.* 2007; Morris, Lenski, and Zinser 2012). For example, high light *Prochlorococcus spp.* form fewer photosystem subunits presumably to decrease nitrogen constraint in the higher light conditions (MacGregor-Chatwin *et al.* 2019) while they also have cut all genes for phycobilosomes from their genome over the span of their evolution (Ulloa *et al.* 2021; Urbach *et al.* 1998). It is key to note that these proteins are known to be especially nitrogen and metal heavy (Barynin *et al.* 2001; Brill and Williams 1961), similar to catalase proteins (Chelikani, Fita, and Loewen 2004; Barynin *et al.* 2001). Thus *Prochlorococcus* may have ‘lost’ or ‘cut’ the genes for phycobilosomes and catalase to minimize ‘wasted’ genetic space (Morris, Lenski, and Zinser 2012; Morris, Papoulis, and Lenski 2014) and reduce the amount of cellular material required for growth. Thus allowing survival of *Prochlorococcus* over the course of the cyanobacteria’s 3.5 million year evolutionary span in such low nutrient environments (Zhang *et al.* 2021; Zamocky *et al.* 2012).

Competition theory did not explain fully why *Synechococcus* can continue to grow in the oligotrophic regions despite *Prochlorococcus* being better adapted for the nutrient conditions (Hutchinson 1967) until recently when Masuda *et al.* (2023) quantitatively showed that *Prochlorococcus* and *Synechococcus* are able to coexist by taking advantage of the differences in ammonia and nitrate concentrations in the oligotrophic gyres and genetic nitrogen usage repertoire (Garcia-Fernandez, de Marsac, and Diez 2004). Genetic and physical streamlining offers the distinct trade-off that in cutting 'extraneous' genes in order to decrease cell size, the cell is reducing its ability to perform functions that could become necessary in a changing environment (Kettler *et al.* 2007; Swan *et al.* 2013; Giovannoni, Cameron Thrash, and Temperton 2014; Giovannoni *et al.* 2005). For example, *Prochlorococcus* is smaller than *Synechococcus* and thus can acquire nutrients more efficiently (Berube *et al.* 2018) but also lacks the gene for most efficient H₂O₂ detoxifying enzyme in cyanobacteria, a bifunctional catalase-peroxidase, *katG* (Bernroither *et al.* 2009; Williams 1928; Keilin and Hartree 1943). As *Prochlorococcus* did not retain catalase, a key enzyme in the protection against H₂O₂ (Biller, Berube, *et al.* 2015; Berube *et al.* 2015, 20; Morris, Papoulis, and Lenski 2014),

Prochlorococcus must rely on other cyanobacterial species (like *Synechococcus*) or heterotrophic bacteria (like *Alteromonas spp.*) in this environment for H₂O₂ detoxification function.

H₂O₂ STRESS IN PHYTOPLANKTON

Reactive oxygen species (ROS) are an inevitable side effect of marine aerobic life (Imlay 2013; Morris, Lenski, and Zinser 2012; Hansel and Diaz 2021; Diaz and Plummer 2018). ROS can arise from biotic processes like photosynthesis (Banerjee *et al.* 2013; Mackey *et al.* 2013; Bailey and Grossman 2008) and respiration (Bond, Hansel, and Voelker 2020; Hayyan, Hashim, and AlNashef 2016; Seaver and Imlay 2001) as well as from abiotic processes such as photocatalytic H₂O₂ production via sunlight and dissolved organic carbon (Cooper *et al.* 1988; Zinser 2018a; Morris, Rose, and Lu 2022). Short lived ROS like OH[•] or O₂^{•-} are charged and thus more unstable (Cooper *et al.* 1988; Zinser 2018b; Morris, Rose, and Lu 2022) and reactive to than O₂ or H₂O₂; meaning that unless OH[•] or O₂^{•-} are created in the cell, they do not pose a significant threat to microbial cell health as they cannot easily cross the membrane and generally react too quickly (Imlay 2003; 2013; Seaver and Imlay 2001; Möller *et al.* 2019; Morris *et al.* 2011). H₂O₂ is created down-stream of O₂^{•-} reactions through the enzyme superoxide dismutase (SOD). It detoxifies O₂^{•-} to H₂O₂ and is found ubiquitously in aerobic microbes (Ighodaro and Akinloye 2018; Miller 2012). While *Prochlorococcus* only contains one type of SOD (Latifi, Ruiz, and Zhang 2009), other ocean microbes contain various SOD enzymes with different metallic centers thus giving a range of catalytic events across broader electron (e⁻) charge ranges (Boden *et al.* 2021; Miller 2012). H₂O₂ also forms when electron transport, either through the photosystem, or the aerobic metabolic chain, gets overloaded from errant or excess electrons Aharonovich, Sher, D., 2016. Thus creating [•]OH or O₂^{•-} that then damage cellular material or are detoxified to H₂O₂. Transcriptional responses of *Prochlorococcus* to co-culture with a marine *Alteromonas* indicate *Prochlorococcus* interacts differentially with separate strains and these differences may involve putative info or ROS chemicals (Imlay 2013; 2003; Zamocky *et al.* 2012).

Extracellular H₂O₂, however, can persist in the environment for hours (Zinser 2018a; 2013; Petasne and Zika 1997; Avery *et al.* 2005) as abiotic decomposition is relatively slow and

largely dominated by thermal decomposition (Hong *et al.* 2009). Extracellular H₂O₂ can cross the cellular membrane as it is uncharged and dipolar, similar to water molecules (Tjell and Almdal 2018). While H₂O₂ is important in iron and other key trace metal charge cycling, if it is not detoxified, H₂O₂ interacts and damages lipids, DNA, proteins, and cellular material (Imlay 2003; Latifi, Ruiz, and Zhang 2009; Morris 2011). These damages may compile leading to cellular death (Laloi and Havaux 2015; Imlay 2003; Curtin, Donovan, and Cotter 2002).

H₂O₂ is created abiotically from extracellular chromatic dissolved organic material capturing electrons displayed by ultraviolet (UV) and visible light in the surface ocean (Cooper *et al.* 1988; Morris *et al.* 2016; Zhu *et al.* 2022) and from rain events (Vaughan and Blough 1998; Willey, Paerl, and Go 1999). Dark production of H₂O₂ also occurs in abiotic and sea waters below the mixed surface layer indicating more complex mechanisms at work (Sutherland, Wankel, and Hansel 2020; Yuan 2004; Hopwood *et al.* 2017; Zinser 2018b). With open oceans having high incident light (reaching up to 2000 μmol quanta of electrons $\text{m}^{-2} \text{s}^{-1}$ at the surface (Morris *et al.* 2011)), there is a detectible diel or production cycle (Zinser 2018b; Imlay 2003; Blot *et al.* 2011; Cooper *et al.* 1988; Nishiyama, Allakhverdiev, and Murata 2006). Different wavelengths of light create different amounts of H₂O₂ giving complex patterns in water due to the wavelength's differential refraction rates and H₂O₂ production (Zhu *et al.* 2022). H₂O₂, if not detoxified, will damage DNA, proteins, and cellular material that it contacts until the cell can no longer function (Imlay 2003; Latifi, Ruiz, and Zhang 2009; Morris 2011). For photosynthetic organisms, H₂O₂ poses a particular threat to photosystem two (PSII) as electrons are both key to PSII function and easily move from reactive species like H₂O₂ (He and Häder 2002; Latifi, Ruiz, and Zhang 2009; Nishiyama, Allakhverdiev, and Murata 2006; Takahashi and Murata 2008). D1 is one of the two protein components in the reaction center of PSII responsible for binding P680 and various electron receptors (Campbell *et al.* 1998; Maxwell and Johnson 2000; Szilárd, Sass, and Vass 2002). It is key to photosynthetic energy storage. D1 is routinely damaged and replaced during photosynthesis due to the high reactivity of this process (Maxwell *et al.* 1994; Soitamo, Havurinne, and Tyystjärvi 2017; Takahashi and Badger 2011). H₂O₂ inhibits photosynthesis and the regeneration of D1 in PSII by blocking the synthesis of new D1 subunits (Nishiyama, Allakhverdiev, and Murata 2006; Takahashi and Badger 2011). In this direct way, H₂O₂ can lead

to decreased photosynthetic efficiency and photoinhibition in photosynthetic organisms like *Prochlorococcus* and *Synechococcus*.

STRATEGIES TO MITIGATE H₂O₂

H₂O₂ is diffusible through the cell membrane and aquaporin channels (Bienert *et al.* 2007), so cells must get rid of H₂O₂ they create through cellular respiration and photosynthesis (Curtin, Donovan, and Cotter 2002; Nishiyama, Allakhverdiev, and Murata 2006; Ziegelhoffer and Donohue 2009; Zinser 2018b), as well as environmentally sourced H₂O₂. Many phytoplankton possess enzymes to detoxify H₂O₂ as enzymatic decomposition of H₂O₂ is much faster (Alfonso-Prieto *et al.* 2009; Zinser 2018a) than abiotic processes, but others have lost one or more of the enzymes needed to detoxify ROS fully (Bengtsson-Palme *et al.* 2014; Regelsberger *et al.* 2002; Bernroither *et al.* 2009). Cellular detoxification of H₂O₂ canonically is attributed to catalase, and to a lesser extent, peroxidase enzymes (Ighodaro and Akinloye 2018; Williams 1928; Schellhorn 1995; Sato *et al.* 1992; Petigara, Blough, and Mignerey 2002). Thus, organisms which lack catalase but contain peroxidase, such as *Prochlorococcus*, are more vulnerable to their environmental H₂O₂ conditions. With the inherent leakiness of H₂O₂ across membranes (Bienert *et al.* 2007; Tjell and Almdal 2018), detoxification of H₂O₂ cannot be inherently for just one cell but inadvertently benefits the entire biological community within that environment. Consequently, if an organism detoxifies all of the H₂O₂ in its cell, the laws of diffusion and chemical reaction balance will dictate that the internal absence of H₂O₂ will draw externally sourced H₂O₂ into the cell (Omar *et al.* 2022). This is typically described as a 'leaky function' (Blot *et al.* 2011, 201; Cooper *et al.* 1988; Imlay 2013; Latifi, Ruiz, and Zhang 2009). This implies that organisms capable of 'cheating' by not making a catalase enzyme while still getting the benefit of catalase's function, will do so (de Mazancourt and Schwartz 2010; Estrela, Morris, and Kerr 2016; Allison 2005; Ehrlich, Kath, and Gaedke 2020). This evolutionary drive for genetic streamlining, in *Prochlorococcus* for example, has been termed the "Black Queen Hypothesis" (Morris, Lenski, and Zinser 2012). It states that if a function is leaky while also being energetically costly to perform and maintain, the function will only be executed by the organisms in a community that 'get stuck with the job', much like the Queen of Spades in a game of Hearts (Morris, Lenski, and Zinser 2012). The oligotrophic gyres of the ocean have both very

low nutrient influx and high light incidence, giving a harsh combination of nutrient deprivation and high rates of H_2O_2 formation that microbes must endure (Diaz and Plummer 2018; Zinser 2018b). *Prochlorococcus* is the most numerically abundant photosynthetic organism in oligotrophic waters and is estimated to perform half of the carbon fixation there, yet it cannot live in monoculture with the level of H_2O_2 found in its natural environment. Clearly *Prochlorococcus* has evolved a dependence on other microorganisms, allowing it to survive its environment in mixed-culture communities, but not in monocultures (Biller, Berube, *et al.* 2015; Berube *et al.* 2015; Morris *et al.* 2011; Morris, Lenski, and Zinser 2012).

MODELING SURFACE OCEAN MICROBIAL COMMUNITIES

As oligotrophic environments contain a plethora of abiotic and biotic variables, understanding the drivers of marine microbial growth, biogeochemical cycling, and the structure of oligotrophic microbial communities is difficult (Dutkiewicz, Follows, and Bragg 2009; Follows and Dutkiewicz 2011; Hutchinson 1967). Mathematical models are often used to quantify parameters and interactions within these communities that are problematic to isolate or test with traditional experimental methods (Saba *et al.* 2010). This also creates the opportunity to use such models as predictive frameworks for organisms that are difficult to study in their natural environment conditions. Previous mathematical models that describe oceanic cyanobacterial distributions have been either correlative models (Flombaum *et al.* 2013) that assume only abiotic factors like temperature and light are drivers of microbial abundance, or deterministic models (Dutkiewicz, Follows, and Bragg 2009; Follows *et al.* 2007; Ward *et al.* 2017) that explore competitive outcomes between microbes using population traits and nutrient supply (Dutkiewicz, Follows, and Bragg 2009; Flombaum *et al.* 2013; Follows *et al.* 2007; Ward *et al.* 2017; 2012). However, there has yet to be a model that considers the influence of H_2O_2 dynamics and nutrient balances on large-scale oceanic microbial distributions using these deterministic methods. Correlative models that assume abiotic factors like temperature and light are drivers of microbial abundance innately contain the light and temperature dependencies of H_2O_2 concentration, and further, cell death via H_2O_2 , yet do not track or represent it plainly. This thesis aims to add deterministic value to marine global model regarding H_2O_2 .

Current biochemically resolved circulation models of Earth's oceans (Carroll *et al.* 2020; Ward *et al.* 2012) agree with niche models and limited data (Flombaum *et al.*, 2013; Partensky, Blanchot, *et al.*, 1999) indicating that *Prochlorococcus* dominates in the most oligotrophic marine surface regions (Dutkiewicz, Follows, and Bragg 2009; Follows and Dutkiewicz 2011; Ward *et al.* 2017). While *Prochlorococcus*'s streamlining-induced specialization regarding nutrients has been incorporated (Dutkiewicz, Follows, and Bragg 2009; Masuda *et al.* 2023; Ward and Follows 2016) into empirical, predictive models, the known phytoplankton death processes of *Prochlorococcus* have been less noted (Anderson, Diou-Cass, and Harvey 2018; Hinson *et al.* 2023). The inclusion of phototrophic levels (Dutkiewicz, Follows, and Bragg 2009; Dutkiewicz, Scott, and Follows 2013) and predation like grazers (Follett *et al.* 2022) and viruses (Carlson *et al.* 2022; Talmy *et al.* 2019) has begun for global predictions, yet explicit representations of chemical impediments to cyanobacteria, like H₂O₂, have been ignored. The main goal of this thesis to provide a basal framework to allow for the inclusion of H₂O₂ into said global models.

GOALS OF THIS DISSERTATION

In aquatic systems, the organic particulate matter (X, here including cellular matter at the pico and nano level) follow a similar mass balance to that of dissolved material, in that they are governed by a mass balance for advection (κX), diffusion ($\mathbf{u}X$), and biological sources and sinks or losses (S_{BX}), along with an addition loss (sinking) rate of ωx , as the particles themselves are large enough to effect movement calculations (Dutkiewicz, Follows, and Bragg 2009; Follows and Dutkiewicz 2011; Ward *et al.* 2012). With respect to X as carbon, nitrogen, phosphorus, and iron

$$\frac{\partial X}{\partial t} = \underbrace{S_{BX}}_{\text{Biological reactions on X}} - \underbrace{\nabla \cdot (\mathbf{u}X)}_{\text{advection}} + \underbrace{\nabla \cdot (\kappa X)}_{\text{diffusion}} - \underbrace{\frac{\partial \omega x X}{\partial z}}_{\text{sinking along axis Z}}$$

The ultimate goal of the dissertation is to add H_2O_2 dynamics into the complex biological S_{Bx} term. S_{Bx} is the total balance of biological inputs and outputs of carbon in the global ocean with regards to all X molecules of interest cycled through biomatter. Linking H_2O_2 -based detoxification and damage to the microbes considered in S_{Bx} allows for the connection of H_2O_2 concentrations to global predictions of X, be it carbon, nitrogen, or iron. This thesis follows the stepwise methods seen in Figure 1.2 for showing what it takes to go from laboratory to global model scales. While phytoplankton dynamics are incorporated (S_{Bx}) into global models for quantification of their impact on global chemical cycles, the known H_2O_2 dependencies within phytoplankton communities are not explicitly represented. The aim of this dissertation is to provide a first attempt at quantifying (Chapter 2), evaluating (Appendices A, B, and C) and projecting (Chapters 3, 4) the differential impacts of H_2O_2 on phytoplankton monoculture abundances and resultant community dynamics when in concert with *in situ* nutrient limitation.

Boyd *et al.* (2018) suggests that the challenge of climate change on ocean modeling requires integrating information and methods from multiple approaches. This thesis embraces the combination of techniques from various microbial laboratory, computer science, math modeling, and ocean ecology to get a broad scale understanding of global changes wrought by H_2O_2 . We confront several laboratory experiments with ordinary differential equations representing monoculture batch culture cell dynamics with or without H_2O_2 to analyze the innate dependencies of *Prochlorococcus*, and fellow ocean microbes, on H_2O_2 interactions within our framework. Model parameters are difficult to explicitly measure within a laboratory/environmental experiment alone due to difference in lab vs. global scales (Saba *et al.* 2010). And the relatively small amount (~2%) of the ocean's total global area that has been sampled for microbial dynamics is well recognized (Carroll *et al.* 2020) and is thus an impediment to accurately modeling the microbes within marine ecosystems. This project introduces simple mathematical representations of the time dependent cell abundance and nutrient or H_2O_2 dynamics for multiple members of *Prochlorococcus*' oligotrophic community and take a stepwise modeling approach, iterating over specific data sets to recover abiotic, biotic, and H_2O_2 - based parameters in Chapter 2. We parameterized these models with laboratory culture data to ensure that our projections are rooted in the biology driving this complex ecology (Figure 1.2, a, 'Chapter 2' section). We build from prior deterministic models that explore

competitive outcomes between microbes using population traits and nutrient supply (Follows *et al.* 2007; Ward *et al.* 2017; Buitenhuis *et al.* 2012; Edwards *et al.* 2012; Passarge *et al.* 2006) and explore H_2O_2 as an explicit parameter that may influence feedbacks of H_2O_2 dynamics and nutrient balances on large-scale oceanic microbial distributions starting in Chapter 3 (Figure 1.2, b, Chapter 3 competition and coexistence scenarios). Then trade-offs between efficient nutrient acquisition and maintaining H_2O_2 detoxifiers are considered using resource competition theory to assess how nutrient supply and H_2O_2 concentration gradients exert control on the selection of microbial communities. Thus, a framework starts to explicitly resolve how the community interactions of *Prochlorococcus* may be shaped by their H_2O_2 dependencies, with the overall goal of illuminating drivers of large-scale (global) distributions of *Prochlorococcus* biogeography in Chapter 4. *Prochlorococcus* is governed by two opposing forces: the evolutionary push to streamline in order to outcompete all for nutrients and the need for detoxification of unavoidable reactive species created within their environment. This dissertation model set starts with monoculture growth and H_2O_2 culture models with later extrapolation to more complex competition and global model formulations. It continues with mirroring the natural way in which *Prochlorococcus* community structure appears from singular interactions. And concludes by attempting to quantify the ecological tradeoff that *Prochlorococcus* faces *in situ* through the projection of *Prochlorococcus* biogeographies resultant from differing assumptions about how natural *Prochlorococcus* populations balance the interaction of nutrient competition and H_2O_2 detoxification requirements in the oligotrophic ocean.

a)

Chapter 2

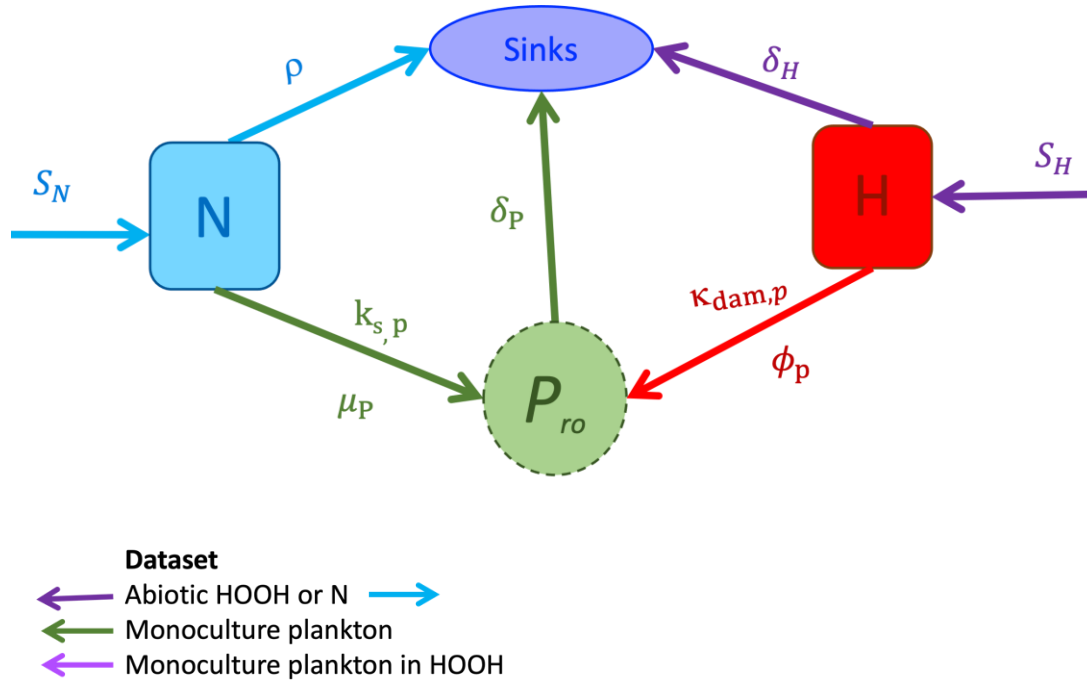


Figure 1.2 Roadmap of the stepwise model formation using divergent H_2O_2 phenotypes in dominant plankton. (a) Chapter 2 uses multiple laboratory datasets to isolate different monoculture parameters for growth and H_2O_2 interactions. Using parameters found for different microbial species in Chapter 2, (b) Chapter 3 explores competition and coexistence outcomes between these species in various nutrient and H_2O_2 supplies. Following this intuition, (c) Chapter 4 utilizes a global model framework to visualize predicted biogeographies of globally important microbial analogues to those chosen for Chapter 2.

b)

Chapter 3

Competitive Exclusion

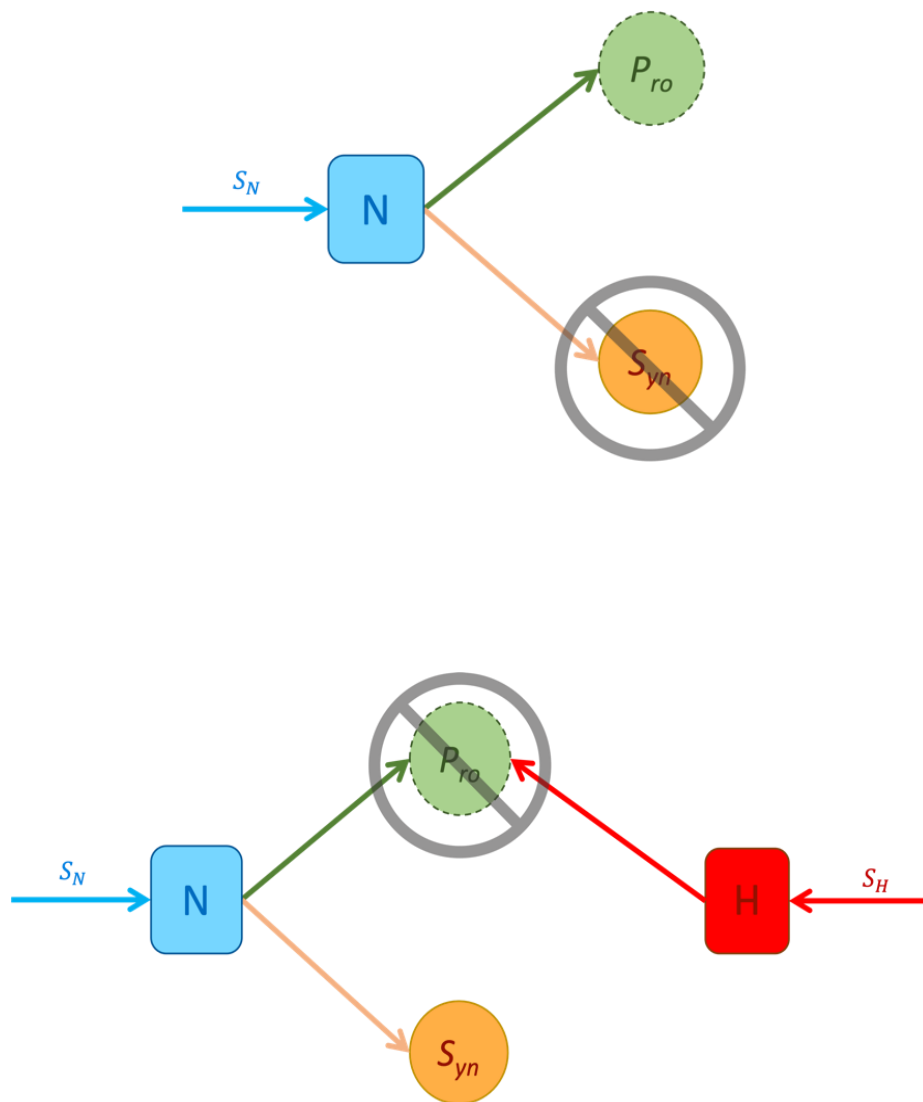
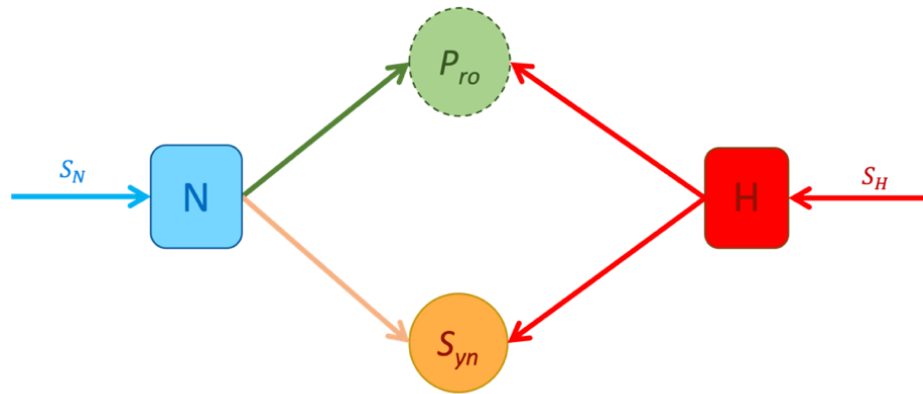


Figure 1.2 continued

Coexistence



$$\phi_B = 0$$

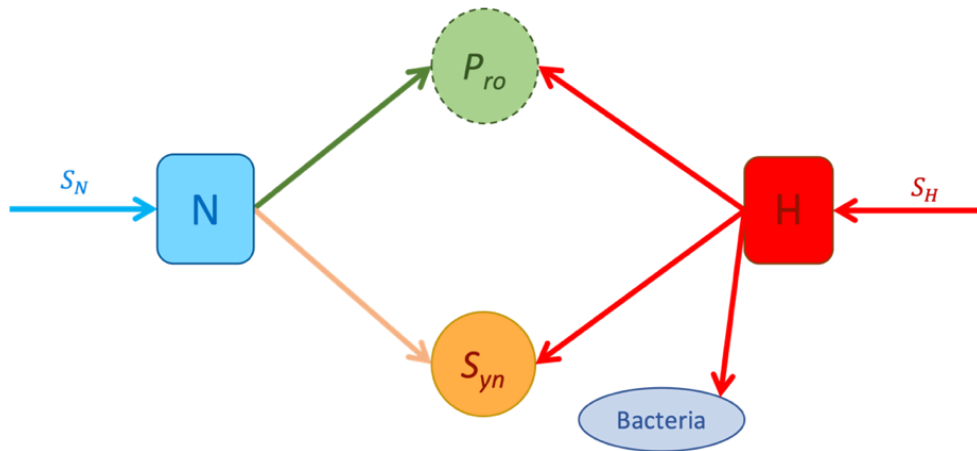


Figure 1.2 continued

c)

Chapter 4

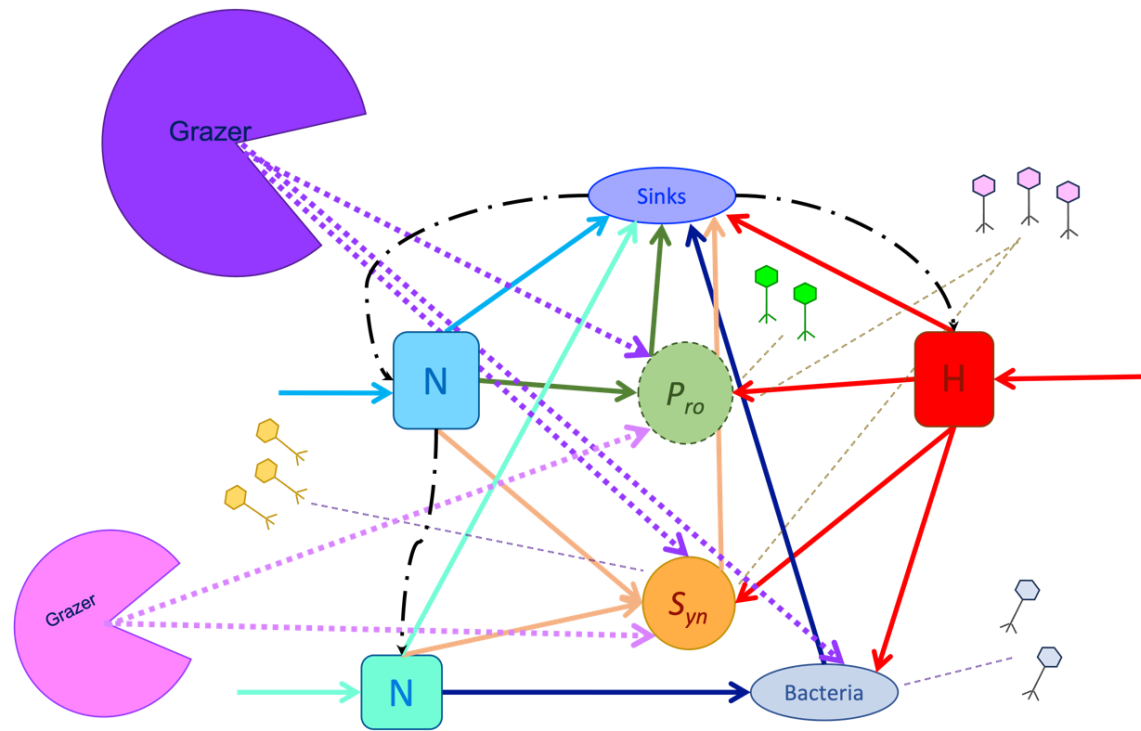


Figure 1.2 continued

Chapter 2 QUANTIFICATION OF THE COUPLED DYNAMICS OF CELL GROWTH AND HYDROGEN PEROXIDE (H_2O_2) IN LABORATORY BATCH CULTURE EXPERIMENTS

Author(s): D.K.M., E.B., M.A.G., B.C.C., E.R.Z., and D.T.

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Author contribution statements: Written and edited by D.K.M., M.A.G., and D.T. with feedback from E.R.Z, and B.C.C. Experiments were designed by B.C.C., E.B., and E.R.Z.; experiments were performed by B.C.C. and E.B.

ABSTRACT

Prochlorococcus, the smallest and most numerous phytoplankter of the oligotrophic surface ocean, requires coexisting ‘helper’ microbes to detoxify hydrogen peroxide (H_2O_2) produced in the surface ocean. In this chapter, we present coupled ordinary differential equation models of the dynamics of multiple oligotrophic plankton grown in different levels of H_2O_2 . We evaluate and fit our model to monoculture time series data of peroxide-sensitive *Prochlorococcus* and peroxide-resistant *Synechococcus* and bacteria. Our fits provide estimates of H_2O_2 cell damage and detoxification rates in these ecologically dominant microbes. Our results indicate that catalase containing microbes, like *Synechococcus* and known heterotroph helpers (*Alteromonas*), have high rates of detoxification and low rates of H_2O_2 mediated cell death. In contrast, the catalase-negative *Prochlorococcus* has high rates of H_2O_2 mediated cell death and low rates of cell-specific detoxification. Our results quantify the possible trade-off between H_2O_2 detoxification and cell death due to the presence of detoxifying enzymes. The use of differential equation models to quantify parameters underlying microbe- H_2O_2 dynamics provides a basis for ecosystem models to explore the impact of reactive oxygen species on cell mortality and microbial community composition.

INTRODUCTION

Oceans cover 70% of the earth and (Raven and Falkowski 1999), outside the inputs of coastal and cold water upwelling inputs (Field 1998; Bristow *et al.* 2017), are largely composed of nitrogen limited ‘nutrient deserts’ (Ustick 2022). Two globally dominant genera of cyanobacteria, *Prochlorococcus* and *Synechococcus* (Flombaum *et al.* 2013) perform ~50% of marine carbon fixation, serving as the basal step of the food chain (Field 1998; Bristow *et al.* 2017; Follows and Dutkiewicz 2011). *Prochlorococcus* is the most numerous phytoplankter genus of the ocean (Kent *et al.* 2016; Zinser *et al.* 2006; Biller, Berube, *et al.* 2018; Berube *et al.* 2018; Johnson 2006; Zinser and Martiny 2023) and numerically dwarfs its sister genus *Synechococcus* in nutrient deplete gyres (Partensky *et al.* 1996). Predictions of future *Prochlorococcus* distributions show numerical dominance of *Prochlorococcus*; again at mid to low latitudes throughout much of the surface open ocean (Flombaum *et al.* 2013), despite future shifting environmental pressures (Dutkiewicz *et al.* 2015; Hutchins and Fu 2017). The numerical dominance of *Prochlorococcus* in these areas is thought to be due to its minimal nutrient

requirements and high surface area to volume ratio associated with its smaller cell (0.6 – 0.8 μm vs 0.6 – 2.0 μm) and total genome lengths (1.5 - 2.5 Million basepairs (Mbp) vs > 2.4 Mbp) as compared to *Synechococcus* (Giovannoni, Cameron Thrash, and Temperton 2014; Roca *et al.* 2003; Dao 2013; Partensky and Garczarek 2010). One striking aspect of *Prochlorococcus*' streamlined genome is its evolutionary loss of catalase, a key enzyme in the protection against the reactive oxygen species (ROS) hydrogen peroxide (H_2O_2) that is present in surface ocean environments (Bernroither *et al.* 2009; Biller, Berube, *et al.* 2015; Berube *et al.* 2015; Morris *et al.* 2011; Morris, Papoulis, and Lenski 2014).

The monofunctional catalase (and bifunctional catalase-peroxidase) enzyme has been shown to be extremely efficient in the face of H_2O_2 spikes whether isolated from mammalian cells, *Escherichia coli*, or *Synechococcus spp.* (Curtin, Donovan, and Cotter 2002; Ravishankar, Rao, and Ramasarma 1995; Alfonso-Prieto *et al.* 2009; Zamocky *et al.* 2012). The function of catalase specifically is expedited by the closeness of the binding sites and protein side chains helping stabilize H_2O_2 during the reaction (Alfonso-Prieto *et al.* 2009; Bernroither *et al.* 2009). It is found in all domains of life (Zamocky *et al.* 2012) including most cultured, aerobic microbial species (Bernroither *et al.* 2009; Harada *et al.* 2021; Kawasaki *et al.* 1997). Despite being found in all kingdoms of life, catalases are isolated infrequently in natural ocean samples (Bernroither *et al.* 2009). In contrast, monofunctional peroxidases, are found across the tree of life and are also widespread, including within *Prochlorococcus*. The pervasiveness of peroxidases and relative rarity of catalases suggest a functional copy of the latter enzyme is costly to express. Catalases and peroxidases both detoxify H_2O_2 to H_2O and another non-toxic molecule (Bernroither *et al.* 2009; Ighodaro and Akinloye 2018; Regelsberger *et al.* 2002). And proteins within these groups have separately been shown to both be constitutive and stress-induced regulation (Brown 1995; Venkatesan *et al.* 2007; Schellhorn 1995; Sato *et al.* 1992). Further peroxidase and catalase require metals to operate properly (Pinnix *et al.* 1994; Regelsberger *et al.* 2002; Bernroither *et al.* 2009; Tovmasyan *et al.* 2015), thus a warming ocean may influence all detox mechanisms of H_2O_2 in microbes. Thus *Prochlorococcus*'s lack of a catalase (Morris, Rose, and Lu 2022; Zhu *et al.* 2022; Goldstone and Voelker 2000) is all the more striking given that abiotic production from a single diel cycle results in ambient H_2O_2 concentrations ten times higher than those shown to kill *Prochlorococcus* (Morris 2011).

Without the ability to robustly detoxify H_2O_2 proficiently, *Prochlorococcus* is left itself to be dependent on other species for this vital detoxification function (Morris 2011; Morris *et al.* 2008; 2011; Morris, Lenski, and Zinser 2012; Morris 2015). H_2O_2 , unlike most other reactive oxygen species, is uncharged and thus can readily diffuse across cell membranes (Ohno and Gallin 1985; Omar *et al.* 2022; Möller *et al.* 2019; Morris, Lenski, and Zinser 2012; Seaver and Imlay 2001). Facilitated by aquaporins (Bienert *et al.* 2007), the internal and external cellular environments can equilibrate in less than 1 second after external peroxide addition (Antunes and Cadenas 2000). Exogenous H_2O_2 that enters bacterial cells is detoxified quickly (Alfonso-Prieto *et al.* 2009; Seaver and Imlay 2001) in organisms that contain catalase enzymes, serving as an important sink of endogenous and exogenous H_2O_2 (Morris 2011). Bacteria with catalases can therefore provide a benefit to their community through exogenous H_2O_2 detoxification (Morris 2011; Morris, Lenski, and Zinser 2012; Ma and Eaton 1992). As a result, *Prochlorococcus* and other globally dominant, genetically streamlined, and catalase-deficient organisms like SAR11 (Giovannoni 2017; Grote *et al.* 2012), are essentially obligate symbionts, dependent on other catalase containing microbes (Morris, Lenski, and Zinser 2012). While data suggests the transmission and expression of singular genes do not have exorbitant energetic costs, (Lynch and Marinov 2015), catalase is a large, metal (iron or manganese) containing enzyme (Barynin *et al.* 2001; Chelikani, Fita, and Loewen 2004). Catalase also has a high turnover rate, requiring renewal of protein structures after activity (Nicholls 2012; Cao *et al.* 2003). High enzyme production and maintenance costs of catalase for *Prochlorococcus*, when combined with the low nutrient supplies in oligotrophic waters and the presence of other catalase expressing community members (e.g. *Synechococcus* and *Alteromonas spp*), may explain the evolutionary loss of catalase in *Prochlorococcus* (Morris, Lenski, and Zinser 2012; Morris 2015; Morris *et al.* 2016; Morris 2011; Ma *et al.* 2018).

In situ measurements cover only a small percentage of the ocean so our current understanding of *Prochlorococcus*, and other picoplankton (Visintini, Martiny, and Flombaum 2021), abundances and distributions are explored through global models. Ecosystem (Carroll *et al.* 2020; Ward *et al.* 2012) and statistical niche models (Flombaum *et al.* 2013; Luo *et al.* 2015; Carroll *et al.* 2020) calculate abundance differently but both indicate that *Prochlorococcus* dominates in the most oligotrophic regions of the ocean (Dutkiewicz, Follows, and Bragg 2009;

Ward *et al.* 2017; Follows and Dutkiewicz 2011). Realistic patterns of *Prochlorococcus* biogeography are recreated in ecosystem trait models that assume *Prochlorococcus* has superior competitive ability for ammonium (Dutkiewicz, Follows, and Bragg 2009; Masuda *et al.* 2023; Ward and Follows 2016). More recent modeling studies indicate that *Prochlorococcus* loss processes, such as viral predation and grazing, are also important influences on *Prochlorococcus* biogeography (Follett *et al.* 2022; Carlson *et al.* 2022). In contrast, the role of H₂O₂ dynamics on dominant oceanic microbial abundances has not been modeled, despite observed susceptibilities of the same plankton when in cultivated settings (Morris *et al.* 2011).

Here, we present a coupled ordinary differential equation (ODE) model for microbial and H₂O₂ dynamics. As our model illustrates, coupled ODEs are well suited to quantification of cell-H₂O₂ dynamics. As microbial populations grow and die, their ability to detoxify exogenous reactive oxygen species changes. Our ODE model explicitly quantifies changes in population sizes and H₂O₂ concentration. By fitting our model to laboratory time-series data using a Bayesian Monte Carlo Markov Chain (MCMC) approach, we obtain estimates of cell specific death and detoxification rates for globally important genera. Finally, we illustrate how our ODE model can be used to explore the impact of H₂O₂ on cell mortality and community composition.

METHODS

Below we describe the system of ordinary differential equations (ODEs) we used to model the population dynamics of bacteria across a range of H₂O₂ values. Special cases of our model were fitted in different permutations of laboratory community assays using a Bayesian Metropolis-Hastings MCMC to estimate the posterior distributions of the relevant model parameters (See Figure 2.1 for a model schematic and Table 1 for a definition of all parameters) (Metropolis *et al.* 1953). All computer code is provided in a GitHub repository: https://github.com/DKMcCullough/HOOH_dynamics. Below is a detailed description of both the model equations and data used to estimate model parameters.

MODEL EQUATIONS

The dynamics of phytoplankton, *i*, abundance (P_i , cells mL⁻¹) and H₂O₂ concentration (H, nmol mL⁻¹) were modeled with a set of coupled ordinary differential equations (ODEs):

Monoculture Model Equation Set

$$\frac{dP_i}{dt} = \underbrace{\mu_i P_i}_{\text{net growth rate of plankton}_i (P_i)} - \underbrace{\kappa_{dam,i} P_i H}_{\text{net loss of } P_i \text{ due to } H} \quad (2.1)$$

$$\frac{dH}{dt} = \underbrace{S_H}_{\text{H}_2\text{O}_2 \text{ supply rate}} - \underbrace{\delta_H H}_{\text{abiotic decay}} - \underbrace{\phi_{max,i} P_i H}_{\text{biotic detoxification by plankton } i} \quad (2.2)$$

All parameter and state variable definitions and units are provided in Table 1.

Phytoplankton grow at constant rate μ_i (day^{-1}). The main rate of cell death is assumed to be dependent on H_2O_2 concentration and a mass action coefficient $\kappa_{dam,i}$ ($\text{mL nmol}^{-1} \text{day}^{-1}$). H_2O_2 is supplied at rate S_H ($\text{nmol mL}^{-1} \text{day}^{-1}$), and subject to first order abiotic decay at rate δ_H (day^{-1}), and cell contact dependent detoxification set by mass action coefficient $\phi_{max,i}$ ($\text{mL cell}^{-1} \text{day}^{-1}$).

LABORATORY DATA

H_2O_2 Spike Assays: We measured the temporal dynamics of H_2O_2 in cell free, abiotic media illuminated AMP-J, filter sterilized artificial seawater for cyanobacteria according to Ma *et al.* (2018) in the oligotrophic ocean spiked to 400nM H_2O_2 . We measured the exogenous H_2O_2 concentration using an acridinium ester chemiluminescence injection protocol (King *et al.* 2007; Morris *et al.* 2011) in abiotic and growth media. Each fluorescence reading was transformed to H_2O_2 nM mL^{-1} data via a linear reference curve made in Microsoft Excel following Morris and Zinser (2013) and Morris *et al.* (2011) protocols. This was done for readings from both the Control (0nM H_2O_2 added) or Spiked (400nM H_2O_2 added) assays at the appropriate time points.

Phytoplankton culture assays: We grew *Prochlorococcus* MIT9215 and *Synechococcus* CC9605 in batch cultures at 24° C (Moore *et al.* 2007) in Percival incubators (Percival, Boone, IA) that allowed for gradual increase and decrease of cool white light to simulate sunrise and sunset with peak midday light intensity of 150 mmol quanta $\text{m}^{-2}\text{s}^{-1}$ on a 14 hr:10 hr light:dark cycle in AMP-J media for all biomass assays (Calfee 2021; Hennon *et al.* 2018; Knight and

Morris 2018). We measured cell densities of *Prochlorococcus* and *Synechococcus* using different autofluorescent properties in flow cytometric assays (Campbell, Nolla, and Vaultot 1994; Calfee, Glasgo, and Zinser 2022). Following published methods, heterotrophs were counted through plating assays as concurrent H_2O_2 measurements were taken (Calfee, Glasgo, and Zinser 2022). All biotic data can be found at the Biological and Chemical Oceanography Data Management Office (Zinser, Talmy, and Calfee 2023).

FITTING PROCEDURE

Our ODE model as defined Equations 1 has five unknown parameters: maximum plankton growth rate (μ_i), H_2O_2 -induced cellular death rate ($\kappa_{dam,i}$), cell-mediated H_2O_2 detoxification rate ($\phi_{max,i}$), abiotic H_2O_2 supply rate (S_H), and abiotic H_2O_2 decay rate (δ_H). Our goal was to find the combination of parameters that best explained observed H_2O_2 and cell abundance dynamics in each laboratory population dataset. We chose to use the Metropolis-Hastings algorithm (Metropolis *et al.* 1953; Hastings 1970), a widely used Monte Carlo Markov-Chain (MCMC) method. We took a Bayesian approach to parameter quantification, thereby assuming that each parameter is itself a distribution with inherent uncertainty. A brief summary of our key assumptions of the fitting procedure is included below. A full explanation of the fitting procedure in Appendix A, Supplementary Information Chapter 2, section A1.

The goal of our model fitting was to sample and summarize the posterior probability distribution of the model parameters given the model and data. Let D be a given experimental dataset and let θ be the set of parameters of interest (e.g., for the full model $\theta = (\mu, \kappa, \dots)$). The following describes a procedure to estimate the posterior probability distributions of the parameters θ given the data D or $P(\theta | D)$.

Using Bayes theorem, the probability of a given parameter set θ given dataset D is:

$$(2.2) \quad P(\theta|D) = \frac{P(D|\theta)P(\theta)}{P(D)}$$

where $P(\theta)$ represents the prior distribution for our parameters. For simplicity, we used an improper, uninformative prior of $P(\theta) = 1$. Further we let the data and associated noise guide further narrowing of the prior $P(\theta)$ through the fitting procedure. Observational uncertainty and

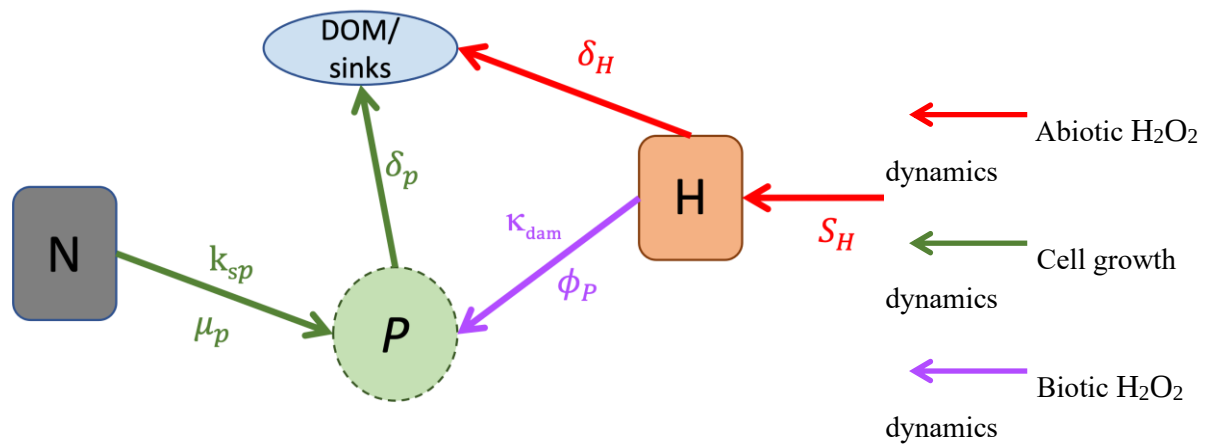


Figure 2.3 Stepwise model construction allows for data constraint on individual parameters within a coupled ODE model. Colored shapes represent measurable pools of Nutrients (N), Phytoplankton (P), H₂O₂ (H), and sinks in a well-mixed batch culture. Arrow colors indicate pathways of H₂O₂ and nutrient flow.

Table 1 Monoculture Parameter and variable definitions and units

Symbol	Description	Units
μ_i	Net growth rate	day ⁻¹
$\kappa_{dam,i}$	H ₂ O ₂ specific rate of cell death	mL nmol ⁻¹ day ⁻¹
S_H	H ₂ O ₂ supply rate	nmol mL ⁻¹ day ⁻¹
δ_H	H ₂ O ₂ decay rate	day ⁻¹
$\phi_{max,i}$	Cell specific rate of H ₂ O ₂ detoxification	mL cell ⁻¹ day ⁻¹
P_i	Plankton (i) Cell abundance	cells mL ⁻¹
H	H ₂ O ₂ concentration	nmol mL ⁻¹

other noise in our data is generally distributed log normally around the ‘true’ state of the system. Under this assumption of Log(N) error, the likelihood function, $L(\theta)$, is defined as follows:

$$(2.3) \quad P(D|\theta) = L(\theta) = \exp(-\chi^2)$$

Using $y_{i,D}$ to represent data point i , such as H_2O_2 concentration or cell abundance, and $y_{i,\theta}$ to represent our model’s predicted value given a set of parameter values θ :

$$(2.4) \quad Pr(D|\theta) \propto \exp\left(\sum_i \frac{(\ln(y_{i,D}) - \ln(y_{i,\theta}))^2}{\sigma_{y,i}^2}\right)$$

where $\sigma_{y,i}$ quantifies the noise in our measurements. For simplicity of our model fittings, we set $\sigma_{y,i}$ equal to the standard deviation of our log transformed measurements across technical and biological replicates (see Appendix A, Supplementary Information Chapter 2).

EXPERIMENTAL APPROACH:

Abiotic H_2O_2 production and decay parameters, S_H , and δ_H , were constrained first via cell-free dynamics of hydrogen peroxide. Then the modeled cyanobacteria population dynamics without added H_2O_2 to constrain growth rates, μ_i . Finally, the predictions of a model were compared with dynamic H_2O_2 with a range of laboratory culture data spanning catalase positive and catalase negative organisms to constrain biotic H_2O_2 -related parameters of damage ($\kappa_{dam,i}$) and detoxification ($\phi_{max,i}$).

RESULTS

ABIOTIC MEDIA ANALYSES

We first set out to model abiotic dynamics in a control system without H_2O_2 additions. In Figure 2.2a, we present both the laboratory data (pink symbols) and model fit (red line) for H_2O_2 in cell free medium with zero addition of H_2O_2 are presented. Here, we set *Prochlorococcus* cell concentrations (P_i) to zero in Equation 2.1. In Figure 2.2 b, c we present histograms of our posterior samples, highlighting the mean and surrounding uncertainty of our model-based estimates of H_2O_2 supply rate, S_H , and decay rate, δ_H . Our data and model both showed rising

H₂O₂ concentration despite no H₂O₂ addition. This is consistent with prior studies indicating illuminated growth media produces H₂O₂ (Jeffrey Morris and Zinser 2013).

Using the same cell free model, we captured and represented H₂O₂ dynamics and parameter distributions for the 400nM H₂O₂ spiked medium (Figure 2.3). Our posterior samples for the abiotic production rate were slightly lower, but within a similar range to the zero-addition treatment in Figure 2.3. Across treatments, the decay rate was generally less than 0.05 day⁻¹. This hints to the model's capture of H₂O₂ dynamics that are agnostic of light like those mentioned in (Sutherland, Wankel, and Hansel 2020). Thus, our model captures H₂O₂ spike assay dynamics in cell-free culture conditions at multiple starting spike levels, providing an important constraint on the rate of H₂O₂ supply and decay in growth media. We use these basal parameter ranges to define background abiotic H₂O₂ dynamics when Equation 2.1 was considered in the presence of microbial cells.

PHYTOPLANKTON BATCH CULTURE DYNAMICS WITHOUT HYDROGEN PEROXIDE

Being now able to account for relevant abiotic processes, we next considered how microbial dynamics may be impacted by H₂O₂. Figure 2.4 shows our model trained and compared with *Prochlorococcus* strain MIT9215 in exponential growth.

The model provides a satisfactory fit to the population density change over time (Figure 2.4a) allowing retrieval of posterior samples of two cell growth-related parameters: the initial *Prochlorococcus* concentration (P_0 , Figure 2.4b) and the exponential growth rate (μ_i Figure 2.4c).

PHYTOPLANKTON BATCH CULTURE DYNAMICS WITH AMENDED HYDROGEN PEROXIDE

Having quantified *Prochlorococcus* growth without H₂O₂ addition, we then compared our model in Equations 2.1 with our microbial abundance and H₂O₂ concentration data. In Figure 2.5 below we present the dynamics of *Prochlorococcus* in 400nM spiked H₂O₂. Our results clearly indicate that *Prochlorococcus* died once H₂O₂ was added to growth medium (Figure 2.5a). We kept the previously quantified exponential growth rate μ_i (Figure 2.5c) steady from the model's initial fitting of *Prochlorococcus* in zero H₂O₂. Having trained our model on cellular

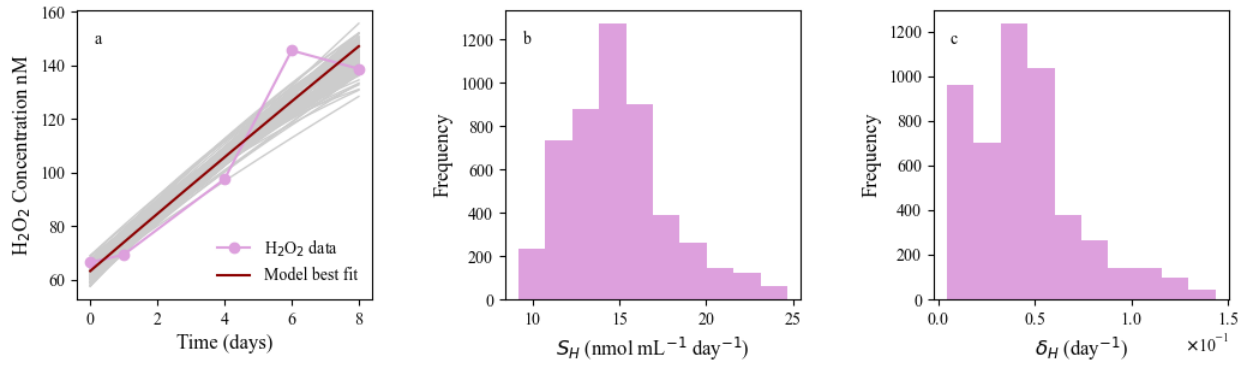


Figure 2.4 Abiotic growth media modeled in zero H_2O_2 spike

Model-data comparison for cell free media with zero H_2O_2 spike assay a) laboratory H_2O_2 measurements (connected pink symbols), model fit (red line), and model uncertainty (grey lines) b,c) histograms representing uncertainty in estimates of H_2O_2 supply rate (S_H , nmol mL⁻¹ day⁻¹) and decay (δ_H , day⁻¹).

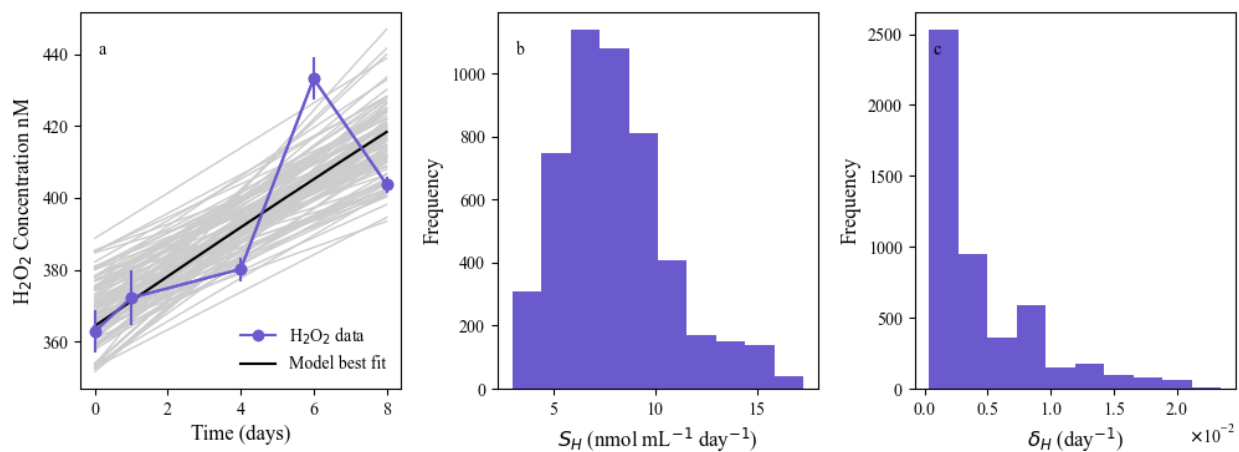


Figure 2.5 Abiotic spiked media modeled in 400nM H₂O₂ spike

Model-data comparison for cell free media with 400 nM H₂O₂ spike assay a) laboratory H₂O₂ measurements (connected blue symbols), model fit (black line), and model uncertainty (grey lines) b,c) histograms representing uncertainty in estimates of H₂O₂ supply rate (S_H , $\text{nmol mL}^{-1} \text{day}^{-1}$) and decay (δ_H , day^{-1}) respectively.

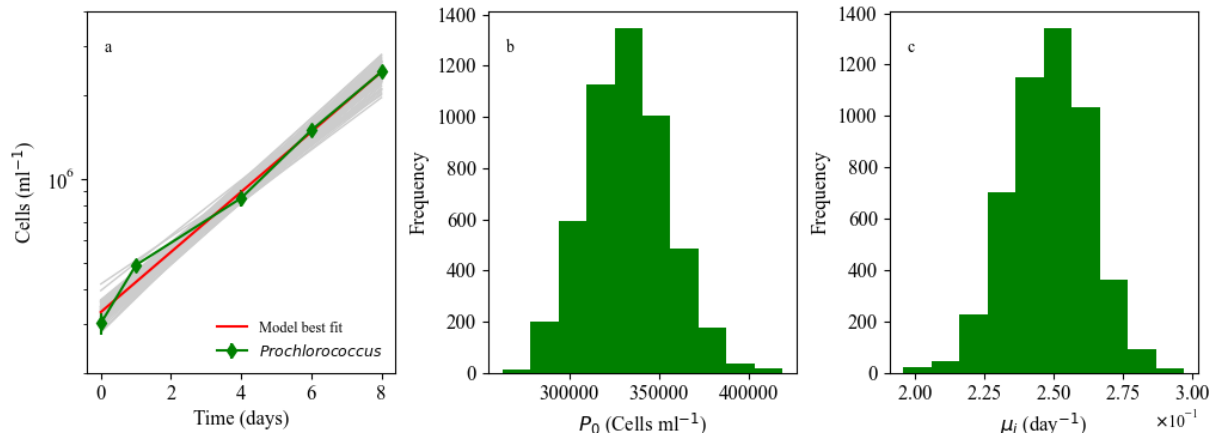


Figure 2.6 Monoculture *Prochlorococcus* dynamics modeled in growth media without additional H₂O₂. Laboratory batch culture dynamics of *Prochlorococcus* (green line) and model searches (grey lines) with best-fit solution (red line) with zero H₂O₂ addition (a). Also shown are parameter posterior distributions for the initial population size, P_0 (b) and the exponential growth rate, μ_i (c). *i.e.* The range of possible model solutions (grey) and best fit (red line) shown in section a are represented in the width and medians of parameter, P_0 in b and μ_i in c, value histograms.

media with the addition of a H_2O_2 spike, we can quantify two H_2O_2 -based parameters: the H_2O_2 specific rate of cell death, $\kappa_{dam,i}$ (Figure 2.5c), and the cell-specific rate of detoxification, $\phi_{max,i}$ (Figure 2.5f). Importantly, our model predicts vanishingly small values for *Prochlorococcus* $\phi_{max,p}$ in Figure 2.5f indicating negligible detoxification of exogenous H_2O_2 ($\phi_{max,p} \sim 0$). This tracks with previous laboratory studies that signified *Prochlorococcus* cannot grow alone in exogenous H_2O_2 above 400nM (Ma 2015; Morris 2011).

MODELING SYNECHOCOCCUS AND H_2O_2 DYNAMICS

We follow the same methodology from *Prochlorococcus* analyses to parameterize our *Synechococcus* monoculture models. Figure 2.6 shows the exponential growth dynamics of *Synechococcus* without H_2O_2 addition. Our model was able to adequately capture these dynamics (Figure 2.6a), allowing us to quantify the initial cell concentration (S_0) and the exponential growth rate (μ_i) (Figure 2.6 b, c). Unlike catalase-deficient *Prochlorococcus*, the catalase positive *Synechococcus* strain CC9605 grew well in the presence of H_2O_2 (Figure 2.7a). Using our model, we were also able to capture these different dynamics (Figure 2.7a for *Synechococcus* vs. Figure 2.5a for *Prochlorococcus*) allowing us to quantify the initial cell concentration (S_0) and a smaller H_2O_2 specific rate of cell death, $\kappa_{dam,i}$ (Figure 2.7 b,c). Similarly, we were also able to capture the concurrent dynamics of *Synechococcus* CC9605 cell growth and drawdown of H_2O_2 concentration (Figure 2.7d) as well as the parameters that represent the initial H_2O_2 concentration (H_0) and *Synechococcus* cell-specific rate of detoxification, $\phi_{max,s}$ (Figure 2.7e,f, respectively).

After noting the ability of our model to capture the divergent H_2O_2 phenotypes of catalase negative *Prochlorococcus* and a catalase positive *Synechococcus* cyanobacteria, we analyzed further monoculture strains. *Alteromonas*, a noted ‘contaminant’ microbe from *Prochlorococcus* strains (Biller, Coe, *et al.* 2015) was of particular interest due to prior reports of its ‘helper’ status in stressful environments (Biller, Coe, *et al.* 2018; Hennon *et al.* 2018; Morris *et al.* 2008). In Figure 2.8 we present the relationship between the H_2O_2 damage rates ($\kappa_{dam,s}$) and H_2O_2 detoxification rates ($\phi_{max,i}$) for microbes (i) in our data. The associated curves and parameter histograms for each model run are shown in Appendices A and B. Our coupled model framework finds that organisms with a high rate of cell-specific detoxification have low rates of cell death.

Simultaneous quantification of detoxification ability ($\phi_{max,i}$) and damage rate ($\kappa_{dam,i}$) trait parameters from different microbes allows us to capture the potential patterns between parameter value and ecological function leading to an idea of the H₂O₂ trait space available to ocean microbes using our model.

DISCUSSION

In this chapter we present an ODE based model of concurrent dynamics of H₂O₂ and microbial abundance which we parameterized using laboratory data. Our model adequately captured H₂O₂ dynamics over a range of ecologically relevant H₂O₂ inputs (spikes) and their differential impacts on closely related cyanobacteria. Our modeling allowed us to quantify two key parameters that describe the impact of H₂O₂ on cells ($\kappa_{dam,i}$) and the impact of detoxification on H₂O₂ concentration ($\phi_{max,i}$). Quantification of these parameters captured well-documented differential H₂O₂ impacts on phytoplankton cells. Catalase positive *Synechococcus* (*Prochlorococcus*' most closely related, but genomically and physically larger, family member) does not show the noted H₂O₂ toxicity sensitivity that most *Prochlorococcus* cultures do in laboratory assays (Chan 2008; Morris 2011; Calfee 2021).

Using the closely related but oppositely genotyped cyanobacteria *Prochlorococcus* and *Synechococcus*, along with known 'helper' and catalase containing heterotroph *Alteromonas*, we demonstrated our model's ability to capture the duality of divergent H₂O₂ detoxification phenotypes from environmentally important microbes using our model. Our ODE based approach allowed us to quantify parameters in a manner that also accounts for changes in H₂O₂ concentration over the course of the experiment. Because many marine ecosystem models (e.g. that of (Fasham, Ducklow, and McKelvie 1990)) are also based on differential equations, the parameters we have identified ($\kappa_{dam,i}$, $\phi_{max,i}$) can be used to evaluate the impacts of H₂O₂ on microbial community composition and carbon cycling within these more complex models.

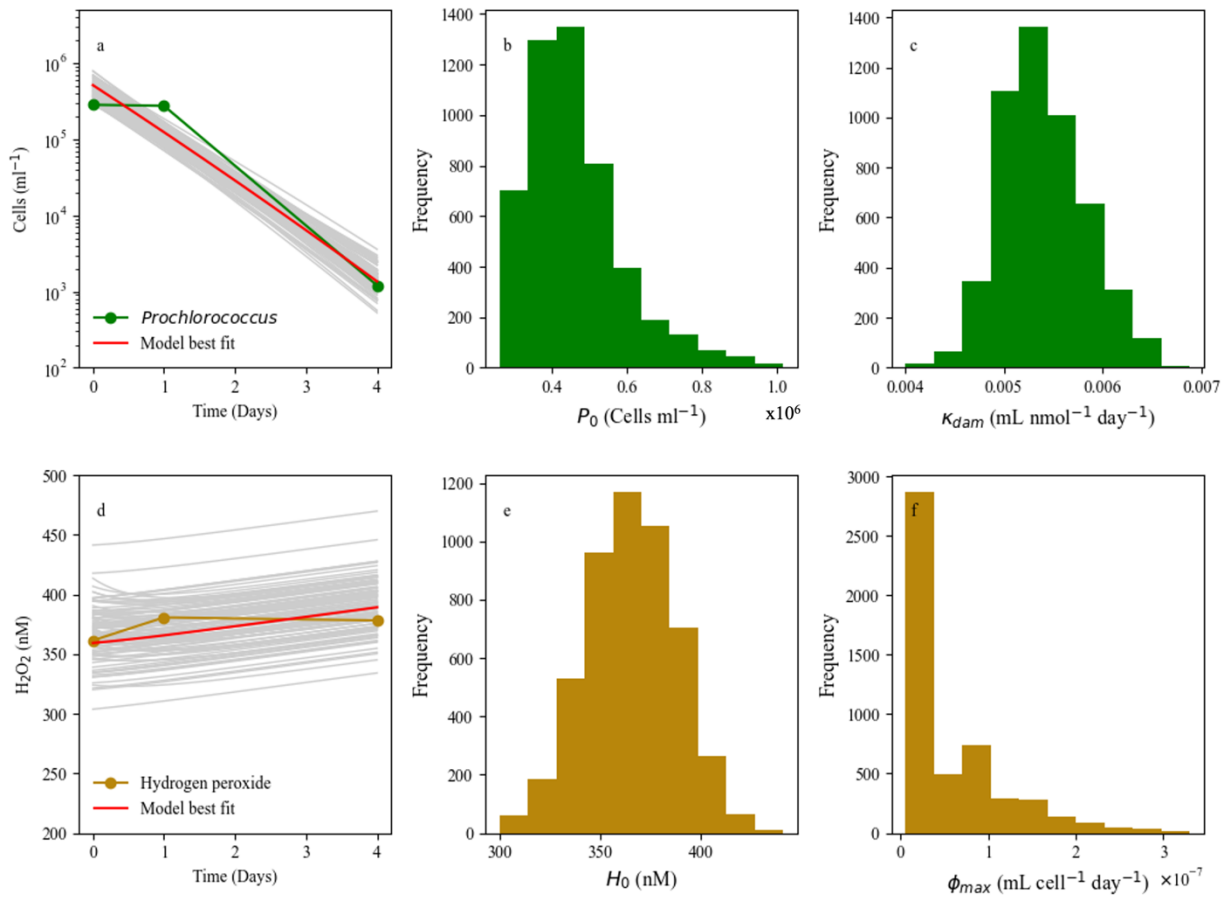


Figure 2.7 Monoculture *Prochlorococcus* modeled in H_2O_2 spiked media.

Coupled laboratory batch culture dynamics of *Prochlorococcus* (2.7a) and H_2O_2 (2.7d) data alongside model best-fit (red line) and searched (grey) combinations in laboratory assays containing 400nM H_2O_2 . To the right of each (P or H) line's dynamics plot, histograms of retrieved possible values for: the initial value of *Prochlorococcus*, P_0 (b) or H_2O_2 , H_0 (e) and the controlling parameter for each line's dynamics: $\kappa_{dam,p}$ for the damage rate of H on P (c) and $\phi_{max,p}$ for detoxification rate of H by P cells (e) are each shown.

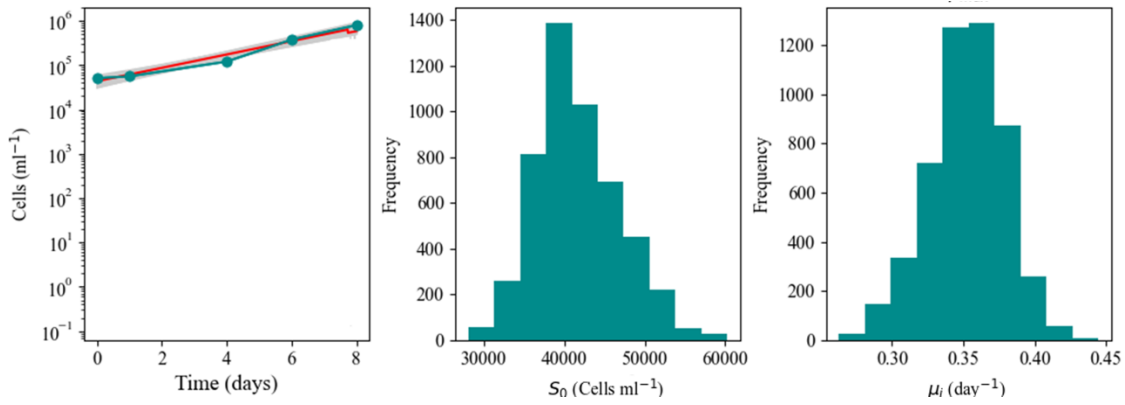


Figure 2.8 Monoculture *Synechococcus* CC9605 modeled in growth media

Laboratory batch monoculture dynamics of *Synechococcus* CC9605 data (blue line) and model searches (grey lines) with best-fit solution (red line) in growth media with zero additional H₂O₂ (a). Also shown are histograms displaying the values retrieved for initial cell concentration of *Synechococcus* (S_0) and exponential growth rate (μ_i) (b,c, respectively).

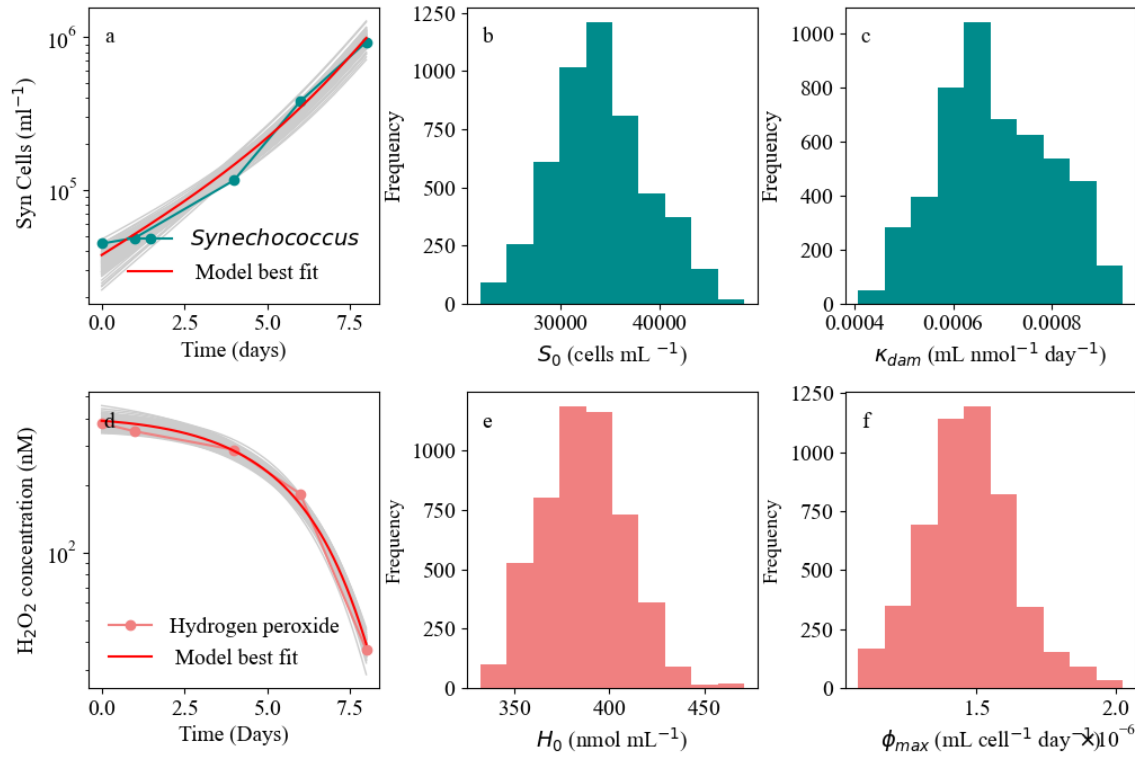


Figure 2.9 Monoculture *Synechococcus* CC9605 modeled in H_2O_2 spiked media.

Coupled laboratory batch culture dynamics of *Synechococcus* CC9605 (a) and H_2O_2 (d) data alongside searched parameter combinations (grey) in batch culture containing 400nM H_2O_2 . To the right of each (S or H) line's dynamics plot, histograms of retrieved possible values for: the initial value of *Synechococcus*, S_0 (b) or H_2O_2 , H_0 (e) and the controlling parameter for each line's dynamics: $\kappa_{dam,s}$ for the damage rate of H on S (c) and $\phi_{max,s}$ for detoxification rate of H by S cells (f) are each shown.

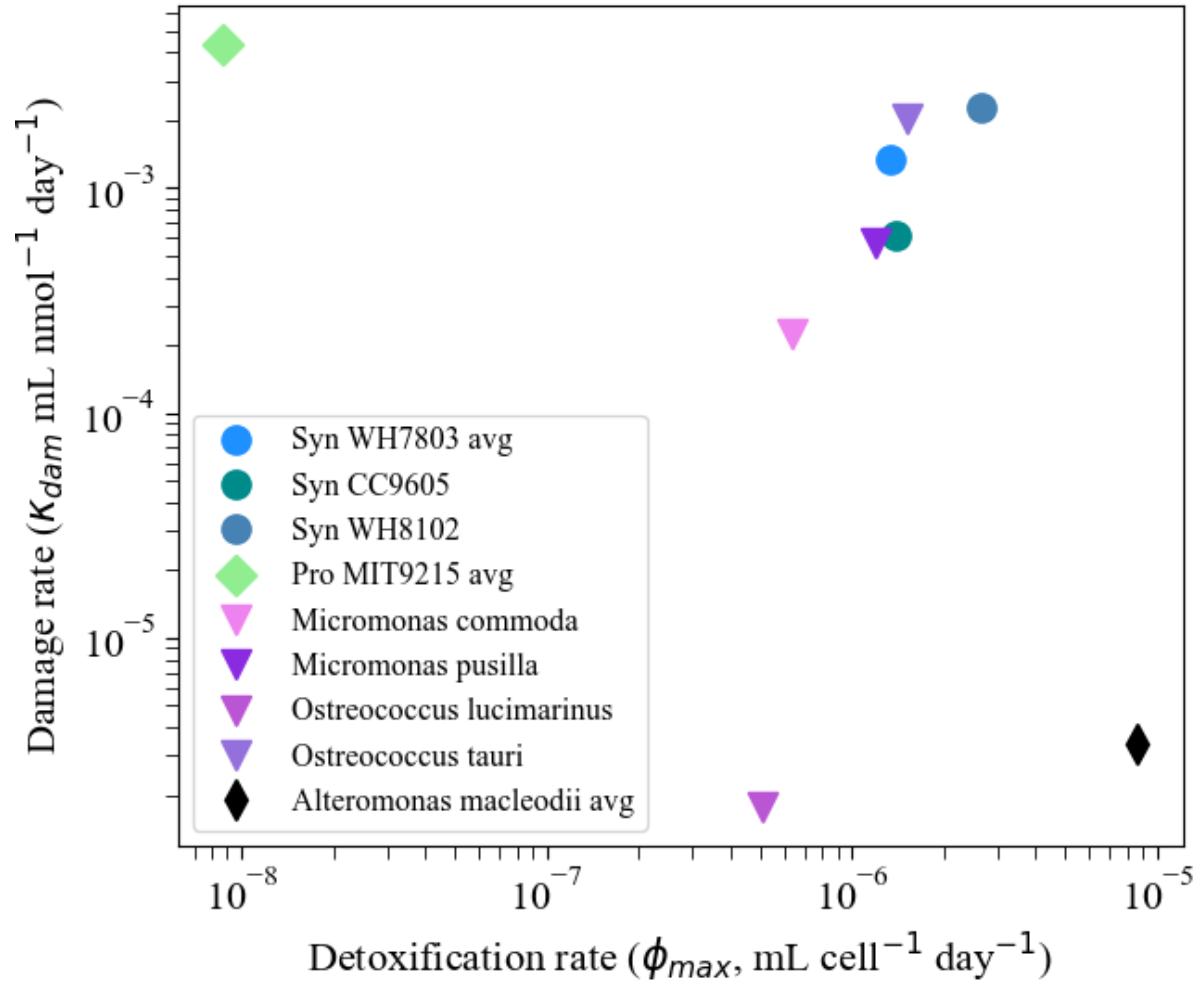


Figure 2.10 Recovered H₂O₂ trait parameters from different picoplankton during H₂O₂ spike assays. H₂O₂ detoxification rate ($\phi_{max,i}$, mL cell⁻¹ day⁻¹) and H₂O₂ induced rate of cell death ($\kappa_{dam,i}$, mL nmol⁻¹ day⁻¹) for different cyanobacterial (*Prochlorococcus* and *Synechococcus*), heterotrophic bacterial (*Alteromonas*), and picoeukaryotic (*Micromonas spp* and *Ostreococcus spp.*) monoculture batch cultures. ‘avg’ delineation indicates multiple monoculture data sets for this species were individually modeled and then averaged. Dynamic models showing the retrieval of these parameters can be found in Appendices A and B.

A limitation of our study is that the laboratory conditions do not fully replicate conditions cells experience in the open ocean. For example, the modeled experiments introduced single spikes of H_2O_2 which while consistent with rain inputs for surface waters (Willey, Paerl, and Go 1999; O’Sullivan *et al.* 1999), is different from the continual H_2O_2 photoproduction in natural systems that follow a diel cycle associated with changes in light and dissolved organic carbon (DOC) concentration (Zinser 2013; 2018b; Zhu *et al.* 2022). Use of chemical buffers to control H_2O_2 production (Zinser 2013) is one way to more closely mimic natural H_2O_2 production, and could be accounted for via alterations of our H_2O_2 production parameter S_H . To constrain our model with experimental data, we require synchronized time-dependent measurements of both H_2O_2 and cell abundance, which were only available in the spike assay data used here. Fitting our model to experiments with chemical buffer would provide a more realistic and general test of this framework. But we do not anticipate this would fundamentally change any of our main findings regarding detoxification and cell death parameters. Future models may wish to consider using diel photoproduced H_2O_2 with cell growth within chemostats to allow for analyses under nutrient restricted and nutrient-tracked assays. This would allow for more realistic estimates of *in situ* H_2O_2 and associated cell dynamics in oligotrophic surface waters where ammonium is often below detectible levels.

Relative to the cyanobacteria modeled here, many heterotrophic bacteria (see, Appendices A and B) such as *Alteromonas*, are known to detoxify H_2O_2 very effectively (Morris *et al.* 2011). Our modeling approach is readily extended to quantify cell-specific rates of detoxification among heterotrophic microbes known to grow with (Babić *et al.* 2017; Aharonovich and Sher 2016) and help (Morris *et al.* 2008; Morris 2011; Biller, Coe, *et al.* 2018; Hennon *et al.* 2018; Zinser 2018a). *Prochlorococcus* in the open ocean. It is unclear why the detoxification rate of *Alteromonas* is ten times higher than *Synechococcus*. *Prochlorococcus* and *Synechococcus* are on similar size ranges, however *Synechococcus* and *Alteromonas* both contain genes for catalase so a combination of allometry and genetic capability may be involved. Further, the ODEs used in our model account for changing cell density, but assume a fixed cell state, *i.e.* we do not account for changing transcription levels of detoxification genes that occur in microbial populations in response to increased reactive oxygen or other environmental pressures (Chan 2008; Cao *et al.* 2003; Kawasaki *et al.* 1997; Schellhorn 1995; Klanian and Preciat 2017;

Aharonovich and Sher 2016; Alonso-Sáez *et al.* 2023; Poretsky *et al.* 2009; Ottesen *et al.* 2014; Mulo, Sakurai, and Aro 2012).

This chapter focuses mainly on the inclusion of catalase in the genome as a binary defining trait. However, a wide array of H₂O₂ detoxification enzymes exist in nature (Tripathi, Bhatt, and Dietz 2009; Bengtsson-Palme *et al.* 2014; Ighodaro and Akinloye 2018). While peroxidases, specifically glutathione peroxidase, have been reported to show faster whole cell-H₂O₂ detoxification rates than isolated catalase rates, it is generally accepted that catalase is the most efficient H₂O₂ detoxification enzyme available to aerobic organisms (Nicholls 2012; Ohno and Gallin 1985). This, along with the genetic closeness of *Prochlorococcus* and *Synechococcus* and stark differential peroxide susceptibility (Morris 2011) led to catalase being the focus of this first presentation of our model. However with catalase and peroxidase showing different tolerances to temperature stress (Omran 1980), differentiation between primary, secondary, and tertiary detoxification mechanisms (Ighodaro and Akinloye 2018) may be important for modeling future ocean microbes (Ma 2015). Further training of our model with data from a wider set of taxonomic groups and relevant detoxification genes would expand the ecological relevance of our modeling framework.

The batch culture conditions used for our laboratory cultures are not reflective of the nutrient limiting conditions prevalent in the oligotrophic systems where *Prochlorococcus* thrives. However, it is difficult to lower nitrogen enough to assess relevant *in situ* *Prochlorococcus* low nitrogen conditions (Masuda *et al.* 2023; Calfee 2021) and global microbial modeling framework (Dutkiewicz, Follows, and Bragg 2009), for which these ODEs were developed, incorporates nutrient fluxes on worldwide inputs. Applying our model to *Prochlorococcus* growing in chemostat conditions – where nutrients are more likely to be limiting - under a range of nitrogen inputs would be a powerful additional test of its applicability. Regardless, the authors do not anticipate that this would qualitatively modify the main finding that catalase positive cyanobacteria tend to have far higher rates of cell specific detoxification than *Prochlorococcus* and have significantly diminished rates of cell death associated with H₂O₂ damage.

Continued excretion of dissolved organic carbon (DOC) by *Prochlorococcus* (Calfee, Glasgo, and Zinser 2022; Grossowicz *et al.* 2017; Roth-Rosenberg *et al.* 2021; Ofaim *et al.*

2021) may augment H₂O₂ production from light (Zhu *et al.* 2022) and other sources. Our model did not explicitly resolve the sensitivity of H₂O₂ production to DOC concentration, but this did not prevent it from providing a satisfactory description of the dynamics of H₂O₂ through batch culture experiments. However, DOC mediated production of H₂O₂ is likely to be an important process in any environmentally relevant model of H₂O₂ dynamics. Further analysis of H₂O₂ production in spike assays (Calfee 2021) and chemical buffers or natural DOC (Zinser 2013; Morris 2011) may elucidate and constrain differences between laboratory and ocean observations. Light and DOC are both currently incorporated within the a global plankton modeling framework (Dutkiewicz, Follows, and Bragg 2009), thus both factors can be incorporated into our model, providing an avenue for further investigation of their importance and impact on microbial dynamics.

Despite its limitations, our model is trained with laboratory data and can quantitatively constrain physiological rate constants. We find that very closely related cyanobacteria displayed a wide range of detoxification rates, indicating this is an evolutionarily variable trait, even among close genera. *Synechococcus* strains are usually less contaminated (Chan 2008; Morris *et al.* 2008) with H₂O₂-detoxifying heterotrophs when isolated than *Prochlorococcus* (Morris *et al.* 2008; Chisholm *et al.* 1988; Moore *et al.* 2007), which tracks with Black Queen Logic, and suggests *Synechococcus* does not require helpers to grow *in situ* (Morel *et al.* 1993).

Our model provides a basis on which to explore the mechanisms driving these interdependencies now and in future oceans. Climate change is changing carbon and nitrogen flux of the ocean (Friedlingstein *et al.* 2006; Sokolov *et al.* 2009; Beman *et al.* 2011; Le Quéré *et al.* 2009) while altering marine temperature (Fu *et al.* 2007) and precipitation (Hutchins and Fu 2017) inputs which directly affect H₂O₂ concentrations (Morris, Rose, and Lu 2022; Zhu *et al.* 2022; Zinser 2018b; Ahmad *et al.* 2015; Blough and Zepp 1995; Vaughan and Blough 1998). Already, noted plankton groups are projected to differ in the future marine environment (Carey *et al.* 2012; Henson *et al.* 2021; Dutkiewicz *et al.* 2015; Knight and Morris 2020; Beman *et al.* 2011). We anticipate that quantification of H₂O₂-detoxification and susceptibility in multiple catalase positive and negative plankton groups will help future analyses exploring the impacts of multiple stressors oligotrophic microbial communities experience in a changing world.

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Chapter 3 EVALUATION OF H_2O_2 AS A COMMUNITY
COMPOSITION MODERATOR WITHIN OLIGOTROPHIC
PLANKTON DYNAMICS

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Author contribution statements: Written and edited by D.K.M. with feedback from D.T.

ABSTRACT

Prochlorococcus the smallest known free-living autotroph, and *Synechococcus*, the closest extant relative known to *Prochlorococcus* perform approximately 50% of marine carbon fixation. Yet laboratory experiments indicate that streamlining-induced loss of vital hydrogen peroxide (H_2O_2), a type of harmful reactive oxygen species, detoxication genes has led to *Prochlorococcus* requiring ‘helper’ microbes to survive the daily environmental H_2O_2 produced in surface waters. The oligotrophic ocean contains high concentrations of H_2O_2 due to photoproduction via pigmented detrital material as well as rain events. *Prochlorococcus* is the most numerically abundant photosynthetic organism in the oligotrophic ocean and is estimated to perform half of the carbon fixation there, yet it cannot live in monoculture at the level of H_2O_2 that accumulates abiotically in a single diel cycle of its natural environment. *Synechococcus* shows marked differences in recovered H_2O_2 monoculture batch assays parameter (See Chapter 2 work). These paradoxical controls over *Prochlorococcus* community dominance are the focus of Chapter 3. We investigate the effect of divergent H_2O_2 phenotypes on modeled phytoplankton community structure under a single nutrient competition framework. We build upon accepted nutrient competition theories and known H_2O_2 impacts on two autotroph species, one catalase negative, P (*Prochlorococcus* analogue), and one catalase positive, S (*Synechococcus* analogue) are used to investigate microbial population composition available to a community level under different ammonium (N) or H_2O_2 (H) conditions. We show that selective resistance to a community threat in microbial populations confers competitive advantages depending on the environmental conditions. We recover distinct phytoplankton assembly predictions for oligotrophic growth when considering two key H_2O_2 model parameters. The tradeoff between growth advantage in low N and higher H_2O_2 detoxification rate controls the toggle between competitive and coexistent behavior in this P, S system. Interestingly, leaky detoxification from *Synechococcus* alone only allows for coexistence at high N or low H supply rates. Bacterial detoxification along with *Synechococcus* detoxification H_2O of was needed in this model framework to recover *Prochlorococcus* and *Synechococcus* coexistence on a single N within ecologically relevant oligotrophic low N or H supply rates. This competition model provides clarity on scenarios in which H_2O_2 exerts ecological pressure on globally important microbes.

INTRODUCTION

Phytoplankton, through their growth and death dynamics, control the nutrient flows and subsequent composition of the community determines the way in which nutrients cycle through a marine system (Behrenfeld *et al.* 2005; Mullin, Sloan, and Eppley 1966; Saba *et al.* 2010). The oligotrophic oceans, while low in nutrient influx both from continents or deep sea (Altabet, Robinson, and Walstad 1986; Dong *et al.* 2022; Karl 1999), supports a diverse range of plankton (Visintini, Martiny, and Flombaum 2021; Buitenhuis *et al.* 2012; Partensky *et al.* 1996; Partensky, Blanchot, and Vaultot 1999; Venrick 1982). *Prochlorococcus*, first isolated in the 1980's (Chisholm *et al.* 1988) has since been shown to dominate oligotrophic surface waters (Partensky *et al.* 1996; Partensky, Hess, and Vaultot 1999) and grow alongside its closest extant relative *Synechococcus* (Partensky, Blanchot, and Vaultot 1999; Rabouille, Edwards, and Zehr 2007). Efficiency of nutrient usage due to genomic and physical streamlining is touted as allowing *Prochlorococcus* to dominate (Read *et al.* 2017; Giovannoni *et al.* 2005; Giovannoni, Cameron Thrash, and Temperton 2014; Beaulieu *et al.* 2008) over sister genera while usage of ammonium over nitrate (Rippka *et al.* 2000) as a nitrogen source has been shown to allow coexistence between *Prochlorococcus* and *Synechococcus in situ* (Morris *et al.* 2008; Rippka *et al.* 2000). Despite their prevalence, *Prochlorococcus* was historically difficult to culture (Rippka *et al.* 2000) until diluted helper bacteria, formally considered 'contaminants,' were found to help *Prochlorococcus* grow when added back to *Prochlorococcus* in dilute coculture (Morris *et al.* 2008; Rippka *et al.* 2000). This 'help' was shown to come in the form of environmental H₂O₂ drawdown via detoxification by whole cells, pyruvate, or exogenous purified catalase (Morris *et al.* 2008; Ma 2015; Zinser 2018a).

Prochlorococcus's loss of catalase-peroxidase (Chelikani, Fita, and Loewen 2004; Alfonso-Prieto *et al.* 2009) due to genomic streamlining and reduction renders it vulnerable to H₂O₂; as little as 400 nM H₂O₂ spike is lethal in monoculture, which is the equivalent of half a day's abiotically accumulated H₂O₂ concentration in *Prochlorococcus*'s native, surface oligotrophic, environment (Morris 2011; Ma *et al.* 2018). In contrast *Synechococcus*, a catalase containing sister genus, does not share this H₂O₂ sensitive phenotype (Morris *et al.* 2016). Ambient marine H₂O₂ rests under 200 nM H₂O₂, below the demonstrated lethal limit for *Prochlorococcus* (Zinser 2013; Powers and Miller 2014). This is despite daily photochemical

H₂O₂ production from DOC (Zhang, Del Vecchio, and Blough 2012; Willey, Paerl, and Go 1999; Avery *et al.* 2005; Goldstone and Voelker 2000) and periodic rain events increasing ROS production rates (Willey, Paerl, and Go 1999; Morris, Rose, and Lu 2022; Harvey 1925). Thus something in the ocean is constantly removing this H₂O₂. A growing host of literature (Morris, Rose, and Lu 2022; Zinser 2018a; Ma *et al.* 2018; Knight and Morris 2018) has shown that H₂O₂ is both important and differentially constraining to community members in open ocean systems.

Our previous work (Chapter 2) in monoculture analyses quantified observed H₂O₂ susceptibilities in *Prochlorococcus* and *Synechococcus* parameters representing detoxification and damage (Figure 3.2). Membrane permeability of H₂O₂ defines the damage and detoxification of it as leaky functions. Meaning the benefits of the functions are available to organisms other than the creator of the function within the system (Morris 2015). Leaky functions have been shown to alter the competitive advantage of microbes in the system, allowing for coexistent rather than exclusive behaviors (Adkins-Jablonsky *et al.* 2021; Morris, Lenski, and Zinser 2012). Functions done within the cell that do not create or catabolize diffusible products generally are non-leaky. Non-leaky functions seem evolutionary more advantageous for strict microbial competition scenarios, especially in diffuse environments; with the noted selection against cheaters, or those taking advantage of other's leaky functions, like iron siderophore production (Manck *et al.* 2022; Butaitė *et al.* 2017). Cheater microbes are selected against due to lack of a necessary function, but survive due to the lower cost living while successfully cheating off of others that have this function (Allison 2005; Morris, Papoulis, and Lenski 2014; Butaitė *et al.* 2017; Adkins-Jablonsky *et al.* 2021). Nutrient restricted environments select for the leakiness of in some functions (Sexton and Schuster 2017) and the presence of leaky functions may significantly impact community competition and community physical structure (Estrela, Morris, and Kerr 2016; Wang *et al.* 2021). H₂O₂ detoxification is notably among the leaky function group and has been experimentally shown to augment microbial interactions (Omar *et al.* 2022; Morris 2015; Zinser 2018a; Kim *et al.* 2016; 2021; Purkan *et al.* 2010). In this Chapter, we add to this study through a computational analysis of how the leakiness of H₂O₂ detoxification and impacts microbial community structure using previously (Chapter 2) recovered *Prochlorococcus*, *Synechococcus*, and heterotrophic bacteria H₂O₂ parameter values.

While both *Prochlorococcus* and *Synechococcus* grow in the oligotrophic gyres, they differ markedly in predicted biogeography (Flombaum *et al.* 2013). They differ both in temperature optima as well as depth profiles due to nutrient availability (Partensky, Blanchot, and Vaulot 1999). Both genera are composed of large pangenomes (Delmont and Eren 2018; Ahlgren and Rocap 2012; Sohm *et al.* 2016; Babić *et al.* 2017) that are divided into coexistent ecotypes (Garczarek *et al.* 2007; Sohm *et al.* 2016), with these microbial sisters grouped into traits regarding ammonium (N) and H₂O₂ (H). *Synechococcus* generally can access more different nitrogen species than *Prochlorococcus* (Moore *et al.* 2002) yet *Prochlorococcus*'s streamlined size is accredited with its superior nutrient affinity for ammonium (Bentkowski, Van Oosterhout, and Mock 2015; Garcia-Fernandez, de Marsac, and Diez 2004). The genetic differentiation of utilization capabilities in *Prochlorococcus* and *Synechococcus* have been well documented, however it was recently, 2023 with Masuda *et al.*, that it was quantitatively confirmed that *Prochlorococcus* and *Synechococcus* strains may coexist by using ammonium or nitrite/nitrate as their main nitrogenous nutrient in the oligotrophic surface ocean (Masuda *et al.* 2023). However, this, and prior, global, models of *Prochlorococcus* fail to incorporate the documented differences in *Prochlorococcus* and *Synechococcus* regarding H₂O₂ in laboratory cultures (Morris *et al.* 2011). A regime based solely on differential nutrient utilization is neglectful of other known differences between *Prochlorococcus* and *Synechococcus*, particularly their differential H₂O₂ responses, which have been extensively documented experimentally in laboratory culture.

Our previous (Chapter 2) model used a simple Monod-based representation of H₂O₂ dynamics to quantify H₂O₂ effects on *Prochlorococcus*, *Synechococcus*, and heterotrophic bacterial 'helper.' Though found almost ubiquitously with *Prochlorococcus* (Partensky, Hess, and Vaulot 1999; Flombaum *et al.* 2013), *Synechococcus* is not the most effective helper of *Prochlorococcus*; a picobacterial heterotroph, *Alteromonas*, provides more effective H₂O₂ detoxification. Thus, *Synechococcus* detoxification efficacy and its contribution as a marine community impactor is uncertain. The interesting tradeoff between the differential ammonium affinity and H₂O₂ detoxification of *Prochlorococcus* and *Synechococcus* has been implicated in allowing for coexistence between these two organisms (Morris, Lenski, and Zinser 2012; Morris 2015). Using monoculture parameters isolated in Chapter 2, we assess the quantitative intuition

for how protection against H_2O_2 , along with resource competition, may influence *Prochlorococcus* and *Synechococcus* within an *in silico* competition framework. Competition and cooperation are notoriously difficult to detangle, and the fact that interrelatedness of organism can either hinder or help cooperation activities further muddles microbial studies (West *et al.* 2006; Wiens 1977; Beckett and Weitz 2017). Armstrong and McGhee (1980) as well as Tilman (1982) showed that multiple species can survive on one resource with additional external forcings from the environment (Armstrong and McGhee 1980; Tilman 1982). With the relative closeness of *Prochlorococcus* and *Synechococcus*, we expect them to operate similarly in a nutrient model as they share a fair amount of base cell machinery for processing the nutrients that they can both acquire (Garcia-Fernandez, de Marsac, and Diez 2004; Baer *et al.* 2017). Bacterial helper interactions are initially ignored to evaluate *Synechococcus* only effects on *Prochlorococcus* biogeography. The model is first used to investigate how P and S, *Prochlorococcus* and *Synechococcus* analogous in our *in silico* analyses, would compete for a single nutrient with the previously determined (Chapter 2) H_2O_2 susceptibilities. Second, we consider how H_2O_2 damage impacts P and S as inhibition of one species via ‘external lethal inhibitor’ that does not affect the other species has been found to promote stable coexistence on a single nutrient when another other species is not susceptible (Bar *et al.* 2020; Tilman 1982; Lenski and Hattingh 1986).

We then include bacterial helper interactions, with *Alteromonas* as a model heterotrophic bacterial helper, ‘B’, (Biller, Coe, *et al.* 2015; Hennon *et al.* 2018), to evaluate how a two vs. three microbe community impacts the outcomes of a single nutrient system. Using appropriate model forcings to explore environmental and biological stimuli, differential stable community structures in oligotrophic systems are recovered. *Alteromonas* is a prevalent (García-Martínez *et al.* 2002; Ivars-Martínez *et al.* 2008) and an active member of the marine community (Henríquez-Castillo *et al.* 2022). It is equipped to survive H_2O_2 with an active copy of catalase (Morris *et al.* 2016; Wietz *et al.* 2022) as well as different metabolism and iron acquisition (iron is a common base metals of H_2O_2 cycling enzymes (Chelikani, Fita, and Loewen 2004; Ighodaro and Akinloye 2018)) than *Prochlorococcus* or *Synechococcus*. Thus, how bacterial detoxification affects competition between the two cyanobacteria for a single nutrient is also evaluated. Figure 3.1 described the overall competitive framework used to evaluate the possible ramifications of

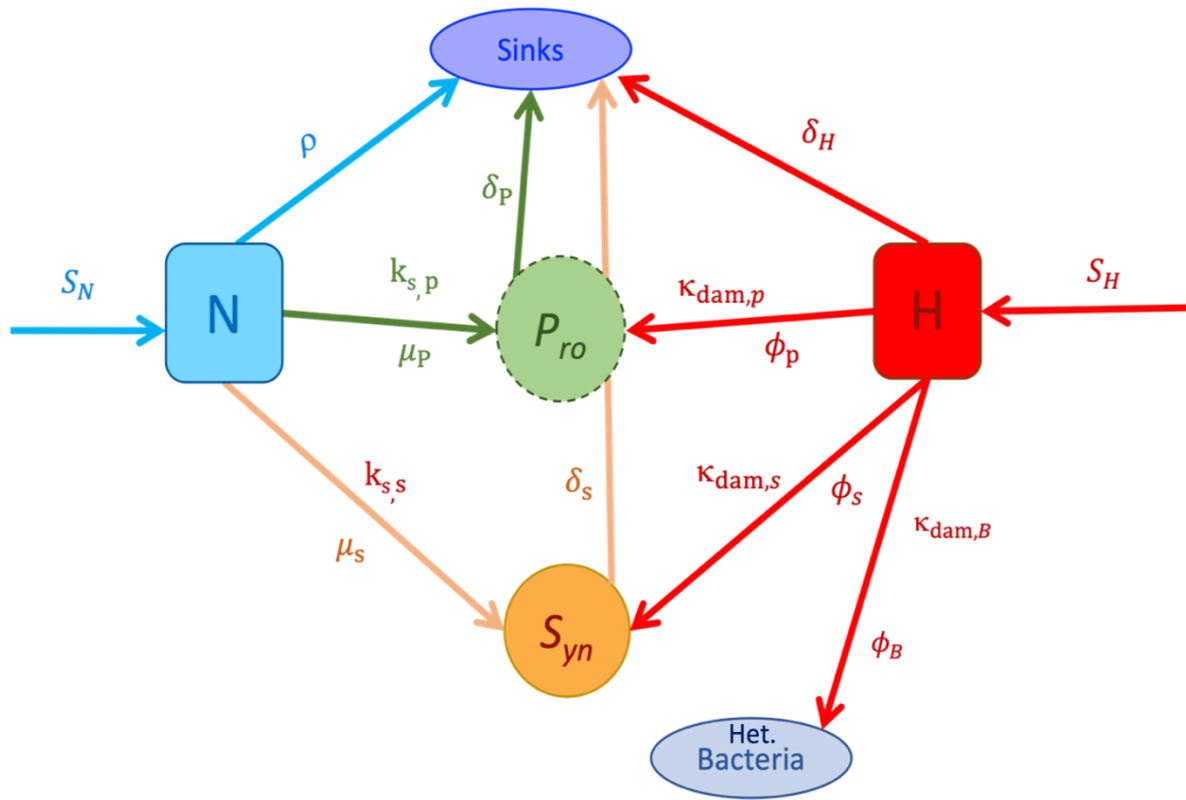


Figure 3.1 Parameters impacting *Prochlorococcus* and *Synechococcus* growing on ammonium in H₂O₂. Schematic showing overall model formulation of the ODEs shows two microbes, *Prochlorococcus* (Pro) and *Synechococcus* (Syn) grow from a singular ammonium pool (N) with external H₂O₂ (H) production. The heterotrophic bacterial helper (B) does not compete for ammonium here. Parameters (symbols) control the flux (arrows) between measurable pools of P, S, N, and H. Differential inclusion of H₂O₂ parameters within this framework allows this model to evaluate how 4 scenarios (cases) impact the competitive advantage of these microbes over one another (a microbial Thunderdome).

differential H₂O₂ trait occurrence within a nutrient competition framework based on parameters from monoculture assessment of *Prochlorococcus* and *Synechococcus*.

METHODS

We developed a coculture model that builds from Chapter 2 parameters but adds source and basal sink terms to N, representing ammonium. This ammonium, which both *Prochlorococcus* and their close relative *Synechococcus* can access at differing degrees (Garcia-Fernandez, de Marsac, and Diez 2004; Masuda *et al.* 2023), serves as the only N term in this system. This single nutrient system, along with only two plankton with different ammonium (and eventually H₂O₂ traits) allows for the examination of growth and detoxification tradeoffs in a simple yet quantitative way. Thus, allowing for the analysis of *Prochlorococcus* and *Synechococcus* dynamics from a community lens while isolating the influences of H₂O₂ and nitrogen on this community. Because this model was built in a stepwise manner, this took only slight augmentation of monoculture parameters to bring new control to its ability for hypothesis testing. Using three cases of a competition model, we assess how the parameters modeled in monoculture may influence long term community structure within a two - microbe system. Specifically, cases evaluate if *Prochlorococcus* H₂O₂ damage is deadly *in situ*, if said damage is ameliorated by *Synechococcus* and what bacterial detoxification adds to that of *Synechococcus* in a community situation.

MODEL

A competition system (Dynamic Model Equation Set: Equations 3.1-3.5) of ordinary differential equations (ODEs) is used to model the population dynamics of cyanobacteria in a range of H₂O₂ conditions. Special cases of this full system of ODEs were used to show differential community permutations possible from environmentally relevant (Chapter 2) parameters, visualized in Figure 3.2, inclusion according to our methods schematic (Figure 3.1). All computer code is provided in a GitHub repository:

https://github.com/DKMcCullough/Pro_vs_Syn.

Dynamic Competition Model Equation Set

$$\frac{dN}{dt} = \underbrace{\lambda_N}_{\text{Abiotic N supply}} - \underbrace{\delta_N N}_{\text{abiotic N decay}} - \underbrace{\frac{\mu_{P_i} N}{N + k_{S_{P_i}}} P_i}_{\text{P uptake/use of N}} - \underbrace{\frac{\mu_{S_i} N}{N + k_{S_{S_i}}} S_i}_{\text{S uptake/use of N}} \quad (3.1)$$

$$\frac{dH}{dt} = \underbrace{\lambda_H}_{\text{H}_2\text{O}_2 \text{ supply}} - \underbrace{\delta_H H}_{\text{abiotic decay}} - \underbrace{\phi_{S_i} S_i H}_{\text{detox via Syn}} - \underbrace{\phi_{B_i} B_i H}_{\text{detox via Bacteria}} \quad (3.2)$$

$$\frac{dP_i}{dt} = \underbrace{\frac{\mu_{P_i} N}{N + k_{S_{P_i}}} P_i}_{\text{Growth of P on N}} - \underbrace{\delta_{P_i} P_i}_{\text{innate cell death}} - \underbrace{k_{\text{dam}_{P_i}} P_i H}_{\text{H}_2\text{O}_2 \text{-based cell loss}} \quad (3.3)$$

$$\frac{dS_i}{dt} = \underbrace{\frac{\mu_{S_i} N}{N + k_{S_{S_i}}} S_i}_{\text{Growth of S on N}} - \underbrace{\delta_{S_i} S_i}_{\text{innate cell death}} \quad (3.4)$$

$$\frac{dB_i}{dt} = \underbrace{\mu_{B_i} B_i - \delta_{B_i} B_i}_{\text{Growth and death of heterotrophic bacteria}} \quad (3.5)$$

The dynamics of a dissolved ammonium (N , nmol mL^{-1}), catalase negative or positive phytoplankton analogues (*Prochlorococcus* (P_i , cells mL^{-1}) and *Synechococcus* (S_i , cells mL^{-1})) as well H_2O_2 (H , nmol mL^{-1}) were modeled with a composite set of coupled ordinary differential equations (3.1-5) shown above. N is supplied at a rate represented by λ_N (or S_N) and has an abiotic depletion rate represented by δ_N . The biotic depletion of N through uptake and growth is based on Michaelis-Menten (Michaelis and Menten 1913) kinetics with μ , μ_{P_i} , representing the maximum growth rate, and the ratio of N uptake/affinity rate α (α_{P_i}), and μ_{P_i} , as the saturation constant ks_i (Monod 1949; Michaelis and Menten 1913) for both *Prochlorococcus* and *Synechococcus* ODEs. The innate death rate of either cell type in in batch culture, represented as $\delta_{P,i}$ and $\delta_{S,i}$, was set to basal rate of 0.2 per day to encapsulate natural cell death, grazer, and viral predation in culture. (See monoculture dynamics in growth media for *Synechococcus* and *Prochlorococcus* in Chapter 2 for further δ information.) For our *in silico* analysis this δ represents not only death found in monoculture laboratory assays but death imposed from sources not quantified here *i.e.* viral loss, grazer predation, toxin effects. However, a fully combined death term is yet unquantified *in situ*, but literature shows that viruses and grazers split death evenly (J A Fuhrman and Noble 1999). Thus, prior modeling in Chapter 2 and literature review guides this choice. The abiotic H flux is controlled by λ_H (or S_H), the supply rate of H , and δ_H , loss rate of H while $\phi_{\max,s}$, represents the biotic degradation in our system of H via S (or B) according to the detoxification ability of each plankton (i) captured in the $\phi_{\max,i}$ parameter on Figure 3.2. $k_{\text{dam},P}$ serves here as a H_2O_2 - specific cellular damage/loss rate to our cell population and is implemented on P according to the values on Figure 3.2 patterns. $\phi_{\max,P}$ is negligible here and set to 0 for better constraint on S (*Synechococcus*) impacts on H pools through detoxification. These parameters are explored in ‘non-leaky’ and ‘leaky’ cases of the model described below.

EXPERIMENTAL APPROACH

A fictional set of ‘Non-leaky’ H_2O_2 detoxification cases are set up. This is done with either supply of H_2O_2 (λ_H or S_H) as zero or non-zero. $S_H = 0$ or $S_H > 0$ competition outcomes of both cases were evaluated using the *Prochlorococcus* (P) and *Synechococcus* (S) analogues’ survival patterns after 1 year under either environmental (S_H) regime. In both scenarios, P and S microbes are specialized for their own survival. P is given a greater nutrient uptake (α_i) value but

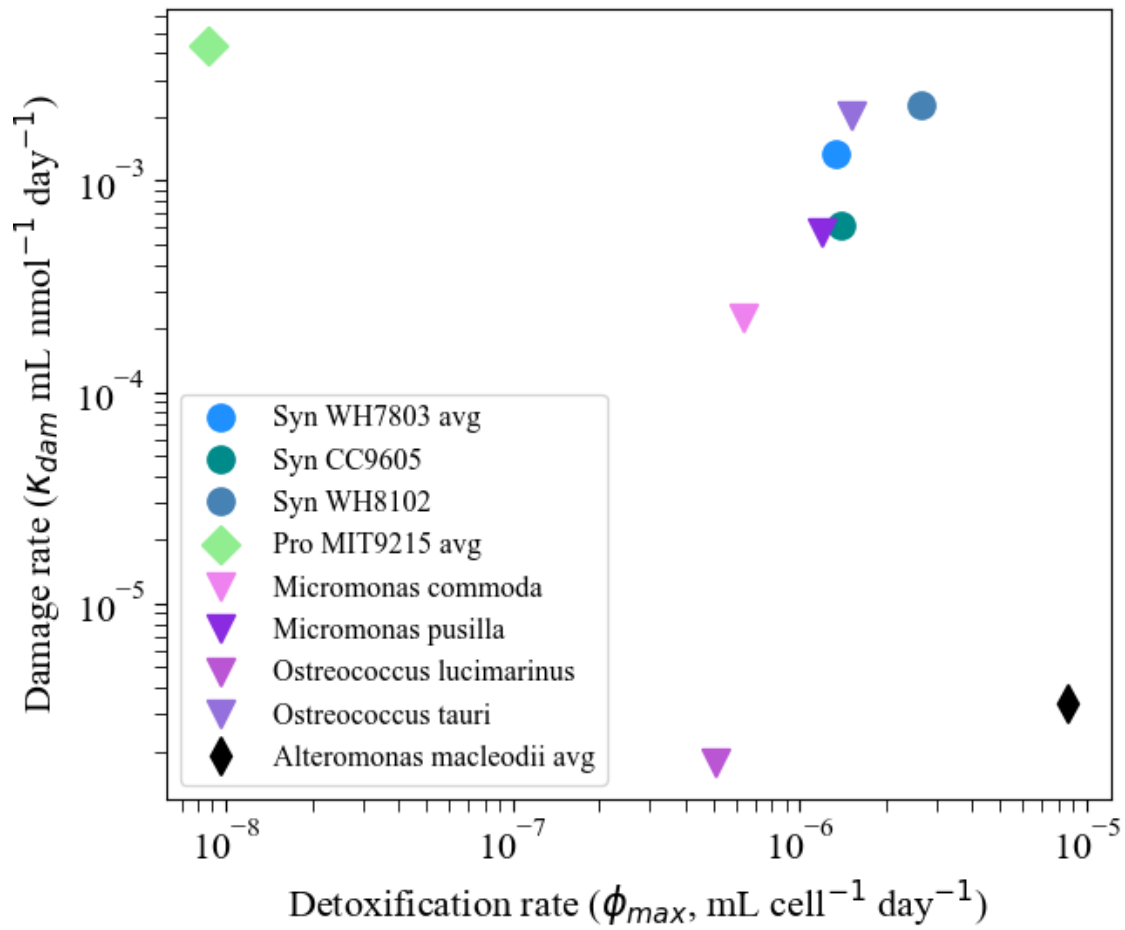


Figure 3.2 Quantitatively recovered tradeoffs found in the coupling of H₂O₂ detoxification rate ($\phi_{max,i}$, mL cell⁻¹ day⁻¹) and H₂O₂ induced rate of cell death ($\kappa_{dam,i}$, mL nmol⁻¹ day⁻¹) for different plankton (i) grown in monoculture batch conditions. Each symbol represents a single plankton species, i.

Table 2 Competition model parameter and variable definitions and units

Symbol	Description	Units
μ_i	Max cell growth rate of plankton, i (S,P or B)	day ⁻¹
δ_i	Innate death rate of plankton, i (S, P, or B)	day ⁻¹
$\kappa_{dam,i}$	Hydrogen peroxide specific rate of cell death	mL nmol ⁻¹ day ⁻¹
λ_N or S_N	Nutrient supply rate	$\mu\text{mol mL}^{-1} \text{ day}^{-1}$
ρ	Abiotic nutrient loss rate	$\mu\text{mol mL}^{-1} \text{ day}^{-1}$
λ_H or S_H	Hydrogen peroxide supply rate	nmol mL ⁻¹ day ⁻¹
δ_H	Abiotic hydrogen peroxide decay rate	day ⁻¹
$\phi_{max,i}$	Cell specific rate of H ₂ O ₂ detoxification	mL cell ⁻¹ day ⁻¹
P_i	Cell abundance of <i>Prochlorococcus</i> analogue	cells mL ⁻¹
S_i	Cell abundance of <i>Synechococcus</i> analogue	cells mL ⁻¹
B_i	Cell abundance of heterotrophic bacteria analogue	cells mL ⁻¹
H	Hydrogen peroxide concentration	nmol mL ⁻¹

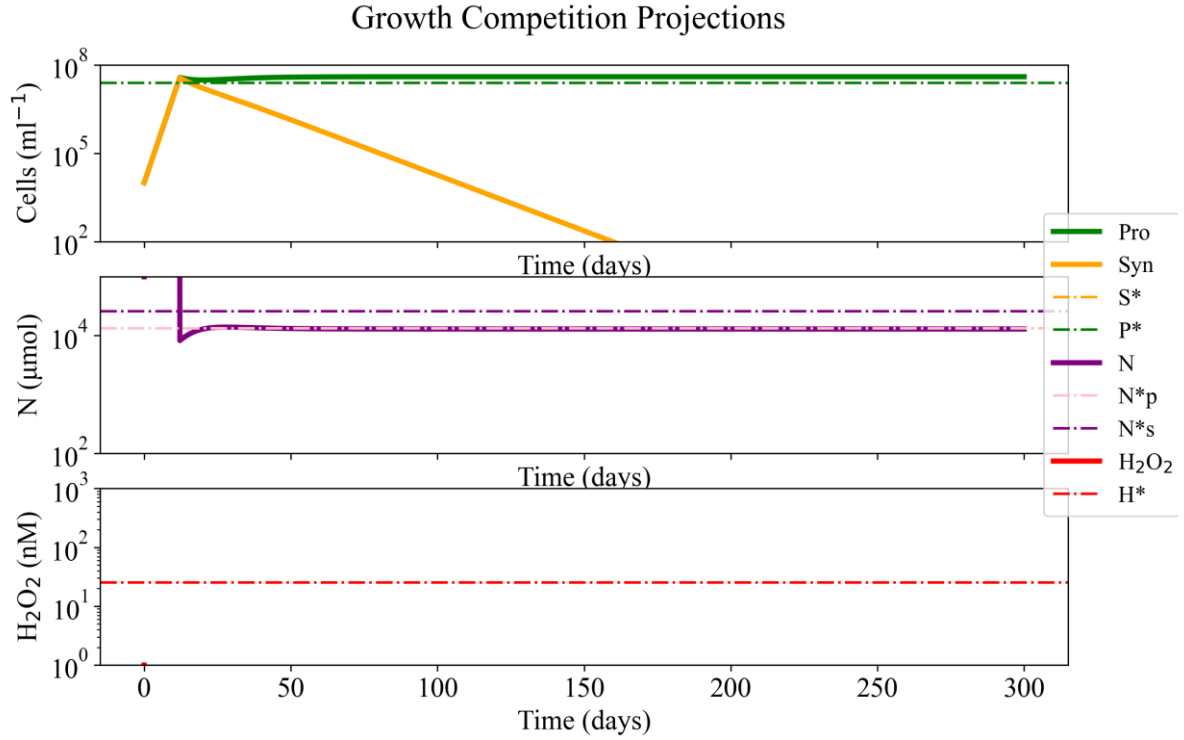


Figure 3.3 Dynamics of *Prochlorococcus* and *Synechococcus* in zero H_2O_2

Dynamics of *Prochlorococcus* (green) and *Synechococcus* (yellow) analogues growing on a single nutrient (purple) in batch culture with zero H_2O_2 in solution. We calculate the equilibrium constants for *Prochlorococcus* and nutrients predicted from our set of initial parameters. In this case (no H_2O_2 damage to cells ($\kappa_{dam,p}$) and no biotic H_2O_2 detoxification ($\phi_{max,S}$ or $\phi_{max,B}$)) we see the green P cells drawing N down to N^* and reaching P^* steady state while S is outcompeted to extinction. The * equilibria values for these and other scenarios are calculated in Appendix B, section B2.

suffers accordingly with H_2O_2 susceptibility ($\kappa_{dam,p}$) . S is able to survive H_2O_2 ($\kappa_{dam,s} \sim 0$) but acquires nutrients at a slower rate than P. ($\alpha_p < \alpha_s$ giving a lower k_s constant. (Aksnes and Egge 1991; Michaelis and Menten 1913)). Figure 3.5 shows this case extrapolated across an array of nutrient and H_2O_2 supplies, S_N and S_H respectively.

A second case, delineated ‘Leaky case’, was evaluated for long term for coexistence with *Prochlorococcus* and *Synechococcus* abundances. Here S is given *Synechococcus*’ $\phi_{max,S}$ value from Figure 3.2. Essentially the full model set is used with $\phi_{max,B}$ or B equal to zero. With the use of realistic $\phi_{max,S}$ values, coexistence is viable but unlikely as it occurs at high nutrient and high S_H values only of $\phi_{max,S}$ and bacterial abundances effects on to *in situ Prochlorococcus* vs. *Synechococcus* (*Prochlorococcus/Synechococcus* (*Pro/Syn*)) competition outcomes are explored using various contour maps.

Lastly, we explore the full model set with the addition of B detoxification. We analyze the effect of bacterial abundance and detoxification on *Prochlorococcus* and *Synechococcus* competition. Heterotrophic Bacteria, B_i , (cells mL^{-1}) here are not modeled as growing from supplied N but rather as a stable population that functions solely to reduce the H_2O_2 standing concentration via its own $\phi_{max,B}$ detoxification. We calculate $\phi_{max,B}$ from a 2-hour detoxification curve from EZ55 growth in H_2O_2 spiked culture following Chapter 2 protocol. (Monoculture time dependent dynamics shown in Appendix B) We then follow up with a secondary bacterial analysis with EZ55 as the lone B detoxifier. This is for the ‘leaky’, more realistic, H_2O_2 case.

For each of these cases, base analytical approaches (equilibrium calculations, time-dependent numerical analysis, sensitivity studies to H and N supplies) were used in congress with ranges of believable (retrieved in Chapter 2 and represented in Figure 3.2) parameters to investigate the effect each H_2O_2 and underlying assumptions phytoplankton community structure.

RESULTS

From Chapter 2 monoculture analyses, Figure 3.2 shows the concurrent values of ϕ and $\kappa_{dam,p}$ recovered. These will be used for non-leaky (*Prochlorococcus* $\kappa_{dam,p}$ and

Prochlorococcus ϕ_{max} (~ 0) values) and leaky (inclusion of *Prochlorococcus* damage ($\kappa_{dam,p}$) and *Synechococcus* detoxification ($\phi_{max,s}$) cases.

NON-LEAKY DYNAMICS

We first assure that our models align with known convention (Button 1998) that *Prochlorococcus*' superior affinity (higher α bundled into lower k_s constant) allows it to outcompete *Synechococcus* without H_2O_2 . We extrapolate our ODE models out for 150 or 300 days to simulate competition over approximately a half or a full year. We find that our competition converges on the eventuality of *Prochlorococcus* analogue, P, reaching an equilibrium state and the *Synechococcus* analogue, S, slowly being outcompeted to extinction (Figure 3.3). The equilibrium solutions for the system shown in dotted lines and calculated (*) equations' outputs are shown below said dynamics. Calculations of equilibria shown are located in Appendix B. In this model P's greater nutrient affinity (α), P outcompetes S to exclusion. However, this matches neither *in situ* or laboratory data (Calfee, Glasgo, and Zinser 2022; Knight and Morris 2018; Visintini, Martiny, and Flombaum 2021; Partensky, Blanchot, and Vaulot 1999) so H_2O_2 parameter impacts are added starting with $k_{dam,i}$ in P for case 2 in Figure 3.4.

The dynamics resulting from analysis of the 'non-leaky' case, (*i.e.*, the inclusion of H_2O_2 damage without the inclusion of community H_2O_2 detoxification) when S_H is non-zero dynamics are shown in Figure 3.4. P's greater nutrient affinity (α) allows P to outcompete S, but the concurrent higher $\kappa_{dam,p}$ rate leads to significant P population loss when not ameliorated by biotic detoxification mechanisms within the environment (*i.e.*, B or S cell based detoxification, $\phi_{max,i}$). The dynamics and equilibria both assert that high nutrient affinity of P alone is not enough to outcompete with S when H_2O_2 is unbounded by biological detoxification. We see a flipping in the dynamics between Figure 3.3 and Figure 3.4 controlled solely by the addition of $\kappa_{dam,p}$ to the P equation, showing P indeed requires external H_2O_2 detoxification to survive and outcompete S via nutrient affinity. This matches culture data where helpers are required to grow *Prochlorococcus* at ecologically relevant H_2O_2 production rates (Morris *et al.* 2011).

When competition of *Prochlorococcus* and *Synechococcus* analogues is assessed across an array of S_H and S_N values, only two outcomes are observed. This allows us to investigate how

cells may react to traveling from the gyres towards the coast (higher H_2O_2 production (S_H) and higher N inflow (S_N) to be found near the coast) or traveling down the water column (higher N inflow (S_N) and unknown (S_H) impacts (decreased photoproduction of H_2O_2 with depth although other H_2O_2 sources seem to change with depth (Sutherland, Wankel, and Hansel 2020; Hopwood *et al.* 2017)). In Figure 3.5, along a matrix of inputs, the end state concentration of S to total plankton (*Synechococcus* and *Prochlorococcus* analogues) in the system is calculated. Thus, when P outcompetes S due to nutrient affinity, the fraction is 0 and the graph is green. When S wins due to H_2O_2 killing P, the fraction equals 1 and the graph is yellow. Despite a wide range of starting parameter values (S_H and S_N), *Prochlorococcus* and *Synechococcus* competition has only two options for outcome within this non-leaky model framework. Either *Prochlorococcus* kills off *Synechococcus* when N is too limited for S to survive or H_2O_2 is too high for *Prochlorococcus* to survive. Thus, *Prochlorococcus* (P) or *Synechococcus* (S) alone can grow on the ammonium (N) of a non-leaky ocean system. There is no coexistence found under this single nutrient regime.

LEAKY DYNAMICS INCLUDING SYNECHOCOCCUS DETOXIFICATION

The ‘leaky case’ of our model involving only *Synechococcus* (S) cell-based detoxification of H_2O_2 is evaluated in Figure 3.6 dynamically. This mirrors known dynamics of H_2O_2 easily flowing across cellular membranes, meaning that if a cell increases or decreases their internal concentration, the extracellular concentrations of H_2O_2 will be impacted as well. This exploration of community H_2O_2 detoxification shows the combination of nutrient competition and innate leakiness of H_2O_2 detoxification that results in a coexistent dynamic with a steady state coexistence of *Prochlorococcus* and *Synechococcus* around their respective N^* s in the equilibria. N^*_s is the nutrient concentration that gives replacement numbers for *Synechococcus*; N^*_p likewise is the nutrient concentration for *Prochlorococcus* to have replacement numbers possible *in situ*. The nutrient acquisition ability of *Prochlorococcus* and *Synechococcus* determine these equilibria N^* values and thus we can use our framework to compare dynamics prediction to mathematically tabulated equilibria. (See Appendix B for full equilibria calculations.)

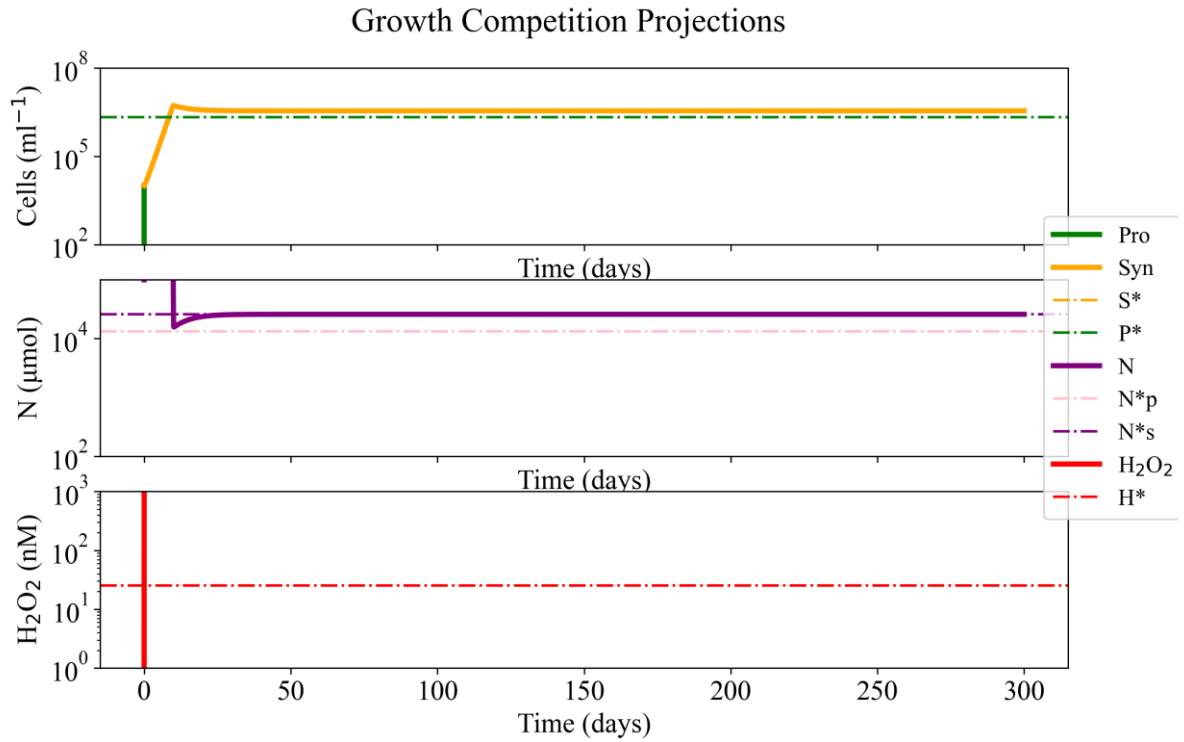


Figure 3.4 Dynamics of *Prochlorococcus* and *Synechococcus* in non-leaky H₂O₂ world

The top graph depicts the dynamic model solutions for *Prochlorococcus* and *Synechococcus* analogue abundances in solid green or yellow lines as predicted with steady H₂O₂ input into solution. Steady state solutions for said models are visualized in dotted lines on the same axes. The middle plot shows the modeled nutrient concentration over time for the dynamics (solid) and steady state (dotted) purple lines. The bottom plot shows H₂O₂ dynamics over time.

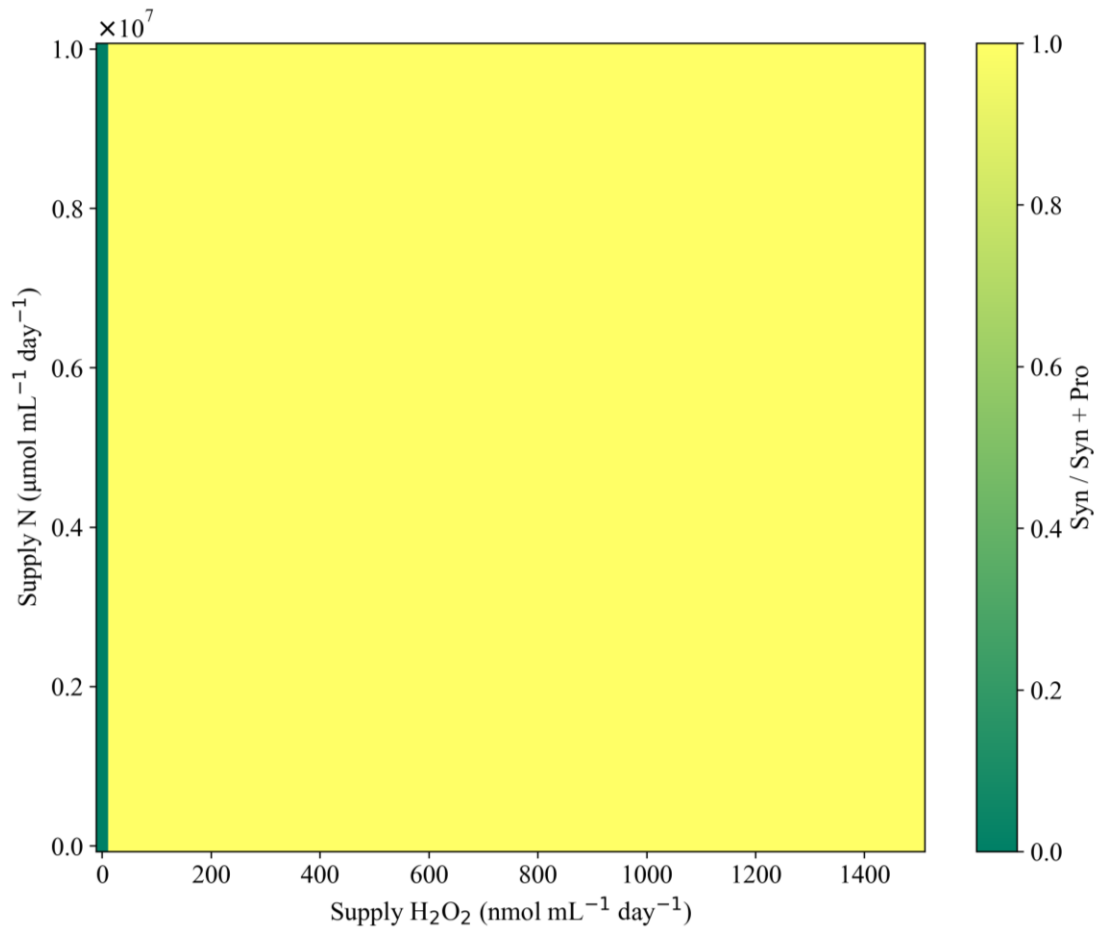


Figure 3.5 Evaluation of non-leaky model dynamics across a range of S_H and S_N

Non-leaky contour map of *Prochlorococcus* and *Synechococcus* competition outcomes across nutrient and H_2O_2 inputs. Dark green represents *Prochlorococcus* dominated (end state) competition via exclusion while bright yellow represents *Synechococcus* – dominated competitive exclusion. There is no yellow-green here, meaning only *Prochlorococcus* or *Synechococcus* exists at the end of these projections. The difference between the green and yellow portions of the contour are determined by the replacement numbers of *Prochlorococcus* on the nutrient available vs the damage incurred from the H_2O_2 input.

a. Leaky Model Projections: S_N $8.5 \times 10^6 \mu\text{mol mL}^{-1} \text{ day}^{-1}$ and S_H $2.5 \times 10^2 \text{ nmol mL}^{-1} \text{ day}^{-1}$

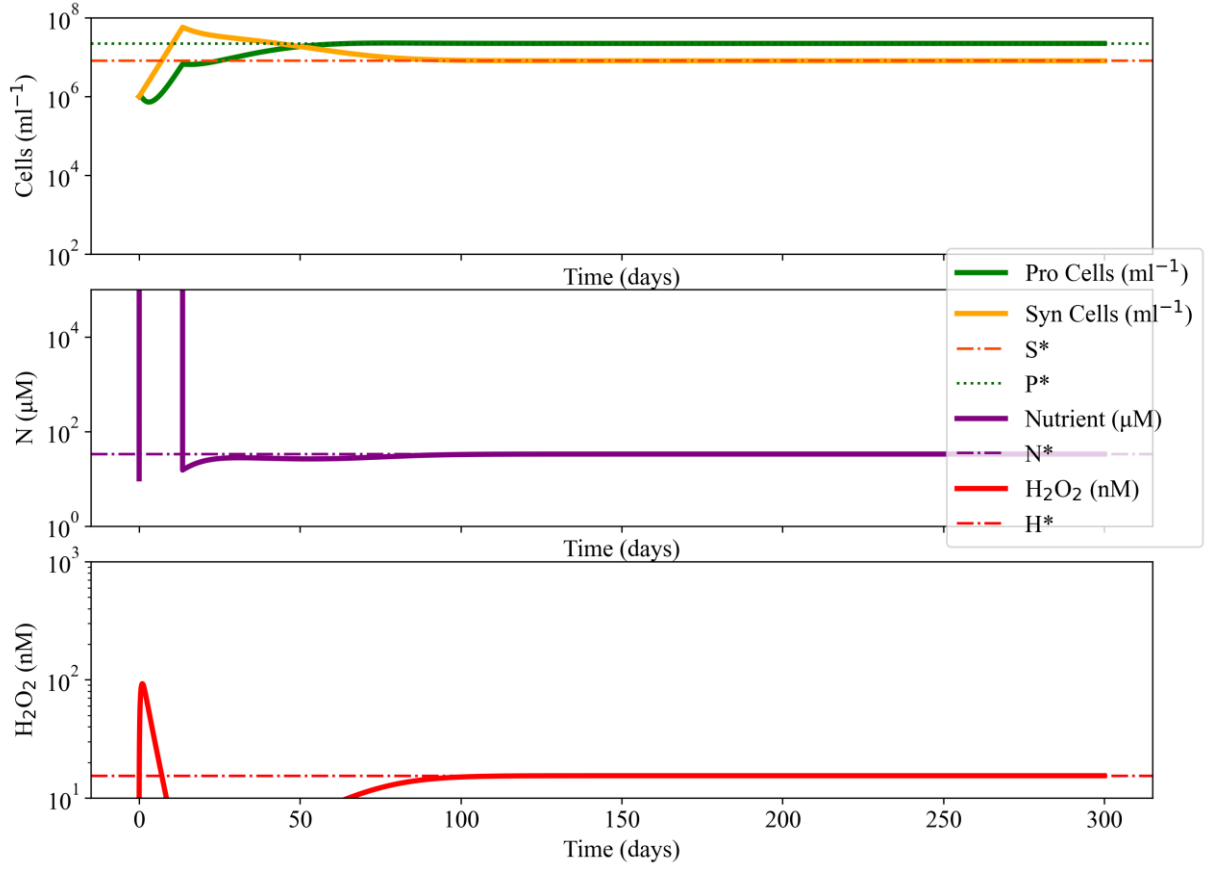


Figure 3.6 Dynamic outcomes for H, N, P and S leaky case model iterations under different H supplies. 3.6a, 3.6b, and 3.6c depict different dynamic model outputs for P, S, N, and H from increasing H inputs under case 3, S leaky H_2O_2 detoxification regime. The top graph of a,b or c each depicts dynamics model solutions for *Prochlorococcus* (P) and *Synechococcus* (S) analogue abundances in green or yellow as predicted solid lines. The associated steady state solutions predicted from these parameter values are visualized in dotted lines on the same axes. The middle plot shows the modeled nutrient concentration over time for the dynamics (solid) and steady state (dotted) purple lines. The bottom plot shows H_2O_2 dynamics over time. S_N is kept constant for all scenarios (a,b,c) as is *Synechococcus* detoxification ($\phi_{\max,S} = 2.0 \times 10^{-6}$). Despite this single parameterization, differentially dominated coexistence scenarios occur in P and S communities based on value S_H . Using only *Synechococcus* detoxification ($\phi_{\max,S}$) allows coexistence along a small (200-900 $\text{nmol mL}^{-1} \text{ day}^{-1}$) range of S_H .

b. Leaky Model Projections: $S_N 8.5 \times 10^6 \mu\text{mol mL}^{-1} \text{ day}^{-1}$ and $S_H 4.5 \times 10^2 \text{ nmol mL}^{-1} \text{ day}^{-1}$

Growth Competition Projections in SN 8000000.0 Sh 450

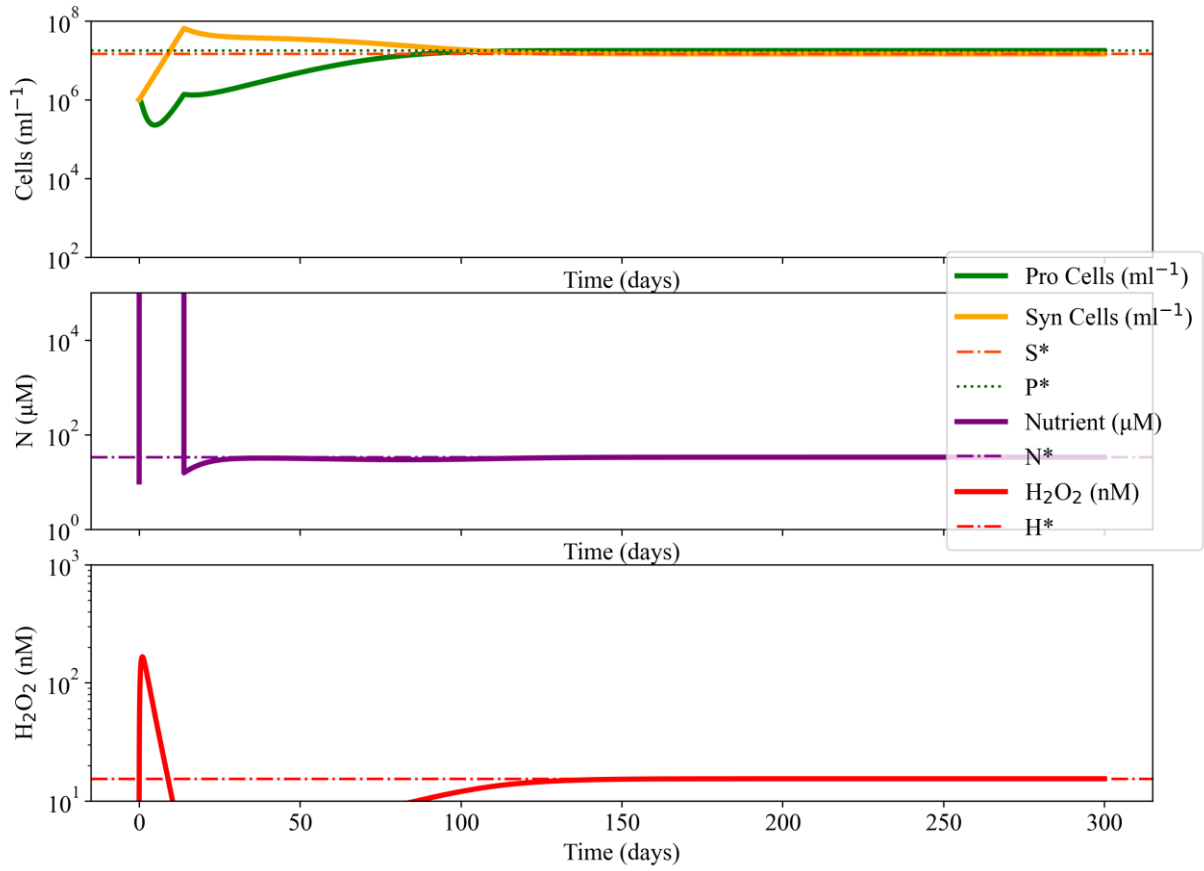


Figure 3.6 continued

c. Leaky Model Projections: S_N $8.5 \times 10^6 \mu\text{mol mL}^{-1} \text{ day}^{-1}$ and S_H $8.5 \times 10^2 \text{ nmol mL}^{-1} \text{ day}^{-1}$

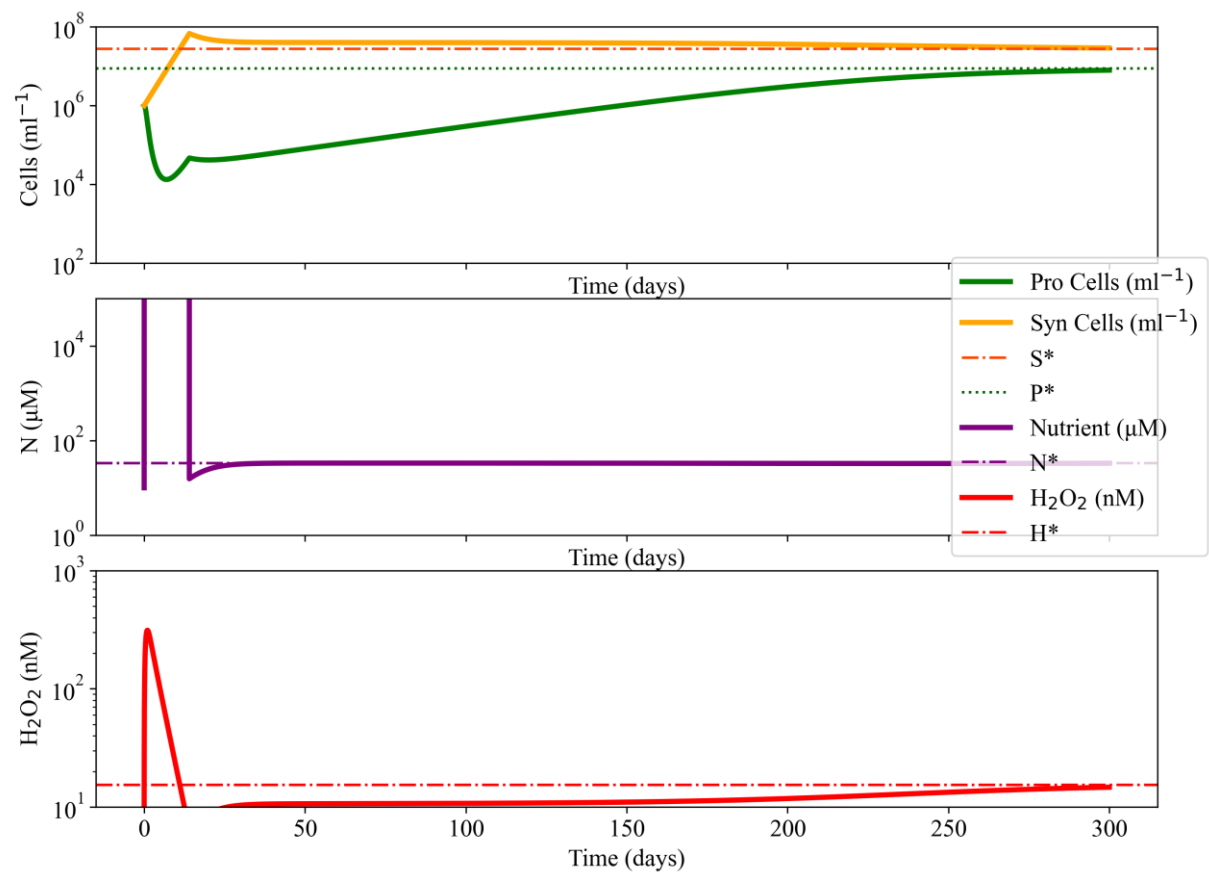


Figure 3.6 continued

Further evaluation of the effect of detoxification ($\phi_{max,i}$) value on the contour outcome gives the range of maps seen in Figure 3.7. (The range of $\phi_{max,S}$ varies from values isolated from *Synechococcus spp* modeled in Chapter 2 or heterotrophic levels of detoxification modeled in Appendix A, Shown in Figure 3.2).

Outcomes of the leaky detoxification via *Synechococcus* on a system of *Prochlorococcus* and *Synechococcus* are projected across nutrient and H_2O_2 supplies in Figure 3.7. Green indicates *Prochlorococcus* dominated competitive exclusion while yellow indicates *Synechococcus* dominated exclusion. Yellow-green (notably absent from the leaky cases) represents various coexistence ratios of *Prochlorococcus* and *Synechococcus*. Dynamics like those pictured in the dynamics of Figure 3.6a-c, give these yellow-green colors. These ranges of coexistence between P and S *in silico* do not occur when S does not provide enough detoxification to give a rate ($\phi_{max,S}$) above 0.

These heat maps show that coexistence is possible but only at high nutrients that give unrealistic (Partensky, Hess, and Vaulot 1999) abundance values for *Prochlorococcus* and *Synechococcus* analogues. The nutrient supply (S_N) of the oligotrophic ocean is unconstrained however given that dynamics in Figure 3.6 have abundances too high along with these overly large N values, we do not think this dynamic alone is controlling dynamics in the ocean. Further, preliminary *in situ* HEPES buffer (Morris *et al.* 2016) and HOTS cruise spike assay data produced S_H values in our current model of <50. These models currently do not include DOC or light explicitly despite both being tied to the creation of more H_2O_2 (Cooper *et al.* 1988; Zinser 2013). Thus, it is unknown how closely these artificial supply rates correlate to values given by Zhu *et al.* (2022) or Powers and Miller (2014).

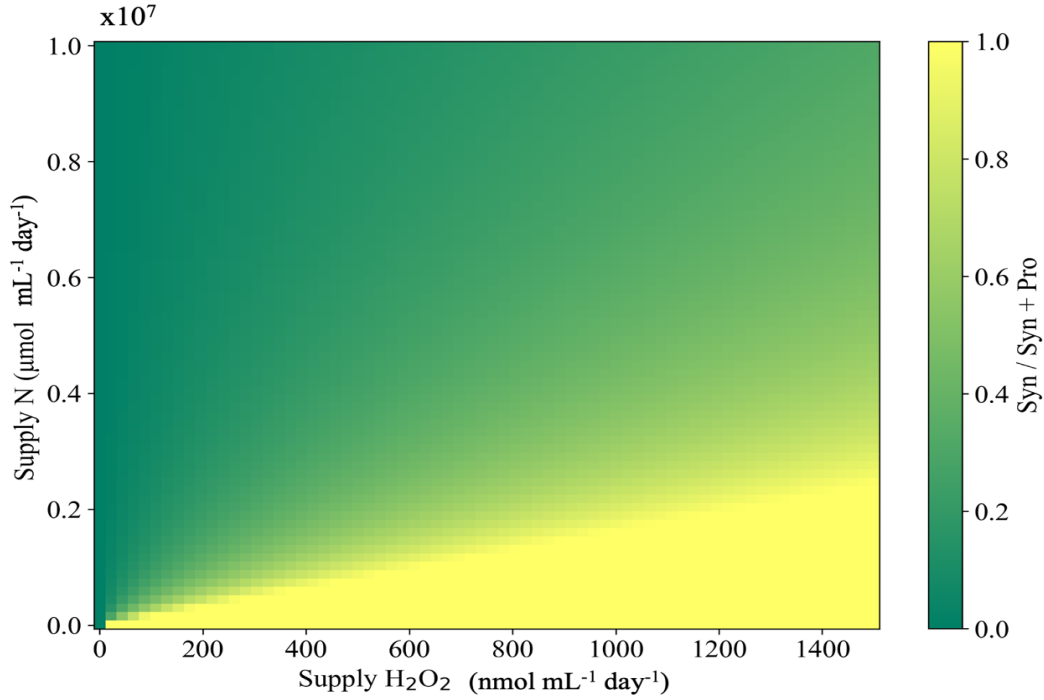


Figure 3.7 $\phi_{\max,S}$ effects on Prochlorococcus and Synechococcus coexistence in dynamics and equilibria contours. Dynamics ODEs equilibrium (*) equations are projected for various H_2O_2 detoxification parameterizations of *Synechococcus*. The non-zero value of $\phi_{\max,S}$ allows coexistence and controls the extent to which it can occur along a continuum of S_N and S_H values. 0 to 1500nM range for *in situ* H_2O_2 is relevant for surface microbes, especially with rain inputs. S_N range was tabulated to be give cellular values close to *in situ* values. The average recovered *Synechococcus* $\phi_{\max,S}$ from Chapter 2 is represented by the middle $\phi_{\max,S}$ (2.0×10^{-6}) value in this figure. Under case 3 of our model's regime (leaky detoxification from only *Synechococcus* cells), coexistence is available only at high S_N or low S_H values. * equilibria are calculated in Appendix B2. Comparison with Dynamic contours are shown in Appendix B, Section B3.

$$\phi_{\max,S} = 8.0 \times 10^{-6} \text{ mL cell}^{-1} \text{ day}^{-1}$$

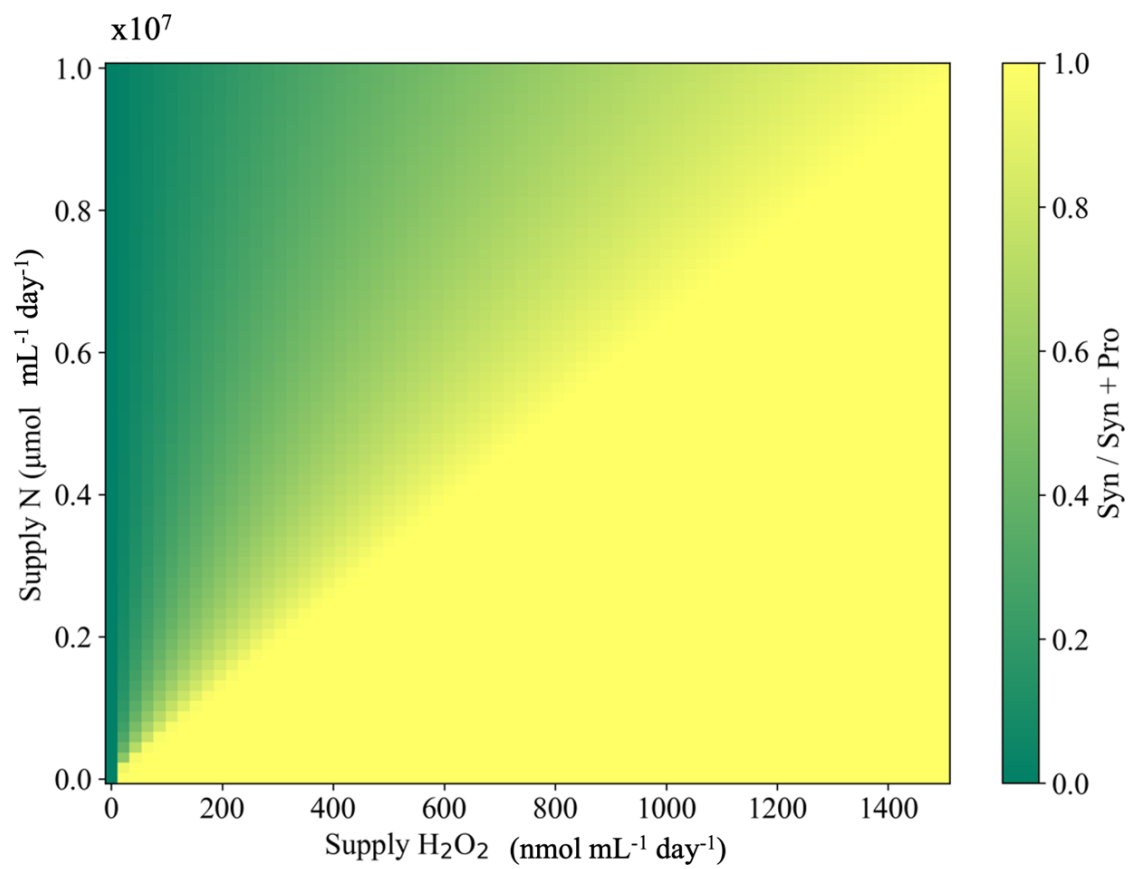


Figure 3.7 continued

$$\phi_{\max,S} = 2.0 \times 10^{-6} \text{ mL cell}^{-1} \text{ day}^{-1}$$

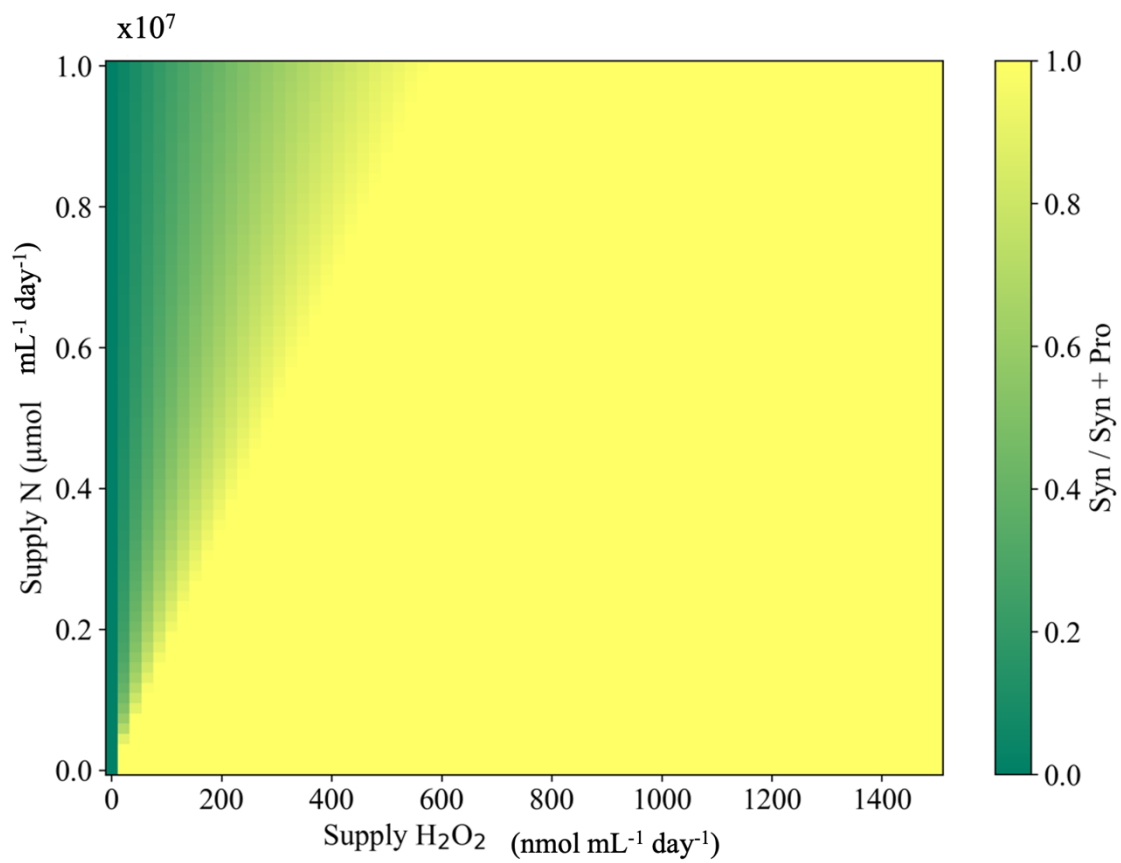


Figure 3.7 continued

$$\phi_{\max, S} = 0.5 \times 10^{-6} \text{ mL cell}^{-1} \text{ day}^{-1}$$

LEAKY DYNAMICS INCLUDING *SYNECHOCOCCUS* AND BACTERIAL DETOXIFICATION

Bioinformatic comparative analysis of all known bacterial genomes has been found ‘surprisingly small’ amount of detoxification genes in marine microbes (as compared to cultured bacteria). In bacteria, catalase is ‘almost completely absent’ (Bengtsson-Palme *et al.* 2014; Morris *et al.* 2016) while peroxides are found to be abundant in marine isolates. Thus it seems not all H₂O₂ detoxification enzymes are equally as prone to being cut from populations (Bernroither *et al.* 2009). *Prochlorococcus* has retained multiple peroxidases, but *Synechococcus* and other plankton have retained catalase (Bernroither *et al.* 2009). Bacterioplankton groups Alpha(α –)proteobacteria and Gamma(γ –)proteobacteria compose ~50% of average total marine bacterial genomic reads (Barberán and Casamayor 2010). Heterotrophic classes eclipse phototrophic groups in all eubacterial abundance size fractions and locations sampled in ocean samples (Henríquez-Castillo *et al.* 2022) while in coastal studies, γ –proteobacteria were shown to compose ~70% of microbial genetic reads, with distinct *Vibrio* and *Alteromonas* isolates found (Hagström, Pinhassi, and Zweifel 2000). *Alteromonas macleodii* (γ –proteobacteria: Alteromonadales: Alteromonadaceae: *Alteromonas* : *spp* (Wietz *et al.* 2022), is ubiquitous in temperate marine waters and increases in proportion to the total amount of free living bacteria along the water column down to ~ 3500m the surface (García-Martínez *et al.* 2002). EZ55 (Morris *et al.* 2008), an isolated strain of *A. macleodii* similar to ones found *in situ* in Henríquez-Castillo *et al.* (2022), has been characterized (Baumann *et al.* 1984) and isolated from ‘contaminated’ *Prochlorococcus* marine samples (Biller, Berube, *et al.* 2015). Other γ –proteobacteria that have also been investigated for reactive oxygen detoxification (Schellhorn 1995; Morris, Papoulis, and Lenski 2014) include model organisms *Vibrio* spp. and *E. coli* (García-Martínez *et al.* 2002). Thus we choose the heterotrophic γ –proteobacteria as the basis of our heterotrophic bacteria (B) population and explore it further its effect on our model further with the noted EZ55 bacterial helper.

Adkins-Jablonsky *et al.* (2021) suggest that the higher cost of a leaky function (how much that cost affects the helper) determines if said ‘helper’ is the sole helper or if the leaky functions more spread out between multiple helpers. B is initially modeled as representative heterotrophic eubacterial concentration of surface waters (Wigington *et al.* 2016; Zinser, Talmy, and Calfee 2023) and used with calculated detoxification $\phi_{max,i}$ (Chapter 2, *Alteromonas* avg.

parameters) as a steady draw on H₂O₂ input (i.e., it is not growing on the N source for which P and S compete). For all ‘leaky’ model runs: B is $1 \times 10^{+5}$ and $\phi_{max,B}$ is 4×10^{-4} . This ϕ value comes from the modeling of EZ55, a laboratory strain of *Alteromonas*. The coculture assays from which these parameters came are shown in Figure 3.2 and Appendix A. EZ55, an isolate of catalase positive *Alteromonas* had a roughly calculated ϕ_{max} of 4×10^{-4} mL cell⁻¹ day⁻¹. This is ten times that of *Synechococcus*. We evaluated EZ55 ϕ_{max} as a main control of coexistence for *Prochlorococcus* and *Synechococcus* through the detoxification of H₂O₂. (Modeling of this bacterial ϕ_{max} is found in Appendix B.) Dynamic and contour plots show different EZ55 abundance scenarios to evaluate possible effect and possible ‘super helper – EZ55’ effect on *Prochlorococcus* and *Synechococcus* competition.

Bacterial cell specific H₂O₂ detoxification, $\phi_{max,B}$, is ten times higher than that of *Synechococcus* and thus allows coexistence between S and P at lower S_N and higher S_H values as compared to $\phi_{max,S}$ alone. Greater ranges of coexistence are permitted across large areas of an environment when bacteria and *Synechococcus* are both detoxifying H₂O₂. With $\phi_{max,B}$ bringing standing H₂O₂ concentrations down to a lower level, *Synechococcus* detoxification ($\phi_{max,S}$ in Figure 3.8) is then enough to help save *Prochlorococcus* while also itself surviving on low nutrients that *Prochlorococcus* otherwise outcompetes it for. $\phi_{max,B}$ is a rough estimate from one species (EZ55, *Alteromonas* sp.) so we encourage future studies to explore and characterize the detoxification range of marine bacterial populations. Further, heterotrophic bacteria (B) biomass was not tied to N here in Figure 3.8. Thus $\phi_{max,B}$ acts as a very steep abiotic H₂O₂ sink in this model formulation. This is continued in the models of Chapter 4. However, we note that further biotic analysis of H₂O₂ and nutrient impacts on picoheterotrophs along with broad bacterial population responses, are needed to model these heterotrophic microbes and resultant H₂O₂ detoxification more robustly in future studies.

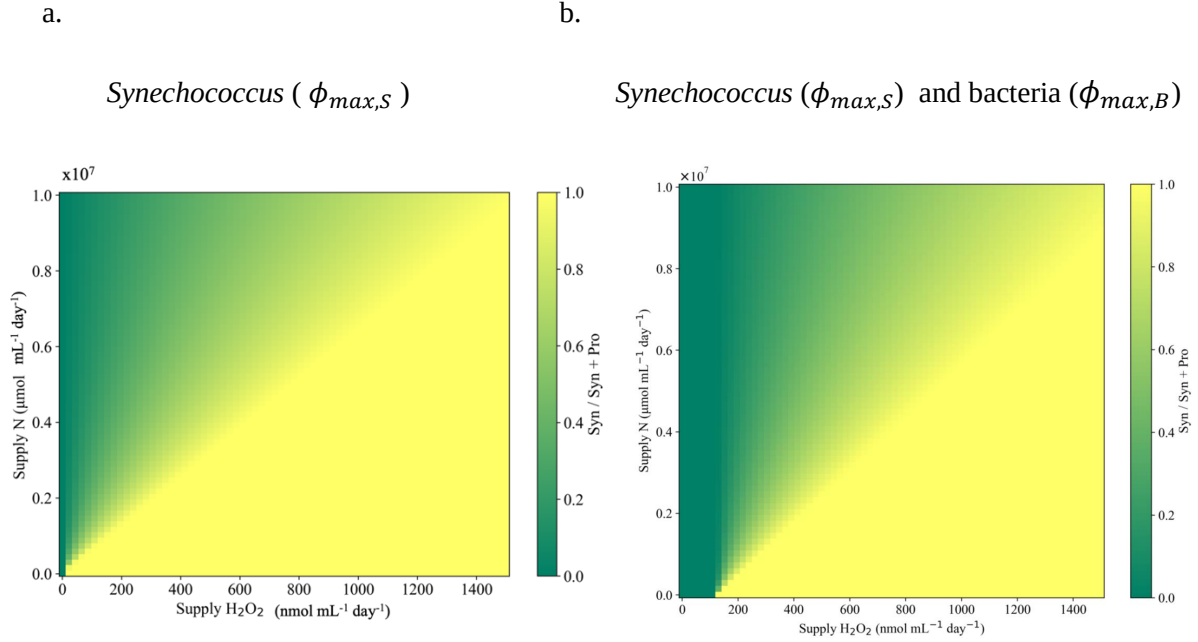
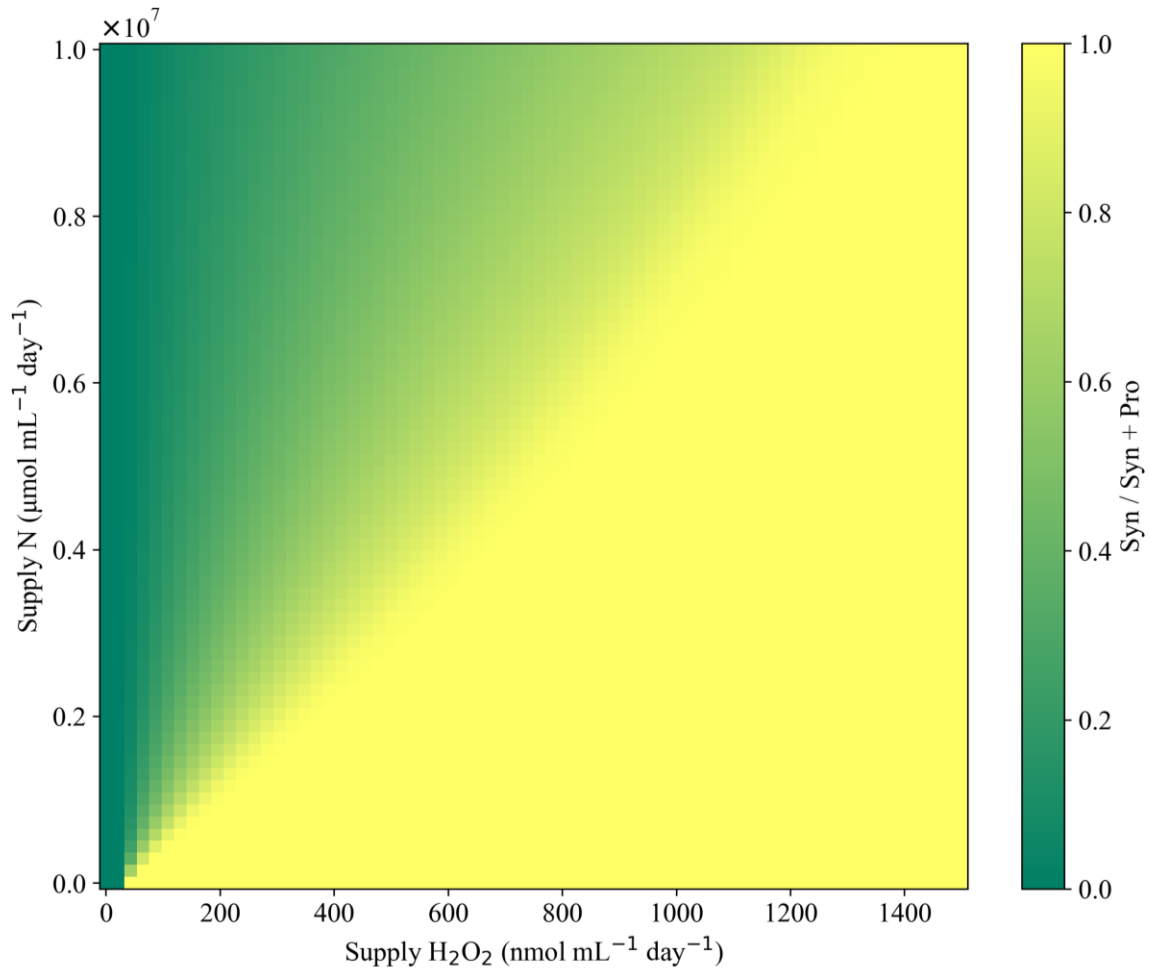


Figure 3.8 Different coexistence landscapes available dependent upon who is detoxifying H.

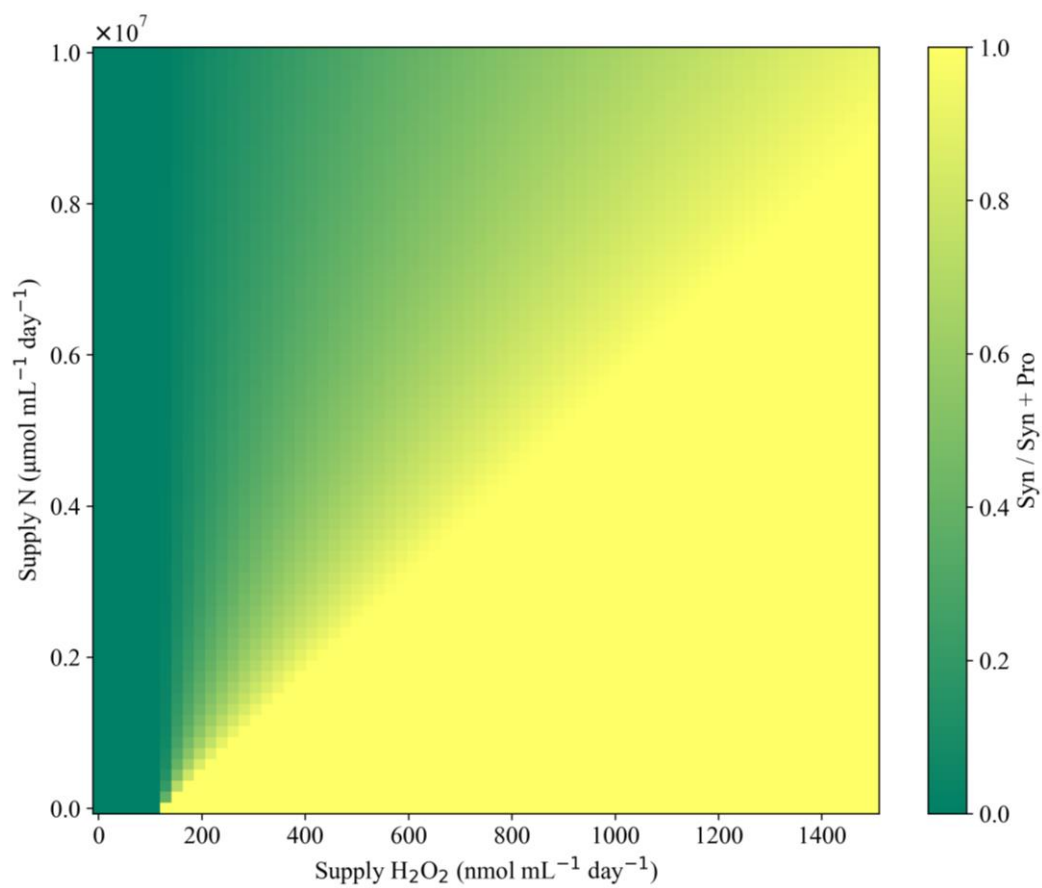
Heat map contours for dynamic competition outcomes between *Prochlorococcus* and *Synechococcus* over a range of S_N ($\mu\text{mol mL}^{-1} \text{ day}^{-1}$) and S_H ($\text{nmol mL}^{-1} \text{ day}^{-1}$) inflows. We compare here two different detoxification regimes; *Synechococcus* detoxification ($\phi_{max,S}$) one or *Synechococcus* and heterotrophic bacterial detoxification ($\phi_{max,B}$). *Synechococcus* detoxification ($\phi_{max,S}$) is 2.0×10^{-6} in both contour plots but bacterial detoxification ($\phi_{max,B}$) is either 0 (a, left) or 4.0×10^{-4} (b, right).



$$\phi_{\max,B} = 2.0 \times 10^{-5} \text{ mL cell}^{-1} \text{ day}^{-1}$$

Figure 3.9 Contour outputs across a range of S_N and S_H under different heterotrophic detoxification ($\phi_{\max,B}$) regimes

Dynamics ODEs (left) or equilibrium solution equations (right) are projected along a biomass ratio contour plot indication $\text{Syn}/(\text{Pro}+\text{Syn})$. These competitions are evaluated over a range of S_N ($\mu\text{mol mL}^{-1} \text{ day}^{-1}$) and S_H ($\text{nmol mL}^{-1} \text{ day}^{-1}$) inputs. Each row represents a different parameterization (value) of stationary phase heterotrophic Bacterial (B) culture H_2O_2 detoxification ($\phi_{\max,B}$).



$$\phi_{\max, \text{B}} = 8 \times 10^{-5} \text{ mL cell}^{-1} \text{ day}^{-1}$$

Figure 3.9 continued

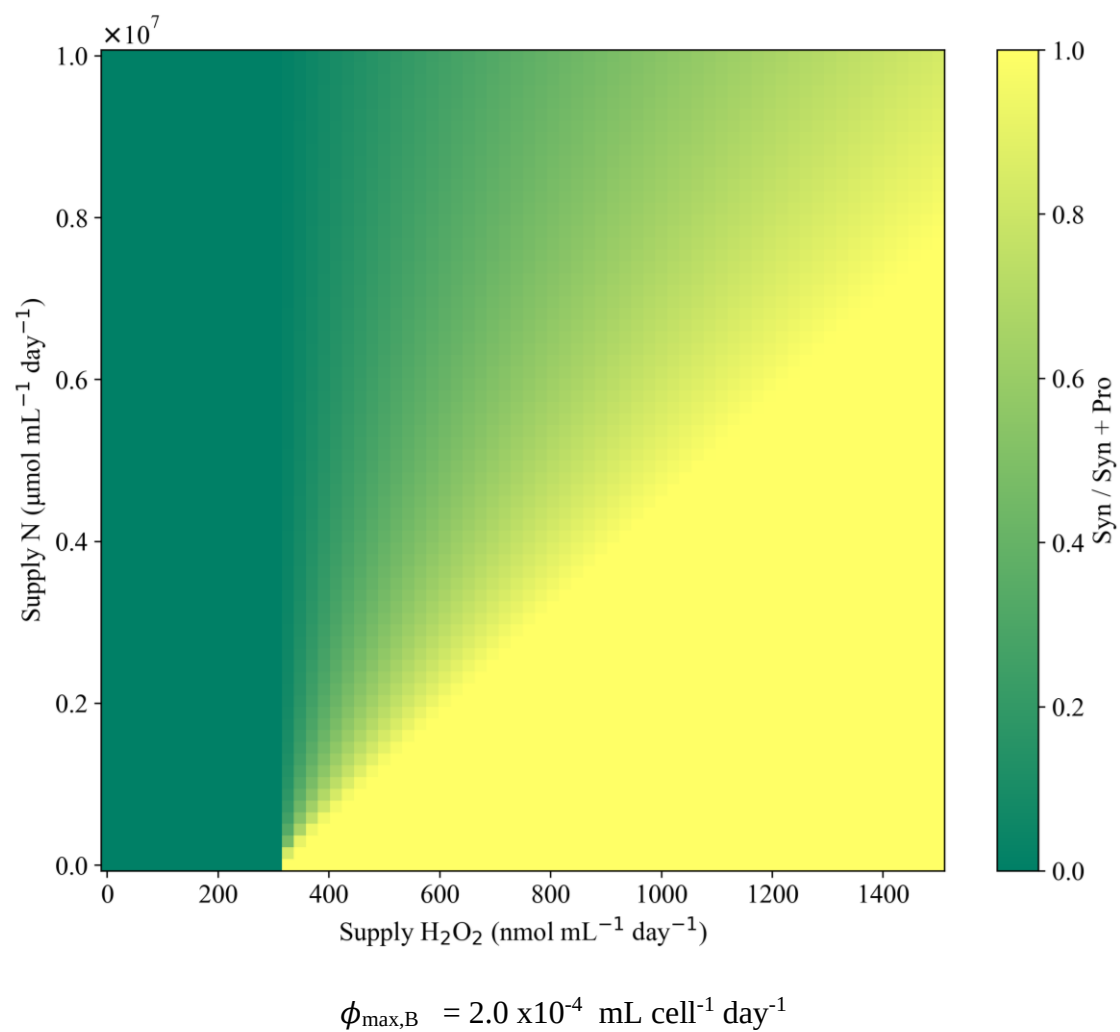


Figure 3.9 continued

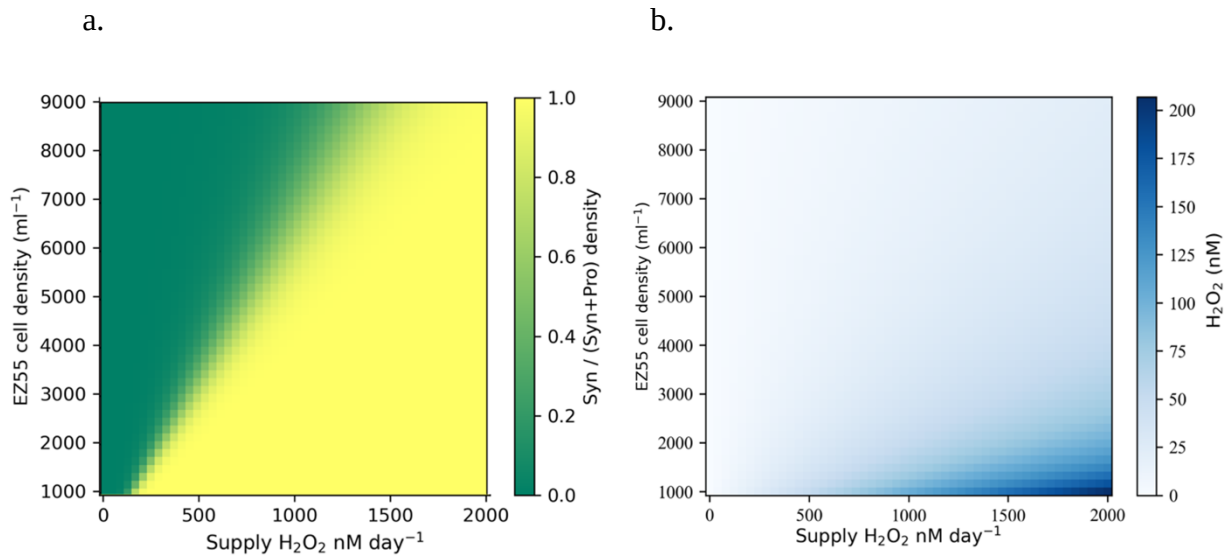


Figure 3.10 *Prochlorococcus* and *Synechococcus* competitive outcomes as a result of EZ55 cell density. a. Competition outcomes of P and S across a range of EZ55 (cell mL⁻¹) and S_H (nmol mL⁻¹ day⁻¹) values. Dark Green represents *Prochlorococcus* dominated (end state) competitive exclusion while yellow represents *Synechococcus* – dominated competitive exclusion. Yellow-green represents various coexistence ratios of *Prochlorococcus* and *Synechococcus*. b. Right contour shows how EZ55 concentration, $\phi_{\max,D}$, and S_H input parameters work together to create standing stock H₂O₂ concentrations that affect the *Prochlorococcus* and *Synechococcus* equilibria. Note here that H₂O₂ concentrations hover between 10 and 200nM, much like daily ambient H₂O₂ seen *in situ* (Morris *et al.* 2016; Zhu *et al.* 2022; Hopwood *et al.* 2017).

DISCUSSION

Resource competition theory (Tilman 1982; Grover 1997) indicates that when two or more organisms attempt to coexist in an environment on one nutrient, the better competitor organism should eventually outcompete the other, unless the competitor develops the ability to utilize other nutrient pools (Armstrong and McGehee 1980; Hardin 1960; Hutchinson 1967). The full extent of resource partitioning in the ocean and associated microbial community composition is far from understood (Hutchinson 1967; Bier *et al.* 2015; Litchman *et al.* 2006; Saba *et al.* 2010). While differential nutrient acquisition has long been considered and modeled as an important driver of ecological niches, Berube *et al.* (2023) and Calfee *et al.* (2022) showed that cross feeding of nitrogen (N) or carbon (C) molecular species in oligotrophic genera may sustain coexistence (Berube *et al.* 2023; Calfee, Glasgo, and Zinser 2022)). Here we show that H_2O_2 pressure should also be considered a niche-expanding mechanism and thus be modeled accordingly. We first evaluated the hypotheses that superior nutrient acquisition mechanisms, devoid of other forcings, were enough to allow *Prochlorococcus* to competitively exclude community members for nutrients. Our initial modeling shows that nutrient affinity indeed controls our system in the absence of H_2O_2 (zero for supply ($S_H = 0$) and initial conditions of H_2O_2 ($H(0) = 0$)) and *Prochlorococcus* dominates. However, when we put *Prochlorococcus* in an environment where there is a cost to nutrient affinity in the form of H_2O_2 susceptibility (*i.e.*, catalase loss from evolutionary streamlining) it is clear, that nutrient success alone is not sufficient to allow survival when H_2O_2 is non-zero, as is true in the oligotrophic ocean. While *Prochlorococcus* has optimized its ability to obtain ammonia, *Synechococcus* can utilize sources of nitrogen, such as nitrate, that *Prochlorococcus* cannot access (Garcia-Fernandez, de Marsac, and Diez 2004) thus this *in silico* scenario allowed for the isolation of interactions that cannot be isolated directly from *in situ* sampling. Different *Prochlorococcus* (Berube *et al.* 2023) or *Synechococcus* ecotypes (Masuda *et al.* 2023) can coexist on different N sources (with NO_2 or NO_3 adding niche room for coexistence), with the phenomenon modeled by Masuda alongside ecologically relevant data. Our results provide context to the competition dynamics that allow coexistence on a shared ammonium (N) source.

Non-leaky H_2O_2 detoxification causes the predicted dynamic outcomes to switch from competitive exclusion of *Synechococcus* to the competitive exclusion of *Prochlorococcus*. In this

case, ‘non-leaky’ detox of H_2O_2 keeps *Synechococcus* safe but does not allow *Prochlorococcus* to reap the benefit of community detox. (This is done practically with *Synechococcus* ODE dynamics containing no H_2O_2 -based damage rate ($\kappa_{dam,s}$) and the ODE controlling H (See equations 3.1-3.5) having no biotic detoxification term.) Thus, *Synechococcus* is ‘agnostic’ to H_2O_2 and leaves *Prochlorococcus* to be damaged by the external H_2O_2 . Despite *Prochlorococcus*’ superior nutrient affinity, this burden of toxic H_2O_2 is too much for *Prochlorococcus* to overcome. Omar *et al.* (2022) showed that diffusional and spatial properties of the oligotrophic environment make it unlikely that microbes receive H_2O_2 detoxification help directly via cell contact but rather through diffusive interactions. Thus, we suspect our current form of inadvertent H_2O_2 detoxification is a good fit biologically and mathematically for this system. We assessed our non-leaky framework’s ability to capture *Prochlorococcus* and *Synechococcus* competitive interactions across ranges of nutrient and H_2O_2 inputs, reminiscent of a sampling transect across the surface marine environment. Despite the varied inputs, only two community outcomes were found to be possible withing a non-leaky H_2O_2 framework.

Detoxification of H_2O_2 however is known to be a leaky process, where environmental H_2O_2 is detoxified by *Prochlorococcus*’s microbial community members. We were required to allow for leaky community H_2O_2 detoxification, first from *Synechococcus* alone, in case 3, and then from heterotrophic bacteria, in case 4, that were quantified with Chapter 2 methods ($\phi_{\max,S}$ and $\phi_{\max,B}$). Inclusion of these allowed for cell-based drawdown ambient H_2O_2 concentrations in the model, allowing for *Prochlorococcus* to survive and compete with its community members via greater nutrient acquisition ability. We first attempted to do this in the leaky model case 3 with *Synechococcus* ‘help’ alone ($\phi_{\max,S}$), but the detoxification efficiency of *Synechococcus* was not high enough to allow coexistence of *Prochlorococcus* and *Synechococcus* analogues in a biologically reasonable range of ammonium and H_2O_2 concentrations. An additional heterotrophic bacterial helper (B), an *A. macleodii* analogue with associated detoxification rate ($\phi_{\max,B}$), was able to drawdown H_2O_2 and allow *Prochlorococcus* to survive and coexist with *Synechococcus* at lower ranges of N and higher ranges of H.

A. macleodii is an ecologically important marine community member. *A. macleodii* produces a photoreactive siderophore on a scale that have been modeled to significantly

influence global iron patterns (Manck *et al.* 2022). Metatranscriptomic analysis of marine waters also suggested *Alteromonas* iron specialization and carbon metabolism are closely tied (Henríquez-Castillo *et al.* 2022), meaning this microbe may also affect its overall draw down of *in situ* H_2O_2 concentrations via prevention of iron based, Fenton-like, reactions (Zinser 2018b; Zhang, Del Vecchio, and Blough 2012). *A. macleodii* is a diverse genera with a pangenome composed of 7819 total genes (Nayfach *et al.* 2016) that is divided into 35 species as of November 2022 (Wietz *et al.* 2022). Surface and deep species of *A. macleodii* differ in organic C or N usage (Biller, Berube, *et al.* 2015; V. Klochko *et al.* 2012) and pH optima (Ivanova *et al.* 2015). This mirrors the differentiation regimes of *Prochlorococcus* and *Synechococcus* genera through nitrogen, light, and temperature (Delmont and Eren 2018; Johnson 2006; Kujawinski *et al.* 2023; Sohm *et al.* 2016). Thus, we expect H_2O_2 influences the community composition between these organisms over a wide range of environments within the ocean. Chapter 4 will delve into a global circulation model's extrapolation of our H_2O_2 framework to explore natural variation of H_2O_2 and nutrient sources in the global ocean.

CONCLUSIONS

This exploration into parameter theoretical space is used to show that microbial detoxification of exogenous H_2O_2 is a key toggle for *Prochlorococcus* niche space and is key in allowing coexistence in singular nutrient scenarios and thus allows microbial dynamics to switch between competitive exclusion and coexistence. The streamlined nature of *Prochlorococcus* is touted as the way in which it dominates current marine biogeography (Visintini, Martiny, and Flombaum 2021; Flombaum *et al.* 2013; Partensky, Hess, and Vaulot 1999). However, the downside, mainly H_2O_2 – based lethality, of its streamlined nature is not currently quantitatively established. We use the base ODE models from Chapter 2 and evaluate the effect of H_2O_2 on *Prochlorococcus* and *Synechococcus* analogs in a competition framework. We first use values isolated from quantitative modeling methods in Chapter 2 and expand with bacterial detoxification and abundance stock values to analyze the different possible community structures available under relevant H_2O_2 and nutrient inputs. Predicted decreases in overall average cell size of the surface ocean microbes (a predicted outcome for future ocean nutrient inputs due to climate change (Dutkiewicz *et al.* 2015; Litchman *et al.* 2006)) is modeled to change the downward concentration and flux of carbon to the sequestration deep ocean (Levin and Le Bris

2015; Falkowski 1997; Hutchins and Fu 2017) through changing community composition augmenting internal mixed layer fluxes (Ducklow, Quinby, and Carlson 1995; Dutkiewicz, Scott, and Follows 2013; Barreto Filho *et al.* 2022; Henson *et al.* 2021; Wagner and Adrian 2009; Beman *et al.* 2011; Dutkiewicz *et al.* 2015; Traving, Clokie, and Middelboe 2014; Lomas and Lipschultz 2006) and decreased mixing between surface mixed layer and deep ocean layers (Sallée *et al.* 2021; Wang *et al.* 2023). Thus future oceans changes are set to augment the carbon storage function of the ocean (Boyd 2015). Models predict that heterotroph : autotroph ratios increase with temperature and even more so if heterotrophs are modeled to have ‘relevant’ higher enzyme sensitivity (Archibald *et al.* 2022). Since heterotrophs are key to the detoxification of H_2O_2 for the current dominant cyanobacteria of the ocean, and chemistry and temperature of future oceans favors longer reactive oxygen species lingering times (Morris, Rose, and Lu 2022), we find this study’s timing apt.

Chapter 4 MODELING THE IMPACT OF H_2O_2 TRAITS ON MARINE BIOLOGY AT A GLOBAL SCALE

Author(s): D.K.M., E.A.C., and D.T.

Author contribution statements: Darwin model implementation lead by E.A.C., written by D.K.M. with guidance from D.T. and E.A.C.

ABSTRACT:

Global models attribute approximately 50% of marine phytoplankton primary production, and accordingly 25% of Earth's total primary carbon primary fixation function, to the cyanobacterial sisters *Prochlorococcus* and *Synechococcus*. However, *Prochlorococcus* and *Synechococcus* have differential H₂O₂ interactions that have been verified *in situ* and via laboratory assays but are yet to be incorporated into global plankton models. In this chapter, *Prochlorococcus*, *Synechococcus*, and *Alteromonas* again are used as model organisms with differential cellular susceptibilities to and impacts on environmental H₂O₂. Basal supply and decay rates of H₂O₂ are inserted into a globally resolved biological trait framework. Following the community composition lens of Chapter 3, this global model is used to evaluate H₂O₂ impacts on phytoplankton biogeography in several cases. Without helper detoxification, diel concentrations of H₂O₂ input can kill off *Prochlorococcus* across all latitudes allowing *Synechococcus* to numerically dominate the globe at all locations. *Synechococcus* detoxification of environmental H₂O₂ is then added and found to significantly alter *Prochlorococcus* and *Synechococcus* interactions, allowing for coexistence rather than exclusion. *Alteromonas* (heterotrophic bacterial)-based detoxification further shapes community composition allowing for *Prochlorococcus* to fully dominate low latitudes while *Synechococcus* overtakes *Prochlorococcus* at higher latitudes. This presents a biogeographic output for *Prochlorococcus* and *Synechococcus* that are consistent with current understanding of plankton *in situ* biogeographies. Using different H₂O₂ parameter combinations layered sequentially into a global plankton competition framework, we quantitatively show that H₂O₂ impacts large scale microbial biogeographical patterns and their stability.

INTRODUCTION:

With marine production controlling 50% of global carbon flux (Field 1998; Raven and Falkowski 1999), microbial phytoplankton are the key link between ocean chemistry and biology (Azam *et al.* 1983; Falkowski, Fenchel, and Delong 2008). *Prochlorococcus* and *Synechococcus* combine to facilitate 50% of this marine flux (Partensky, Blanchot, and Vaulot 1999; Flombaum *et al.* 2013). Only a small percentage of the ocean has currently been sampled and models are used to visualize hypothesized population densities of microbes based on noted microbial traits (Buitenhuis *et al.* 2012). Modeling plankton, using growth and death traits quantified from

environmentally relevant environmental scenarios in laboratory experiments, is a key bridge in understanding how the physical environment of the ocean and the microbial life within it interact on large scales. This is sometimes done at the species level (Larkin *et al.* 2023) however the number of microbes known vs. culturable microbes is low (Barton *et al.* 2010; Fuhrman and Campbell 1998; Kaeberlein, Lewis, and Epstein 2002; Barberán and Casamayor 2010). Thus, direct laboratory to *in situ* extrapolation is difficult. Furthermore, modeling ocean dynamics with the full microbial diversity of the ocean would be computationally intractable. Other model frameworks consider genera of plankton constrained with laboratory optima of temperature and light growth extrapolated across global temperature and light patterns (Flombaum *et al.* 2013; Visintini, Martiny, and Flombaum 2021). While mirroring vertical and latitudinal distributions in the water column, these representations do not allow direct analysis of the underlying chemical and biological processes. For direct mechanistic connections between cellular growth and environmental conditions, we utilize a Massachusetts Institute of Technology (MIT) global plankton modeling framework (Follows *et al.* 2007). In Chapter 3 we explored cell based H_2O_2 parameters and their impact the community dynamics of picoplankton. In Chapter 4 we use these parameters and extrapolate to global scale investigation of community plankton dynamics via the implementation of a phytoplankton H_2O_2 trait model within the MIT General Circulation Model (gcm) (<https://darwinproject.mit.edu/research/>).

The ocean's physical dynamics imposed by the MIT General Circulation Model are then funneled into the MIT 'Darwin' plankton model (Follows *et al.* 2007). This trait model that uses Monod nutrient (Monod 1950) uptake that scales allometrically, meaning cell size determines with nutrient uptake, maximum growth, and nutrient quotas of each microbe (Edwards *et al.* 2012). This mirrors the choice in Chapter 3 to link nutrient affinity to size, with *Prochlorococcus* having a smaller size and associated higher nutrient affinity. Nutrient and temperature traits of globally important groups like Diatoms, Flagellates, and *Prochlorococcus* and *Synechococcus* are represented (Follows *et al.* 2007; Dutkiewicz, Follows, and Bragg 2009).

Global H_2O_2 has previously been represented in photochemical production models as linear (Powers and Miller 2014) or non-linear (Zhu *et al.* 2022) functions of light and water's physical properties. However, these models were implemented and compared to abiotic H_2O_2

dynamics. Omar *et al.* (2022) further investigated H_2O_2 as it, and its diffusive properties, pertain to differently sized cells (Omar *et al.* 2022, 20). While they determined direct cell-to-cell detoxification is unlikely in the diffuse marine ecosystem, community impact of batch species could be significant.

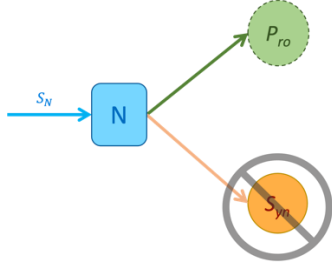
The future ocean is predicted to change in ways (increased temperature, increased stratification, pH decrease, CO_2 influx, nitrification decrease (Wilson *et al.* 2022; Dutkiewicz *et al.* 2015)) that are expected to widen the nutrient deplete areas of the ocean as well as increase production of reactive oxygen species (Friedlingstein *et al.* 2006; Raven and Falkowski 1999; Fu *et al.* 2007; Beman *et al.* 2011; Diaz and Plummer 2018; Dutkiewicz *et al.* 2015). Thus, we find it timely to investigate how cellular interactions with H_2O_2 recovered from Chapter 2 impact community dynamics. To start, this chapter details how Chapter 2 parameter ranges are converted to global parameters with further analyses interaction seen in Chapter 3 to investigate cellular impacts of H_2O_2 in a global model. Figure 4.1a below shows the four cases of our model we investigate within the Darwin framework which we show in a simplified rendition in Figure 4.1b.

METHODS:

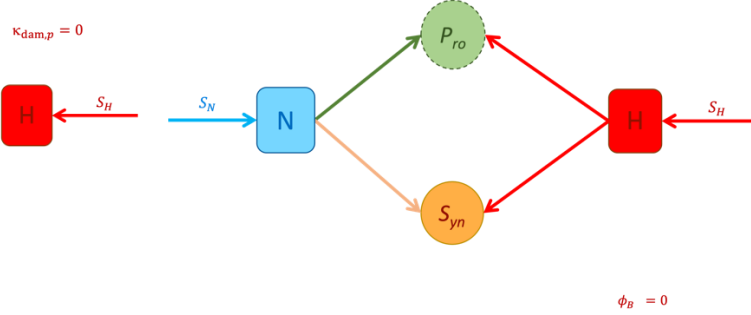
Figure 4.1 depicts the path this chapter takes to continue analyzing H_2O_2 impacts on plankton dynamics. Panel A represents the different community composition outcomes analyzed using the H_2O_2 model for two microbes under nutrient competition using monoculture derived parameters from Chapter 2. Panel B represents a simplified view of the ocean that is still computationally and mathematically dense. We utilize prior global frameworks created to handle plankton dynamics calculations across ocean basins to further investigate impacts of H_2O_2 on ocean plankton. The ‘Darwin’ framework tracks microbial cell concentrations (abundances) and nutrient concentrations using mass balance equations on a global framework. Thus, it allows for the quantification of chemical flow between the pools of microbes and nutrients of interest across global scales.

a.

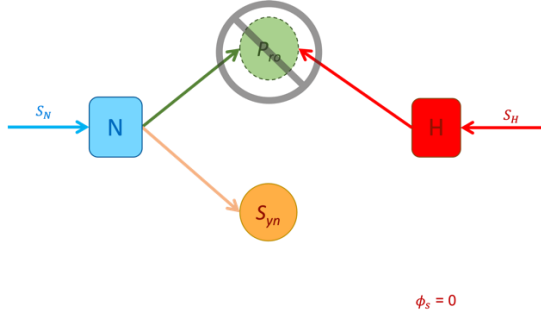
Case 1



Case 3



Case 2



Case 4

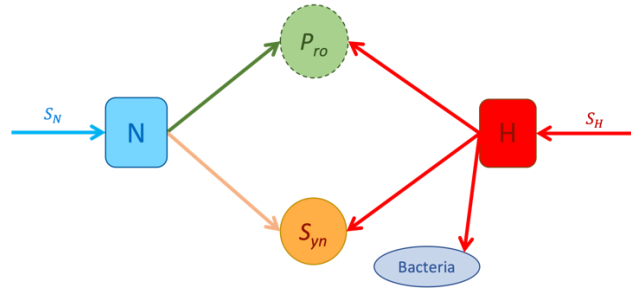


Figure 4.1 Extrapolating from four dimensionless plankton competition models to an *in situ* global plankton model influenced by realistic physical inputs. Stepwise (a -->b) model construction accounting for interactions of phytoplankton directly impacted or mediated by H_2O_2 . This model formulation accounts for measurable pools of nutrients (N), H_2O_2 , (H) or cells of *Prochlorococcus* (Pro), *Synechococcus* (Syn), and heterotrophic bacterial helper (Bacteria) represented by bubbles. The arrows represent the fluxes of carbon, nitrogen, *etc.* between these pools. The fluxes of this model are controlled by parameters retrieved from monoculture data analyses in Chapter 2.

b.

A 'simple' ocean

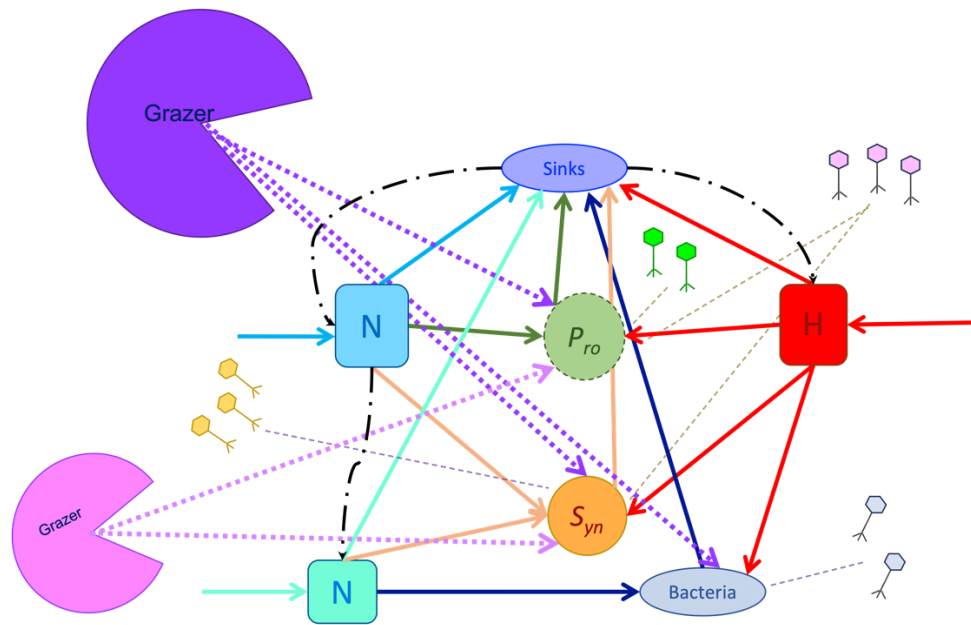


Figure 4.1 Continued

Generic tracers in the Darwin, labeled X (representing elements such as carbon, nitrogen, phosphorus, or iron), are influenced by advection (κX), diffusion (uX), and biological sources and sinks (S_{BX}) along with an additional loss (sinking) rate of ωx , that only applies to particles that are large enough to effect movement calculations (such as particulate organic matter or large phytoplankton). Z axis refers to axis of measured water column depth here.

$$\frac{\partial X}{\partial t} = \underbrace{S_{BX}}_{\text{Biological reactions on X}} - \underbrace{\nabla \cdot (uX)}_{\text{advection}} + \underbrace{\nabla \cdot (\kappa X)}_{\text{diffusion}} - \underbrace{\frac{\partial \omega x X}{\partial z}}_{\text{sinking along Z axis}}$$

Different tracers, representing single nutrients or complex microbial cells, are integrated for 10 years along on a 1 degree x 1 degree resolution of the globe allowing for the evaluation of each tracers' biogeographies (Follows *et al.* 2007). The first 9 years here are treated as a training (or burn in) period and we visualize patterns and dynamics that appear in the 10th, and final, year of the model. Darwin includes global temporal dynamics of temperature and light which are key to ROS production and were incorporated in the new H₂O₂ tracer dynamics (described below) for this global model. A Darwin model framework involving 6 plankton and 4 zooplankton each with unique growth traits implemented over the global ocean on chemical tracers such as carbon, ammonium, and iron (https://gitlab.com/darwinproject/gud/-/tree/gud/verification/monod_eccov3_6+4?ref_type=heads) was utilized. This implementation included offline ocean currents and initialization files for published Darwin tracers. This version of Darwin's trait framework forms the basis our novel model implementation that incorporates H₂O₂ abiotic and biotic interactions into multiple plankton tracers on a global scale. In this way, observed H₂O₂ impacts on plankton growth were explicitly tied to global predictions of plankton abundance. This allowed for the investigation of H₂O₂ based parameters and assumptions on globally dominant plankton genera *Prochlorococcus*, *Synechococcus*, and *Alteromonas* on a global scale.

MODEL AND EXPERIMENTAL APPROACH

We now document the changes that were made to the Darwin code, beginning with the insertion of our simple H₂O₂ model into a global framework. In the following equation reports the chemical and biological modification to the H₂O₂ tendency term (encapsulated within S_{Bx} in the above general mass-balance). Advective and diffusive effects on [H] are treated in the MITgcm (<https://darwinproject.mit.edu/research/>) and are not described further in this document.

$$[H] = [H] + ((QuantumYield)(DOC)(PAR_{total}) - abioticHdecay [H] - biotic_{detox}[H]) * timestep$$

H concentration abiotic H production rate abiotic H decay rate biotic H detoxification rate

Here, H₂O₂ production is now a linear function (Powers and Miller 2014) of dissolved organic carbon (DOC) and Total Photosynthetically Available Radiation (PAR). The abiotic decay of H₂O₂ was merely converted from recovered Chapter 2 findings, and the biotic detoxification term is described below. The timestep parameter controls the time step for integration and was set to three hours for all simulations. H₂O₂ production was initiated at year 1 in the Darwin burn in period with a starting concentration of 0 and augmented abiotic H₂O₂ supply and decay parameters. The H₂O₂ dynamics were allowed to stabilize over 9 years. The 10th year within the Darwin model is then used for analysis of mean yearly H₂O₂ dynamics and projected resultant impacts on biotic tracers.

CASE 1 – NONLEAKY WORLD, H₂O₂ NO-IMPACT

The tracers for *Prochlorococcus* and *Synechococcus* in Darwin were hijacked to allow for H₂O₂ trait parameter tradeoff analysis. For case 1, and all following cases, *Prochlorococcus* and *Synechococcus* were set to different size classes, with *Prochlorococcus* being the smaller, to allow Darwin's allometry-based framework inflict a growth cost on *Synechococcus* for retaining detoxification enzymes. Detoxification of exogenous H₂O₂ was neglected by setting biotic_detox in the [H] tracer equation to zero. Nutrient distributions were initialized from previous Darwin simulations. The tracers of *Prochlorococcus* and *Synechococcus* were then simulated over 10 years. This non-leaky H₂O₂ assumption, case 1, allows for the analysis of Chapter 3 competition frameworks with a set of global H₂O₂ projections. With no H₂O₂ damage term implemented on *Prochlorococcus*, the cost of higher cell size (*Synechococcus*) and innate differences in nitrogen

uptake and utilization traits (de Marsac *et al.* 2001; Moore *et al.* 2002) are expected to control the competitive outcome (Masuda *et al.* 2023).

CASE 2 – NON-LEAKY WORLD, H₂O₂ DAMAGE ON *PROCHLOROCOCCUS* IMPLEMENTED

The monoculture *Prochlorococcus* damage rate ($\kappa_{dam,p}$), isolated in Chapter 2 work, was converted to Darwin's units and shown as the cell-based H₂O₂ damage ($\kappa_{dam,p}$) in the equation for *Prochlorococcus*'s global tracer, denoted [P]. Table 4.1 shows the monoculture to Darwin conversions. The *Synechococcus* analogue was left agnostic to H₂O₂ as it was given neither H₂O₂ damage ($\kappa_{dam,s}$) nor H₂O₂ detoxification ($\phi_{max,s}$) parameters. H₂O₂ dynamics were dominated by abiotic supply and decay rates described above. The *Prochlorococcus* was simulated to change with time as follows:

$$[P] = \underbrace{[P]}_{\text{P concentration}} + \underbrace{(\text{DarwinGrowthFunctions} - \text{Kdam}(j) * \text{photoTempFunc}(j) * [H] * [P])}_{\text{P growth rate} - \text{P death rate due to H}} * \text{timestep}$$

CASE 3 - LEAKY WORLD, *SYNECHOCOCCUS* DETOXIFICATION IMPLEMENTED

The monoculture *Synechococcus* cell-specific detoxification rate ($\phi_{max,s}$) isolated from Chapter 2 was converted to Darwin's units, seen in Table 4.1, and inserted into the [H] tracer equation within the biotic detoxification parameter, $\phi_{max,i}$. The 'photoTempFunc', a function included within the Darwin framework, ensures that reactions with the rate $\phi_{max,i}$ only occur in ranges of ecologically relevant temperatures (*i.e.*, temperatures at which biotic growth can occur). The *Prochlorococcus* tracer was enacted as before in case 2. All community members were then projected forward through time for 10 years to analyze resultant community composition.

$$[\text{biotic}_{\text{detox}}] = [\text{biotic}_{\text{detox}}] + \underbrace{(\text{Phi}(j) * \text{photoTempFunc}(j) * [H])}_{\text{S detoxification rate of H}} * X$$

In the Darwin code, this line appears within a for loop that sums over plankton types, *j*. *Synechococcus*, but not *Prochlorococcus*, is part of this plankton type (*j*).

CASE 4 - LEAKY WORLD, SYNECHOCOCCUS AND BACTERIAL DETOXIFICATION

Lastly, heterotrophic bacterial (bacteria in equation) effects on detoxification, and thus *Prochlorococcus* biogeography were assessed. Biotic decay, from case 3, was given a second $\phi_{max,i}$ term, this one reliant on a steady state heterotrophic bacterial concentration ((2500 cells per mL)) and recovered (within Chapter 2) *Alteromonas* H₂O₂ detoxification ($\phi_{max,B}$) parameter values. With the lack of constraint on dynamics *in situ* heterotrophic bacterial concentrations (and corresponding lack of bacterial tracers within the Darwin framework), we enact only a steady concentration of heterotrophic bacteria, meaning no growth or innate death parameters are associated with the bacterial tracer. This also means the detoxification through bacteria does not draw down ammonium here, mirroring work done in Chapter 3), but acts outside of the *Prochlorococcus* and *Synechococcus* competition dynamics, acting on the H₂O₂ of the system alone. *Prochlorococcus* damage and *Synechococcus* detoxification is implemented as in case 3.

$$\text{bacterial}_{\text{detox}}[H] = \underbrace{[\text{bacterial}_{\text{detox}}] + (\text{Phi}(j) * \text{photoTempFunc}(j) * [H])}_{\text{bacterial detoxification rate of H}} * X$$

$$[H] = [H] + \left\{ \begin{array}{l} ((\text{QuantumYield})(\text{DOC})(\text{PAR}_{\text{total}}) - \text{abioticHdecay}[H]) \\ - \text{biotic}_{\text{detox}}[H] - \text{bacterial}_{\text{detox}}[H] \end{array} \right\} * \text{timestep}$$

$\left. \begin{array}{l} \text{H}_2\text{O}_2 \text{ abiotic rates} \\ \text{H}_2\text{O}_2 \text{ biotic rates} \end{array} \right\}$

As mentioned previously, Chapter 2 and 3 considered cells as their own entities while Darwin operates in moles of carbon, thus conversions were necessary to get recovered parameters into usable forms in this Darwin framework. The *Prochlorococcus* and *Synechococcus* plankton types within a published Darwin framework tracking 6 phytoplankton types globally were utilized to parallel the work performed non-spatially in Chapter 3. Cellular rates and parameter ranges from Chapter 2 were transformed into carbon-based rates to detoxification or damage scalars to be implemented via binary detoxification/damage parameters respectively. The values and conversions of Chapter 2 laboratory parameters to global model parameters for the Darwin framework are shown in Table 3. The binary state (on or off) of each global model parameter for investigative Cases 1- 4 is indicated by the color blocking in Table 4.

RESULTS:

Following the cases laid out in Figure 4.1, we analyze the effect of stepwise inclusion of H_2O_2 parameters delineated in Table 4. In Figure 4.2 we show our predicted H_2O_2 abiotic concentrations buildup via dissolved organic material (DOM), light, and recovered H_2O_2 production parameter values (from Chapter 2) using the Darwin framework that works on top of MIT global circulation models. This means that H_2O_2 concentration is now subject to spatial/temporal patterns from physical models. Thus, our model allows for the analysis of H_2O_2 as it impacts of global nutrient and chemical fluxes. Note that DOM within Darwin is a combined term composed of DOC (Dissolved organic carbon) and other dissolved organic nutrients that were not tracked within original laboratory cultures from Chapter 2.

CASE 1 – NONLEAKY WORLD, H_2O_2 NO-IMPACT

We first evaluated H_2O_2 implementation on a non-leaky world, or a world where H_2O_2 does not cross the cell membrane. Thus, we produce H_2O_2 (Figure 4.3) but the microbes are not affected by it (no biotic damage term, $k_{\text{dam},i} = 0$) and are not affecting H_2O_2 either (no biotic detoxification term, $\phi_{\text{max},i} = 0$). This means that competition between microbes should be governed solely by their nutrient and temperature functions in Darwin. Specifically with regards to the differing size classes of *Prochlorococcus* and *Synechococcus* analogue implemented to account for *Synechococcus* growth disadvantage due to catalase production.

Prochlorococcus should numerically dominate *Synechococcus* in areas where ammonium superiority would give a growth advantage. This is seen in Figure 4.3 alongside a H_2O_2 plot that remains unchanged between Figure 4.2 as there is no H_2O_2 -based drawdowns on any plankton groups' cell concentration. *Prochlorococcus* global concentration predictions, and all other tracer predictions except for DOC, thus remain largely unchanged between Darwin iterations without H_2O_2 and this first case of our H_2O_2 model. (Only minute differences in predictions occur here due to use of DOC in H_2O_2 production.

CASE 2 – NONLEAKY WORLD, H_2O_2 DAMAGE ON *PROCHLOROCOCCUS* IMPLEMENTED

We next implement our retrieved monoculture damage rate for *Prochlorococcus* in a *Prochlorococcus* analogue in the global model. The flux of H_2O_2 across membranes here is only

Table 3 Monoculture laboratory parameters conversion to global Darwin parameters

Monoculture Name	Monoculture units	Darwin Name	Value	Units in Darwin
-----NA-----	-----	H ₂ O ₂ _quantum_yield	3.0 x10 ⁻¹⁰	
K _{dam} <i>Prochlorococcus</i>	ml(cell) ⁻¹ (day) ⁻¹	H ₂ O ₂ _grp_Kdam	6.4x10 ⁻⁵	m ³ (mmol) ⁻¹ (s) ⁻¹
φ _{max} abiotic	(day) ⁻¹	H ₂ O ₂ _abiotic_detox	5.8 x10 ⁻⁸	(s) ⁻¹
φ _{max} bacteria	ml(cell) ⁻¹ (day) ⁻¹	H ₂ O ₂ _bacterial_detox	5.8 x10 ⁻⁶	m ³ (mmol) ⁻¹ (s) ⁻¹
φ _{max} <i>Synechococcus</i>	ml(cell) ⁻¹ (day) ⁻¹	H ₂ O ₂ _grp_Phi	1.39 x10 ⁻⁴	m ³ (mmol) ⁻¹ (s) ⁻¹

Table 4 Global parameter inclusion into four global model cases

Case	H ₂ O ₂	Abiotic detoxification	K _{dam} <i>Prochlorococcus</i>	ϕ_{max} <i>Synechococcus</i>	ϕ_{max} Heterotrophic bacteria
1	yes	yes	no	no	no
2	yes	yes	yes	no	no
3	yes	yes	yes	yes	no
4	yes	yes	yes	yes	yes

allowed for cells damaged by H_2O_2 . Again, *Synechococcus* and other (heterotrophic) bacterial tracers are agnostic to H_2O_2 . When projecting these results below, we see that the damage from H_2O_2 decimates *Prochlorococcus* across the globe allowing for *Synechococcus* to achieve what many humans strive for; world domination. However non-leaky detoxification is not possible practically for microbes in the ocean as H_2O_2 crosses cell membranes and is produced in the sunlit surface ocean.

CASE 3 - LEAKY WORLD, *SYNECHOCOCCUS* DETOXIFICATION IMPLEMENTED

We next transition to a leaky world, as we know H_2O_2 can cross biological and artificial phospholipid membranes (Antunes and Cadenas 2000; Möller *et al.* 2019; Tjell and Almdal 2018). Implementing detoxification found in Chapter 2 for the *Synechococcus* analogue, we view a global model of a two-microbe system in H_2O_2 below in Figure 4.5. With *Synechococcus* alone conducting detoxification, *Prochlorococcus* biogeography is expanded back to a range reminiscent of *in situ* studies. Not only does *Prochlorococcus* survive in marine environments unavailable to it in Figure 4.4., it does so with the implementation of monoculture H_2O_2 damage and detoxification ($\phi_{max,i}$) parameters retrieved in Chapter 2. Thus, monoculture H_2O_2 interactions, parameterized in laboratory cultures, have the potential to impact global microbial populations. Note that while *Prochlorococcus* does grow in the lower latitudes, the encroachment of *Synechococcus* into the northern Atlantic regions does not match *in situ* data (Buitenhuis *et al.* 2012; Partensky, Blanchot, and Vaultot 1999).

CASE 4 - LEAKY WORLD, *SYNECHOCOCCUS* AND BACTERIAL DETOXIFICATION

Lastly, bacterial detoxification along with *Synechococcus* biotic detoxification was analyzed with respect to model outcomes. Figure 6 shows these results. With bacterial detoxification implemented as a $\phi_{max,B}$ and B^* , with B^* here representing the assumed equilibria (stationary phase concentration) of heterotrophic bacterial population. This additional detoxification essentially reduced the standing stock of H_2O_2 that *Synechococcus* then has to detoxify in order to compete to coexist with *Prochlorococcus*. Bacterial detoxification (without *Synechococcus*) is visualized in the Chapter 4 Supplementary Information of Appendix C. While *Synechococcus* detoxification alone seems to save *Prochlorococcus* in this model framework, the amount of *Prochlorococcus* recovered with bacteria and *Synechococcus* is greater than that of

Synechococcus detoxification alone. With bacteria like *Alteromonas* absent from biogeochemical models, this basal detoxification using monoculture parameters and assumed stationary bacteria are the limit of this study.

DISCUSSION:

Chapter 4 adds realism to this modeling story with the addition of physical water movements, seasonal variation, and global spatial resolution. We use the Darwin framework that transports biological tracers, affected by light, nutrient, and temperature fields, by currents, $\mathbf{u}$, and mixing coefficients, κ , using the MIT ocean circulation model. Cases 1 – 4 shown in Table 4 are investigated using *Prochlorococcus* and *Synechococcus* analogues made to differ in nutrient affinity using Darwin 's built in allometric scaling. Initial model runs, case 1, were used to ensure that under a zero H_2O_2 production regime (Figure 4.3), a superior nutrient affinity (*Prochlorococcus* analogue) ensures competitive advantage in marine systems. This H_2O_2 map is projected from case 1 of the methods mentioned above. Brighter red colors represent higher H_2O_2 within ocean waters. These projected values are from between the 25 and 30m depth slice (27.5 meters is the midpoint) of the MITgcm giving the approximate mixed layer concentration of H_2O_2 of the globe.

H_2O_2 global production was implemented as a function of our recovered (Chapter 2) H_2O_2 production rate, Dissolved organic material (DOM) concentrations, and light influx for the surface ocean. More accurate quantum yields of H_2O_2 take into account the wavelengths of light, but this is not yet feasible for this model within the Darwin framework as PAR is a composite variable. However the global map of 1 year the results of our projected H_2O_2 geography in Figures 4.2a and 4.2b respectively give similar patterns and ranges to more complex photochemical models (Zhu *et al.* 2022). With H_2O_2 production solved, the biological connections between H_2O_2 and *Prochlorococcus* or *Synechococcus* were inserted first with damage and then detoxification rates. By inserting damage rates (recovered from Chapter 2) without implementing detoxification as well, we simulate a 'selfish detoxification' world. This allows for Figure 4.5 to give the resultant 'flipped' biogeography to that of known oceanic measurements (Partensky, Blanchot, and Vaulot 1999) , while the implementation of both damage and detoxification gave a more realistic world view in Figure 4.6. The abiotic decay,

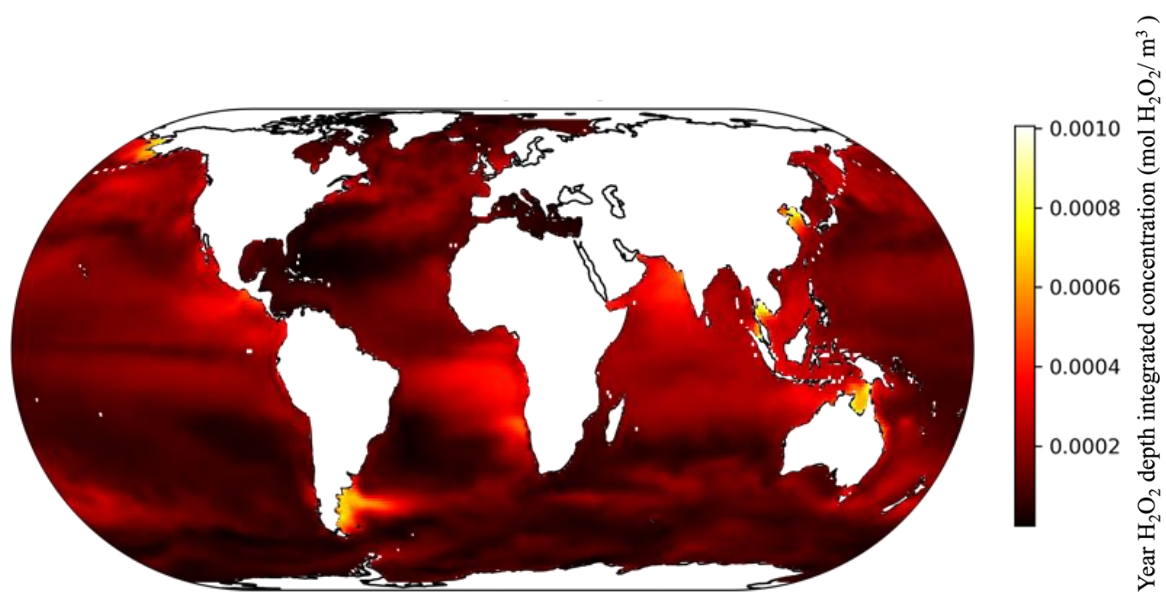


Figure 4.2 Mean temporally aggregated H_2O_2 concentration from last year of a 10 year global circulation model

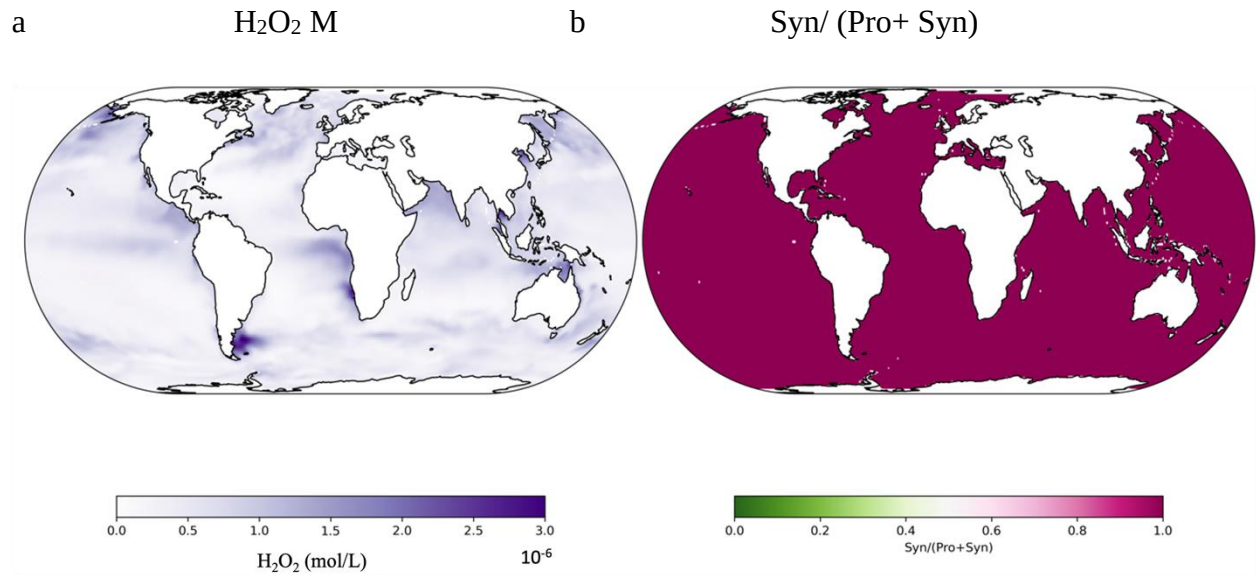


Figure 4.3 Nutrient Competition in a non-leaky world

Annual mean H_2O_2 (a, left) and the ratio of *Synechococcus* (*Syn*) over *Prochlorococcus* (*Pro*) and *Synechococcus* (*Syn*) (b, right) from the last year (9 or 10) in case 1 of our model. With the nutrient tradeoff of H_2O_2 detoxification incorporated but not H_2O_2 impact, *Synechococcus* (in red) is outcompeted nearly everywhere by *Prochlorococcus* (in green). Thus, if H_2O_2 is not directly tied to the biology, *Prochlorococcus* in this model format is projected to numerically dominate over *Synechococcus* at lower latitudes via its superior ammonium affinity.

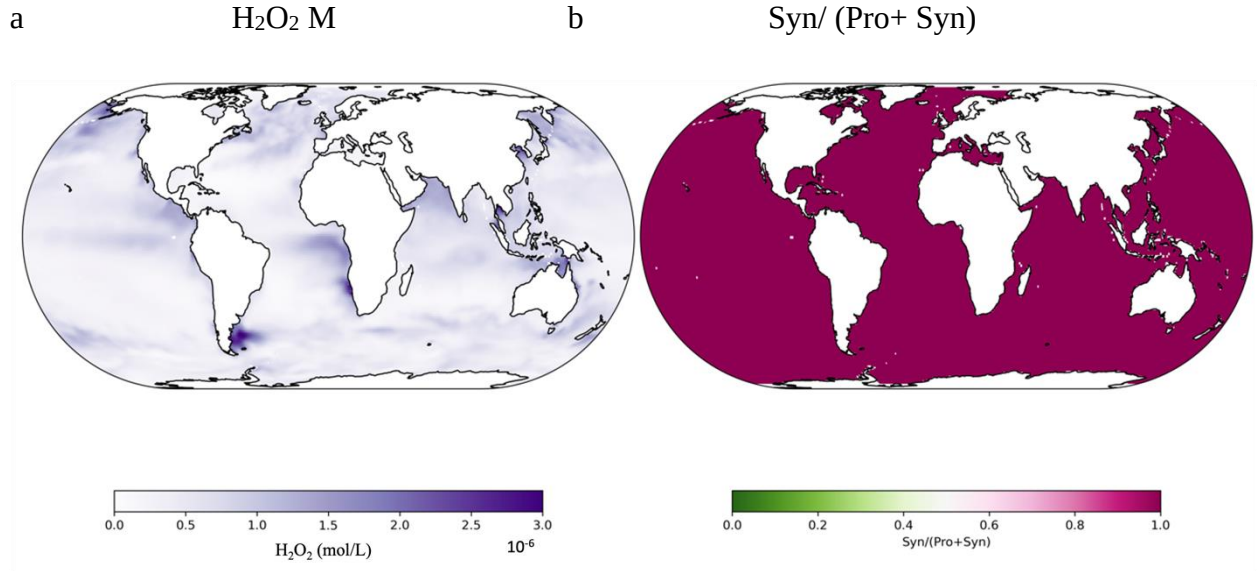


Figure 4.4 Unilateral H_2O_2 damage in a non-leaky world

Annual H_2O_2 (left, a) and (right, b) *Synechococcus* (Syn) over *Prochlorococcus* (Pro) and *Synechococcus* (Syn) ratio predicted from 1 year if H_2O_2 influx ($S_H > 0$) and damage ($\kappa_{\text{dam},i}$) but no biotic detoxification ($\phi_{\text{max},i}$) is allowed. *Synechococcus* survives H_2O_2 test while *Prochlorococcus* is killed everywhere despite its superior nutrient affinity. Leading to a world devoid of *Prochlorococcus* cell biomass.

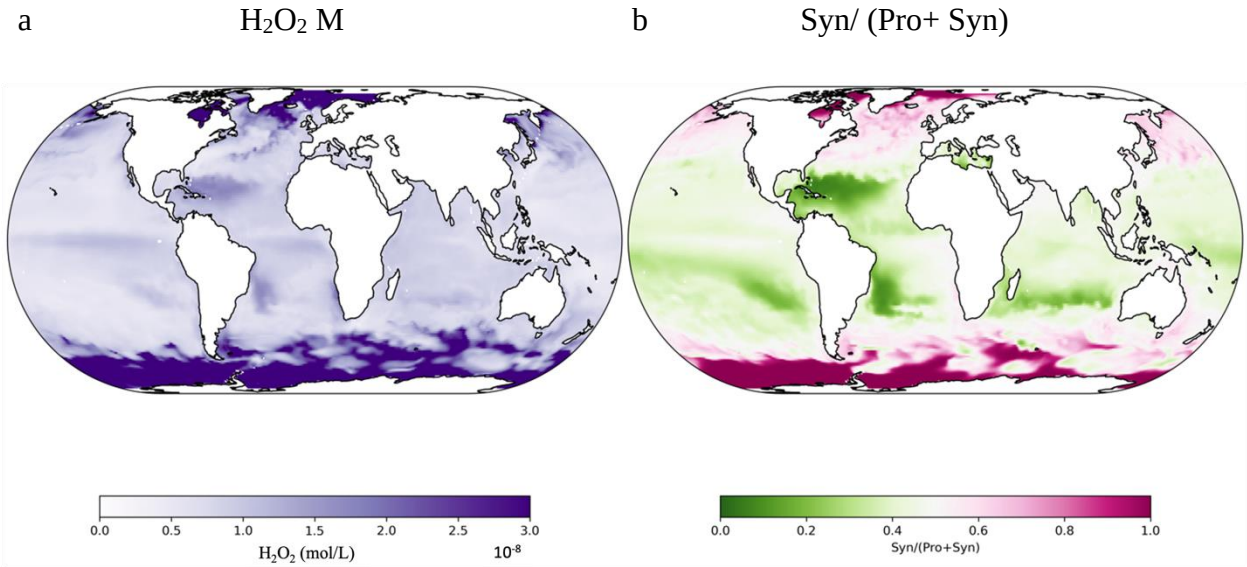


Figure 4.5 Leaky H_2O_2 dynamics using monoculture *Prochlorococcus* and *Synechococcus* parameters. Annual mean H_2O_2 (left,a) and (right,b) *Prochlorococcus* and *Synechococcus* from year 9 of our model case for H_2O_2 impact with biotic detoxification implemented as well. *Prochlorococcus* is saved from obliteration through the implementation of a biotic H_2O_2 detoxification parameter ($\phi_{max,i}$) provided by *Synechococcus* cells ($\phi_{max,s}$) in this case 3 model run. Heterotrophic bacteria are neglected in this case (#3).

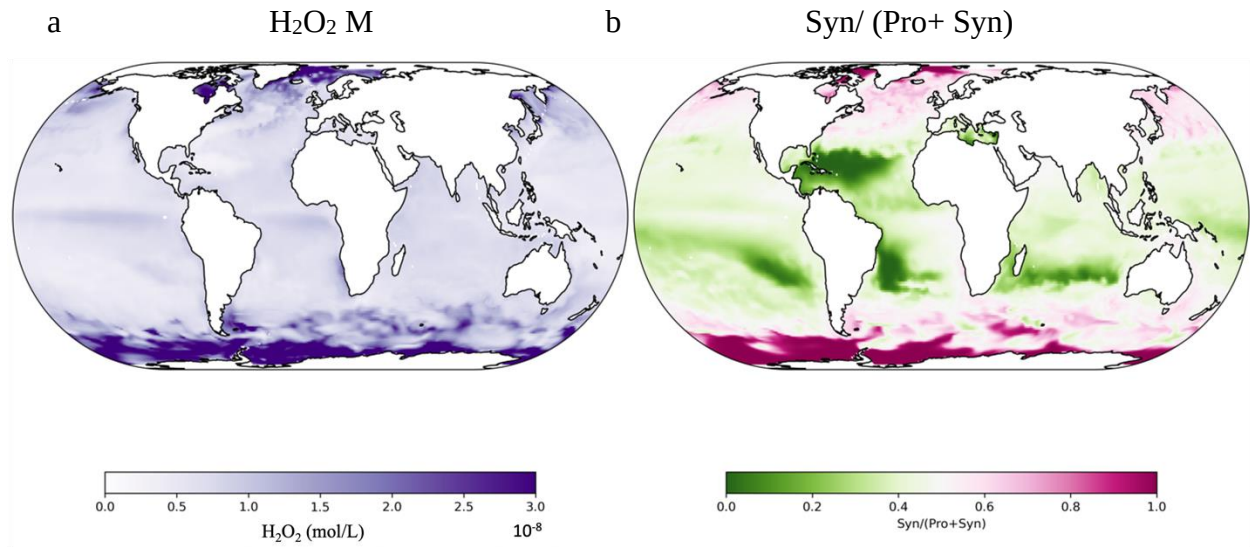


Figure 4.6 Leaky H₂O₂ dynamics using Bacterial and *Synechococcus* detoxification parameters.

H₂O₂ (left, a) and (right, b), *Prochlorococcus* and *Synechococcus* ratio predictions incurred from *Synechococcus* and bacterial detoxification both occurring within a global framework.

Prochlorococcus is further saved by H₂O₂ detoxification parameter from *Synechococcus* ($\phi_{max,S}$) and bacterial detoxification ($\phi_{max,B}$) inclusion, while *Synechococcus* biomass is also bolstered (in the southern ocean) by the additional (heterotrophic bacteria) detoxification.

other than bacterial inclusion in case 4, is not linked to cell waste. Dead cells and particulate matter pools of material may act as significant reactive oxygen sinks. But as these are unquantified currently on monoculture levels, we identify this for future studies.

Prochlorococcus biogeography is commonly modeled to be primarily based on the intersection of environmental nitrogen, temperature, and light forcings (Flombaum *et al.* 2013; Rabouille, Edwards, and Zehr 2007). Higher ammonium affinity allows *Prochlorococcus* to numerically dominate low latitude and oceanic gyres (Partensky, Blanchot, and Vaulot 1999, 201; Partensky and Garczarek 2010) over other phytoplankton genera (Dutkiewicz, Follows, and Bragg 2009). Previous models indicate *Prochlorococcus* and *Synechococcus* community composition is influenced by differential light and nutrient or temperature optima (Moore, Goericke, and Chisholm 1995; Mella-Flores *et al.* 2012; Lipschultz 1995). Light and temperature stress are both implicated in reactive oxygen species mediated cell damage (Fu *et al.* 2007; Ma *et al.* 2018). With multiple intersecting environmental stresses weighing on cyanobacteria, H₂O₂ detoxification amelioration has important environmental stress relieving effects on *Prochlorococcus* growth. Ma (2018) investigated the effect of H₂O₂ on biogeochemistry with regard to temperature, showing that microbial helper populations were essential to recover *Prochlorococcus* biogeography reflected in oceanic data (Partensky, Blanchot, and Vaulot 1999; Ma *et al.* 2018). This study expands upon possible bacterial cell effects on *Prochlorococcus* though with evaluation of plankton cell-specific (Chapter 2 isolated) detoxification ($\phi_{max,i}$) of environmental H₂O₂.

Decreasing pH of the ocean is projected to increase ocean reactivity (Ahmad *et al.* 2015; Hopwood *et al.* 2018) while increased CO₂ also has been shown to increase H₂O₂ production (Klanian and Preciat 2017; Hennon *et al.* 2018). Further, warmer temperatures are predicted decrease oceanic nitrogen flux (Beman *et al.* 2011; Sallée *et al.* 2021) and augment community composition through cascading changes in nutrient structure (Dutkiewicz *et al.* 2014, 201; 2015). This implies that as pCO₂ rises and oceans warm (Dutkiewicz, Scott, and Follows 2013; Fu *et al.* 2007) H₂O₂ detoxification requirements of *Prochlorococcus* may be simultaneously more profound and less addressed via the community.

Prior H₂O₂ models have shown depth and spectra resolved H₂O₂ quantum yields from seawater (He and Häder 2002; Zhu *et al.* 2022; Cooper *et al.* 1988). This study omits the noted effects of UV spectra on H₂O₂ as our current laboratory data made it impossible to isolate specific wavelength based H₂O₂ parameters. Instead, the study utilizes the Darwin total PAR as the main light controlling H₂O₂ production in the global model. Further (dissolved organic carbon) DOC and light effects on H₂O₂ are not constrained in monoculture (Chapter 2) here before insertion (S_H term here originated from spike assay analyses in Chapter 2). Use of these models on H₂O₂ producing or inert media buffers, like HEPES and TAPS buffers, in Appendix A, (Zinser 2013; Zigler *et al.* 1985; Lepe-Zuniga, Zigler, and Gery 1987) or natural waters (Morris *et al.* 2016; Goldstone and Voelker 2000; Powers and Miller 2014) that produce H₂O₂ could further constrain the production terms in this model format to start accounting for this drawback. Dark production has been noted for various reactive oxygen species (Sutherland, Wankel, and Hansel 2020; Zinser 2013) but this is ignored here as the diel light cycle produces significantly higher H₂O₂ fluxes (Cooper *et al.* 1988) and more readily matches laboratory methods and prior H₂O₂ ocean studies (Morris, Rose, and Lu 2022; Zinser 2018b). This study also ignores direct H₂O₂ deposition from atmospheric production and diffusion (O’Sullivan *et al.* 1999), rain events (Willey, Paerl, and Go 1999; Hopwood *et al.* 2018; Morris, Rose, and Lu 2022), and terrestrial runoff effects (Zhang, Del Vecchio, and Blough 2012) which have all been noted as significant sources of surface water H₂O₂. This model uses binary inclusion of model parameters in cases 1- 4 but there are an array of H₂O₂ detoxification mechanisms and an even larger array of ways in which cells have been shown to augment the number, regulation, or combination of these detoxification mechanisms throughout their lifecycles (Gómez-Baena *et al.* 2001; Kawasaki *et al.* 1997; Morris *et al.* 2016, 20; Rai *et al.* 2021; Sato *et al.* 1992; Venkatesan *et al.* 2007; Schellhorn 1995). This range of control on possible H₂O₂ parameter values at the microbial cell level are ignored here for simplicity. Further, another iteration of Darwin, using intercellular fluxes has been published, but as exact H₂O₂ detoxification mechanisms have not been mechanistically traced within *Prochlorococcus* cells as extensively they have been in *E.coli* (Seaver and Imlay 2001) or neutrophils (Ohno and Gallin 1985), this remains out of our framework’s scope. To our knowledge, none of these reported H₂O₂ influences are incorporated

into large scale ecosystem or biogeochemical model. Using Darwin, we have assessed biologically measured impacts of H_2O_2 on dominant microbes in the oligotrophic ocean.

This model does not account for ecotype or strain variability within any of the microbes chosen here. The Darwin framework does allow for multiple microbes within a group to scale a shared trait allometrically so later inclusion of this intricacy may be feasible. However, more monoculture constraint on the connection between detoxification gene presence within a cell and the cell's detoxification ability is needed for clarity here. Furthermore, we chose microbes of importance for Chapter 2 basal modeling. However, the Darwin model framework we utilized only includes 10 plankton (6 phytoplankton, 4 zooplankton) total, meaning it fails to capture a variety of ocean complexity. The picoeukaryotic (Appendix B) and picoheterotrophic (*Alteromonas* in Appendix A) bacteria that seem to detoxify most for *Prochlorococcus*, are among the dynamics lost with this model implementation despite their various documented impacts on *Prochlorococcus*' growth (Biller, Coe, *et al.* 2018; Guillou *et al.* 2001, 20; Follett *et al.* 2022; Weissberg, Aharonovich, and Sher 2023, 20). Different heterotrophs have documented differential susceptibilities to H_2O_2 (Bond, Hansel, and Voelker 2020, 202), thus these need to be investigated along with picoeukaryotes that grow alongside *Prochlorococcus* (Visintini, Martiny, and Flombaum 2021).

CONCLUSIONS:

The aim of Chapter 4 was to insert the cell based H_2O_2 parameters that were isolated and estimated in Chapter 2 and investigate the community dynamics those parameters predict in Chapter 3 on a global scale via the phytoplankton trait-based model Darwin. With the exploration of H_2O_2 trait parameters, differences in H_2O_2 susceptibilities on model organisms from laboratory to global scales are analyzed. Through differential implementations of the full model framework, global repercussions of two recovered H_2O_2 parameters ($\phi_{\max,i}$ and $k_{\text{dam},i}$) on the microbiology of the ocean were further investigated. Outcomes in this chapter echoed non-spatial model conclusions; that the combination of H_2O_2 leaky damage and H_2O_2 leaky detoxification can allow for coexistence rather than competitive exclusion in our model.

Chapter 5 CONCLUSIONS AND FUTURE WORK

Author(s): D.K.M., D.T.

Author contribution statements: Written and edited by D.K.M. with feedback from D.T.

Prochlorococcus is the smallest yet most numerous phytoplankton of the oligotrophic surface ocean (Flombaum *et al.* 2013; Partensky, Blanchot, and Vaulot 1999, 199). Its dominance of marine microbial communities is primarily attributed to its superior nutrient affinities (Masuda *et al.* 2023; Partensky and Garczarek 2010) induced from genetic and physical streamlining. *Prochlorococcus*'s closest genetic relative, *Synechococcus*, dominates growth at higher latitudes and lower depths as compared to its streamlined sister (Flombaum *et al.* 2013, 201). Lab experiments indicate that streamlining-induced loss of fundamental hydrogen peroxide (H_2O_2) detoxication genes, like *katG* encoded catalase-peroxidase, has led to *Prochlorococcus* requiring coexisting 'helper' microbes, such as *Synechococcus* (Bernroither *et al.* 2009; Biller, Berube, *et al.* 2015; Morris *et al.* 2011), survive the daily environmental H_2O_2 produced (Morris, Rose, and Lu 2022; Zhu *et al.* 2022) surface waters. Current phytoplankton global models attribute 25% of global production to *Prochlorococcus* (Flombaum *et al.* 2013) but exclude H_2O_2 as a source of microbial death.

H_2O_2 production may be directly impacted by the higher pCO_2 , higher temperatures, and lower pHs predicted to occur with changing climate and increasingly stratified upper ocean. Microbial nutrient structures will be further impacted by the decreased atmospheric nitrogen deposition along with decreased upwelling rates (Gruber and Galloway 2008; Altabet, Robinson, and Walstad 1986, 19). Nutrient pressures alone would indicate the future ocean selecting for *Prochlorococcus*-like microbes (Martiny *et al.* 2022; Voss *et al.* 2013). However, as H_2O_2 inputs may increase, while nutrient depletion could simultaneously decrease community 'helper' populations, additional investigation is required to further the understanding of this system. Current biochemically resolved circulation models of Earth's oceans (Carroll *et al.*, 2020; Ward *et al.*, 2012) agree with thermal niche and nutrient models that *Prochlorococcus* dominates in the most oligotrophic marine surface regions (Dutkiewicz, Follows, and Bragg 2009; Follows and Dutkiewicz 2011; Ward *et al.* 2017). However explicit representations of H_2O_2 , impacts on these cyanobacteria have been ignored. This thesis aims to serve as a basal step in filling this need.

This thesis presents a stepwise methodology for the construction of a microbial model framework. Laboratory data was used to constrain monoculture parameters that we then manipulate across modeled nutrient and H_2O_2 landscapes in order to investigate these as drivers

of microbial community composition. Divergent phenotypes of different picoplankton are quantifiable H_2O_2 using this model. This is the first iteration of quantifying the biogeographical impact of H_2O_2 on various plankton found in the surface ocean. The tradeoffs between growth advantage (*Prochlorococcus* and other streamlined competitors) and H_2O_2 detoxification (*Synechococcus* or heterotrophic bacteria) were found to control the toggle between competitive and coexistent behavior in this model framework. The leakiness of H_2O_2 detoxification was found to be a key driver of coexistence both in both the dimensionless Chapter 3 and the spatially and temporally resolved Chapter 4. Thus, quantitative ecological evaluation of microbes impacted by H_2O_2 can be performed at the community level using this model framework.

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APPENDICES

APPENDIX A SUPPLEMENTARY INFORMATION CHAPTER 2

A1 PARAMETER FITTING PROCEDURE

The goal of our fitting procedure was to search for probability distributions that maximize the probability of each parameter being true, given the model and the experimental data. Let a given experimental dataset be labelled D , and a set of parameters be θ . In the following, we describe a procedure to estimate the probability of the parameters given the observations, which may be written $P(\theta | D)$. The following is true for any specific choice of data (e.g. *Prochlorococcus* cell densities through time, main text Figure 2.4a) and corresponding parameter set (e.g. $\theta = \{P_0, \mu_{max}\}$, main text Figure 2.4b,c). Using Bayes theorem, the probability of a given parameter set given the observations is:

$$(A1 \text{ eq1}) \quad P(\theta | D) = \frac{P(D|\theta)P(\theta)}{P(D)}$$

In practice, it is difficult to directly find the set θ which agrees with the probability in Equation S1. The Metropolis-Hastings algorithm approximates the set θ with a random walk procedure. Consider first assuming a given parameter set, which we call θ_{old} . The essence of the random walk algorithm is to consider the feasibility of this parameter set by comparing it to a competing set of parameters, which we call $\theta_{proposed}$. By Equation A1 eq1, the ratio of $P(\theta_{proposed}|D)$ to $P(\theta_{old}|D)$ may be written as

$$(A1 \text{ eq2}) \quad \frac{P(\theta_{proposed}|D)}{P(\theta_{old}|D)} = \frac{P(D|\theta_{proposed})}{P(D|\theta_{old})} \frac{P(\theta_{proposed})}{P(\theta_{old})}$$

Note that $P(D)$ does not appear in Equation A1 eq2, which is helpful as this quantity is difficult to ascertain empirically. Equation A1 eq2 requires knowledge of $P(D|\theta)$, the probability of the data for a given parameter set, and $P(\theta)$, the probability of a given parameter set independent of anything else. Before defining these mathematically, we focus attention on procedural aspects of the Metropolis-Hastings algorithm.

The Metropolis-Hastings algorithm generates each new parameter set following a random walk procedure:

$$(A1 \text{ eq3}) \quad \mu_{prop} = \mu_{old}e^a \quad \tau_{prop} = \tau_{old}e^b \quad \varphi_{prop} = \varphi_{old}e^c \quad \varphi_{prop} = \varphi_{old}e^d$$

where a, b, c and d are independent random numbers drawn from a normal probability distribution with mean zero and standard deviation 0.05. The precise choice of standard deviation modifies the computational efficiency of the algorithm but should not change the results (Robert and Casella 2010). If the new parameter set is more probable than the old parameter set, then the ratio of $P(\theta_{proposed}|D)$ to $P(\theta_{old}|D)$ (Equation A1 eq2) will be greater than one. If this is the case, then we can adopt this parameter set by relabeling it θ_{new} . We then generate another $\theta_{proposed}$ with Equation 7 (this time θ_{new} becomes θ_{old}), and repeat this procedure iteratively until the random walk navigates to the set that best describes the probability in Equation A1 eq1. A subtle but key additional feature of the Metropolis-Hastings algorithm is to search parameter space by occasionally letting worse parameter sets be adopted. It does this by accepting the new parameter set if:

$$(A1 \text{ eq4}) \quad \frac{P(\theta_{proposed}|D)}{P(\theta_{old}|D)} e^{a+b+c+d} \geq r$$

where r is drawn randomly from a uniform distribution within the range [0,1]. This prevents parameter set convergence on local minima and helps the algorithm search for global minima, and thus global optima for parameter values. Note that the exponential term in Equation A1 eq4 helps the algorithm compensate for the asymmetry of the lognormal distribution, and its tendency to favor large values over small values. If the ratio in Equation A1 eq4 is greater than r, then it must also be greater than one, and hence improvements to the parameter set are always accepted. However, every so often, the ratio in Equation A1 eq4 will be less than one but greater

than r . Here, poorer parameter sets are still opted for allowing for the search of some ‘bad’ parameter sets as well. The algorithm still converges upon optimal parameter sets since there is a stronger tendency to select improved parameter sets over poorer ones.

We now return to the unknowns in Equation A1 eq2: the probability of the data being true with respect to a given parameter set (i.e., $P(D|\theta)$), and the probability of each parameter set being true, independent of anything else (i.e., $P(\theta)$). In practice, these are typically quantified by a likelihood, and a set of priors, respectively. The likelihood of the data being true for any proposed parameter set is calculated with a likelihood function, $\mathcal{L}(\theta)$, which is defined as follows:

$$(A1 \text{ eq5}) \quad P(D|\theta) = \mathcal{L}(\theta) = \exp(-\chi^2)$$

Where χ^2 is the squared sum of differences between log-transformed hydrogen peroxide and cell abundances and the corresponding model estimate, evaluated at θ (labelled here $y_{i,m}$):

$$(A1 \text{ eq6}) \quad \chi^2 = \sum_i \frac{(\overline{\ln(y_{i,j})} - \ln(y_{i,m}))^2}{\sigma_{y,i}}$$

In Equation A1 eq6, $y_{i,j}$ represents the population size at time-point $i=1,n$, and replicate $j=1,m$, and $\overline{\ln(y_{i,j})}$ is the average of replicates after log-transformation. The parameter $\sigma_{y,i}$ is an uncertainty value, which sets the confidence with which we believe each datapoint is true. The specific choice of $\sigma_{y,i}$ was estimated for each dataset separately by analyzing the standard deviation among replicates or triplicates of log-transformed hydrogen peroxide and cell abundance data (see Section A2 and Figures A1-A3).

The probability of a given parameter set being true, ($P(\theta)$ in Equation A1 eq1), is set by priors, which we have the freedom to choose. We assumed uninformative priors by neglecting $P(\theta)$, allowing all parameter values to be equally feasible before the fitting procedure. For each model-data fit, the random walk was run for 100,000 steps in parallel from two chains. The initial 50,000 iterations of the random walk were discarded as ‘burn in’ period or training runs.

A2 QUANTIFYING UNCERTAINTY IN EXPERIMENTAL DATA

The Metropolis-Hastings algorithm takes information about the uncertainty of each experimental datapoint to quantify parameter posterior distributions. Our likelihood function (Equations A1 eq5 and A1 eq6) assumes log-transformed data are normally distributed. This assumption was based on our observation that strong correlation between standard deviation and mean of biological replicates (Figure A2.1, left column) largely disappeared after log-transformation (Figure A1, right column). Strong positive correlation between standard deviation and mean is an indication that a multiplicative process generates variance in biological replicates. The lack of clear structure in the relationship between standard deviation and mean after log-transformation suggests a log-normal distribution may be an appropriate description of variance. The log-normal distribution is widely used to model variance in biological systems for theoretical and empirical reasons (Crow and Shimizu 1987; Koch 1966; Limpert, Stahel, and M. 2001).

Figure A1 gives the analysis of the relationship between standard deviation and mean among biological triplicates for raw (left column) vs log-transformed (right column) a,b) *Prochlorococcus* abundance data c,d) *Synechococcus* abundance data and e,f) Hydrogen peroxide concentrations. There is greater positive correlation between mean and standard deviations in untransformed data, which is largely removed after log-transformation. We interpret this as evidence of multiplicative noise-generating processes and assume log-transformed data are normally distributed.

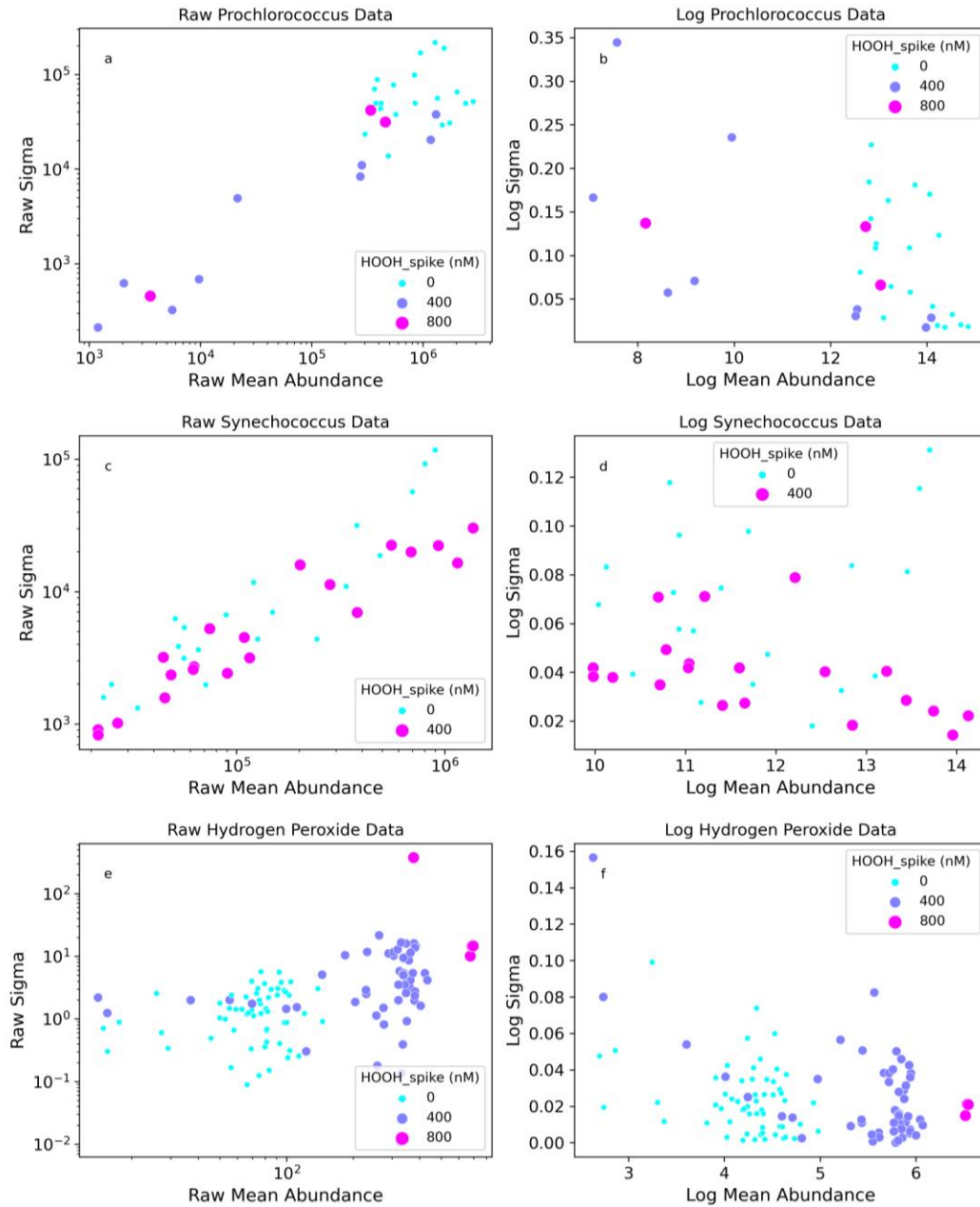


Figure A 1 Standard deviation and mean analysis among biological triplicates for raw (left) vs log-transformed (right) Prochlorococcus abundance, Synechococcus abundance and H_2O_2 (HOOH) data.

We first assumed that the uncertainty parameter, $\sigma_{y,i}$ could be constrained by the standard deviation among biological replicates or triplicates:

$$(A2 \text{ eq1}) \quad \sigma_{y,i}^2 = \sum_j \left(\ln(y_{i,j}) - \overline{\ln(y_{i,j})} \right)^2$$

where $y_{i,j}$ represents the population size at time-point $i=1,n$, and replicate $j=1,m$, and $\overline{\ln(y_{i,j})}$ is the average of replicates. Within each dataset, we initially constructed a 99% confidence interval on the uncertainty parameter, by assuming that the standard deviation follows a chi-squared distribution (Sheskin 2003):

$$(A2 \text{ eq2}) \quad \sqrt{\frac{m-1}{\chi_{\frac{\alpha}{2}, m-1}^2}} < \sigma_{y,i} < \sqrt{\frac{m-1}{\chi_{1-\frac{\alpha}{2}, m-1}^2}}$$

where α is the desired confidence level which in our case is $1-0.99=0.01$. Due to the high-level of uncertainty associated with sample size $m=2$ or $m=3$ (as is the case for biological replicates or triplicates, respectively), Equation S9 led to unrealistically wide confidence intervals for the standard deviations. We therefore estimated the standard deviation across time-points. To do this, we first subtracted the mean of log-transformed replicates from each log-transformed replicate (i.e. $\tilde{y}_{i,j} = \ln(y_{i,j}) - \overline{\ln(y_{i,j})}$). We assumed that each $\tilde{y}_{i,j}$ was normally distributed with mean 0 and unknown variance σ_y which we assumed was equal across time-points (i.e. $\sigma_y = \sigma_{y,i}$ for all $i=1,n$). We then calculated the variance of $\tilde{y}_{i,j}$ and used Equation S9 to again construct 99% confidence intervals, this time however, with $n-1$ degrees of freedom:

$$(A2 \text{ eq3}) \quad \sqrt{\frac{n-1}{\chi_{\frac{\alpha}{2}, n-1}^2}} < \sigma_y < \sqrt{\frac{n-1}{\chi_{1-\frac{\alpha}{2}, n-1}^2}}$$

In Figure A2 2a, we show hydrogen peroxide replicates in media with zero H_2O_2 addition. The blue dots in Figure A2 2b show log-transformed standard deviations among replicates (i.e., $\sigma_{y,i}$). The red-dashed line is the average standard deviation across time-points, and the solid red line is the upper limit of the 99% confidence interval in Equation A2 eq2. The solid red line was used to constrain $\sigma_{y,i}$ in Equation A2 eq3. A different $\sigma_{y,i}$ was calculated for

each dataset D following the same procedure. An example of this for *Synechococcus* cell dynamics is shown in Figure A3.

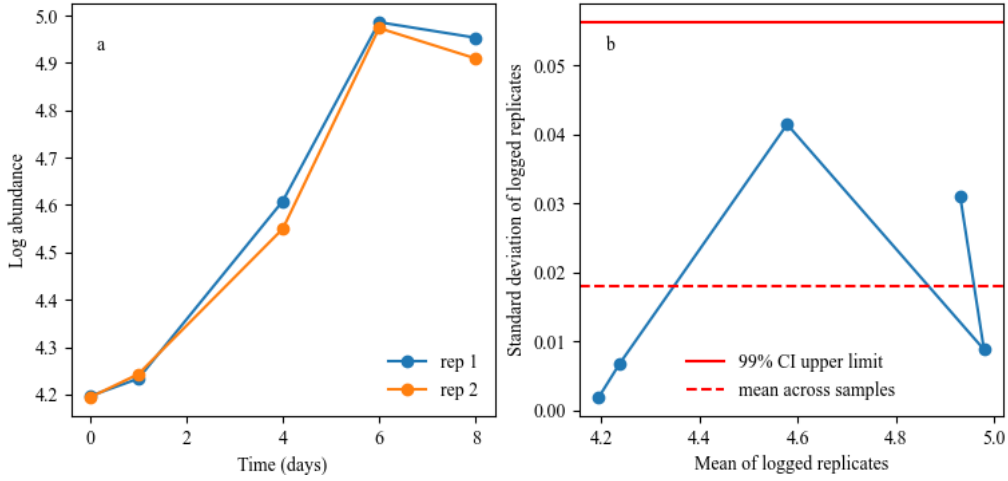


Figure A 2 Error analysis replicates of hydrogen peroxide

Replicates of hydrogen peroxide concentration (a) along with a depiction of different quantities to constrain uncertainty across time-points (b). The red dashed line marked by the upper limit of a 99% confidence interval was determined with Equation A2 eq3 and set the uncertainty parameter $\sigma_{y,i}$ across timepoints in this dataset.

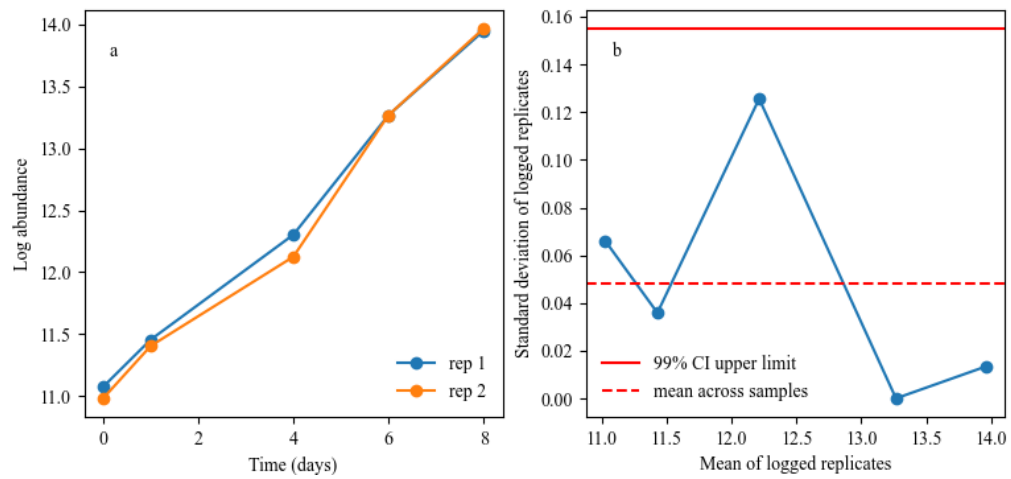


Figure A 3 Error analysis replicates of *Synechococcus*

Replicates of *Synechococcus* abundance (a) along with a depiction of different quantities to constrain uncertainty across time-points (b). All lines and symbols have the same meaning as in Figure A2.

A3 ADDITIONAL EXPERIMENTAL DATASETS

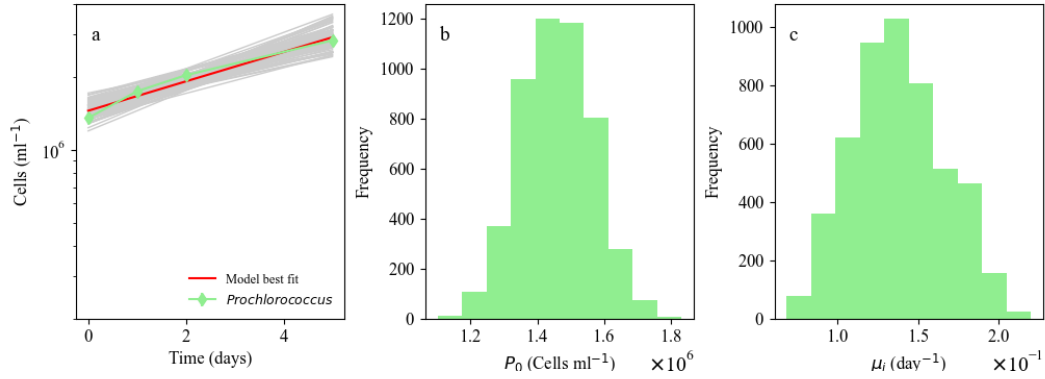


Figure A 4 Monoculture dynamics of *Prochlorococcus* dataset #2 modeled in growth media with 0 additional H₂O₂. Laboratory batch culture dynamics of *Prochlorococcus* (Vol #1) data (blue line) and model searches (grey lines) with optimal solution (red line) in zero H₂O₂ batch culture (a). Also shown are parameter value histograms for the initial cell concentration (P_0) and exponential growth rate (μ_i) (b,c, respectively).

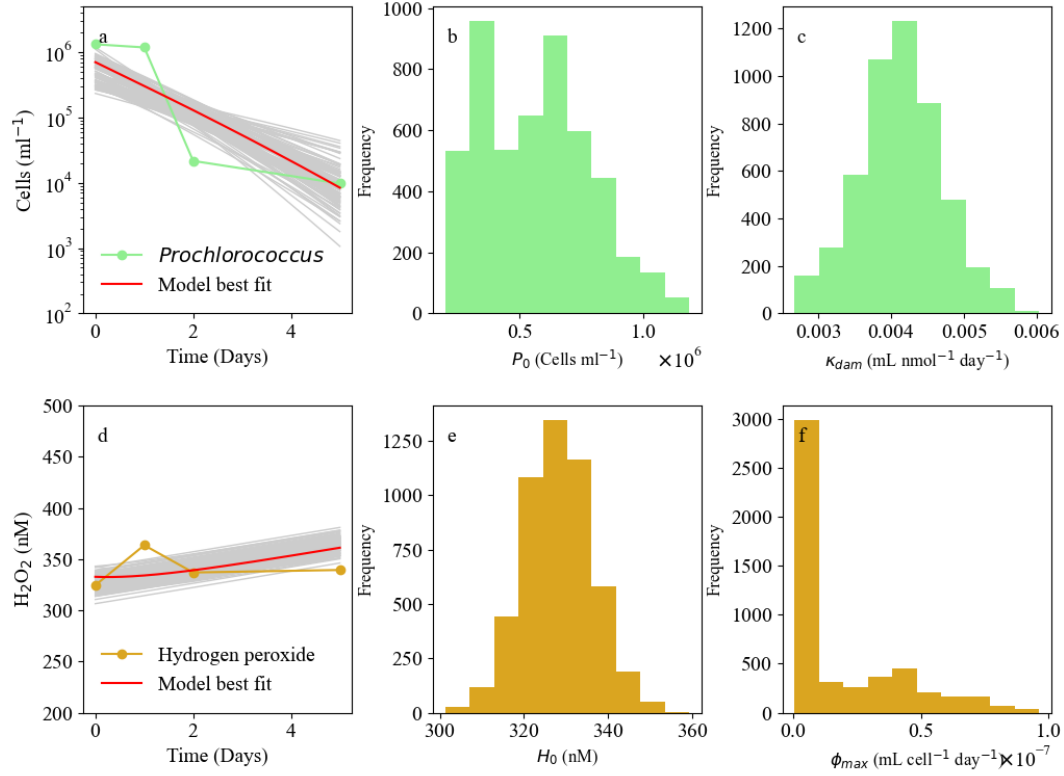


Figure A 5 *Prochlorococcus* and H_2O_2 model from 400nM spike assay repetition

Coupled laboratory batch culture dynamics of *Prochlorococcus* (a) and H_2O_2 (d) data alongside model optimal (red line) and searched (grey) combinations in laboratory assays containing 400nM H_2O_2 . To the right of each dynamics plot we show the initial value of P (b) or H (e) and the controlling parameter for each line: $k_{dam,I}$ for P (c) and $\phi_{max,i}$ for H (f).

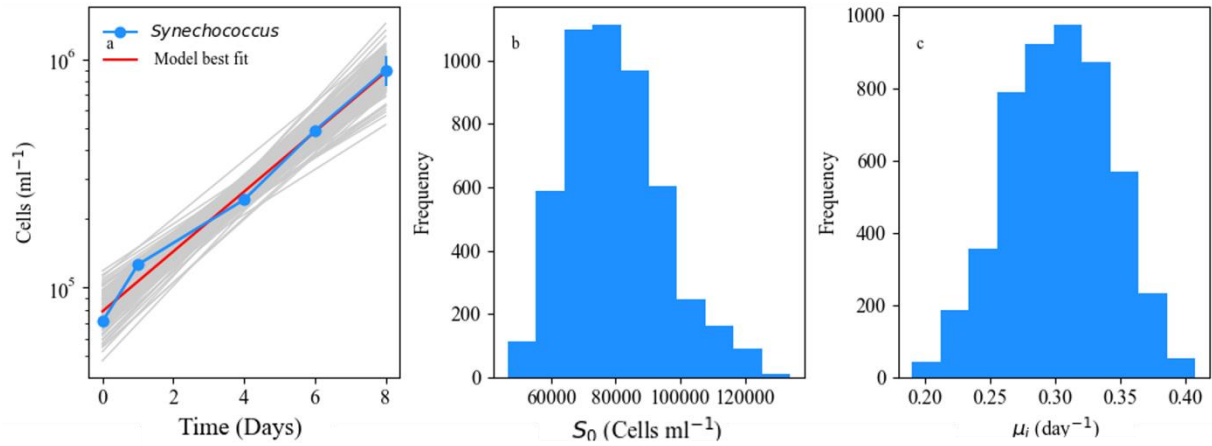


Figure A 6 *Synechococcus* WH7803 (Vol #28) modeled in growth media.

Laboratory batch culture dynamics of *Synechococcus* WH7803 (Vol #28) data (blue line) and model searches (grey lines) with optimal solution (red line) in zero H₂O₂ batch culture (a). Also shown are parameter value histograms for the initial cell concentration (S_0) and exponential growth rate (μ_i) (b,c, respectively).

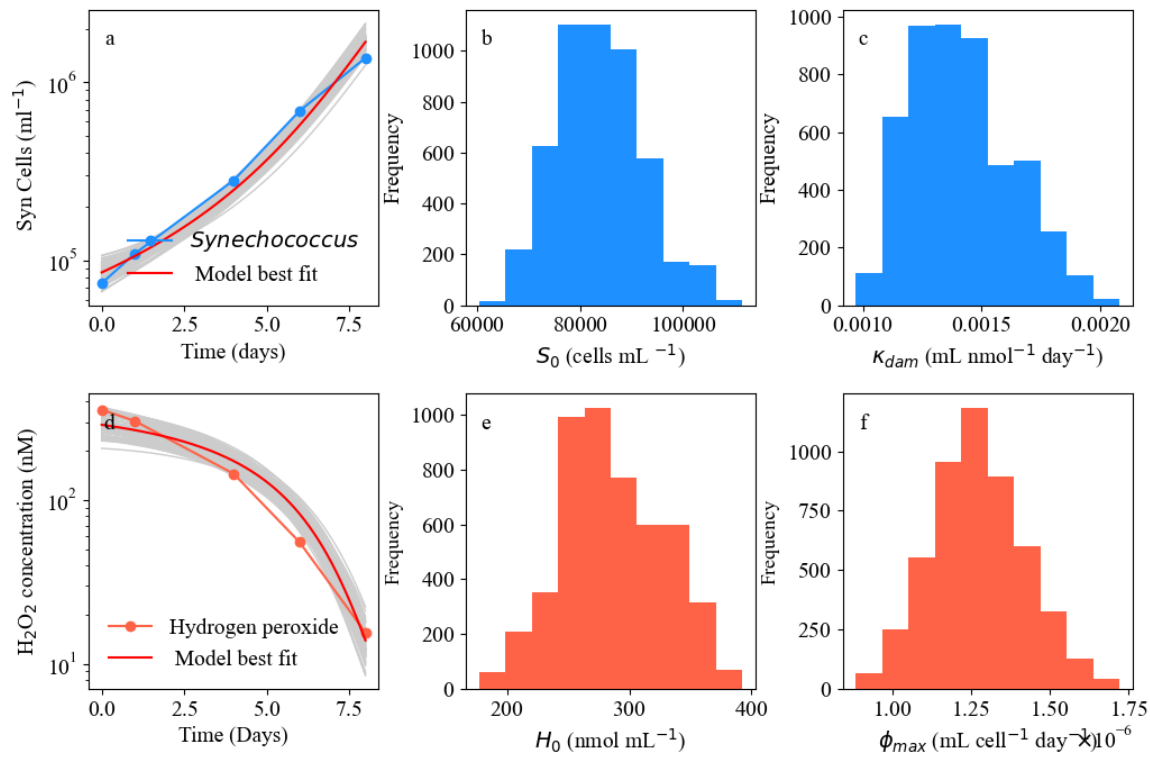


Figure A 7 *Synechococcus* WH7803 (Vol #28) in 400nM H₂O₂ spike assay Coupled laboratory batch culture dynamics of *Synechococcus* WH7803 (Vol# 28) (a) and H₂O₂ (d) data alongside searched parameter combinations (grey) in batch culture containing 400nM H₂O₂. To the right of each dynamics plot we show the initial cell concentration (S_0) and H₂O₂ specific death rate ($k_{dam,i}$) (b,c) and initial hydrogen peroxide concentration (H_0) and cell-specific detoxification rate ($\phi_{max,i}$) (e,f).

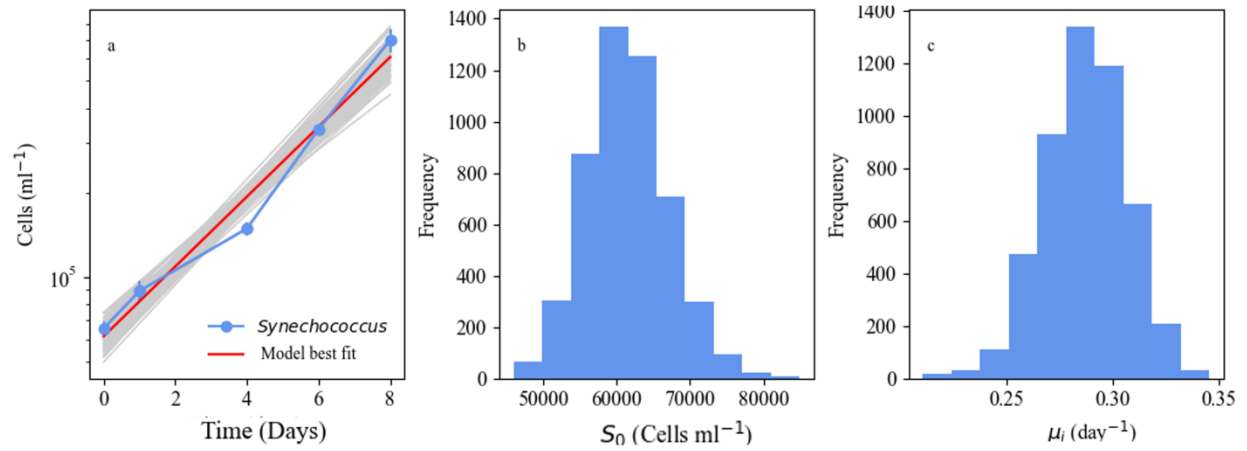


Figure A 5 *Synechococcus* WH7803 (Vol #52) modeled in growth media with 0 additional H₂O₂

Laboratory batch culture dynamics of *Synechococcus* WH7803 (Vol #52) data (blue line) and model searches (grey lines) with optimal solution (red line) in zero H₂O₂ batch culture (a). Also shown are parameter value histograms for the initial cell concentration (S_0) and exponential growth rate (μ_i) (b,c, respectively).

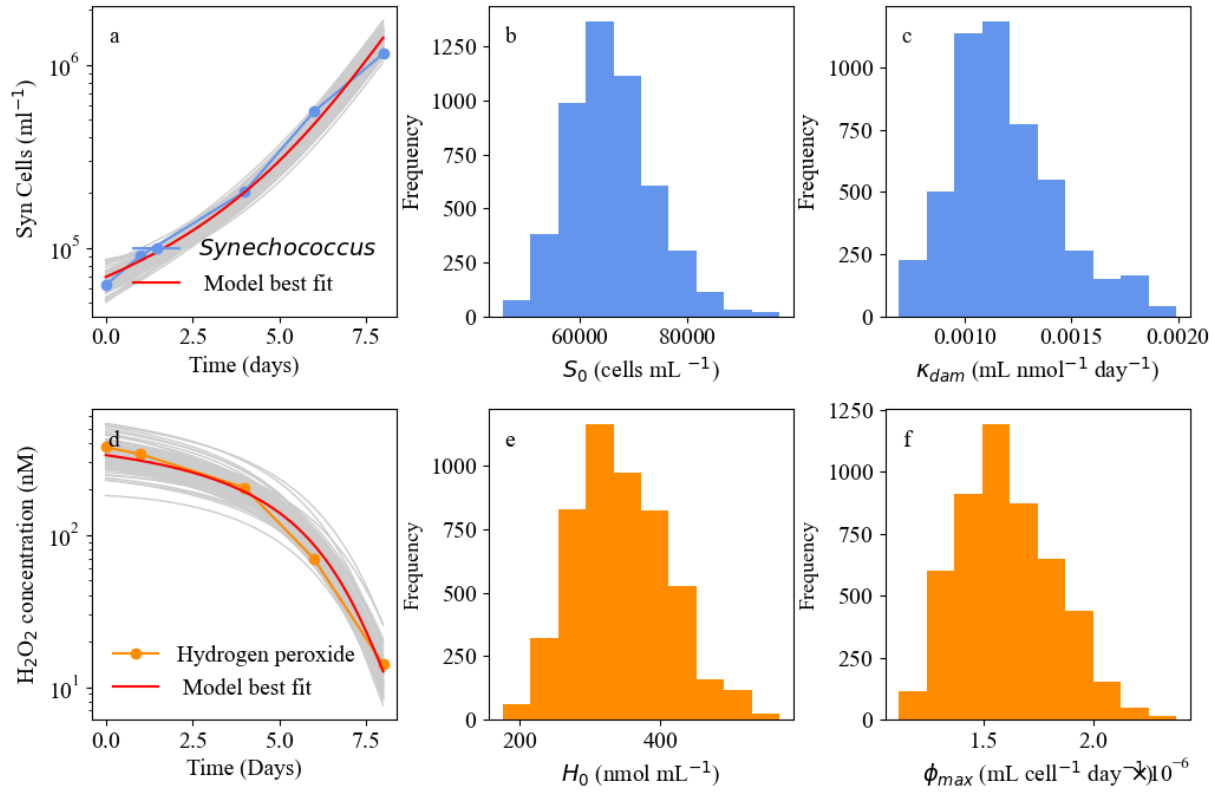


Figure A 6 Modeled *Synechococcus* WH7803 monoculture in 400nM H_2O_2 spike assay

Coupled laboratory batch culture dynamics of *Synechococcus* WH7803 (a) and H_2O_2 (d) data alongside searched parameter combinations (grey) in batch culture containing 400nM H_2O_2 . To the right of each dynamics plot we show the initial cell concentration (S_0) and H_2O_2 specific death rate ($\kappa_{dam,i}$) (b,c) and initial hydrogen peroxide concentration (H_0) and cell-specific detoxification rate ($\phi_{max,i}$) (e,f).

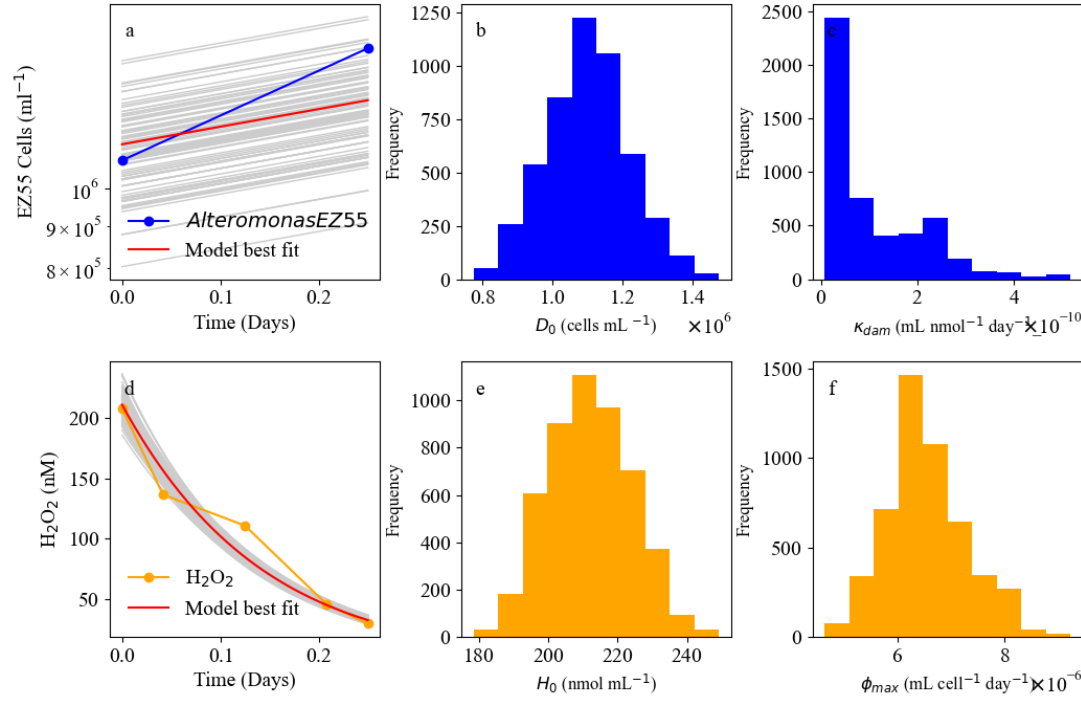


Figure A 7 Heterotroph EZ55 300nM H₂O₂ spiked media dynamics over 2 hours

Coupled laboratory batch culture dynamics of *Alteromonas* EZ55 (a) and H₂O₂ (d) data alongside searched parameter combinations (grey) in batch culture containing 300nM H₂O₂. To the right of each dynamics plot we show the initial cell concentration (D_0) and H₂O₂ specific death rate ($k_{dam,i}$) (b,c) and initial H₂O₂ concentration (H_0) and EZ55 cell-specific H₂O₂ detoxification rate ($\phi_{max,i}$) (e,f).

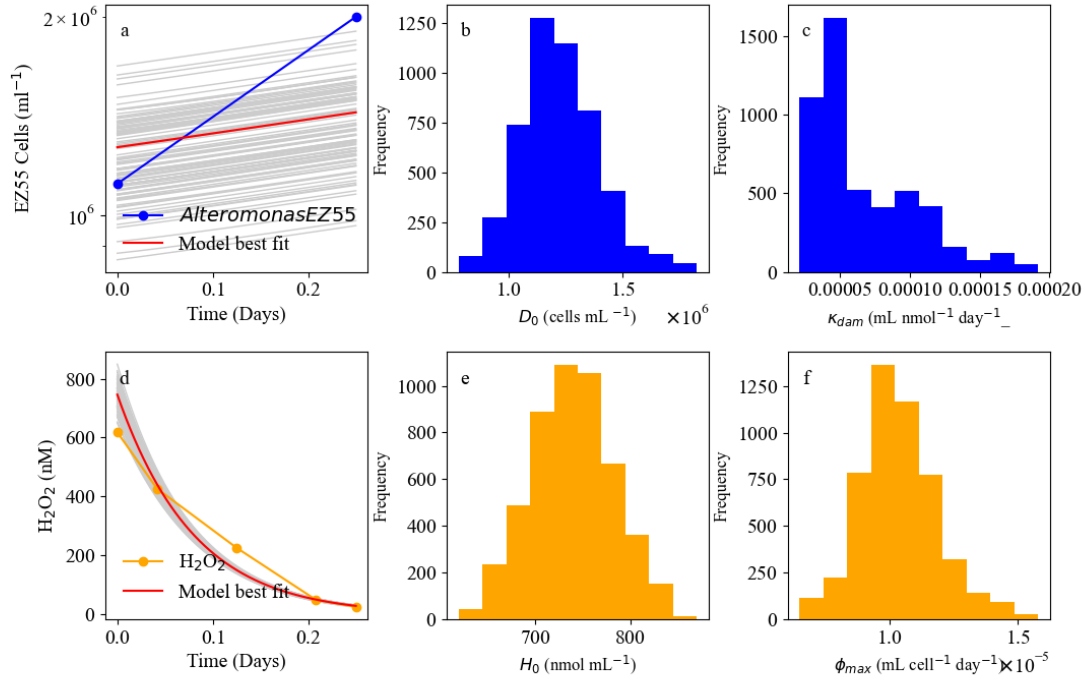


Figure A 8 Heterotroph EZ55 800nM H_2O_2 spiked media dynamics over 2 hours

Coupled laboratory batch culture dynamics of *Alteromonas* EZ55 (a) and H_2O_2 (d) data alongside searched parameter combinations (grey) in batch culture containing 800nM H_2O_2 . To the right of each dynamics plot we show the initial cell concentration (D_0) and H_2O_2 specific death rate ($k_{dam,i}$) (b,c) and initial H_2O_2 concentration (H_0) and EZ55 cell-specific H_2O_2 detoxification rate ($\phi_{max,i}$) (e,f).

A4 MEDIA AND BUFFER H₂O₂ PRODUCTION ASSAYS

HEPES buffer produces H₂O₂ when illuminated at relevant irradiances (Morris 2013) so we use it here to ensure our model may be used for the more environmentally relevant, photochemically produced H₂O₂ dynamics before analyzing the more common spike assay. While the rest of this thesis concerns H₂O₂ spikes as the main source of H₂O₂ in media, the diel nature of *in situ* external H₂O₂ threats drove us to ensure the applicability of our model. We leverage the assays of prior studies (Jeffrey Morris & Zinser, 2013; Morris, 2011) that tested H₂O₂ photoproduction in growth buffers to test our H₂O₂ production term (S_H) and its ability to capture a more realistic photochemical reaction to that of the open ocean (Jeffrey Morris and Zinser 2013). H₂O₂ concentrations from dataset 1 singular TAPS (Figure A12) or HEPES (Figure A13) production were evaluated against the performance of our model from Eq. set 1 in natural seawater. Production here takes on a non-linear production, yet our model manages to capture this. The dashed analytical solution of our model is visualized as well to ensure that our model solution protocol is effective. Similar to our models for H₂O₂ spike assays, the abiotic model captured the HEPES-initiated dynamics in our batch data. Agreement of both model solutions with laboratory data led to our adoption of this formulation for future H₂O₂ modeling regarding media assays. Through this analysis we ensured that 1) chosen ODE set 1 solved to an intelligible solution, shown below, and 2) this solution converged on our supplied data in a similar degree and manner as our fitting method. This step is key in evaluating our integration methods thus allowing a comparison of this data set dynamics to that of an analytical solution set calculated from an abiotic scenario.

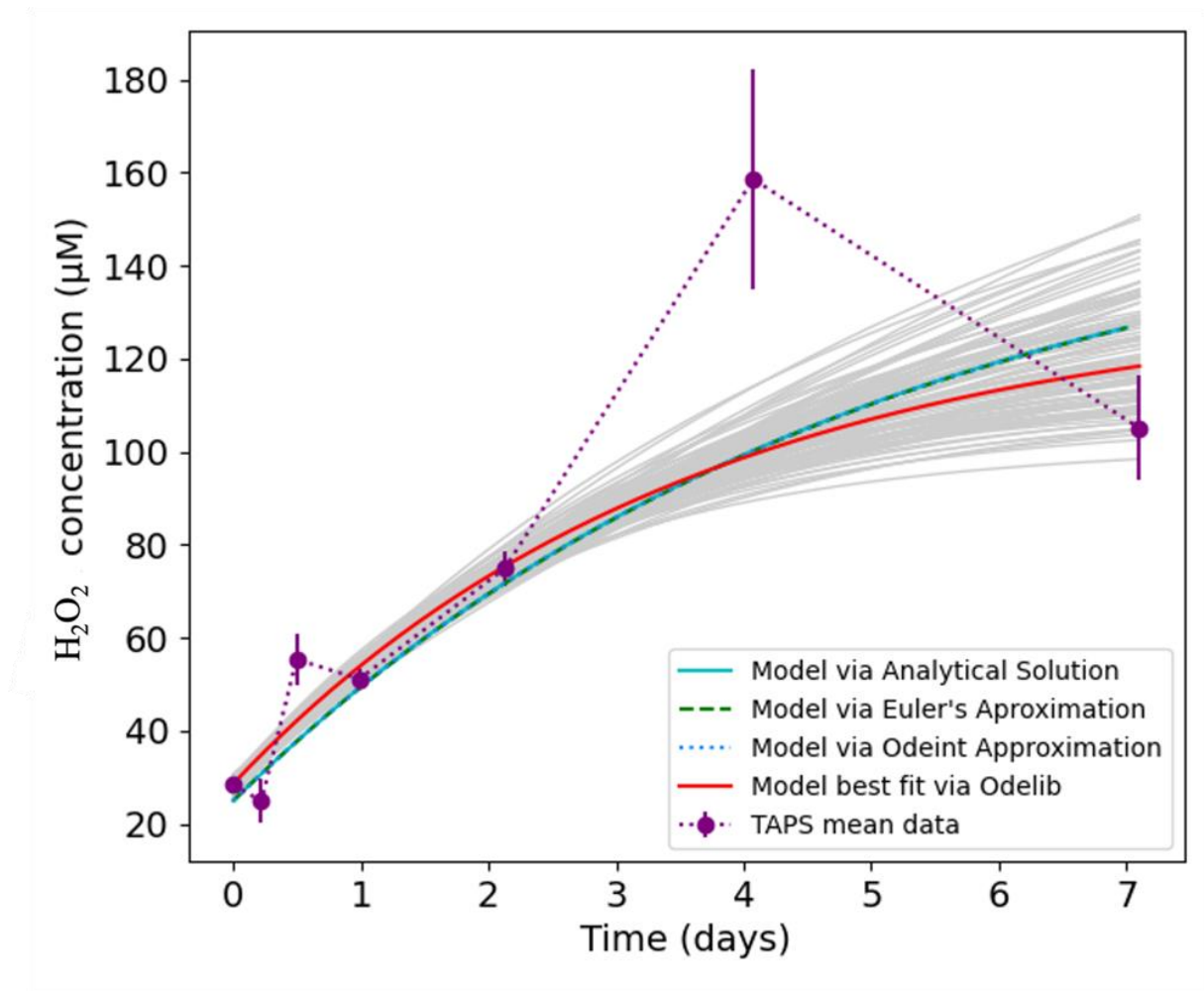


Figure A 9 Modeled H_2O_2 production in TAPS buffer

H_2O_2 measurements (connected purple symbols), sample explored model solutions (grey lines) and optimal model formulation (red line) from 3.75 mM TAPS buffer in batch growth media. Data from Morris *et al.* 2011. This solution is visualized against several fitting methods to ensure reproducibility of analyses.

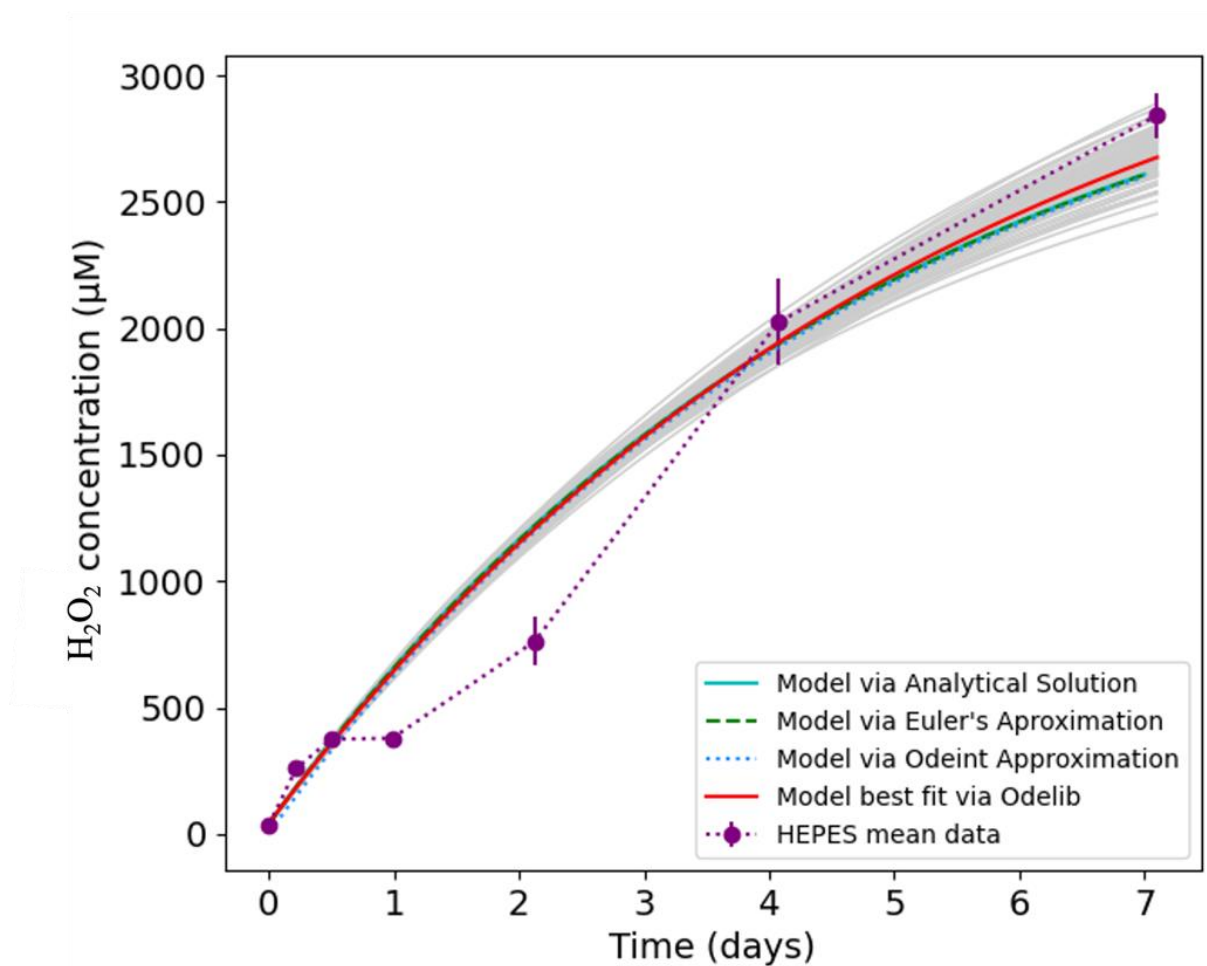


Figure A 10 Modeled H₂O₂ production in HEPES buffer

Visualization of direct HEPES to H₂O₂ conversion following Eq. 1. Both the analytical solution (green solid line) and model solutions via ODEint (cyan dotted line) or ODElib (Red solid) capture the measured H₂O₂ (purple symbols) dynamics of a 3.75mM HEPES buffer assay over 7 days. A singular rate of abiotic decay (δ_H) is assumed here. (Data from Morris *et al.* 2011)

APPENDIX B SUPPLEMENTARY INFORMATION: CHAPTER 3

B1 MONOCULTURE PARAMETER FITTINGS

Monoculture curves fittings for picoeukaryotes mentioned in Figure 3.2 of the main text.

Prochlorococcus and *Synechococcus* spp. and *Alteromonas macleodii* monoculture parameter analyses were shown in the main text or supplementary information (Appendix A) for Chapter 2.

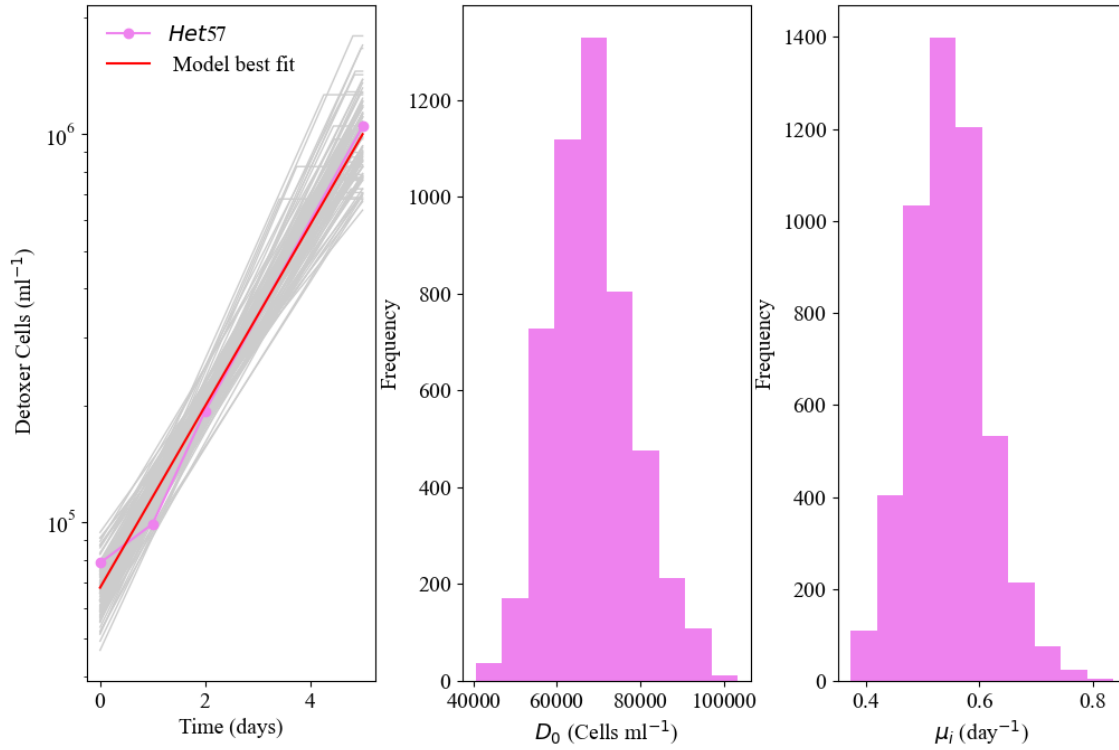


Figure B 11 Monoculture *Micromonas commoda* in growth media with 0 additional H₂O₂

Monoculture laboratory dynamics of the detoxifier (D) *Micromonas commoda* (pink line) and model searches (grey lines) with optimal solution (red line) in zero H₂O₂ batch culture (a). Also shown are parameter value histograms for the initial cell concentration (D_0) and exponential growth rate (μ_i) (b,c, respectively).

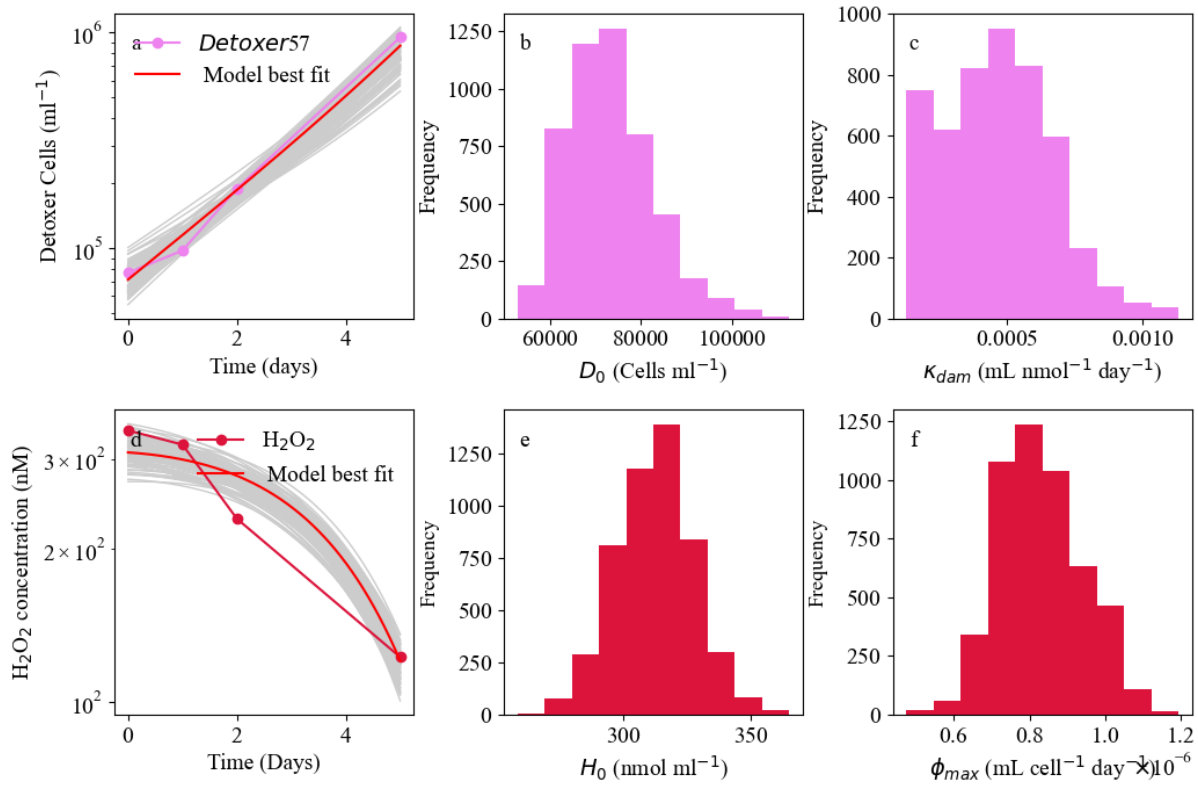


Figure B 2 Monoculture (Detoxer57) *Micromonas commoda* in 400nm H_2O_2 spike Coupled laboratory batch culture dynamics of the detoxifier (D) *Micromonas commoda* (pink line) (a) and H_2O_2 (maroon line) (d) data alongside searched parameter combinations (grey) in batch culture containing 400nM H_2O_2 . To the right of each dynamics plot we show the initial cell concentration (D_0) and H_2O_2 specific death rate ($\kappa_{dam,i}$) (b,c) and initial hydrogen peroxide concentration (H_0) and cell-specific detoxification rate ($\phi_{max,i}$) (e,f).

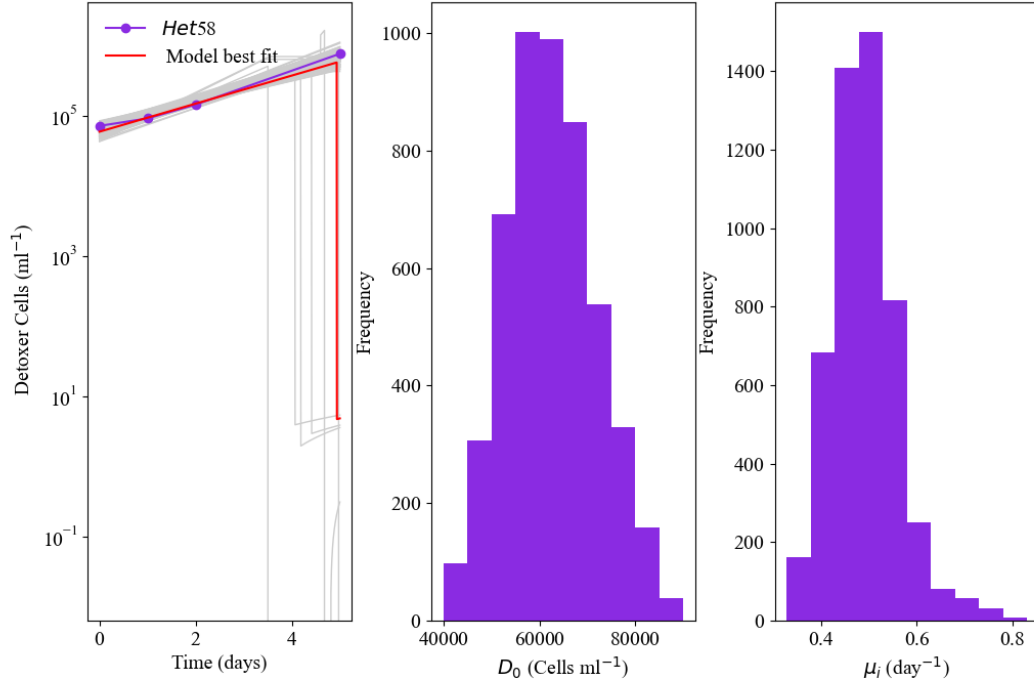


Figure B 3 Monoculture *Micromonas pusilla* in growth media with 0 additional H₂O₂

Laboratory batch culture dynamics of the detoxifier (D) *Micromonas pusilla* with data (purple line) and model searches (grey lines) with optimal solution (red line) in zero H₂O₂ batch culture (a). Also shown are parameter value histograms for the initial cell concentration (D_0) and exponential growth rate (μ_i) (b,c, respectively).

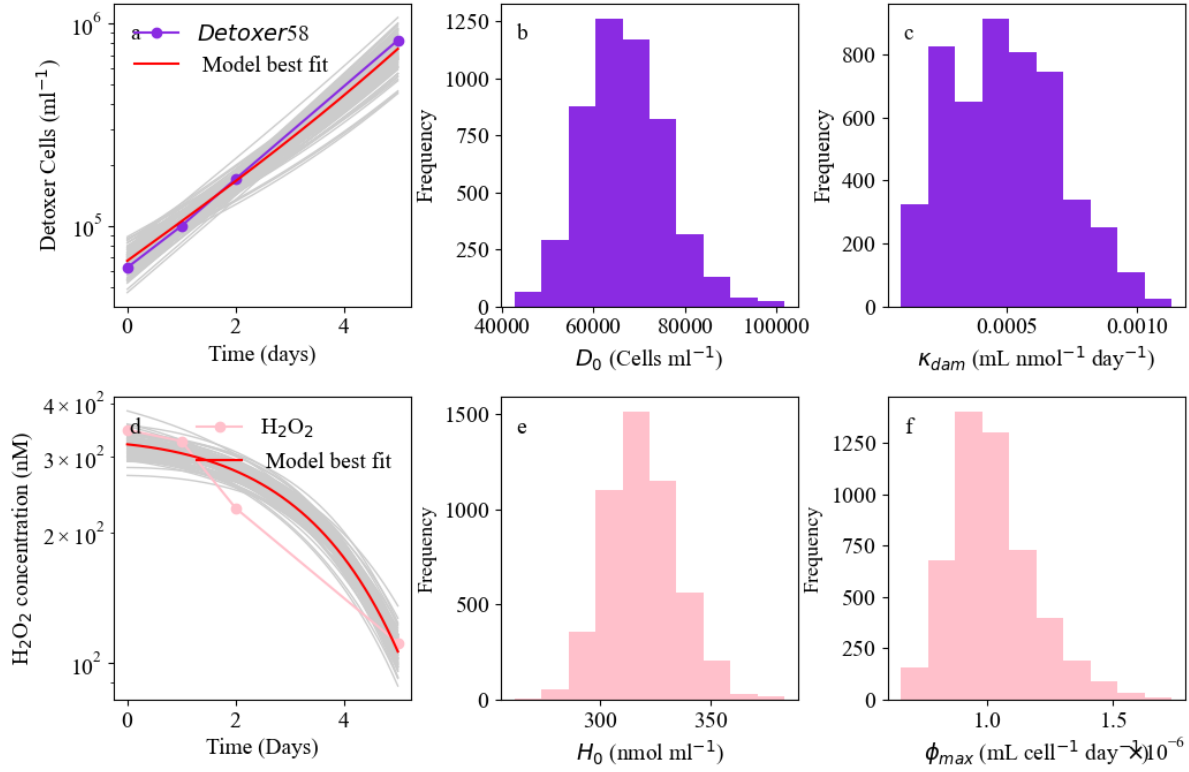


Figure B 4 Monoculture (Detoxer58) *Micromonas pusilla* in 400nM H_2O_2 spike

Coupled laboratory batch culture dynamics of the detoxifier (D) *Micromonas pusilla* (purple line) (a) and H_2O_2 (pink line) (d) data alongside searched parameter combinations (grey) in batch culture containing 400nM H_2O_2 . To the right of each dynamics plot we show the initial cell concentration (D_0) and H_2O_2 specific death rate ($\kappa_{dam,i}$) (b,c) and initial hydrogen peroxide concentration (H_0) and cell-specific detoxification rate ($\phi_{max,i}$) (e,f).

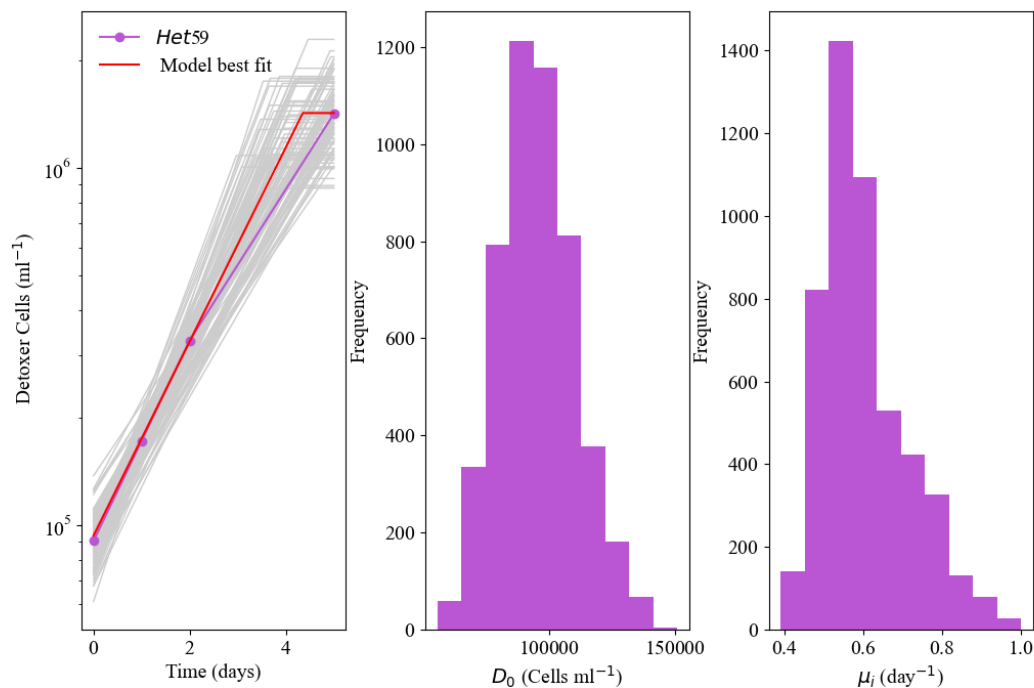


Figure B5 Monoculture *Ostreococcus lucimarinus* in growth media with 0 additional H₂O₂

Laboratory batch culture dynamics of the detoxifier (D) *Ostreococcus lucimarinus* with data (pink line) and model searches (grey lines) with optimal solution (red line) in zero H₂O₂ batch culture (a). Also shown are parameter value histograms for the initial cell concentration (D_0) and exponential growth rate (μ_i) (b,c, respectively).

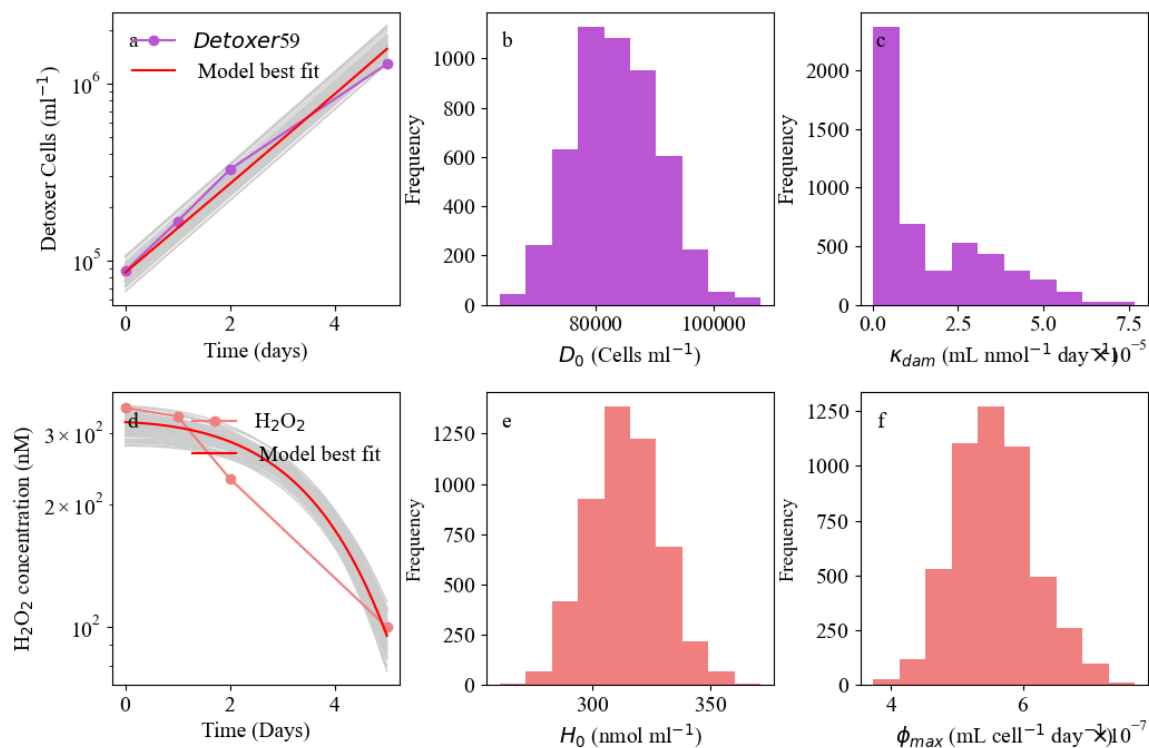


Figure B6 Monoculture (Detoxer59) *Ostreococcus lucimarinus* in 400nM H_2O_2 spike

Coupled laboratory batch culture dynamics of the detoxifier (D) *Ostreococcus lucimarinus* (pink line) (a) and H_2O_2 (orange line) (d) data alongside searched parameter combinations (grey) in batch culture containing 400nM H_2O_2 . To the right of each dynamics plot we show the initial cell concentration (D_0) and H_2O_2 specific death rate ($k_{dam,i}$) (b,c) and initial H_2O_2 concentration (H_0) and cell-specific detoxification rate ($\phi_{max,i}$) (e,f).

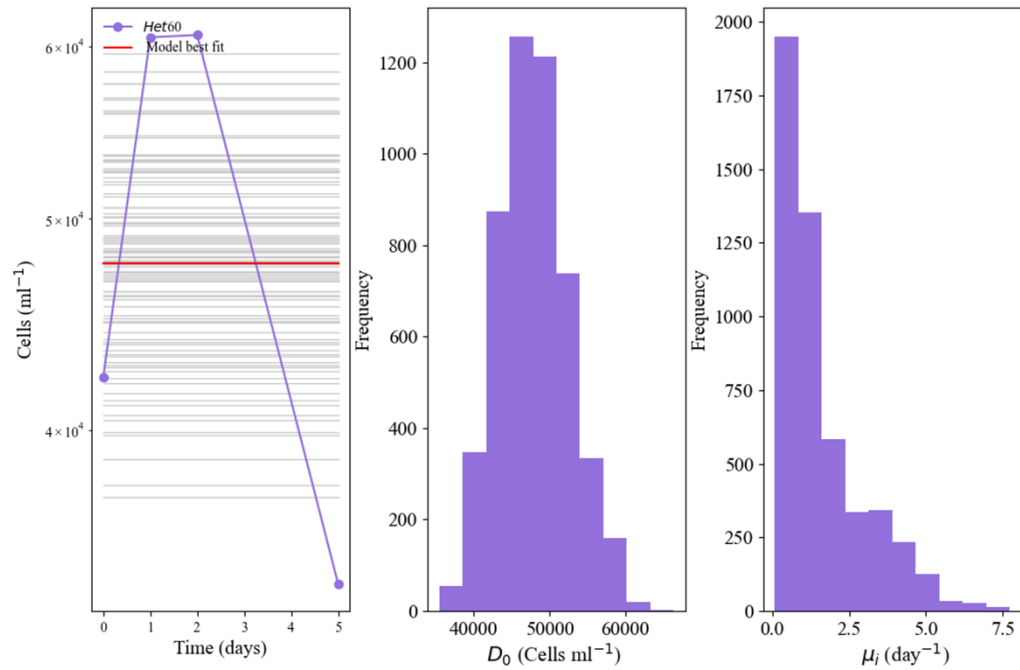


Figure B7 Monoculture *Ostreococcus tauri* in growth media with 0 additional H_2O_2

Laboratory batch culture dynamics of the detoxifier (D) *Ostreococcus tauri* with data (purple line) and model searches (grey lines) with optimal solution (red line) in zero H_2O_2 batch culture (a). Also shown are parameter value histograms for the initial cell concentration (D_0) and exponential growth rate (μ_i) (b,c, respectively).

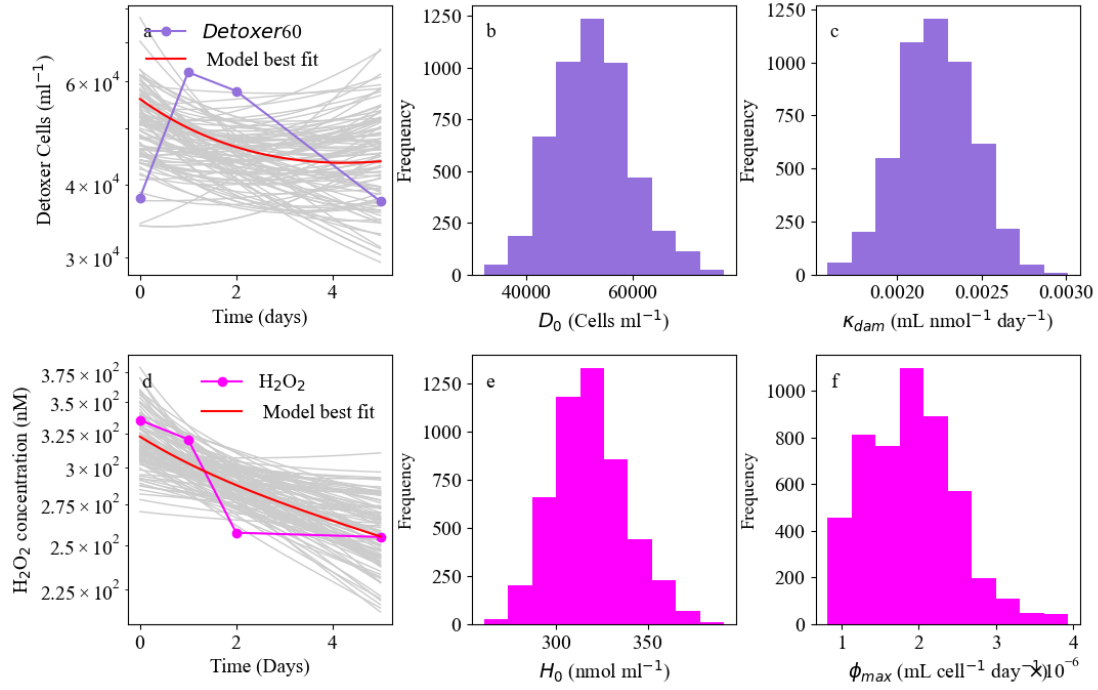


Figure B8 Monoculture Parameter Summary Table Growth Parameters of μ_i (mus) and nutrient affinity (k1s) from assays with 0nM additional H_2O_2 spike and H_2O_2 parameters of cell-specific H_2O_2 detoxification, (phis) $\phi_{max,i}$ and H_2O_2 cell damage, (kdams) $\kappa_{dam,i}$, from assays with 400nM additional H_2O_2 spike.

names	mus	k1s	markers	colors	phis	kdams
Syn WH7803 avg	0 0.258216 Name: k2, dtype: float64	0 7.270547e+21 Name: k1, dtype: float64	o	dodgerblue	0 0.000002 Name: phi, dtype: float64	0 0.001314 Name: kdam, dtype: float64
Syn CC9605	0 0.376705 Name: k2, dtype: float64	0 9.144078e+19 Name: k1, dtype: float64	o	darkcyan	0 0.000002 Name: phi, dtype: float64	0 0.000663 Name: kdam, dtype: float64
Syn WH8102	0 0.002161 Name: k2, dtype: float64	0 3.230279e+08 Name: k1, dtype: float64	o	steelblue	0 0.000003 Name: phi, dtype: float64	0 0.002213 Name: kdam, dtype: float64
Pro MIT9215 avg	0 0.187638 Name: k2, dtype: float64	0 1.089586e+35 Name: k1, dtype: float64	D	lightgreen	0 8.946629e-09 Name: phi, dtype: float64	0 0.004299 Name: kdam, dtype: float64
Micromonas commoda	0 0.389136 Name: k2, dtype: float64	0 0.011513 Name: k1, dtype: float64	v	violet	0 6.894003e-07 Name: phi, dtype: float64	0 0.000205 Name: kdam, dtype: float64
Micromonas pusilla	0 0.535482 Name: k2, dtype: float64	0 2.179717e+25 Name: k1, dtype: float64	v	blueviolet	0 0.000001 Name: phi, dtype: float64	0 0.000753 Name: kdam, dtype: float64
Ostreococcus lucimarinus	0 0.603263 Name: k2, dtype: float64	0 0.077151 Name: k1, dtype: float64	v	mediumorchid	0 4.957278e-07 Name: phi, dtype: float64	0 0.000038 Name: kdam, dtype: float64
Ostreococcus tauri	0 0.530208 Name: k2, dtype: float64	0 12.12721 Name: k1, dtype: float64	v	mediumpurple	0 0.000002 Name: phi, dtype: float64	0 0.002046 Name: kdam, dtype: float64

Figure B9 Monoculture Parameter Summary Table Growth Parameters of μ_i (mus) and nutrient affinity (k1s) from assays with 0nM additional H2O2 spike and H2O2 parameters of cell-specific H2O2 detoxification, (phis) $\phi_{max,i}$ and H2O2 cell damage, (kdams) kdam,i, from assays with 400nM additional H2O2 spike.

B2 EQUILIBRIA

Equilibrium for cases 1-4 calculated with differentiation and S*, P*, H*, N* algebraic substitutions for chapter 3 analyses of long term S,P,N,H dynamics.

Steady State of P and N in 0 supply of H₂O₂ : Equilibria Set 1

$$N^* = - \frac{(\delta_H \delta_P + H^* \kappa_{dam,p}) \mu_P}{\alpha_1 (\delta_H \delta_P + H^* \kappa_{dam,p} - \delta_H \mu_P)}$$

$$P^* = \frac{N^* \delta_H}{\delta_H \delta_P + H^* \kappa_{dam,p}} + \frac{\delta_H \rho \mu_P}{\alpha_P (\delta_H \delta_P + S_H \kappa_{dam,p} - \delta_H \mu_P)}$$

$$S^* = 0 ; H^* = 0$$

Steady States calculated for *Synechococcus* winning completion for N: Equilibria case 2

$$N^* = - \frac{\delta_S \mu_S}{\alpha_2 (\delta_2 - \mu_2)}$$

$$S^* = \frac{N^*}{\delta_S} + \frac{\rho \mu_S}{\alpha_S (\delta_S - \mu_S)}$$

$$H^* = \frac{S_H \alpha_S \delta_S (\delta_S - \mu_S)}{\rho \delta_S \mu_S \phi_S + \alpha_S (\delta_S - \mu_S) (\delta_H \delta_S + S_N \phi_S)}$$

$$P^* = 0$$

Steady States equilibria values calculated for P, S, H, and N in ‘leaky case: Equilibria set 3

$$N^* = -\frac{\delta_S \mu_S}{\alpha_S (\delta_S - \mu_S)}$$

$$H^* = \frac{\alpha_p \delta_S (\delta_p - \mu_p) \mu_S + \alpha_S \delta_p \mu_p (-\delta_S + \mu_S)}{\kappa_{dam,p} (\alpha_S \mu_p (\delta_S - \mu_S) - \alpha_p \delta_S \mu_S)}$$

$$S^* = \frac{(S_H - (\delta_H (H^*)))}{(H^*) \phi_S}$$

$$P^* = \frac{\left(S_N - \left(\frac{\mu_P (N^*)}{k_{S_S} + (N^*)} \right) (S^*) - \rho (N^*) \right)}{\left(\frac{\mu_P (N^*)}{k_{S_P} + (N^*)} \right)}$$

Steady States equilibria values calculated for P, S, H, and N in ‘leaky case: Equilibria set 4

$$N^* = -\frac{\delta_S \mu_S}{\alpha_S (\delta_S - \mu_S)}$$

$$H^* = \frac{\alpha_p \delta_S (\delta_p - \mu_p) \mu_S + \alpha_S \delta_p \mu_p (-\delta_S + \mu_S)}{\kappa_{dam,p} (\alpha_S \mu_p (\delta_S - \mu_S) - \alpha_p \delta_S \mu_S)}$$

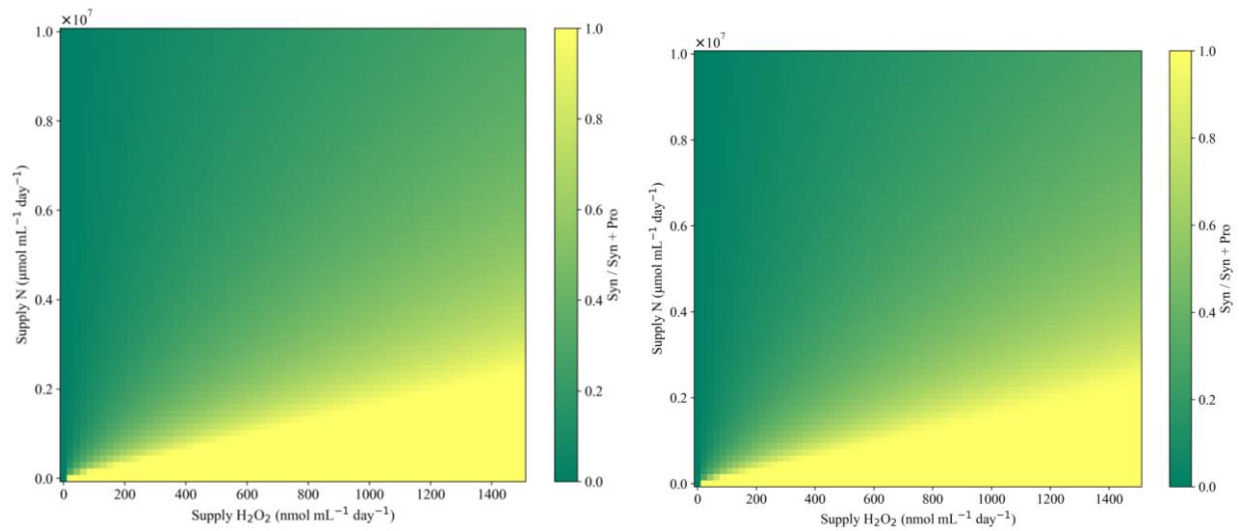
$$S^* = \frac{(-k_{12} \mu_1 (\delta_2 - \mu_2) (H_{supply} \kappa_1 + \delta_1 (\zeta + 1000 \phi_3)) + k_{11} \delta_2 \mu_2 (H_{supply} \kappa_1 + \delta_1 (\zeta + 1000 \phi_3) - \mu_1 (\zeta + 1000 \phi_3))}{(k_{12} \delta_1 \mu_1 (\delta_2 - \mu_2) + k_{11} \delta_2 (-\delta_1 + \mu_1) \mu_2) \phi_2}$$

P *

$$= \left(\frac{(k_{12} \mu_1 (\delta_2 - \mu_2) - k_{11} \delta_2 \mu_2)}{\left(\rho k_{11} \delta_2^2 (-\delta_1 + \mu_1) \mu_2^2 \phi_2 + k_{12}^2 \mu_1 (\delta_2 - \mu_2)^2 (H_{supply} \delta_2 \kappa_1 + \delta_1 (N_{supply} \phi_2 + \delta_2 (\zeta + 1000 \phi_3))) - k_{12} \delta_2 (\delta_2 - \mu_2) \mu_2 \right)} \right. \\ \left. \left(-\rho \delta_1 \mu_1 \phi_2 + k_{11} (-N_{supply} \mu_1 \phi_2 + \delta_1 (N_{supply} \phi_2 + \delta_2 (\zeta + 1000 \phi_3)) + \delta_2 (H_{supply} \kappa_1 - \mu_1 (\zeta + 1000 \phi_3))) \right) \right) \\ \left(/ (k_{11} k_{12} \delta_2 \mu_1 \mu_2 (-\delta_2 + \mu_2) (k_{12} \delta_1 \mu_1 (\delta_2 - \mu_2) + k_{11} \delta_2 (-\delta_1 + \mu_1) \mu_2) \phi_2) \right)$$

B3. EQUILIBRIA AND DYNAMIC CONTOUR PLOTS

Figure B10 below represents dynamics ODEs (left) or equilibrium * equations (right) are projected for various H₂O₂ detoxification parameterizations of *Synechococcus*. The non-zero value of $\phi_{max,S}$ allows coexistence and controls the extent to which it can occur along a continuum of S_N and S_H values. The average recovered *Synechococcus* $\phi_{max,S}$ from Chapter 2 is represented by the middle $\phi_{max,S}$ (2.0×10^{-6}) value in this figure. Under this regime, (leaky detoxification from only *Synechococcus* cells) coexistence is available only at high N or low H. * equilibria are calculated in Appendix B2.



$$\phi_{max,S} = 8.0 \times 10^{-6}$$

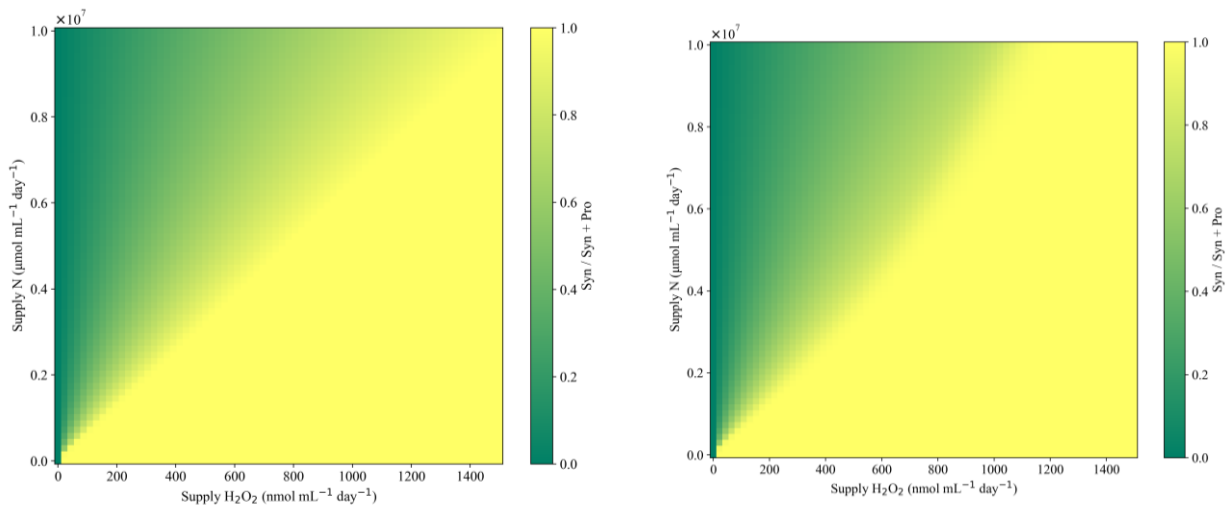


Figure B 10 $\phi_{max,s}$ effects on Prochlorococcus and Synechococcus coexistence in dynamics and equilibria contours. Dynamics ODEs (left) or equilibrium solution equations (right) are projected along a biomass ratio contour plot indication Syn/(Pro+Syn). Each row represents a different parameterization (value) of Synechococcus cell based H₂O₂ detoxification ($\phi_{max,S}$).

$$\phi_{max,S} = 2.0 \times 10^{-6}$$

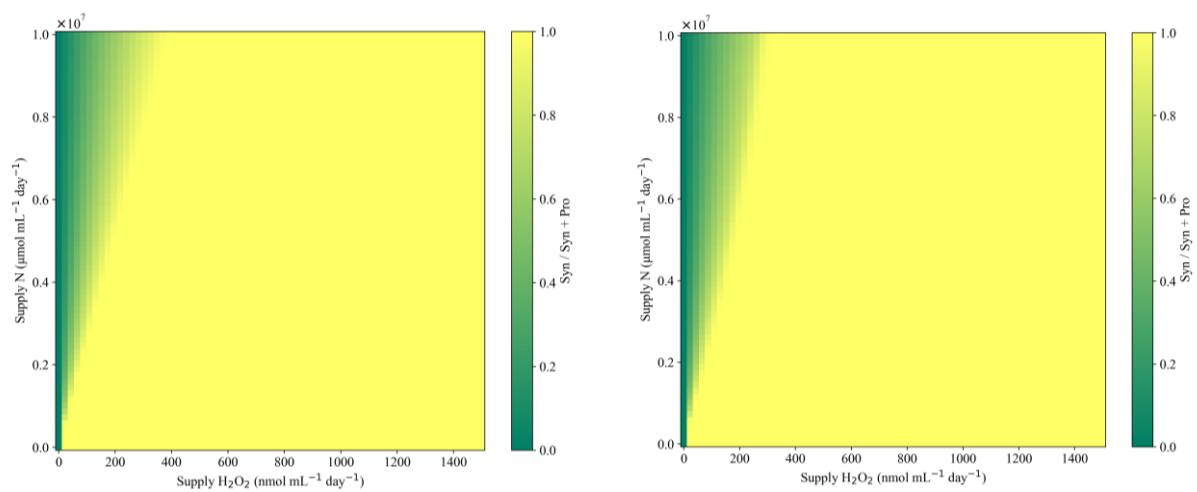
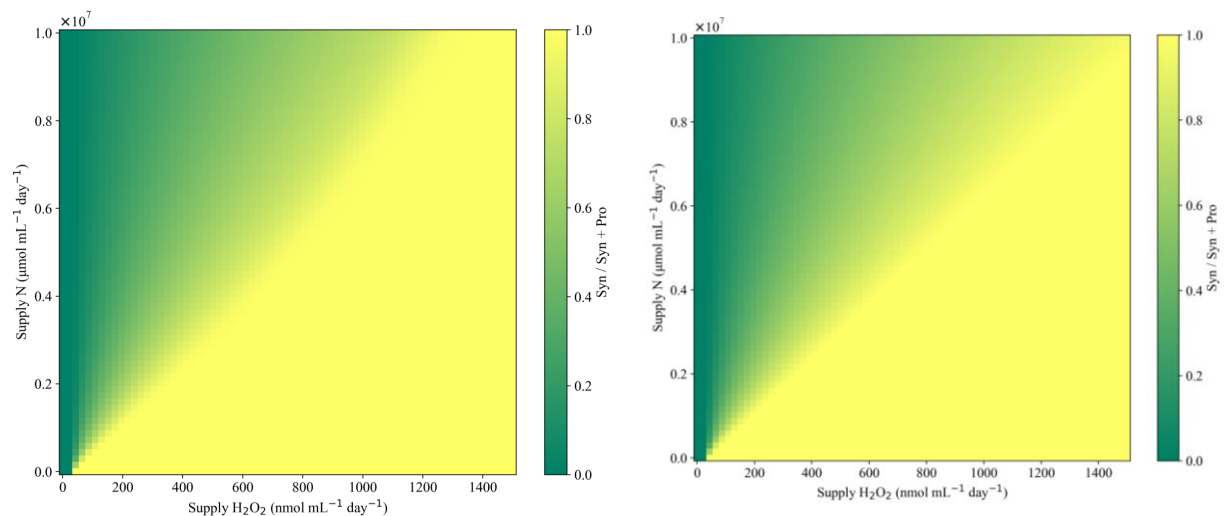


Figure B10 Continued

$$\phi_{max,S} = 0.5 \times 10^{-6}$$



$$\phi_{\max,B} = 2.0 \times 10^{-5}$$

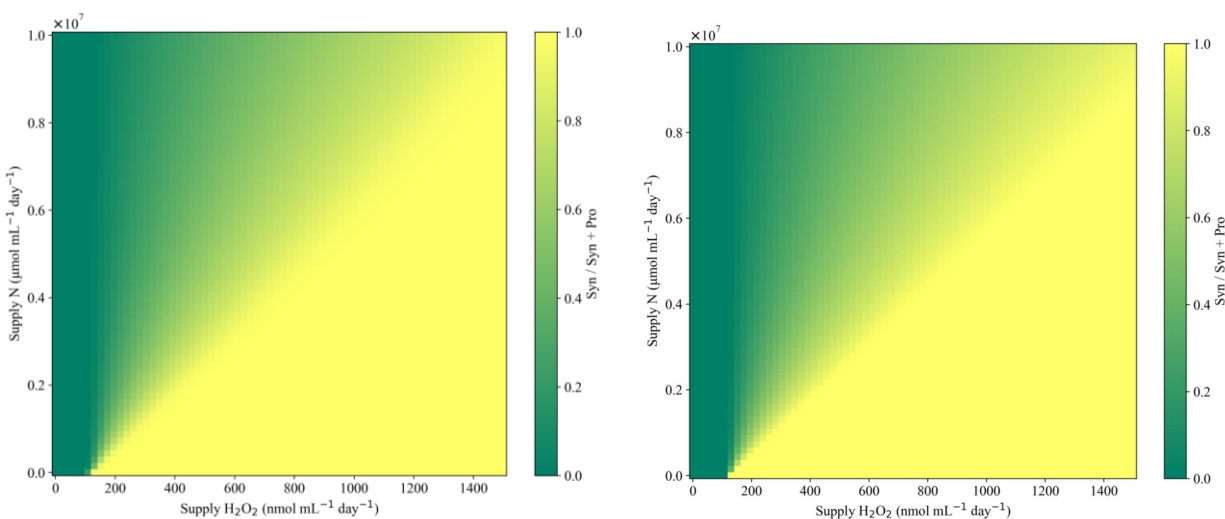


Figure B 11 $\phi_{\max,B}$ effects on Prochlorococcus and Synechococcus coexistence in dynamics and equilibria contours. Dynamic ODEs (left) or equilibrium solution equations (right) solutions are projected along a heat map of S_H and S_N inputs. Color represents the biomass ratio of Syn/(Pro+Syn). Each row represents a different parameterization (value) of a stationary phase heterotrophic bacterial (B) culture's H₂O₂ detoxification rate ($\phi_{\max,B}$). *Synechococcus* detoxification rate ($\phi_{\max,B}$) is held constant at 2.0×10^{-6} . $\phi_{\max,B} = 8 \times 10^{-5}$

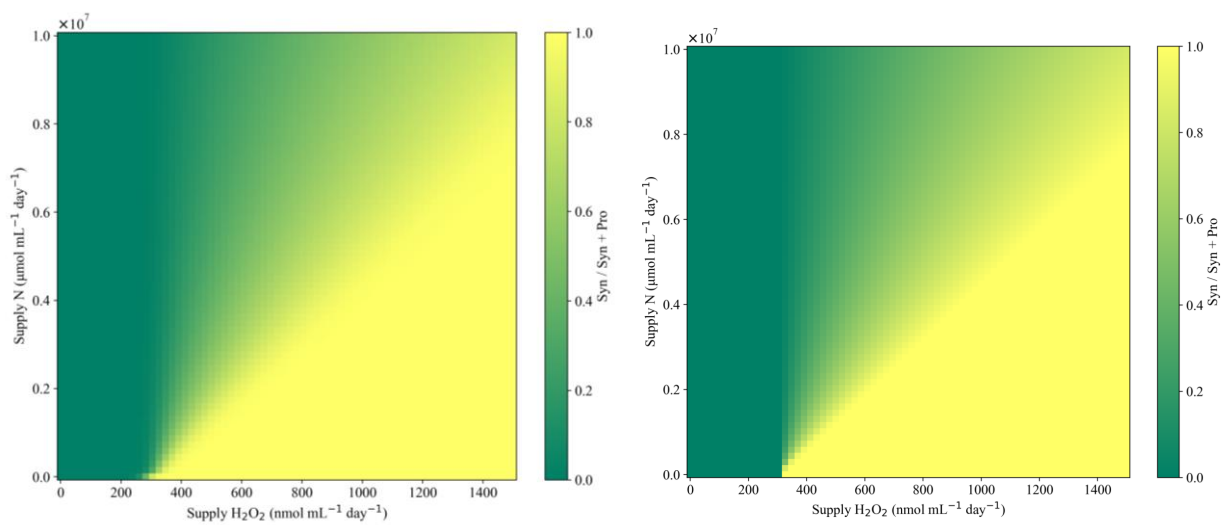


Figure B11 Continued

$$\phi_{\text{max,B}} = 2.0 \times 10^{-4}$$

APPENDIX C SUPPLEMENTARY INFORMATION: CHAPTER 4

Global maps of individual H, P, and S, tracers in Cases 1-4 are shown below.

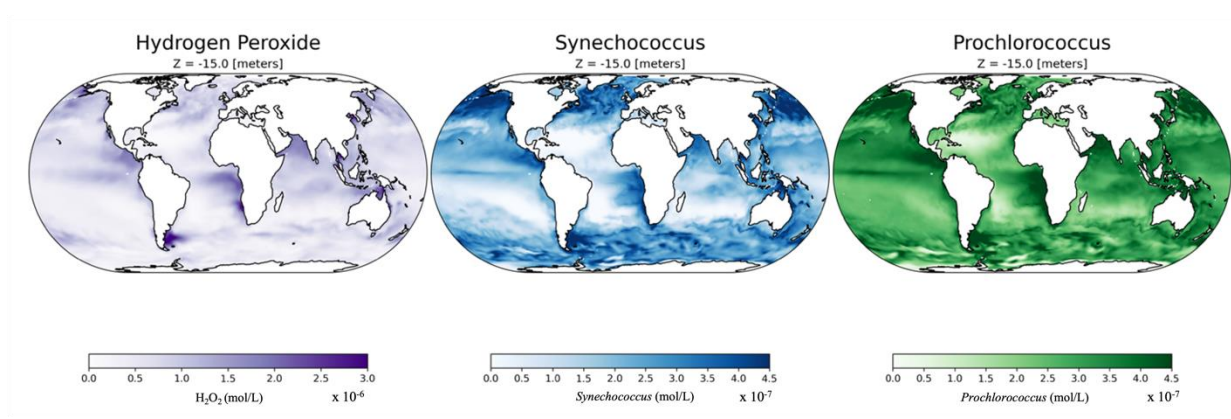


Figure C 1 H_2O_2 , *Prochlorococcus* and *Synechococcus* biogeography in year 9 of Case 1: Non-leaky world, no $k_{\text{dam},p}$.

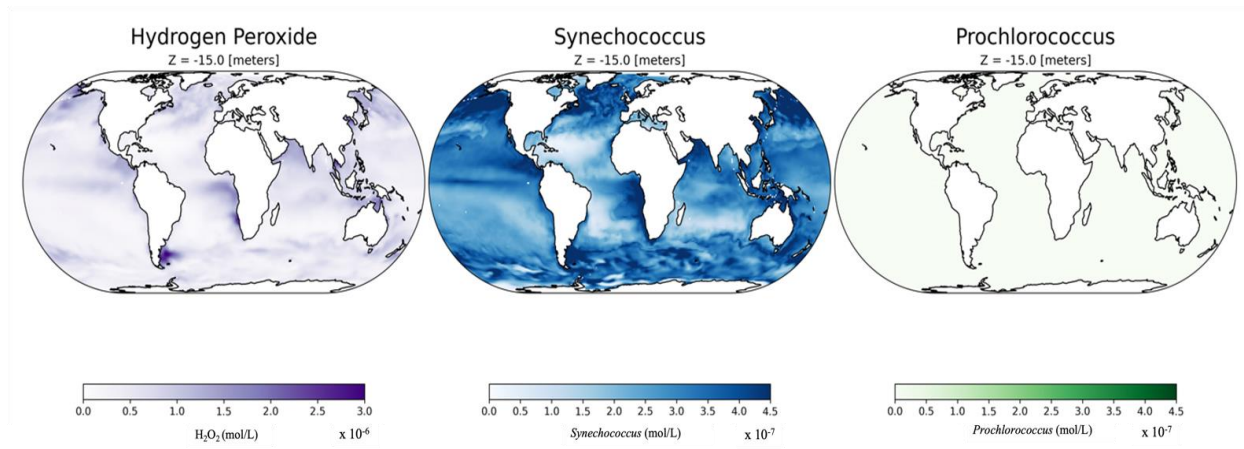


Figure C 2 H_2O_2 , *Prochlorococcus* and *Synechococcus* biogeography in year 9 of Case 2: Non-leaky, no biotic detoxification ($\phi_{\max,i}$).

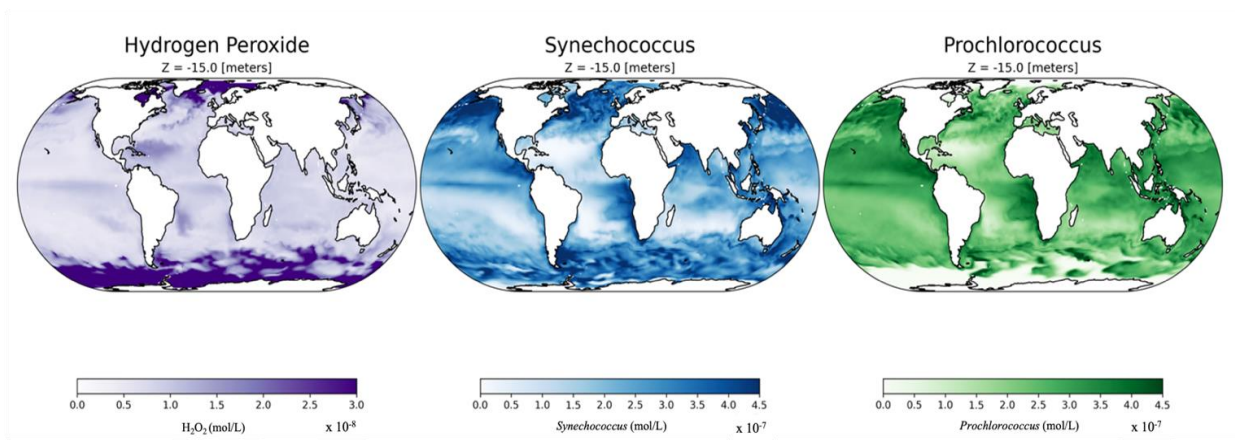
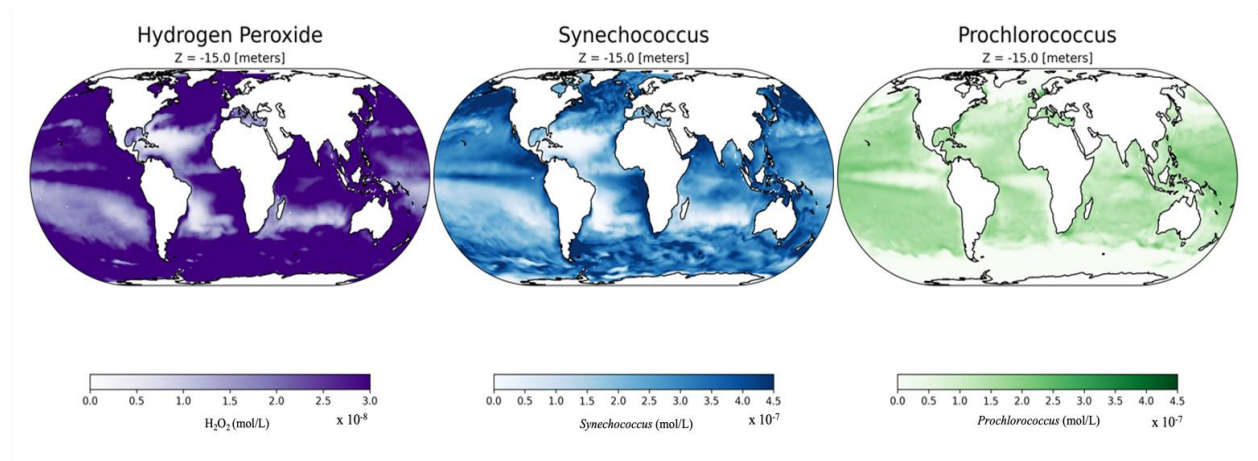


Figure C 3 Projected H_2O_2 , *Prochlorococcus* and *Synechococcus* biogeographies in year 9 of Case 3: Leaky world, *Synechococcus* H_2O_2 detoxification ($\phi_{max,S}$) alone is implemented in the model.



C 4 Projected H_2O_2 , *Prochlorococcus* and *Synechococcus* biogeographies in year 9 of Case 3: Leaky world, heterotrophic detoxification ($\phi_{max,B}$) alone.

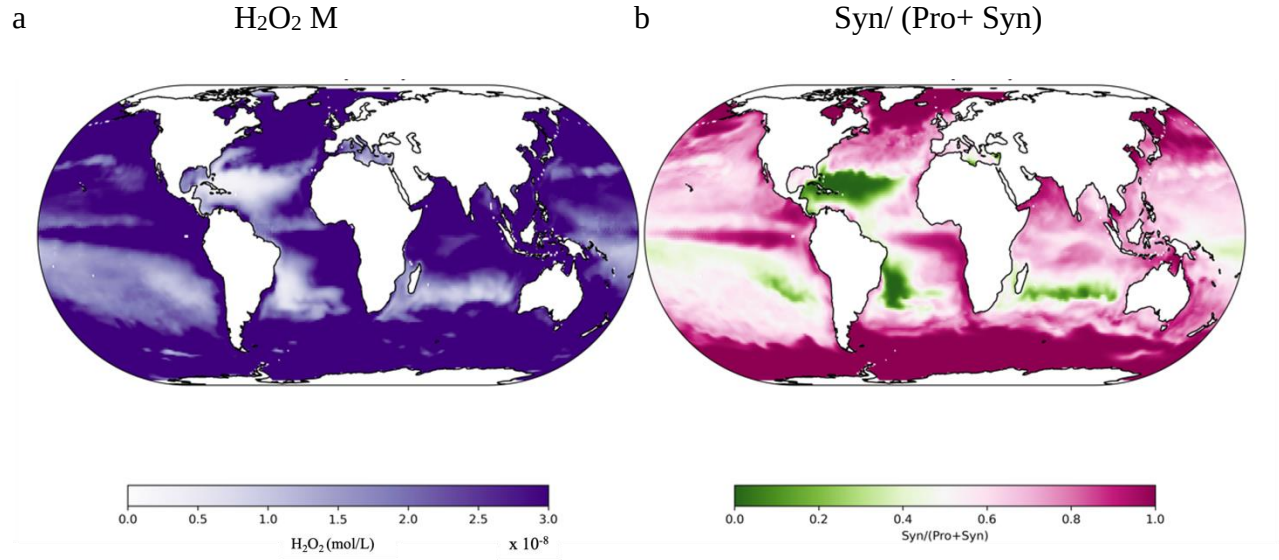


Figure C 5 H_2O_2 biogeographical plot and ratio plot of $(\text{Syn}) / (\text{Syn} + \text{Pro})$ biogeography in Case 3: Leaky world, heterotrophic detoxification ($\phi_{\max,B}$) alone.

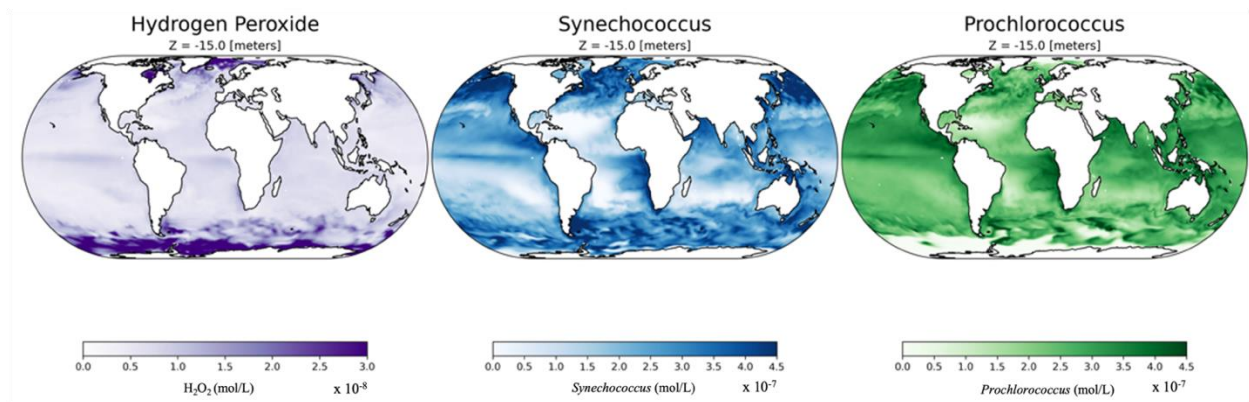


Figure C 6 Projected H_2O_2 , *Prochlorococcus* and *Synechococcus* biogeographies in year 9 of Case 4 with both heterotrophic bacterial and *Synechococcus* H_2O_2 detoxification ($\phi_{\max,S}$ and $\phi_{\max,B}$) incorporated in the model.

VITA

Donna Kathleen “Katie” McCullough was born in Huntsville, Alabama in 1994, two days before a tornado rattled the whole town. She is named after Donna Barnes, the first female head of the Mississippi Appellate State Court and biggest fan of Disney ever born. As the 3rd child to Claire and Calvin McCullough, an electrical and nuclear engineer respectively, she enjoyed a curiosity and educational upbringing. Her eventful childhood with older twin siblings, Charles and Sarah McCullough, was first located in Huntsville, AL and then Ringgold, Georgia. Katie first attended school at Graysville Elementary and then finished her K-12 education at Ringgold Middle and High school. Katie attended the University of North Georgia (Formally North Georgia College and State University) with both academic and music scholarship support. After volunteering at Lumpkin Country Animal Shelter in Dahlonega, Ga and memories of an unfortunate fetal pig dissection, D. Katie changed her major from veterinary sciences to biology. After a year of field reef ecology research parried with a microbiology laboratory prep assistant job, D. Katie found herself fully entrenched in biological research. UNG faculty were instrumental in this growth. After a year sabbatical studying the genetics of yeast subpopulation differentiation Poland under a Fulbright scholarship, Katie enrolled in the University of Tennessee in Knoxville for a PhD in Microbiology. Joining the Talmy lab, she delved into ocean microbiology with computer science and mathematical modeling tools informed by laboratory and *in situ* data.