TITLE

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**Introduction**

**Polar Phytoplankton**

Phytoplankton are photosynthetic microorganisms representing a diverse evolutionary history and ecology [1]. Inhabiting aquatic environments, they can be found in oceans, lakes, rivers, streams, estuaries, and wetlands across the biosphere.

Photolithotrophic growth, a defining characteristic of phytoplankton, is characterized by an increase in biomass without compromising the fitness of successive generations, thereby continuing in perpetuity under the given environmental conditions [2]. The primary inputs are photons, serving as the energy source, and inorganic sources of carbon, nitrogen, phosphorus, sulfur, and other essential nutrients [2]. Phytoplankton growth is constrained by the photic zone, the region with sufficient light for photosynthesis. In water, a decrease in photosynthetically active radiation (PAR) is observed with depth, as light is scattered and absorbed as it passes through the water column [3]. Eventually, light attenuates to the accepted bottom limit of the photic zone, historically established at 1% of surface irradiance, equivalent to 2-20 µmol photons m-2 s-1[2].

Light availability is further constrained in polar regions, presenting unique challenges for phytoplankton growth [4,5]. The radiation that penetrates through the ice is regulated by solar angle, sea ice thickness, and snow depth [6]. The sun's elevation angle undergoes changes attributed to the obliquity, or tilt, of the Earth as it orbits the sun, resulting in phenomena such as the polar night and midnight sun, dependent on the season. The thickness of the ice and snowpack further exacerbates light constraints, with reflective and relatively opaque ice and snow cover limiting the amount of light that permeates these environments [4]. Snow, in particular, is characterized by high light attenuation properties, further reducing the already limited availability of light energy [5].

Despite these constraints, certain psychrophiles demonstrate remarkable adaptability, exhibiting net growth under the ice through photosynthesis at extremely low light levels [4,5]. Across the Arctic, phytoplankton have been reported beneath the sea ice from Resolute Bay to the North of Svalbard, Baffin Bay, and the Greenland, Barents, Laptev, and Chukchi Seas [7]. In 1995, the lower limit of the photic zone was reconsidered as benthic microalgae in the Antarctic were reported photosynthetically active at light levels less than 1 µmol photons m-2 s-1 [8]. Moreover, more recent studies have documented winter phytoplankton growth below >1 m of snow and 1 m of sea ice, corresponding to PAR below 0.15 µmol photons m-2 s-1 [5]. This represents photosynthesis occurring at light levels 1-2 orders of magnitude below the accepted lower limit.

The faint but significant growth during winter under the ice underscores the ability of psychrophilic phytoplankton to maintain intact photosystems throughout the polar night [4]. Further, it offers support for the theoretical minimum light level for phytoplankton growth of 0.01 µmol photons m-2 s-1[2]. Such growth may serve to mitigate cell mortality in the extended darkness of winter, establishing a seeding population for the spring bloom [4].

**Arctic Blooms**

As the ice begins to retreat in the spring, phytoplankton blooms start to appear in the Arctic Ocean. Springs blooms are a major source of annual net primary production in this region, representing the most significant event for carbon export to higher trophic levels or sequestration in the deep ocean [7]. However, the ice-free period is also characterized by the stratification of surface water, which limits the inorganic nutrient supply [6]. Nutrient limitation imposes an upper limit on the size and duration of spring blooms, resulting in the region’s highly shortened productive period [6]. Regardless, spring phytoplankton blooms in the Arctic play key global biogeochemical roles in primary production and carbon cycling.

Phytoplankton are highly efficient primary producers relative to their biomass, owing to their rapid proliferation and photosynthetic activity in all cells, distinguishing them from terrestrial plants [1]. This efficiency is further accentuated by their rapid consumption, ultimately creating a prolific food source for ice-associated zooplankton and amphipods. Therefore, the timing of spring blooms is crucial in shaping food availability and the hatching success of associated zooplankton [5]. As the base of polar food webs, changing phytoplankton dynamics can have repercussions across all trophic levels.

Polar regions play a pivotal role in carbon cycling, representing almost half of the global CO2 sequestration through microbial photosynthesis [9]. Here, phytoplankton form the biological carbon pump, facilitating the drawdown of atmospheric carbon to the ocean's interior [1]. The increased solubility of CO2 at low water temperatures leads to substantial carbon sequestration through deep water formation at the poles, establishing a crucial carbon export pathway from surface waters to the deep ocean [5,7]. Further, substantial CO2 sequestration arises from high river inputs and the resistance of the terrestrial dissolved organic matter entering the Arctic basin to degradation [10].

The dynamics of polar phytoplankton blooms are currently undergoing substantial changes in both total annual productivity and seasonal peaks, primarily driven by climate change [7,11]. In the Arctic, the pace of warming is accelerating, resulting in multifaceted alterations within the marine ecosystem. The warming-induced reduction in sea ice extent and thickness has increased light availability, extending the phytoplankton growing season and expanding their open-water habitats [7]. Simultaneously, escalating freshwater inputs from melting contribute to an increase in vertical stratification, influencing nutrient availability. However, these effects are counterbalanced by heightened storm frequency and increased wind speeds, fostering vertical nutrient mixing [7]. Further, ocean acidification results in reduced calcification and elevated water temperatures both boost metabolic activity for some species while posing challenges for obligate cold extremophiles [7,12].

These climate-driven modifications extend across the atmosphere, cryosphere, and ocean, and greatly alter marine ecological dynamics, including productivity, interspecific interactions, population mixing, and pathogen and disease transmission [7]. Comprehending the adaptations and ecophysiology of psychrophilic phytoplankton becomes imperative in anticipating the consequences of these rapid global changes.

**Psychrophilic Adaptations**

Numerous known adaptations enable phytoplankton to survive in extreme polar environments. Microbes inhabiting sea ice must contend with solar, osmotic, oxidative and nutrient stress [10]. Further, as poikilotherms, they must overcome the severe inhibiting effects of a cold, low-energy environment. Cold temperatures place severe physiochemical constraints on the cellular functions of these organisms, exerting a negative influence on water viscosity, solute diffusion rates, membrane fluidity, enzyme kinetics and macromolecule interactions [10].

As a result of their extreme environments, psychrophilic phytoplankton exhibit high genetic divergence from closely related temperate species. A study of the polar diatom *F. cylindrus* found that approximately 25% of its genome consists of genetic loci with highly divergent alleles [13]. Genes related to catalytic activity, transport, metabolic processes and those integral to membranes were shown to be significantly enriched compared to temperate species, consistent with known microbial adaptations to cold temperatures [13].

To combat the harsh cold, some psychrophiles produce new compounds or alter existing ones. Cryospheric enzyme flexibility is promoted by changes in protein structure, including amino acid substitutions, H-bonds, and salt bridges [10]. Further, synthesizing cold shock proteins minimizes cold denaturation, promoting replication, transcription, and translation under low-temperature conditions [10]. These organisms also possess anti-freeze proteins (AFPs), encoded by numerous genes identified through metagenomic analyses [14]. AFPs are released into the extracellular space, where they act on ice through an adsorption-inhibition mechanism, effectively inhibiting ice recrystallization. As temperatures dip below freezing, AFPs attach to the ice crystal, forcing the ice front to grow between them. This induces a surface curvature of the crystal that shifts the equilibrium vapour pressure, lowering the local freezing point and limiting local ice growth [14].

Beyond novel compounds, polar microbes alter their cell membranes and solutes. First, they utilize cellular-compatible solutes, including sugars, polyols, amino acids, betaine, and DMSP, which reduce intracellular freezing points and maintain enzyme hydration spheres, stabilizing catalytic activity [10]. Additionally, they exhibit high levels of polyunsaturated fatty acids (PUFAs) in their lipid membranes, including cell membrane phospholipids and chloroplast membrane galactolipids [10,12]. The unsaturated bonds contribute to a looser packing of lipids, maintaining membrane fluidity at cold temperatures.

Moreover, many psychrophilic species coordinate multiple metabolic routes to achieve photostasis, a delicate balance between energy input and utilization [12]. During dark periods, they employ the Entner-Doudoroff pathway (EDP). While the EDP provides less energy per molecule of glucose than glycolytic metabolism, it requires fewer resources for enzyme synthesis, representing a strategic trade-off [15]. Using this alternate metabolism enables the phytoplankton to retain the functionality of their photosynthetic apparatus during prolonged dark periods, allowing them to quickly recover upon re-illumination [12].

Much is known about these diverse physiochemical adaptations for surviving cold temperatures and low light. However, possible bioenergetic adaptations in the photosynthetic apparatus of psychrophilic phytoplankton remain understudied.

**Energetic Principles of Photosynthesis**

Oxygenic photosynthesis is the metabolic process by which light energy, water, and carbon dioxide are converted to oxygen and chemical energy. Photosynthesis occurs in specialized organelles called chloroplasts, which are bounded by a two-to-four-membrane envelope and filled with a granular matrix called the stroma [3]. The stroma comprises a concentrated solution of proteins, including the enzymes used in carbon dioxide fixation. Within the stroma are thylakoids, membrane-bound compartments containing pigments and electron carriers. The thylakoid membrane is composed of a polar lipid bilayer, and embedded in it is photosystem II (PSII), a multi-subunit protein complex [3].

PSII consists of 17 transmembrane subunits, three membrane-extrinsic subunits, and more than 40 cofactors, including the Mn cluster, chlorophylls, carotenoids, plastoquinones, and Fe2+. Ca2+, and Cl- ions [16,17]. During photosynthesis, its crucial function is to catalyze light-induced charge separation and water oxidation, transferring electrons from a donor to acceptor molecules and producing molecular oxygen [16].

In PSII, photons are captured by light-harvesting chlorophyll molecules, composed of a central magnesium atom surrounded by cyclic tetrapyrrole [3]. Absorption of a photon by a chlorophyll molecule initiates a transition from the ground state to an electrically excited state. The excitation energy is distributed variably among three pathways: photochemistry, dissipation as heat (DNPQ), and re-emission as fluorescence (ChlF) [18]. The energy directed to photochemistry by antenna pigments undergoes inductive resonance transfer, eventually reaching the reaction center of PSII [3]. The reaction center, P680, is composed of a Chl a heterodimer PD1 and PD2 [17,19].

When P680 is raised to its excited state, P680+, it transfers an electron to an acceptor molecule and congruently withdraws an electron from a donor molecule [3]. On the acceptor side, P680+ first passes an electron to pheophytin (Phe). The electron from reduced Phe is transferred to plastoquinone A (QA), followed by plastoquinone B (QB) [17]. Once QB is fully reduced by receiving two electrons, the electrons are passed to the mobile plastoquinone pool in the lipid phase of the thylakoid membrane [3]. On the donor side, an electron is extracted from the manganese cluster in PSII, thereby reducing a tyrosine residue D1-Tyr-161 (Yz) [20]. P680+ receives an electron from YZ, returning it to its original state, P680.



Figure 1: Pathway of s-state cycling and the electron transfer in PSII. Successive PSII charge separations extract electrons from the Mn cluster, inducing four increasingly oxidized states. After accumulating four oxidizing equivalents, 2 H2O molecules are oxidized to 1 O2.

*S-State Cycling*

The water-oxidizing or oxygen-evolving complex (WOC) of PSII is the active site where water is converted to oxygen and protons [21]. It consists of a metal-oxo cluster with the formula Mn4CaO5. As P680 absorbs successive photons, they induce charge separations, which cause electrons to be extracted from the Mn cluster. Each electron removed from the WOC replaces one lost by the reaction center when an electron is transferred downstream. As consecutive charge separations occur, it induces four increasingly oxidized states, known as s-states [22]. S-states are denoted as S0 to S3, with a transient S4 state which rapidly decays to S0 (Figure 1). Once four photons have been absorbed and the Mn cluster has lost four electrons, it replaces them by oxidizing two water molecules to one molecule of molecular oxygen. Therefore, a complete water oxidation cycle during oxygenic photosynthesis requires the absorption of four photons and the successive accumulation of four oxidizing equivalents [21–23].

As the WOC moves between s-states, it alters the kinetics and free energy of the system [24]. In turn, this changes the partitioning of excitation energy between photochemistry, dissipation as heat, and chlorophyll fluorescence (ChlF). As the energy allocated to ChlF is modulated by the energy allotted to photochemistry and thermalization, the quantum yield of ChlF varies between s-states [18,21]. Therefore, the four s-states are reflected by a four-period oscillation in chlorophyll fluorescence [25].

*Recombination*

During photosynthesis, secondary electron transfer occurs at both the donor and acceptor sides of Photosystem II (PSII), stabilizing separated charges [17]. However, these reactions remain reversible, occasionally resulting in the backflow of electrons through the pathway, referred to as recombination reactions [26]. Recombination plays a pivotal role in photodamage and photoprotection, both harming PSII and proving essential for survival under excess light conditions [27].

At elevated light levels, electron transfer from P680+ is blocked by the total reduction of downstream electron acceptors. Under such conditions, the primary radical pair [P680+Phe-] will recombine, generating the excited triplet chlorophyll 3P680+ [28]. Chlorophyll triplets react with ground-state molecular oxygen to generate singlet oxygen (1O2), a highly damaging, photoinhibitory reactive oxygen species [27]. In contrast, direct recombination events are governed by the redox properties of the QA and Phe electron acceptors. They contribute to photoprotection by competing with triplet chlorophyll formation in the PSII reaction center [26].

Beyond their roles in photodamage and photoprotection, charge recombination reactions may also occur as a wasteful process that reduces photosynthetic energy conversion efficiency [27]. Altering redox potentials of downstream electron acceptors, leading to changes in energy gaps, may represent evolutionary adaptations aimed at maximizing photoprotection and minimizing inefficient back reactions under light-limited conditions [24].

**Study Aims**

This study aims to evaluate if psychrophilic diatoms and green algae have evolved to increase photosynthetic energy conversion efficiency by minimizing inefficient back reactions.

The duration of s-state cycling in a phytoplankton sample may be evaluated by the applications of short, very bright, single-turnover light flashes. As sequential light flashes are applied, PSII is driven synchronously through its S-state cycle [23]. In an idealized sample, the four s-states will be reflected by a periodic oscillation in ChlF. However, recombination reactions represent a loss of charge separation and wasteful slippage in the s-state cycling of an individual PSII. As more recombination events occur, the desynchronization of s-state cycling within a sample will scramble the periodic changes in ChlF, dampening the observed oscillation [25]. An organism exhibiting longer s-state cycling indicates fewer inefficient back reactions and more efficient photosynthetic energy conversion.

By comparing the s-state cycling of psychrophilic and temperate taxa across common conditions, we can determine if psychrophilic diatoms and green algae have evolved increased photosynthetic efficiency and under what conditions they have a significant advantage.

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