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LES RÉPONSES DES MICROALGUES ARCTIQUES ET TEMPÉRÉES AUX PESTICIDES ET À LA LUMIÈRE DIFFÈRENT PAR LEURS CARACTÉRISTIQUES ÉCOPHYSIOLOGIQUES

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PREFACE

During the first year of my master's studies in 2014, I discovered an interest in algal physiology. I decided to continue my research in this field and started my PhD studies in Philippe's lab at UQAM. During my master's studies, I mainly studied the combined effect of heavy metal cadmium (Cd) and light on cyanobacterial of *Synechocystis* PCC6803, indicating that *Synechocystis* after adapted to high light becomes more tolerant to Cd compared to cells grown under low light since *Synechocystis* has different photoprotection mechanisms to respond to Cd and light, which was contradictory with my hypothesis. I read a lot of references to get more knowledge about the effects of light intensity on algal physiology and pollutant toxicity. I learned that light intensity is one of the most important environmental factors for photosynthetic organisms, it is not only the driving force of photosynthesis but also a stress factor affecting both photosystems. Light intensity can affect the physiochemical processes of phytoplankton, such as pigment composition, light energy conservation, photoprotective ability (NPQ), photosystem content, and the toxicity and absorption of toxic pollutants (pesticides and metals, etc.). Indeed, it will finally affect the community composition of phytoplankton in the aquatic environment. In my master's study, I only studied the effect of cadmium on prokaryotic cyanobacteria under different light conditions, but cyanobacteria are different from eukaryotic algae in many aspects, such as the lack of chloroplast and its pigment composition, the mode of action of non-photochemical quenching (NPQ), and energy metabolism. What are the toxic effects of pollutants and the combined effects of light intensity and pollutants on eukaryotic algae? Is there a big difference effect between photosynthetic eukaryotic cells and prokaryotic cells in response to light intensity or pollutant stress?

I started my PhD study in January 2018 with these questions. As far as I know, pesticide pollution in recent years has become a worldwide environmental problem, even in the Arctic region. Through months of literature knowledge reading and discussions with my supervisor, I made a study plan for PhD project to study how different ecophysiological characteristics influence the response of Arctic phytoplankton to pesticides as compared to their temperate counterparts (Chapter 1). Concomitantly, algae experience light fluctuations in the aquatic

ecosystem due to the daily sunset and seasonal change responses, including the extreme environment of the Arctic Ocean due to melting ice. Therefore, I want to understand the response to variable light intensity in photo-adapted Arctic and temperate microalgae exposed to herbicides (Chapter II). After finishing these two chapters, I found that the EC_{50} of operational PSII quantum yield (Φ'_M)-in marine microalgae was very high, and marine ecosystems can not achieve such high pesticide concentrations. However, EC_{50} was a prerequisite for doing mixing experiments and there is no such serious pesticide pollution in natural ocean waters. So, I decided to study single and mixed pesticides and the combined effects of pesticides and light on freshwater microalgae in order to close to the natural situation (Chapter 3).

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T-CN	Temperate *Chaetoceros neogracile*
T-MB	Temperate *Micromonas bravo*
A-MP	Arctic *Micromonas polaris*
A-CN	Arctic *Chaetoceros neogracilis*
XC	xanthophyll cycle
Car	carotenoids
Chl *a*	Chlorophyll a
PS (I or II)	Photosystem I or II
NPQ	Non-photochemical quenching
qE	a fast phase of NPQ
qI	a slow phase of NPQ
qT	a medium phase of NPQ
PQ	plastoquinone
Q_A	primary electron acceptor of PSII
Q_B	secondary electron acceptor of PSII
ETC	electron transport chain
RC	reaction center
Φ_M	maximum PSII quantum yield
Φ'_M	operational PSII quantum yield
ROS	Reactive oxygen species
SOD	superoxide dismutase

CAT	catalase
log*Kow*	the ratio of a chemical's concentration in the octanol phase
LHC	light-harvesting complex
NADPH	reduced from $NADP^+$
Rubisoco	ribulose-1 ,5-bisphosphate carboxylase/oxygenase
VLL	very low light intensity
LL	low light intensity
ML	medium light intensity
HL	high light intensity
CEF	cyclic electron flow

RÉSUMÉ GÉNÉRAL

Les écosystèmes polaires jouent un rôle important dans la production primaire mondiale. Les microalgues de ces régions ont développé des adaptations qui leur permettent de prospérer avec des températures basses en permanence et des variations extrêmes de l'éclairement et de la durée du jour. Par conséquent, leurs caractéristiques éco-physiologiques devraient être différentes des microalgues tempérées. Parallèlement, ces adaptations pourraient modifier leur sensibilité aux polluants abiotiques tels que les pesticides. En effet, nous avons constaté que les microalgues arctiques ont évolué de manière différente pour développer des caractéristiques éco-physiologiques par rapport à leurs homologues tempérés : les microalgues arctiques ont (1) des teneurs en chlorophylle *a* (Chl *a*) et en caroténoïdes (Car) plus faibles et (2) une teneur beaucoup plus élevée NPQ_{max} intrinsèque, (3) une teneur en espèces réactives de l'oxygène (ROS) plus faible et (4) des activités de catalase (CAT) et de superoxyde dismutase (SOD) plus élevées, par rapport aux espèces tempérées. Les paramètres écophysiologiques des tests de toxicité des pesticides standard étaient la croissance, le biovolume cellulaire, la teneur en pigments, l'activité photosynthétique et les mécanismes photoprotecteurs (NPQ, activité enzymatique antioxydante) et la teneur en ROS. Les résultats obtenus ont montré qu'une espèce arctique (*Micromonas polaris*) est plus tolérante à l'atrazine et à la simazine que ses homologues tempérés (*Micromonas bravo*), tandis que l'autre espèce arctique (*Chaetoceros neogracilis*) est plus sensible à ces herbicides par rapport à son homologue tempéré (*Chaetoceros néogracile*). De plus, les deux microalgues arctiques sont plus sensibles au chlorpyrifos (insecticide) que leurs homologues tempérées. Ces différences sont principalement dues à des différences dans les principaux mécanismes de protection entre les microalgues arctiques et leurs homologues tempérées. La taille des cellules, la teneur en pigments, le NPQ et les activités enzymatiques antioxydantes peuvent expliquer cette sensibilité différente aux pesticides.

D'autre part, les algues subissent de légères fluctuations dans l'énergie lumineuse reçue dans l'écosystème aquatique en raison des réponses quotidiennes au coucher du soleil et aux changements saisonniers, y compris l'environnement extrême de l'océan Arctique en raison de la fonte des glaces. Mais on sait très peu de choses sur la réponse des microalgues marines

tempérées et arctiques de photoadaptation à l'exposition aux herbicides. Nous avons donc étudié l'activité photosynthétique, les flux d'énergie PSII, la teneur en pigments, la capacité photoprotectrice (NPQ) et la teneur en ROS dans trois conditions de lumière de culture différentes (40-LL, 100-ML, and 400-HL μmol photons m^{-2} s^{-1}). Nous avons constaté que la croissance et le biovolume cellulaire des microalgues arctiques et tempérées étaient stimulés par l'augmentation de l'intensité lumineuse de la culture, même si l'efficacité de la photosynthèse était réduite. La principale différence entre les réponses de photoadaptation des microalgues arctiques et tempérées était que les deux microalgues arctiques répondent principalement à l'inhibition du transfert d'électrons photosynthétiques sous une haute lumière (HL) en augmentant la taille du pool de PQ, plutôt qu'en régulant l'absorption de la lumière en réduisant la teneur en chlorophylle comme dans microalgues tempérées. Parallèlement, la diatomée de *Chaetoceros* de la région arctique avait des capacités d'acclimatation plus élevées aux variations de lumière que l'algue verte de *Micromonas*. De plus, même le NPQ et la formation de pigments photoprotecteurs (Car) étaient fortement activée après une forte acclimatation à la lumière, mais ces effets inhibiteurs étaient plus forts lorsque les microalgues rencontraient une forte lumière en été ou par une fonte massive de la glace de mer causée par le réchauffement climatique dans les décennies à venir.

À la suite des résultats obtenus dans ces deux parties de ma thèse, j'ai étudié les herbicides binaires sur les microalgues d'eau douce puisque les herbicides n'existent jamais seuls dans les écosystèmes aquatiques. Nos résultats ont montré que la lumière peut affecter le type d'interaction (synergie, antagonisme, addition) des herbicides binaires avec les microalgues d'eau douce, qui est fortement liée à la concentration du mélange de pesticides. De plus, les microalgues d'eau douce acclimatées au HL ont réduit la toxicité de l'atrazine et de la simazine par rapport aux cellules acclimatées au LL, la stratégie protectrice du NPQ joue un rôle important dans ce processus. De plus, les communautés d'algues peuvent être altérées dans les eaux contaminées par les herbicides et les changements de lumière, car les algues vertes ont une forte capacité d'acclimatation à la lumière et une résistance élevée aux herbicides.

Mots clés : Microalgues, *Micromonas*, *Chaetoceros*, Arctique, tempéré, intensité lumineuse, écophysiologie, photosynthèse, pesticides simples/binaires, mécanisme de toxicité, type d'interaction.

ABSTRACT

Polar ecosystems play an important role in global primary production. Microalgae in these regions have evolved adaptations that allow them to thrive with permanently low temperatures and extreme variations in irradiance and day length. Therefore, their ecophysiological characteristics should be different from temperate microalgae. Meanwhile, these adaptations could change their sensitivity to abiotic pollutants such as pesticides. Indeed, we found that Arctic microalgae evolved in a different way to develop ecophysiological characteristics compared to their temperate counterparts: Arctic microalgae have (1) lower chlorophyll *a* (Chl *a*) and carotenoid (Car) contents and (2) a much higher intrinsic NPQ_{max}, (3) lower reactive oxygen species (ROS) content and (4) higher catalase (CAT) and superoxide dismutase (SOD) activities, compared to temperate species. Ecophysiological endpoints of standard pesticide toxicity tests were growth, cell biovolume, pigment content, photosynthetic activity, photoprotective mechanisms (NPQ, antioxidant enzyme activities), and ROS content. The obtained results showed that one Arctic species (*Micromonas polaris*) is more tolerant to atrazine and simazine than its temperate counterparts (*Micromonas bravo*), while the other Arctic species (*Chaetoceros neogracilis*) is more sensitive to these herbicides compared to its temperate counterpart (*Chaetoceros neogracile*). In addition, both Arctic microalgae are more sensitive to chlorpyrifos (insecticide) than their temperate counterparts. These differences are mainly due to differences in the main protective mechanisms between Arctic microalgae and their temperate counterparts. The cell size, pigment content, NPQ, and antioxidant enzyme activities can explain this different sensitivity to pesticides.

On the other hand, algae experience light fluctuations in the aquatic ecosystem due to the daily sunset and seasonal change responses, including the extreme environment of the Arctic Ocean due to melting ice. However, very little is known about the photoadaptation response of temperate and Arctic marine microalgae to herbicide exposure. We therefore investigated the photosynthetic activity, PSII energy fluxes, pigment content, photoprotective ability (NPQ), and ROS content under three different culture light conditions (40-LL, 100-ML, and 400-HL μmol photons m^{-2} s^{-1}). We found that the growth and cell biovolume of Arctic and temperate

microalgae were stimulated with increasing the culture light intensity, even though photosynthesis efficiency was reduced. The main difference between the photoadaptation responses of Arctic and temperate microalgae was that the two Arctic microalgae mainly respond to the inhibition of photosynthetic electron transfer under high light (HL) by increasing the size of the PQ pool, rather than regulating light absorption by reducing chlorophyll content as in temperate microalgae. Concomitantly, the diatom of *Chaetoceros* from the Arctic region had higher adaptation capacities to variations of light than the green algae of *Micromonas*. Furthermore, NPQ and photoprotective pigments (Car) were heavily activated after high light adaptation, but these inhibitory effects were stronger when microalgae encounter high light in summer or massive sea ice melt caused by global warming in the coming decades.

Following the results obtained in these two parts of my thesis, I studied the binary herbicides on freshwater microalgae since herbicides never exist alone in aquatic ecosystems. Our findings showed that light can affect the type of interaction (synergy, antagonism, addition) of binary herbicides with freshwater microalgae, which is highly related to the mixture of pesticide concentration. Moreover, freshwater microalgae adapted to HL reduced the toxicity of atrazine and simazine compared to cells adapted to LL, the protective strategy of NPQ plays an important role in this process. In addition, algal communities may be altered in waters with herbicide contamination and changes in light since green algae have strong light adaptation ability and high resistance to herbicides compared to cyanobacteria.

Keywords: Microalgae, *Micromonas*, *Chaetoceros*, Arctic, temperate, light intensity, ecophysiology, photosynthesis, single/ binary pesticides, toxicity mechanism, interaction type.

GENERAL INTRODUCTION

Background

Arctic ecosystems represent some of the most extreme environmental conditions on the planet. These ecosystems are important for global primary production, accounting for 11% of the world's organic matter pool (Callaghan and Sven 1995). More than 75% of the Arctic phytoplankton biomass is composed of diatoms and small flagellate prasinophytes (*Micromonas* sp.) (Balzano et al. 2012), which thus play an essential role in the Arctic food web (Jardillier et al. 2010). These phytoplankton support the growth of various zooplankton species (mainly copepods) (Kosobokova et al. 2010), for their part, are essential for fish. A cold environment influences phytoplanktonic organisms in many ways, including changes in membrane fluidity (White et al. 2000), nutrient availability, reduced biochemical reaction rates (Wiebe et al. 1992), energy balance between absorption and utilization (Parker and Armbrust 2005), and the ability to reproduce successfully (Margesin 2007). Ecophysiological characteristics of Arctic microalgae could thus be different relative to their temperate counterparts (Lacour et al. 2017) (Chapter I). Furthermore, marine irradiance levels can fluctuate highly due to the variability of ice and snow-cover, rapid shifts in cloud cover, and seasonal changes, sediment loading as a result of temperature increase coupled with riverine and glacial freshwater input (Ardyna and Arrigo 2020). Therefore, algae experience light fluctuations due to the daily sunset and seasonal changes in the extreme environment of the Arctic Ocean. The response to variable light intensity in photo-adapted temperate and Arctic marine microalgae should be different because of this differential exposure to variation (Chapter II).

With respect to pesticides, a significant portion used in agriculture is lost during and after their application to crops due to dispersal processes such as leaching, runoff and spray-drift, leading to their detection in the aquatic environment (Zhang et al. 2018). Some authors have reported that Arctic waters are contaminated with pesticides applied in the southern parts of Canada,

the USA and EU countries due to the long-distance aerial and marine transport of chemicals (Cabrerizo et al. 2019, Ma et al. 2018, Muir et al. 2013). In addition, owing to the accumulation of pesticides over many years in Arctic ice, pesticide concentrations in Arctic waters should increase as accumulated ice starts to melt at an unprecedented rate because of global warming (Cabrerizo et al. 2019, Pućko et al. 2017). It has been advanced that the Arctic ecosystems are probably more sensitive to low levels of pollutants than ecosystems in temperate latitudes (CARC 1990, Kottuparambil et al. 2017). Even though current levels of pollution are considerably lower than in most urban and industrialized areas in the mid-latitudes, transboundary pollution appears to have been increasing over the past few decades (Vorkamp and Riget 2014). Moreover, most of our knowledge about the effect of pesticides is from temperate species. It is therefore essential to better understand the potential pesticide effect on the Arctic microalgae compared to their temperate counterparts (Chapter I).

In aquatic environments, light intensity is one of the critical environmental factors for phytoplankton photosynthesis (Gomes and Juneau 2017). As mentioned, phytoplankton experiences intense fluctuations of light variations due to the daily course of sunlight and seasonal changes (Wagner et al. 2006), and the movement of organisms in the water column also modifies available light intensity for short periods of time, where photon flux may be scarce deep in the water column, or abundant at the surface (Dubinsky and Stambler 2009). Photosynthetic organisms respond to fluctuating light environments by adjusting their physiology, biochemistry, and morphology and these responses occur at different time scales (Bellacicco et al. 2016, Deblois et al. 2013a). Short-term responses (seconds to minutes) such as NPQ quenching (by OCP-related quenching or state transition, or enzyme de/activation) are considered as regulation. Mid-term responses (a few hours to days) represent an acclimation, such as the response observed under high light (increase pigment content and protein synthesis, changes in photosystem stoichiometry and antioxidant contents (Bellacicco et al. 2016). Furthermore, it has been demonstrated that light can affect the toxicity of algae to pesticides (Gomes and Juneau 2017), but very little is known about

the response to variable light intensity in photoadaptation temperate and Arctic marine microalgae exposed to herbicides (Chapter II).

In most polluted aquatic environments, phytoplankton, as primary producers, are exposed to a mixture of chemicals rather than a single chemical (Dupraz et al. 2019a, Magdaleno et al. 2015). Thus, studying the toxicity of single substances may be insufficient since interactions between chemicals can occur. Some studies have demonstrated that a combination of low concentrations of pesticides induces greater inhibition of temperate phytoplankton photosynthesis than the sum of each individually (DeLorenzo and Serrano 2003, Dupraz et al. 2019b). The combination of pesticides may trigger synergistic (a greater effect than additive), antagonistic (a lower effect than additive) or additive (sum of individual pesticide toxicity) effects on phytoplankton physiology (Crain et al. 2008) depending on the stressors' mode of action (Korkaric et al. 2015). As mentioned, light, as one of the important environmental factors for photosynthetic organisms, may affect the toxicity of pesticides, but information on how changes in light affect the toxicity of mixed pesticides on algae is lacking. Therefore, it is urgently needed to characterize the toxicity of mixed pesticides and examine the effects of light variation on the pesticide mixtures as I have done in Chapter III.

Arctic microalgae and their temperate counterparts

Microalgae are usually found in marine and freshwater environments. They are crucial for life on earth as they can perform photosynthesis; they generate around half of the oxygen in the atmosphere and use concomitantly sunlight, carbon dioxide, and other greenhouse gases to grow (Chen et al. 2016). Microalgae are the main primary producers in aquatic ecosystems and form the base of the food chain. They convert dissolved inorganic carbon into organic matter through photosynthesis, which supplies energy to the whole food webs in the ocean. Increasing attention to the ecophysiology of Arctic phytoplankton has increased in recent years since global warming exacerbates Arctic ice melting, which can affect the primary productivity of phytoplankton (Frey et al. 2018, Hoppe et al. 2018). The availability of light and the presence of nutrients are two factors that greatly influence primary productivity. Furthermore, microalgae are highly sensitive to the various pollutants of the aquatic environment (Chen et al. 2016). However, we lack a mechanistic understanding of how these drivers interact with primary producers in the Arctic Ocean. Therefore, research into photosynthesis, physiology and contamination effects in Arctic algae is important to evaluate Arctic ecosystem functioning since current assays have only used temperate strains.

More than 75% of the Arctic phytoplankton biomass is composed of diatoms (*Chaetoceros*) and small flagellate prasinophytes (*Micromonas* sp.) (Balzano et al. 2012, Lovejoy et al. 2007). *Chaetoceros* was first described by Ehrenberg in 1844 and belongs to nanophytoplankton which is especially tiny phytoplankton with sizes from 2 to 20 µm. It is primarily a marine genus and is one of the most abundant and diverse diatom genera in the oceans (Wolf et al. 2018). *Chaetoceros neogracilis* is a representative diatom in the Arctic Ocean and generally occurs as single cells, but can also form short colonies (Balzano et al. 2017). Its temperate counterpart, *Chaetoceros neogracile*, is similarly a marine species. *Micromonas* was the first picoplanktonic species (1.5-3.0 µm) to be described (Lovejoy et al. 2007). It is small unicellular pear-shaped prasinophyte algae without a cell wall and has a single mitochondrion and chloroplast, covering almost half of the cell. Moreover, unlike many marine algae, the genus is distributed widely in both warm and

cold water (Worden et al. 2009). *Micromonas polaris* is the primary pico-eukaryote in the Arctic Ocean (Beaufort Sea) in the summer, but is absent from the temperate area. In the temperate ocean in summer, *Micromonas bravo* dominates the *Micromonas* community, while being absent from polar regions (Gérikas Ribeiro et al. 2020). As mentioned, because of their dominance in the Arctic Ocean, this study examined responses in two Arctic microalgae *M. polaris* and *C. neogracilis*, and their reproductively isolated temperate counterparts *M. bravo* and *C. neogracile* respectively.

Photosynthesis

Photosynthesis is the process used by some bacteria, algae, and plants to produce sugar and oxygen through carbon dioxide, water, and sunlight, via a long series of chemical reactions (Hall and Rao 1999). But it can be divided into two processes, the " photo " section refers to reactions driven by light; " Synthesis " — the production of sugars — is a separate process called the Calvin cycle (Hall and Rao 1999). In this study, we focus on how the " photo " part responds to changes in light intensity, pesticide stress, and the combined effects of light and pesticides in Arctic microalgae compared to their temperate counterparts.

The light reaction begins with light absorption by the light-harvesting complex (LHC) of photosystem II (PSII). Then PSII uses this energy to split water and release an electron and H^+ energy (Young et al. 2015). The electron transfer along a Z-scheme electron transport chain (ETC) from PSII to PSI via cytochrome b_6f complex (Cyt b_6f), ultimately reducing $NADP^+$ to NADPH, thereby converting light energy to chemical energy (ATP), as shown in Figure I (Yamori and Shikanai 2016). This Z-scheme photosynthetic ETC has consisted of a series of electron carriers: plastoquinone (PQ) pool, Cyt b_6f, PC/Cyt c553, and the last $NADP^+$, which are located on the thylakoid membrane. The trans-thylakoid proton gradient (ΔpH) is formed during the electron transport, which serves to produce ATP by ATP synthase, energy used to fix carbon dioxide in the " Synthesis " part (Markou and Muylaert 2016). To transfer light energy absorbed into PSII to electrons, two main PSII components are involved: LHC and the reaction center (RC) (Figure II)

(Minagawa and Takahashi 2004). Chlorophyll *a* (Chl *a*), one of the components of LHC, captures light and transfers it to the PSII RCs, where photosynthetic electron transport begins, to produce chemical energy (NADPH and ATP) (Ayelén 2017).

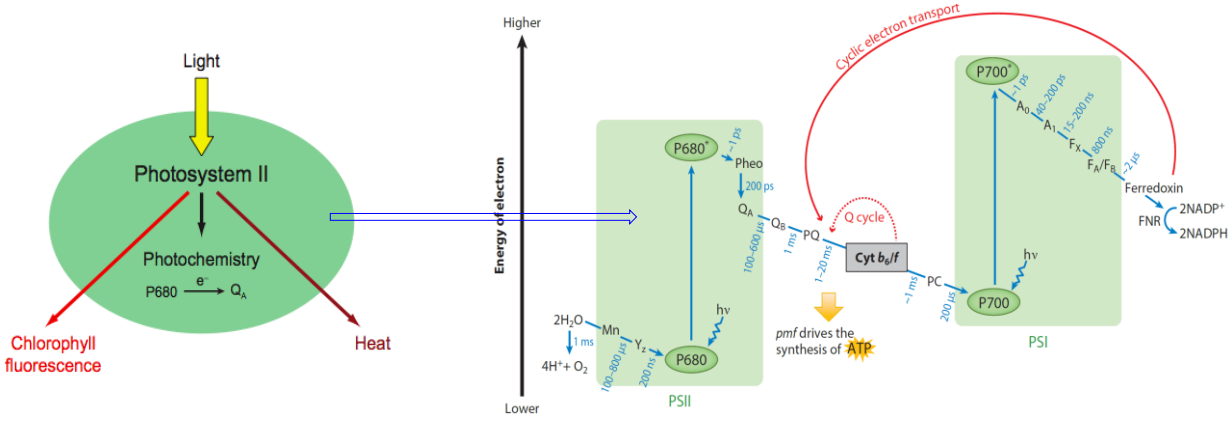

Figure 0. 1. "The Z-Scheme diagram of photosynthesis. The electron transport pathway from water (H_2O) to $NADP^+$. Mn for a manganese complex containing 4 Mn atoms, bound to PSII RC; O_2 for oxygen; H^+ for protons; P680 for the RC chlorophyll (Chl) in PSII: it is the primary electron donor of PSII; Excited (Chl) P680 for P680* that has the energy of the photon of light; Pheo for pheophytin molecule (the primary electron acceptor of PSII; it is like a chlorophyll a molecule where magnesium (in its center) has been replaced by two "H"s); Q_A for a plastoquinone molecule tightly bound to PSII; Q_B for another plastoquinone molecule that is loosely bound to PSII; Cyt b_6f for cytochrome complex; PC for copper protein plastocyanin; P700 for the RC Chl (actually a dimer: two molecules together) of PSI; it is the primary electron donor of PSI; Excited (Chl) P700 for P700* that has the energy of the photon of light; Ao for a special chlorophyll a molecule (primary electron acceptor of PSI); A1 for a phylloquinone (Vitamin K) molecule; FX, FA, and FB are three separate Iron Sulfur Centers; FD for ferredoxin; and FNR for Ferredoxin NADP oxido Reductase (FNR) ". From (Yamori and Shikanai 2016).

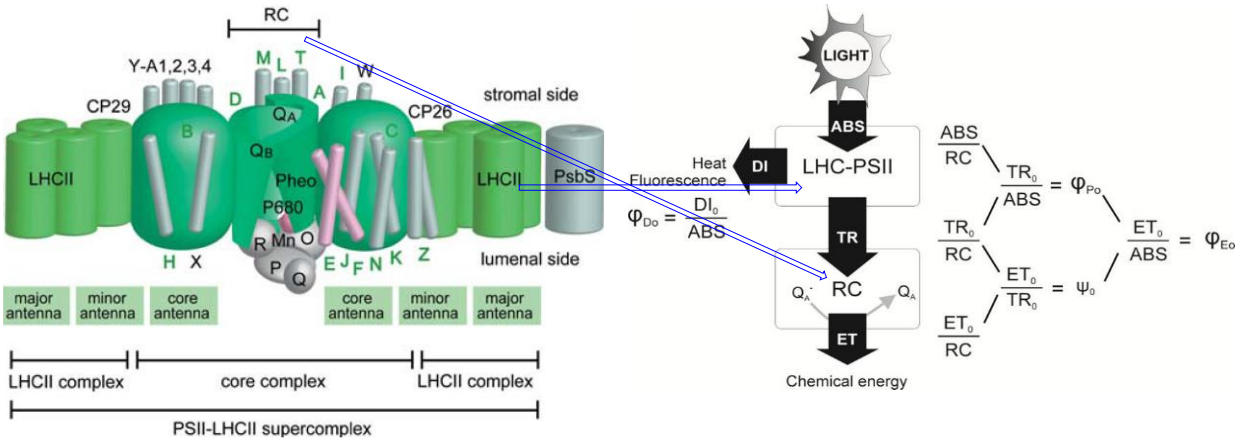

Figure 0. 2. Subunit structure of PSII-LHCII supercomplex on left.

Light intensity

Light is one of the most important environmental factors for the photosynthetic organism's growth and the source of energy for all physiological activities (Edwards et al. 2015). Therefore, changes in light intensity can affect algal photosynthesis and growth (Croteau et al. 2022, Metsoviti et al. 2019), resulting in alterations in their distributions in freshwater ecosystems. Indeed, phytoplankton experiences intense light fluctuations due to the daily sunlight exposure and seasonal changes in aquatic environments (Rumschlag et al. 2020, Wagner et al. 2006), where photon flux may be scarce deep in the water column, or abundant at the surface (Dubinsky and Stambler 2009). Therefore, some species have unique flagella or vacuole structures that allow them to move in the water column to optimize light harvesting (Deblois et al. 2013a).

To cope with the light variations in aquatic environments, photosynthetic organisms have evolved diverse phenotypic adjustments including photoadaptation processes (Deblois et al. 2013b, Handler 2017). Photoadaptation to low or high light environments involves the adjustments of their morphology (size and shape), biochemistry (electron transport chain), and physiology (photosystem apparatus and pigment, etc), and these responses occur at different time scales (Bellacicco et al. 2016, Deblois et al. 2013a). Algal cells can adjust their sizes to better accommodate the light fluctuations, as smaller cells can maintain higher photosynthetic rates under

light limiting conditions without the significant optical packaging effects that reduce photon capture present in larger cells (Wu et al. 2014). However, larger cells may be less susceptible to photoinhibition under excessive light (Key et al. 2010). Furthermore, high levels of light can inhibit the ETC to reduce the biochemical conversion capacity (Bellacicco et al. 2016). On the other hand, light is the driving force of photosynthesis but also a stress factor affecting both photosystems, PSI and II via their RCs. PSII is more sensitive to light-induced damage than PSI when the absorbed light exceeds the light algal needs, with damage rates positively related to the light intensity (Virtanen et al. 2021). Variation in photosynthetic pigments, such as Chl *a*, to adjust the light absorption via photoadaptation can occur (Du et al. 2019). Photosynthetic organisms develop a series of physiological photoprotection mechanisms under light fluctuations, including PSII and PSI electron recycling, photosynthetic and non-photosynthetic pigments, rapid repair of the PSII D1 protein reaction centers (RCs), state transitions, changes in the efficiency of energy transferred from the collection complex to RCs, and NPQ induced by activation of the xanthophyll cycle (XC) (Dong et al. 2016, Hopes and Mock 2015). Among them, NPQ is a major photoprotective mechanism toward light changes (Goss and Lepetit 2015).

Protective measures

1) Non-photochemical quenching (NPQ)

Photosynthetic organisms can be photoprotected by heat dissipation. This thermal dissipation of light energy, termed non-photochemical quenching (NPQ) by chlorophyll fluorescence, is the fastest and most flexible response to cope with excess light among the known photoprotective strategies (Goss and Lepetit 2015, Lacour et al. 2020, Müller et al. 2001). Over-excitation of the photosynthetic pigments on PSII and over-reduction of the ETC caused by stress may lead to the generation of ROS. XC-related NPQ interacts with the PSII antenna complex to transform the LHC antenna into a heat-dissipating state to dissipate the excess energy as heat, and thus NPQ can

prevent potential damages from oxidative stress generated by ROS, with the major target of photodamage being the D1 (PsbA) protein of PSII (Lacour et al. 2020).

There are three factors for the activation of NPQ: 1) de-epoxidized xanthophyll synthesized by the XC, 2) a high proton gradient across the thylakoid membrane, and 3) the LHC proteins (Ruban 2016). NPQ has multiple components and can be divided into at least three different components, each of which is distinguished by various intervals of relaxation in the dark : (1) qE, the energy-dependent quenching is the fastest in terms of induction and relaxation (1-2min), (2) qT, state transitions serve the optimal balancing of the energy distribution between PSII and PSI (5-10min), (3) qI, photoinhibition quenching is directly related to the photoinhibition of PSII under high light condition (hours) (Kress and Jahns 2017), their components are shown in Figure III (Marcello 2017). NPQ in green algae and plants is divided into three types described above, but the definition of NPQ is not similar due to the absence of qT-state transition (Goss and Lepetit 2015, Lavaud and Lepetit 2013). Furthermore, the pigments involved in the NPQ process are different. Diatoms utilize the reversible conversion of diadinoxanthin (Ddx) to diatoxanthin (Dtx) instead of the cycle of zeaxanthin to violaxanthin used by plants and most green algae (Figure IV) (Fernandez-Marin et al. 2021, Goss and Lepetit 2015, Lacour et al. 2018).

Although the composition of NPQ varies among different algae, it is generally believed that the major component of NPQ is qE under most light conditions (Papageorgiou and Govindjee 2014). And qE is controlled by the pH of the thylakoid lumen, thus allowing rapid and flexible regulation of energy dissipation in response to the saturation of photosynthetic electron transport (Lepetit et al. 2017). The activation of qE also requires a quenching site, Psbs, an intrinsic Chl a/b binding protein of PSII complex, supposed to be one of the quenching sites of qE formation in higher plants (Goss and Lepetit 2015). Interestingly, some authors have recently shown that the green algal lineage lacked the qE component of NPQ since the induction of NPQ is independent, which is neither dependent on the formation of pH, nor regulated by XC activity (Christa et al. 2017), showing that not all photosynthetic lineages contain NPQ. Moreover, the qE and qT are quite different between diatoms and green algae, and the mechanism of qE is far to be clear in

diatoms (Matuszynska and Ebenhoeh 2015). On the other hand, when the first two components of NPQ (qE and qT) are not sufficient to protect PSII under high light conditions in higher plants, the degradation of the Dl protein of damaged PSII RCs serves as a signal to trigger the qI component of NPQ (Malnoë 2018). The recovery of qI normally needs several hours since it requires the de novo synthesis of the D1 protein, and it really depends on the extent of the high light stress and species (Malnoë 2018). Furthermore, the qI mechanism of the eukaryotic algae, such as green algae and diatom, is assumed to be the same as higher plants.

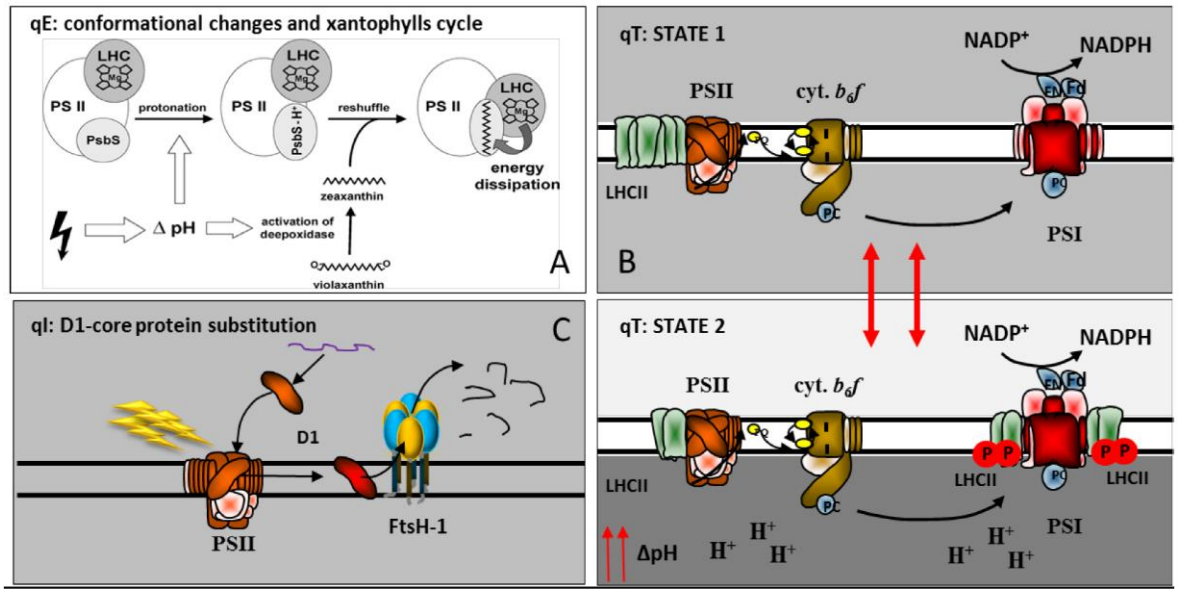

Figure 0. 3. " Non Photochemical Quenching. A) qE component, heat dissipation triggered by Chl-b and performed by carotenoids and conformational changes in antenna. B) qT component, State transitions triggered by ΔpH-dependent phosphorylation of LHCII. C) qI component, degradation of damaged D1-core protein and parallel co-translational substitution ". From (Marcello 2017).

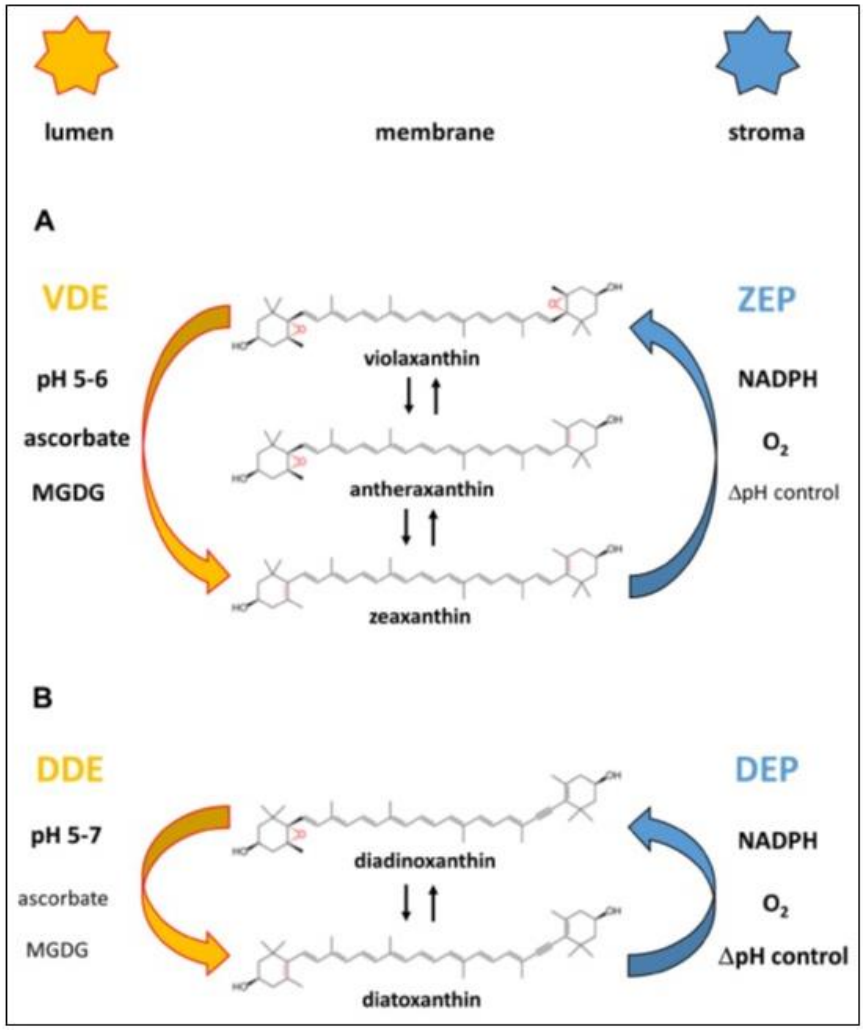

Figure 0. 4. " 'Classical model' of the violaxanthin-VX (A) and diadinoxanthin-DD (B) cycles. The violaxanthin cycle is present in higher plants and green and brown algae, the diadinoxanthin cycle is found in diatoms, haptophytes and dinophytes. The establishment of a proton gradient inhibits diatoxanthin epoxidation (high ΔpH control) and is thus presented in bold type whereas zeaxanthin epoxidation is unaffected by the presence of the transmembrane ΔpH (ΔpH control depicted in normal type). The pH value of the thylakoid lumen which leads to VDE and DDE activation (possibly by VDE or DDE dimerization) and membrane binding is also indicated for the two xanthophyll cycles. MGDG, monogalactosyl diacyl glycerol; VDE, violaxanthin de-epoxidase; ZE, zeaxanthin epoxidase; DDE, diadinoxanthinde-epoxdiase; DEP, diatoxanthin epoxidase " From (Goss and Latowski 2020).

2) Antioxidant system

Reactive oxygen species (ROS), including hydroxyl radicals ($OH^{\bullet}$), superoxide (O_2^{-}), and hydrogen peroxide (H_2O_2) are produced by the imbalance between cellular ROS production and elimination, and this balance may be disrupted under stressful conditions, resulting in a local increase in ROS concentrations, leading to oxidative stress which alters or inactivates biochemical activity (Mittler et al. 2004). Algae have two defensive strategies to deal with ROS before they can damage cellular different components. These are non-enzymatic (eg, carotenoids, α-tocopherols, ascorbic acid, and glutathione) and enzymatic (eg, ascorbate peroxidase, catalase, and superoxide dismutase) antioxidants that protect cells from various stress-induced oxidative stress (Figure V) (Rezayian et al. 2019). The second mechanism involves enzymes that repair and remove damaged macromolecules. The enzymatic antioxidants are essential for detoxifying the harmful effects of ROS (O_2^{-}, H_2O_2, $OH^{\bullet}$) produced during electron transport. However, the non-enzymatic antioxidants because of their precise targets are better at preventing ROS production by transferring the excitation energy ($^1O_2$).

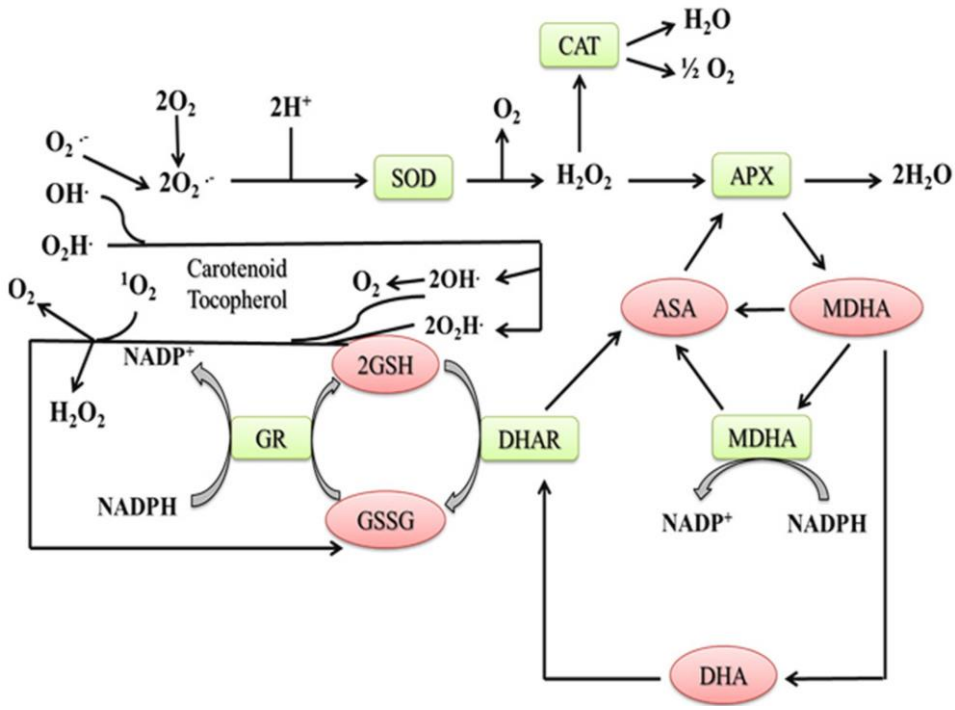

Figure 0. 5. " Enzymatic and non-enzymatic antioxidants in algae. ASC, Ascorbate; APX, Ascorbate peroxidase; CAT, Catalase; DHA, Dehydroascorbate; GSH, Glutathione; GR, Glutathione reductase; GSSG, glutathione disulfide; MDHA, Monodehy-droascorbate; SOD, Superoxide dismutase; DHA, Dehydroascorbate reductase ". From (Rezayian et al. 2019).

Pesticides

The pesticides used in this thesis were chosen based on those detected in Arctic waters from previous field sampling campaigns or based on previous estimates for these ecosystems (Hoferkamp et al., 2010; Weber et al., 2010; Vorkamp and Rigét, 2014). Four of the studied pesticides were chosen from the ones listed as research priorities by the NCAG (National Contaminants Advisory Group) of 2015-2016. Moreover, we also included other typical pesticides found in Arctic waters (chlorpyrifos, diazinon, endosulfan, and lindane) (Hoferkamp et al., 2010; Weber et al., 2010; Vorkamp and Rigét, 2014), and some (atrazine, simazine) found in surface and groundwater near agricultural lands across Québec (Giroux, 2015; Giroux, 2016), due to their potential long-range aerial transport up to the Arctic. We first selected eight pesticides to perform preliminary tests but focused on four pesticides atrazine, simazine, chlorpyrifos, and trifluralin for Chapter I since another four pesticides had no significant effect (5%-10%) on photosynthetic activity. Atrazine and simazine were chosen for Chapters II and III.

Pesticides can be divided into herbicides, insecticides, and fungicides according to their usage. Herbicides of atrazine (2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) and simazine (6-chloro-N, N′-diethyl-1,3,5-triazine-2,4-diamine) are part of the triazine family, which are widely used globally. They have resulted in environmental toxicity and contamination in aquatic ecosystems because of their toxic effect on non-target organisms (Bai et al. 2015). In 2017, atrazine was found in 98% of samples collected at four rivers situated in agricultural regions of Quebec province (Giroux 2019). In the Maumee river, the maximal atrazine concentrations have been detected up to 30 µg/L (0.14 µM), despite typical concentrations being between 0.1–10 µg/L (Sullivan et al. 2009). Furthermore, atrazine and simazine are well-known photosynthetic inhibitor

herbicides that block the photosynthetic electron transfer between PSII and PSI by binding to the Q_B site of D1 protein on PSII RC of photosynthetic organisms (Sun et al. 2020).

The herbicide trifluralin (α,α,α-trifluoro-2-6-dinitro-N-N-dipropyl-p-toluidine) has been used in agriculture since 1963 to control crops and ornamental plants. It has been banned in the European Union since 2008 due to its toxicity for farm workers (Coleman et al. 2020). Trifluralin has low water solubility and is dissipated by photodecomposition, volatilization, and biodegradation. Trifluralin has a high affinity for soil and is relatively immobile. In surface waters of Manitoba, the presence of trifluralin has been occasionally detected at low levels (less than 1 g/L), and its concentrations in streams near areas used herbicide range from 0 to 1.8 µg/L (D. 1992). One study showed that it can specifically bind tubulins in *Chlamydomonas reinhardii*, showing that its major mode of action is the inhibition of cell mitosis of algae and plants (Fernandes et al. 2013). However, the toxic effects of trifluralin are not only limited to this behavior and are not restricted to weedy plant species (Coleman et al. 2020).

The insecticide chlorpyrifos (O, O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate) is developed in the 1960s as a replacement for persistent organochlorine pesticides (Chen et al. 2016). It is increasingly found in aquatic ecosystems due to its intensive agricultural application. It binds to the active site of acetylcholinesterase by forming a stable covalent bond to inhibit the function of the nervous system in insects (Garrido et al. 2019). Furthermore, although chlorpyrifos is not designed to affect plants and algae, it was shown to be toxic to algae (Fernandez et al. 2021). Chlorpyrifos significantly decreased the population growth rate of *Dunaliella tertiolecta* at high concentrations (over 400 µg/L), although toxicity is doubtful at typical environmental concentrations (DeLorenzo and Serrano 2003). The structures of the four pesticides used in this study are shown in Figure VI.

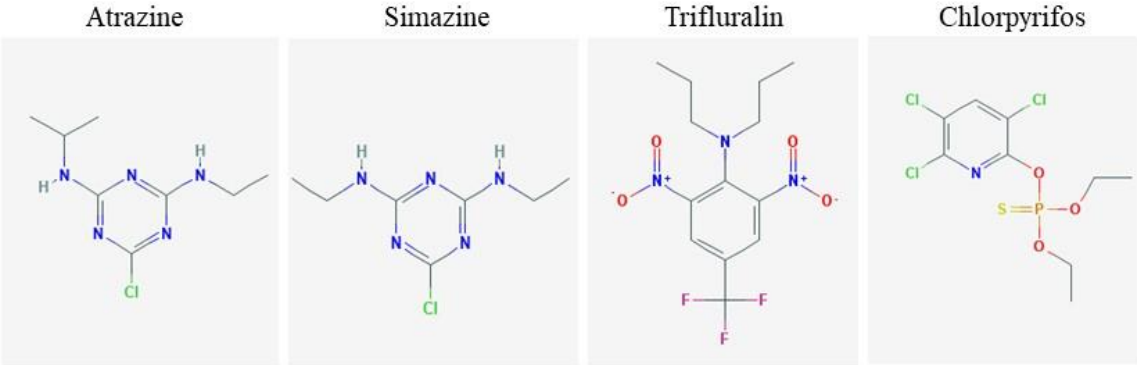

Figure 0. 6. The structures of the four pesticides used in this study: three herbicides (atrazine, simazine, trifluralin) and one insecticide (chlorpyrifos). From https://pubchem.ncbi.nlm.nih.gov/.

The effect of pesticides on algae

Very little is known about the ecophysiological features of Arctic phytoplankton and the potential impacts of pesticides on these organisms. In recent decades, most of our knowledge about ecophysiology and the effect of pesticides has come from temperate algae. In temperate waters, aquatic organisms are affected by various pesticides in the environment, usually impairing their physiology and growth (Hernandez et al. 2017, Singh et al. 2016, Wang et al. 2020), and phytoplankton is especially targeted by herbicides (Vonk and Kraak 2020). Herbicides are ordinarily most toxic to phototrophic organisms, displaying toxicity by inhibiting photosynthesis as with the herbicides atrazine, which inhibits carbon assimilation and affects photosynthetic light reactions (Mofeed and Mosleh 2013). It is reported that the maximal quantum yield of PSII (F_v/F_m) of the diatom *Synura petersenii* declined rapidly in response to atrazine, but thereafter remained stable with no effect on growth (Choi et al. 2012). However, in another diatom, *Navicula pelliculosa* the F_v/F_m over 80 min decreased in response to atrazine and growth was inhibited (Fai et al. 2007). The green algae, *Pseudokirchneriella subcapitata*, had similar half maximal inhibitory concentration (IC_{50}) of atrazine as did *Chlamydomonas reinhardtii* (Vallotton et al. 2008). These results clearly indicate that various species have different sensitivities to atrazine, which is attributed to the different protective mechanisms and the various culture conditions of the

experiment. On the other hand, atrazine and simazine inhibit the photosynthetic ETC from Q_A to Q_B by binding the Q_B site, resulting in a reduction of ATP and NADPH needed for carbon fixation and cell growth (Mofeed and Mosleh 2013). This inhibition of ETC leads to the over-excitation of PSII RC which induces a large amount of ROS formation. The continued accumulation of ROS induces cellular oxidative damage that degrades pigments, proteins, and lipids associated with the photosynthetic apparatus (Singh et al. 2016, Wang et al. 2020). Many studies have demonstrated that biochemical and physiological processes (such as NPQ-photosynthesis strategy and antioxidant system-ROS protection mechanisms) are modified in the presence of pesticides to decrease/avoid oxidative damage caused by ROS to the photosynthetic system (Morgan-Kiss and Dolhi, 2006; Lyon and Mock, 2014; Mock et al., 2017).

The protective measures of phytoplankton are mainly induced to avoid oxidative damage under stressful conditions resulting in the optimization of photosynthesis and growth. These protective mechanisms are mainly related to 1) non-photochemical quenching (NPQ) (Goss and Lepetit 2015), 2) antioxidant system (Medithi et al. 2021), and 3) photoprotective pigments (Car) (Kress and Jahns 2017, Kuczynska et al. 2015), NPQ is the most important strategy among them. In summary, algal species vary quitely in relation to their sensitivity to herbicides, and several factors may explain this species-specific sensitivity including cell size, photosynthetic ability, pigment composition, cellular lipid and protein content (DeLorenzox et al. 2004, Millie et al. 1992, Morgan-Kiss and Dolhi 2012, Singh et al. 2016). Furthermore, it has been discovered that the reactions of algal subcellular to herbicides are likewise class-dependent. In general, bacillariophytes are more tolerant than chlorophytes when comparing the toxicity of herbicides across phyla (Debenest et al. 2010, Singh et al. 2016). On the other hand, some pesticides (such as endosulfan) were proposed to affect respiration by blocking the mitochondrial ETC, resulting in the direct generation of ROS (Gomes and Juneau 2017, Gomes et al. 2017). In addition, even if respiration and photosynthesis are not the main targets of certain herbicides (such as glyphosate),

these compounds might also affect the metabolic pathways (Gomes and Juneau 2017, Gomes et al. 2014).

The impacts of herbicides on phytoplankton have been much more studied than that of insecticides, and some studies showed that insecticides also affect the growth of phytoplankton, photosynthesis, their biovolume, and their pigment and lipid contents (Asselborn et al. 2015, Fernandez et al. 2021, Yadav 2015). Yadav (2015) showed that for *Spirulina platensis* growth, pigment and protein content decreased in response to chlorpyrifos. Asselborn et al. (2015) observed that the biovolume and the lipid concentration of *Ankistrodesmus gracilis* increased after exposure to the chlorpyrifos. Chlorpyrifos also led to drastic changes in the ultrastructure of cells: alteration of the cell shape, the distribution of the crests in the cell wall, and increased size and number of starch granules (Asselborn et al., 2015). The toxic effects of pesticides on physiological processes in temperate algae such as pigment changes, photosynthesis, and growth are well documented. However, this lack of knowledge on the potential impacts of pesticides on Arctic phytoplankton has direct implications for the use of bioassays to detect water pollution in the Northern regions. Indeed, since the algal bioassays performed currently use temperate species, they might be not well suited to investigate contamination of Arctic waters.

The interaction of mixture pesticides on algae

A combination of pesticides may trigger synergistic (a greater effect than additive), antagonistic (a lower effect than additive) or additive (sum of individual pesticide toxicity) interactive effects on phytoplankton physiology depending on the stressors' mode of action (Crain et al. 2008). Although some chemical pairs have different interactions, antagonism is often observed when herbicides with different modes of action (MOA) are combined. Simultaneously, interaction types also vary depending on the study level (population: synergistic, community: antagonistic), and trophic level (heterotrophs: synergistic, autotrophs: antagonistic) (Crain et al. 2008). The MOA of the compounds, as well as their concentrations, exposure times, and assessed

endpoints, were found to influence the interaction effect also to depend on, and on the (Korkaric et al. 2015).

Next, we provide some background for understanding the terminology introduced. If we do not know the specific underlying mechanisms of pesticides, such as molecular action sites and how they affect physiological behavior, it is better to use less-than-additive, additive, and more-than-additive toxicity to describe the interaction effect instead of synergistic, antagonistic or additive (Van Genderen et al. 2015). These terms (less-than-additive, additive, and more-than-additive) can be used to depict the global effect induced by a mixture of pollutants found in the water even if the underlying modes of action are not known.

The effect of environmental factors (light) on algae

The growth conditions of aquatic photosynthetic organisms are expected to be affected by climate change that alters the physical and chemical parameters of the environment, including precipitation, temperature, and incident light properties (Finkel et al., 2010). In aquatic ecosystems, light intensity is one of the critical factors of phytoplankton photosynthesis, which is both the driving force and the influencing factor of photosynthesis (Agarwal et al. 2019, Gomes and Juneau 2017). Phytoplankton experience light fluctuations due to disturbances in water flow and water turbidity across seasons and latitude, where photon flux may be plentiful at the surface, or sparse in the deep water column (Dubinsky and Stambler 2009, Edwards et al. 2015). Photoinhibition may be induced if the light intensity is too high, which will produce ROS accumulation, and finally decrease the photosynthetic activity and growth (Waring et al., 2010; McGinty et al., 2012). On the other hand, if the light intensity is too low, that will reduce nutrient absorption and pigment content, as well as photosynthetic activity, ultimately leading to slow cell growth or even death (Lepetit et al. 2013, Virtanen et al. 2021). Therefore, with changes in the light intensity, photosynthesis and the growth of algae are affected, which may result in an alteration of algal distribution in aquatic ecosystems (Croteau et al. 2022). In addition, many studies reported that

light availability also affects photosystem ratio, pigment contents, electron transport way, lipid contents, and cellular metabolism except growth (Croteau et al. 2022, Edwards et al. 2015, Handler 2017).

To cope with the light fluctuation environment, photosynthetic organisms have evolved diverse phenotypic adjustments including photoadaptation processes (Deblois et al. 2013a, Handler 2017). Photoadaptation to low or high light environments involves the adjustments of their physiology, biochemistry, and morphology, and these responses occur at different time scales (Bellacicco et al. 2016). Physiological photoprotection mechanisms of photosynthetic organisms under light fluctuations include PSII and PSI electron recycling, changes in energy efficiency from the collection complex to RCs, NPQ induced by activation of the XC, state transitions, and repair cycle of the PSII D1 protein RCs (Deblois et al. 2013a, Dong et al. 2016, Lacour et al. 2019). Among them, NPQ is the fastest and most flexible response toward light change (Goss and Lepetit 2015). Morphologically, high light can modify the size of the cells, which may influence their uptake of pollutants (Finkel et al., 2010). In addition, small cells are more susceptible to photoinhibition (Falkowski and Raven 2013).

The combined effect of pesticides and light on algae

Several environmental factors can affect the toxicity of pesticides to phytoplankton, such as temperature and light (Figure VII). For example, several studies have individually demonstrated the effect of light on the toxicity of the pesticide to freshwater algae (Baxter et al. 2016, Deblois et al. 2013a, Wood et al. 2016). Atrazine and light are two environmental factors that can influence phytoplankton photosynthesis, and their interaction effect has been previously conducted (Deblois et al. 2013a, Gomes and Juneau 2017). The early study pointed out that the increased simazine-toxicity of *Anabaena circinalis* for high light photoadaptation was associated with decreased pigment contents (Millie et al. 1992). Another study showed a reduction in the toxicity of atrazine after adaptation to high light. This was mainly due to an increase in the plastoquinone pool under high light condition, which dilutes the binding site for atrazine (Deblois et al. 2013a). However,

the toxicity of atrazine was stronger in non-adapted phytoplankton in high light than in low light since protective strategies (NPQ) are not sufficient (Deblois et al. 2013a). Another study indicated that the interaction of light and herbicide effects had no significant impact at the community level (Wood et al. 2016). On the other hand, changes in light intensity can affect the cell size, which can influence the absorption of pesticides; smaller cells have a relatively high surface to volume ratio with which to enter into contact with the pesticide molecule, resulting in the high uptake of pesticides (Gomes and Juneau 2017, Weiner et al. 2004). All these alterations would lead to modifications in photosynthetic electron transport activity and other processes associated with photosynthesis. Therefore, the presentence of crucial interactions between light and the toxicity of herbicides, and the tolerance to herbicides of photosynthetic aquatic organisms is related to their thermal energy dissipation capacities (NPQ) and their pigment contents. Overall, light fluctuations in the environment appear to have primarily antagonistic or synergistic effects with herbicides on phytoplankton physiology.

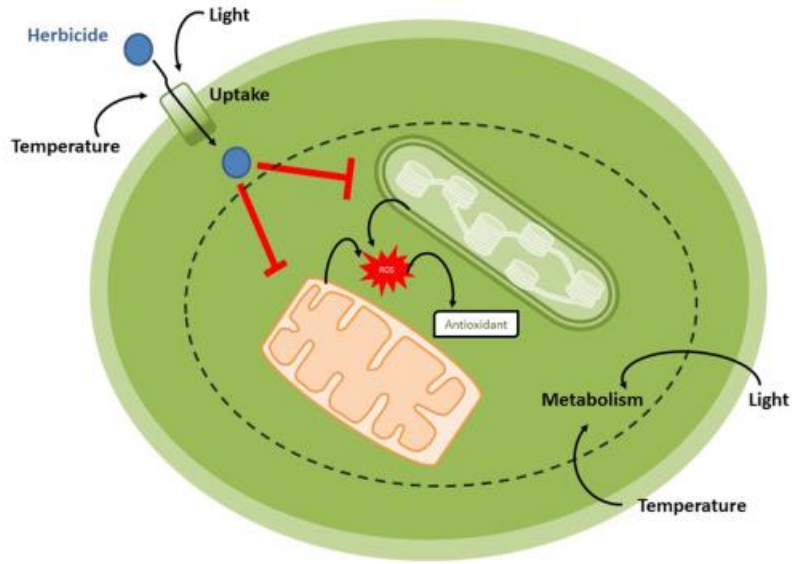

Figure 0. 7. " Changes in temperature and light conditions in water systems over the next century will drive physiological responses of algal and cyanobacterial communities to herbicides, by antagonistic, additive, or synergistic effects with pollutants ". From (Gomes and Juneau 2017).

Thesis objectives

Phytoplankton play a critical role in the Arctic food web and global primary production. However, very little is known about the ecophysiological characteristic and potential impacts of climate change and pesticides on Arctic phytoplankton; even pesticides are found in the Arctic Ocean. This lack of knowledge on the potential impacts of pesticides on Arctic phytoplankton has a direct implication for the use of bioassays to detect water pollution in Northern regions. Indeed, since the algal bioassays performed typically use temperate species, they might be not well suited to investigate contamination in Arctic waters. According to these reasons, the following objectives were defined:

1) It is known that ecophysiological parameters or data of Arctic microalgae can be used for model building to evaluate the change in the community of the Arctic ecosystem. Therefore, we investigated the potential pesticide sensitivity of Arctic microalgae compared to their temperate counterparts in conjunction with their different ecophysiological characteristics with respect to pesticide sensitivity.

2) Algae experience light fluctuations in aquatic ecosystems due to daily sunlight and seasonal changes and the interaction between light intensity and pesticides can occur in water bodies. Therefore, we aimed to compare the response of Arctic and temperate microalgae photoadapted to different light intensities and exposed to herbicides.

3) Since pesticides never exist alone in polluted waters, we studied the effect of mixed pesticides on temperate microalgae and evaluated whether light intensity affects the interaction type (synergistic, antagonistic, and additive) of mixed pesticides in microalgae.

All three chapters are linked together to provide a better understanding of the response mechanisms of pesticides, light, and their combined effects on microalgae. The obtained results can provide basic information on the ecological characteristics of Arctic microalgae and how they respond to light fluctuations and provide data support for building models to evaluate the

phytoplankton biomass and the risk of pollutants in polar ecosystems. Meanwhile, it also provides an interesting trend of species dominance in natural seawater and freshwater ecosystems in the presence of studied pesticides (Rumschlag et al. 2020).

CHAPTER I

PESTICIDES RESPONSES OF ARCTIC AND TEMPERATE MICROALGAE DIFFER IN RELATION TO ECOPHYSIOLOGICAL CHARACTERISTICS

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1.1. Résumé

Les écosystèmes polaires jouent un rôle important dans la production primaire mondiale. Les microalgues ont des adaptations qui leur permettent de prospérer à des températures basses en permanence et à des variations extrêmes d'éclairement et de durée du jour. Leurs adaptations, conduisant à des caractéristiques écophysiologiques différentes par rapport aux espèces tempérées, pourraient également altérer leur sensibilité aux polluants comme les pesticides. Le but de cette étude était de comprendre comment différentes caractéristiques écophysiologiques influencent la réponse du phytoplancton arctique aux pesticides par rapport aux réponses de leurs homologues tempérés. Les paramètres écophysiologiques étaient liés à la croissance, au biovolume cellulaire, à la teneur en pigments, à l'activité photosynthétique, aux mécanismes photoprotecteurs (NPQ, activités enzymatiques antioxydantes) et à la teneur en espèces réactives de l'oxygène (ROS). L'espèce arctique (*Micromonas polaris*) était plus résistante à l'atrazine et à la simazine que son homologue tempérée (*Micromonas bravo*). Cependant, l'autre espèce arctique (*Chaetoceros neogracilis*) était plus sensible à ces herbicides que son homologue tempérée (*Chaetoceros neogracile*). En ce qui concerne la toxicité de deux autres pesticides, les deux microalgues tempérées étaient plus sensibles à la trifluraline, tandis que les microalgues arctiques étaient plus sensibles au chlorpyrifos (insecticide). Toutes les différences pourraient être attribuées à des différences dans les caractéristiques éco-physiologiques des deux groupes de microalgues, qui peuvent être expliquées par la taille des cellules, la teneur en pigment, la teneur en ROS et les mécanismes de protection (NPQ et enzyme antioxydante).

Mots clés : microalgue, *Micromonas*, *Chaetoceros*, écophysiologie, photosynthèse, pesticides, mécanisme de toxicité.

1.2. Abstract

Polar ecosystems play an important role in global primary production. Microalgae have adaptations that enable them to live in low temperature environments where irradiance and day length change drastically. Their adaptations, leading to different ecophysiological characteristics relative to temperate species, could also alter their sensitivity to pollutants such as pesticides. This study's objective was to understand how different ecophysiological characteristics influence the response of Arctic phytoplankton to pesticides in relation to the responses of their temperate counterparts. Ecophysiological endpoints were related to growth, cell biovolume, pigment content, photosynthetic activity, photoprotective mechanisms (NPQ, antioxidant enzyme activities), and reactive oxygen species (ROS) content. The Arctic species (*Micromonas polaris*) was more resistant to atrazine and simazine than its temperate counterpart (*Micromonas bravo*). However, the other Arctic species (*Chaetoceros neogracilis*) was more sensitive to these herbicides than its temperate counterpart (*Chaetoceros neogracile*). With respect to two toxicity of the two other pesticides, both temperate microalgae were more sensitive to trifluralin, while Arctic microalgae were more sensitive to chlorpyrifos (insecticide). All differences could be ascribed to differences in the ecophysiological features of the two microalgal groups, which can be explained by cell size, pigment content, ROS content and protective mechanisms (NPQ and antioxidant enzymes).

Keywords: microalgae, *Micromonas*, *Chaetoceros*, ecophysiology, photosynthesis, pesticides, toxicity mechanism

1.3. Introduction

Arctic habitats are subjected to some of the most extreme environmental conditions on earth. Nevertheless, they provide a major contribution to global primary production and the Arctic Ocean net primary production has increased recently (Ardyna and Arrigo 2020). More than 75% of the Arctic phytoplankton biomass is composed of diatoms and small flagellate prasinophytes (*Micromonas* sp.) (Balzano et al. 2012, Lovejoy et al. 2007), and they thus play an essential role in the Arctic food web (Frey et al. 2018). Microalgae in polar regions have adaptations that enable them to grow well in these regions where temperatures are permanently low, but irradiance and day length are extremely variable (Handler 2017). Microalgae are impacted by cold environments in many ways, including lower enzyme activity (Wiebe et al. 1992), altered membrane fluidity (White et al. 2000), nutrient availability, balancing the usage and absorption of energy (Parker and Armbrust 2005), and the capacity to grow (Margesin 2007). There are differences in taxonomy, genetics, and ecology between Arctic microalgae and their temperate counterparts, while photo-physiology is not well documented (Lacour et al. 2017). Although there have been great developments in the taxonomy, genetics, and ecology of polar phytoplankton, their photo-physiological properties are not yet well documented, and the understanding of aquatic contaminant effects on their ecophysiology is lacking (Lyon and Mock 2014).

The application of pesticides leads to a substantial lost from fields leading to their detection in the aquatic environment, due to leaching, runoff and spray-drift (Larsbo et al. 2016, Zhang et al. 2018). Some authors have reported that Arctic waters are contaminated with pesticides applied in southern regions due to the long-distance aerial and marine transport of chemicals (Cabrerizo et al. 2019, Ma et al. 2018, Muir et al. 2013, Muir and de Wit 2010). Moreover, owing to the accumulation of pesticides in Arctic ice over many years, pesticide concentrations in Arctic waters should increase over time, as accumulated ice is melting at an unprecedented rate due to global warming (Pućko et al. 2017). Chlorpyrifos, diazinon, trifluralin, endosulfan and lindane are some of the typical pesticides found in surface and groundwater near agricultural lands across Canada

and USA (Vorkamp and Riget 2014), as well as in Arctic waters (Hoferkamp et al. 2010, Vorkamp and Riget 2014, Weber et al. 2010).

Among the main classes of pesticides, herbicides are the most widely used. Numerous herbicides have detrimental effects on photosynthesis due to cellular oxidative damage caused by a buildup of reactive oxygen species (ROS). This accumulation promotes lipid peroxidation, which results in the destruction of membranes, such as the photosynthetic ones (Chalifour et al. 2014, DeLorenzo 2001). Insecticides, although not designed to affect plants, have been demonstrated to have toxic effects on the growth, photosynthesis, biovolume, pigment and lipid contents of phytoplankton (Asselborn et al. 2015, Yadav 2015). It is well known that the sensitivity to pesticides of temperate algal species varies considerably, and several factors may contribute to this species-specificity. For example, damage to photosynthetic apparatus caused by pesticides can be minimized by various photoprotective mechanisms, including antioxidant system designed to eliminate the excess ROS (Medithi et al. 2021) and non-photochemical quenching (NPQ) energy dissipation processes related to the capacity to modulate light absorption and dissipate excess energy as heat (Moustakas et al. 2022). Furthermore, cells can also adjust pesticide uptake by modifying their surface to biovolume ratio (Larras et al. 2013, Tang et al. 1998).

Most of our understanding about the physiological characteristics and pesticide effects on microalgae is from temperate phytoplankton species. Indeed, the physiological features and potential impacts of pesticides on Arctic phytoplankton are very scarce (Kottuparambil et al. 2017). However, some have predicted that Arctic ecosystems and their organisms are likely more sensitive to contaminants than those at temperate latitudes (CARC 1990, Kottuparambil et al. 2017). In that case, we would expect that the photo-physiological characteristics and adaptations of Arctic microalgae have evolved in response to their greater potential sensitivity to pesticides. Although there have been many reports on the impacts of pesticides on the physiology and growth of temperate phytoplankton (Singh et al. 2016, Vonk and Kraak 2020), very little is known about their potential impacts on microalgae having extremely low temperatures and rapid environmental change due to global warming such as for Arctic phytoplankton. Moreover, the comparison of the

tolerance mechanisms and ecophysiological characteristics between the phytoplankton of the Arctic with temperate regions is lacking. We thus compared pesticide responses in Arctic microalgae and their temperate counterparts to four main pesticides (atrazine, simazine, trifluralin and chlorpyrifos). We examined which ecophysiological strategies might benefit to Arctic organisms by exploring differences in their sensitivities and response mechanisms. In our study, we found that the Arctic and temperate species have distinct physiological characteristics, which largely determine their different sensitivities to pesticides.

1.4. Materials and methods

1.4.1 Microalgal species and growth conditions

We compared the responses in two temperate species: *Chaetoceros neogracile* (T-CN; CCMP1425), *Micromonas bravo* (T-MB; CCMP1646), and two Arctic species strains *Micromonas polaris* (A-MP; CCMP2099), *Chaetoceros neogracilis* (A-CN; RCC2279). The first three species were purchased from National Contract Management Association (NCMA), while the latter Arctic strain was obtained from the Roscoff culture collections. All species were cultivated in marine L1 medium (Guillard et al., 1993) with a total volume of 100 mL medium in species-specific 250 mL Erlenmeyer flasks. The cultures were grown at 100 μmol photons m^{-2} s^{-1} under a 14:10 h light: dark illumination cycle with daily gentle shaking. Temperate and Arctic species were grown at 18 °C and 4 °C respectively. Algal cells were periodically transferred into fresh medium to maintain their exponential growth phase. The cultures were incubated for at least eight generations under their specific growth conditions. The cell concentrations were measured with a Multisizer 3 Coulter Counter particle analyzer (Beckman Coulter Inc., USA). The growth rate (μ) was determined as follow: $\mu = (\ln N_n) - (\ln N_0)/T$, where μ= Average specific growth rate, N0, Nn indicate cell density (cells/mL) at the beginning of test and the end of the treatment (3 days), T expresses the exposure time (3 days).

1.4.2 Pesticide preparation and treatment

All pesticides (Table 1.1) used in the present study were obtained from Sigma-Aldrich (PESTANAL®, analytical standard, Canada). Pesticides (stock solutions in pure acetone, ≥ 99%) were added to the growth media for the exposure experiments at final acetone concentration never exceeding 0.01% and no measurable effect on the parameters assayed was observed. From the original eight pesticides, the impacts of four of them were further investigated (atrazine, simazine, trifluralin and chlorpyrifos), since the other four pesticides showed no toxicity (clopyralid, metolachlor, endosulfan and lindane) or very little (< 10%) toxicity (endosulfan) on the photosynthetic activity of Arctic and temperate microalgae (data not shown). Concentrations of atrazine and simazine used were 0, 5 µg/L, 25 µg/L, 50 µg/L, 100 µg/L and 250 µg/L; while for trifluralin and chlorpyrifos 0, 200 µg/L and 500 µg/L were used as lower concentrations did not affect any of the tested species. Microalgae were harvested during their exponential growth phase and transferred into 1L Erlenmeyer flasks at a cell density of 2.5×10^5 (*Chaetoceros*) and 2.5×10^6 (*Micromonas*) cells mL^{-1} respectively, and then exposed to different concentrations of pesticides for 72 h. All treatments were done in triplicate. Cell densities and cell biovolumes were assessed at the beginning and the end of the experiment with a particle counter (Multisizer 3 Coulter Counter, Beckman Coulter Inc., USA).

Table 1. 1. The chemical families and mode of action for pesticides used in this study (adapted from www.irac-online.org).

Class	Substance	Chemical family	Mode of action
Herbicide	Atrazine	Triazine	inhibition of photosynthesis at photosystem II
	Simazine	Triazine	inhibition of photosynthesis at photosystem II
	Trifluralin	Dinitroaniline	inhibition of cell mitosis
	Clopyralid	Pyridinecarboxylic acid	inhibit cell division and growth
	Metolachlor	Chloroacetanilide	inhibition biosynthesis of chlorophyll, proteins, fatty acids and lipids
Insecticide	Chlorpyrifos	Organophosphate	inhibition Nervous System (acetylcholine esterase (AChE))
	Lindane	Organochlorine	inhibition Nervous System (GABA receptor)
	Endosulfan	Organochlorine	inhibition Nervous System (GABA receptor)

1.4.3 Pigment measurements

Algal cultures (25 mL) were harvested, under dim green light, 72 h after the beginning of the treatments by gentle filtration on 0.8 µm filter membrane (Polytetrafluoroethylene; Xingya Purifying Materials Factory; Shanghai, China), and placed in 2 mL Eppendorf tubes covered with aluminum foil, then rapidly immersed into liquid nitrogen and kept at -80 °C until analysis. Pigments were extracted by adding 2 mL of acetone 90% to each sample overnight at -20 °C prior to analysis. Ultrasonic probe was used to break the cells (3 W/cm^2 for 20s; Sonic dismembrator Model 100, Fisher Scientific). The extracts were centrifuged at 4 °C for 10 min (10000×g) and the supernatant was kept for quantification of chlorophyll (Chl *a*) and carotenoid (Car). Using Cary 300 UV spectrophotometer (Varian, USA) each extract was scanned between 400–750nm. Independent triplicates were sampled for each culture. The contents of Chl *a* and carotenoids were calculated according to the equations cited in (Jeffrey and Humphrey 1975, Seely et al. 1972).

1.4.4 Fluorescence measurements

The photosynthetic light curves were obtained using a Water-PAM fluorometer (Water-PAM, Walz, Germany) according to Du et al. (2019), with saturation pulses (3000 µmol photons m^{-2} s^{-1}, 800ms) and 8 levels of actinic light intensities (0, 46, 105, 188, 276, 427, 635, 906, and 1207 µmol photons m^{-2} s^{-1}). The samples (3mL) were dark-acclimated for 20 minutes before measurements and all samples were measured at their incubation temperature (4 °C and 18 °C). The maximum (Φ_M) and operational (Φ'_M) PSII quantum yields and the non-photochemical quenching (NPQ) were determined from this light curve. Their evaluation was done using the following equations: $\Phi_M = (F_M – F_0)/F_M$ (Kitajima and Butler, 1975); $\Phi'_M = (F'_M – F_S)/F'_M$ (Genty et al., 1989); NPQ = $(F_M – F'_M)/F'_M$ (Bilger and Björkman,1990). The maximal electron transport rate (ETR_{max}), light saturation coefficient (E_k) and light efficiency use (α) was calculated according to Lacour et al. (2017).

The Plant Efficiency Analyzer (PEA, Hansatech, Instruments Ltd, UK) was used to determine the polyphasic rise in fluorescence transients. Transients were induced by a 2s red (maximal emission at 650 nm) light pulse with 3600 μmol photons m^{-2} s^{-1} (Strasser et al., 1995). The O-J-I-P curves of the microalgae were determined and functional parameters evaluating the PSII energy fluxes under environmental stresses were calculated. All parameter definitions are in the Supplementary Material (Table S1.1).

1.4.5 Reactive oxygen species (ROS) measurement

Intracellular ROS was determined by BD Accuri C6 flow cytometer (Biosciences, San Jose, CA, USA) using the fluorescent dye 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA) (Molecular probes, Eugene, OR, USA) as described in Stachowski-Haberkorn et al. (2013). To eliminate potential signal alterations brought on by pesticide effects on FL1 fluorescence, we presented the results as FL1 ratios (H2DCFDA-stained samples FL1 value divided by non-contaminated samples FL1 values.

1.4.6 Antioxidant enzyme activity measurements

After pesticide exposure (72h), microalgal cultures (50 mL) were centrifuged at 15000×g for 25 min at 4 °C, and the pellet was transferred to a 2 mL microtube covered with aluminum foil, after adding 1 mL extraction buffer and immediately immersed into liquid nitrogen, then each extract was kept at -80 °C until analysis. For each extracted sample enzyme activities were determined with Cary 300 UV spectrophotometer (Varian, USA). Cells were broken with the help of liquid nitrogen, grinding one time, and were then centrifuged at 15000×g for 25 min at 4 °C prior to analysis. Each sample was divided into three replicates for analyzing the superoxide dismutase (SOD) and catalase (CAT) according to (Vitoria et al., 2001) and (Rao et al. 1996) respectively.

1.4.7 Statistical analyses

JMP software 10.0 (SAS Institute Inc) was used for statistical evaluations. Data were verified for normality (Shapiro–Wilk test) and homogeneity (Bartlett test) and then statistically evaluated using either one or two-way analysis of variance (ANOVA). Interactions between pesticide concentrations and different species were considered in 2-way ANOVA. Contrast analysis (Tukey's HSD test) was used when there were significant differences in the response variables between treatments. The EC_{50} (concentration for a 50% of maximum effect) values for response variables (growth, Φ_M, and Φ'_M) were calculated from the nonlinear least-square fits by using the inverse of the regression curve (Juneau et al. 2001).

1.5. Results

1.5.1 Effects of pesticides exposure on cell growth and cell biovolume

1.5.1.1 Effects of atrazine and simazine

The presence of atrazine and simazine for 72h significantly inhibited the growth of all algal species (Tukey's HSD, $P < 0.05$), a growth inhibition that was further exacerbated at increased atrazine and simazine concentration (Fig. 1.1). The growth-EC_{50} for Arctic *C. neogracilis,* temperate *C. neogracile,* temperate *M. bravo* and Arctic *M. polaris* was 143, 86, 52 and 82 µg/L respectively for atrazine, and 166, 171, 69 and 111 µg/L for simazine (Table 1.2). Cellular biovolume of the temperate *C. neogracile* (150 μm^3) was intrinsically nearly three times that of Arctic *C. neogracilis* (50 μm^3), while the biovolume of temperate *M. bravo* was almost the same as Arctic *M. polaris*. The treatment of atrazine and simazine tend to increase the cell biovolumes of all species by 2-12%, although not significantly for all studied species/treatment (Tukey's HSD, $P > 0.05$, Fig. S1.3).

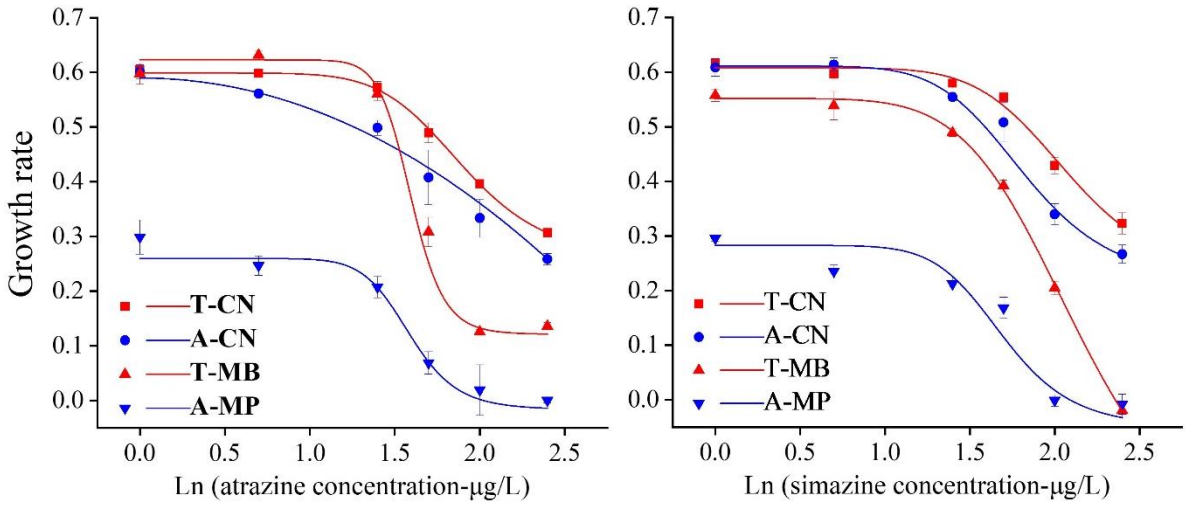

Figure 1. 1. The effects of atrazine and simazine on the growth rate of four species, including (red color) temperate *C. neogracile* (T-CN), temperate *M. bravo* (T-MB), (blue color) Arctic *C. neogracilis* (A-CN) and Arctic *M. polaris* (A-MP) after 72 h exposure. Data expressed as means ± SD (n = 6).

Table 1. 2. The EC_{50} of atrazine and simazine on the growth, the maximal PSII maximum quantum yield (Φ_M), the PSII operational quantum yield (Φ'_M). n.d.= not determined.

Species \ Pesticides	EC_{50}-Φ_M		EC_{50}-Φ'_M		EC_{50}-growth rate	
	Atrazine	Simazine	Atrazine	Simazine	Atrazine	Simazine
T-CN	n.d.	n.d.	66±1.4	142±6.7	275±10.6	306±39.2
A-CN	n.d.	4073±56.3	37±2.1	62±2.1	188±24.3	128±20.7
T-MB	104±5.6	46±1.5	31±2.3	49±1.8	55±3.3	68±2.7
A-MP	156±14.3	703±67.3	36±1.7	46±1.9	75±2,8	111±6.8

1.5.1.2 Effects of trifluralin and chlorpyrifos

The growth of Arctic microalgae (*C. neogracilis*, *M. polaris*) and temperate microalgae (*C. neogracile*, *M. bravo*) was drastically inhibited in the presence of chlorpyrifos at 200 µg/L and 500 µg/L, the exception was for *M. bravo* at 200 µg/L chlorpyrifos (Table S1.2, Tukey's HSD, $P < 0.05$). Overall, Arctic microalgae showed greater decline in their growths than their temperate counterparts in the presence of chlorpyrifos (Table S1.2). The chlorpyrifos treatment induced a significant increase (Tukey's HSD, $P < 0.05$) in the cell biovolume of all species. Overall, the biovolumes of Arctic microalgae increased by more than 100% compared to the temperate counterparts except for the Arctic *C. neogracilis* at 200 µg/L chlorpyrifos (Table S1.1).

The growth of all species was significantly decreased by trifluralin (Tukey's HSD, $P < 0.05$), but Arctic microalgae had smaller growth reductions than did their temperate counterparts in the presence of trifluralin, which is contrary to what was observed in the presence of chlorpyrifos. Cell biovolume of all species except for *M. bravo* increased in the presence of trifluralin (Table S1.1).

1.5.2 Effects of pesticides on pigment contents

1.5.2.1 Effects of atrazine and simazine

Pesticides had various impacts on pigment contents of the four studied microalgae after 72 h treatment. The cellular Chl *a* and Car contents of temperate and Arctic *Chaetoceros* did not show significant changes with increasing atrazine and simazine concentrations, with the exception of simazine at 250 µg/L (Tukey's HSD, $P > 0.05$, Fig. 1.2), resulting in an unchanged Car/Chl *a* ratio. For *M. bravo*, Chl *a* and Car increased slightly at low atrazine concentrations and then significantly decreased at higher concentrations (>100 µg/L, Tukey's HSD, $P < 0.05$, Fig. 1.2), while atrazine did not affect the pigment composition of *M. polaris*. Simazine treatments did not significantly change Chl *a* and Car contents after 72 h of exposure for the temperate and Arctic *Micromonas*

(Tukey's HSD, P >0.05). Under control conditions, Chl *a* for the temperate *Chaetoceros* and *Micromonas* were respectively almost 2.5 and 2.3 times the concentrations of the Arctic *Chaetoceros* and *Micromonas*, and carotenoids 1.5 and 1.2 times higher.

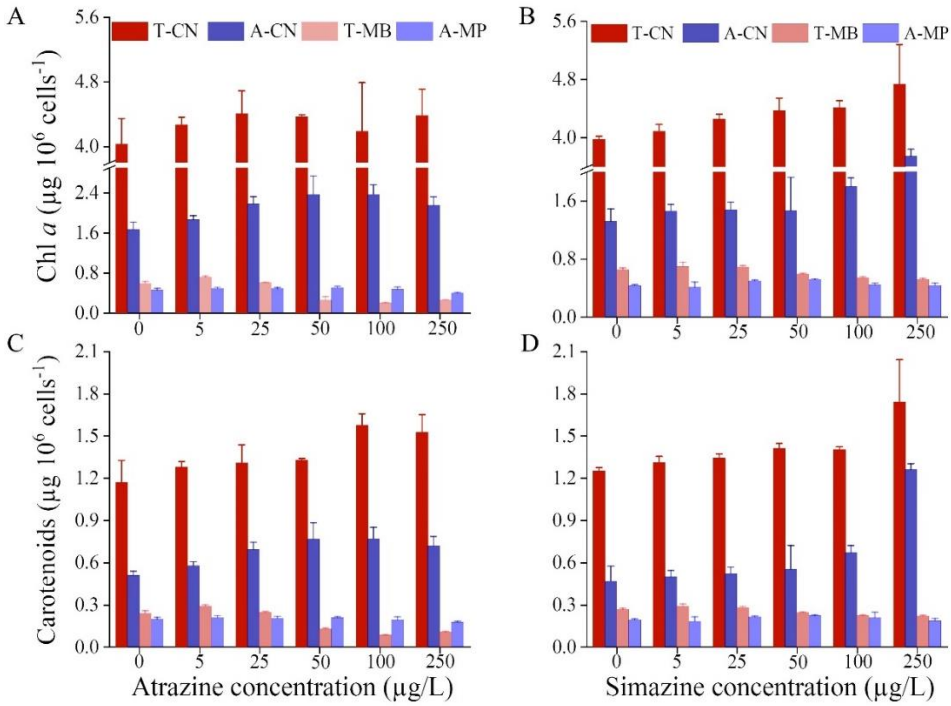

Figure 1. 2. The effects of atrazine and simazine on the pigment concentration of four species, including temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB) and Arctic *M. polaris* (A-MP) after 72 h exposure. Data expressed as means ± SD (n = 6).

1.5.2.2 Effects of trifluralin and chlorpyrifos

Chl *a* and Car contents of both temperate microalgae decreased in the presence of chlorpyrifos (Table S1.1). On the other hand, both Arctic species increased their contents in Chl *a* and Car except for *M. polaris* where the Car was unchanged. Car of both diatoms increased and Chl *a* increased for the lower concentrations of trifluralin, but decreased for the higher concentrations. In contrast, both prasinophytes decreased their Car contents, but Chl *a* content was not affected.

1.5.3 Effects of pesticides on PSII activity and the energy fluxes pathways

1.5.3.1 Effects of atrazine and simazine on PSII activity and the energy fluxes pathways

The maximum PSII quantum yields (Φ_M) of the diatoms (*C. neogracile* and *C. neogracilis*) were not affected by atrazine and simazine except at high concentrations (100 and 250 µg/L; Tukey's HSD, P >0.05) (Fig. 1.3). On the other hand, Φ_M of the two studied prasinophytes (*M. bravo* and *M. polaris*) were reduced (at variable extents) at any tested concentrations except 5 µg/L (Fig. 1.4). The operational PSII quantum yield (Φ'_M) significantly decreased (Tukey's HSD, P < 0.05) with increasing atrazine and simazine concentrations for all studied species (Fig. 1.3 and 1.4). Both Φ_M and Φ'_M of Arctic *C. neogracilis* decreased more than temperate *C. neogracile*. In contrast, Φ_M and Φ'_M of Arctic *M. polaris* decreased less than *M. bravo*. The EC_{50} of Φ'_M and Φ_M also confirmed this result, Φ'_M-EC_{50} of temperate *C. neogracile* and Arctic *C. neogracilis* are respectively 66.2, 36.7 and 142.2, 62.3 for atrazine and simazine; Φ'_M-EC_{50} of temperate *M. bravo* and Arctic *M. polaris* are respectively 30.7, 35.8 and 48.7, 45.8 for atrazine and simazine (Table 1.2). Therefore, similar trend was observed for Φ_M-EC_{50} and the Φ'_M-EC_{50}.

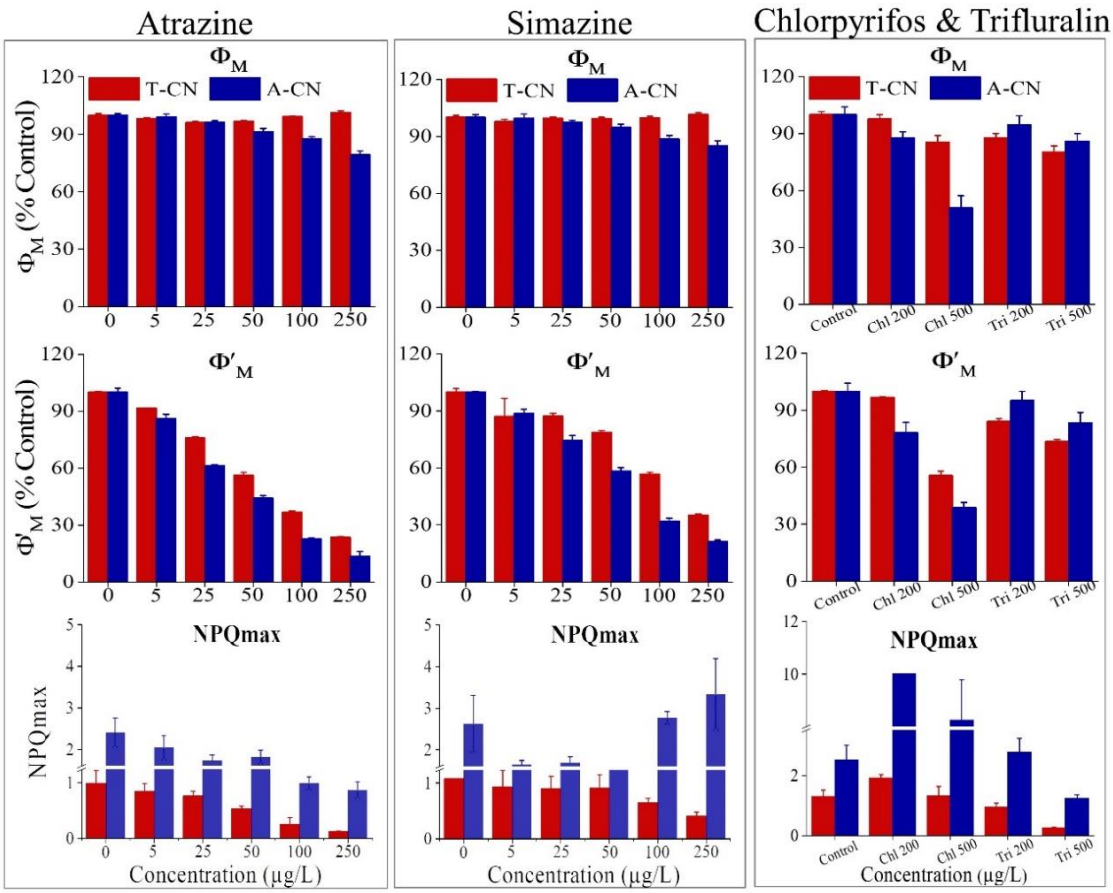

Figure 1. 3. The effects of atrazine, simazine, chlorpyrifos and trifluralin on the PSII maximal maximum quantum yield (Φ_M) and the PSII operational quantum yield (Φ'_M) of temperate *C. neogracile* (T-CN) and Arctic *C. neogracilis* (A-CN) after 72 h exposure. Data expressed as means ± SD (n = 6).

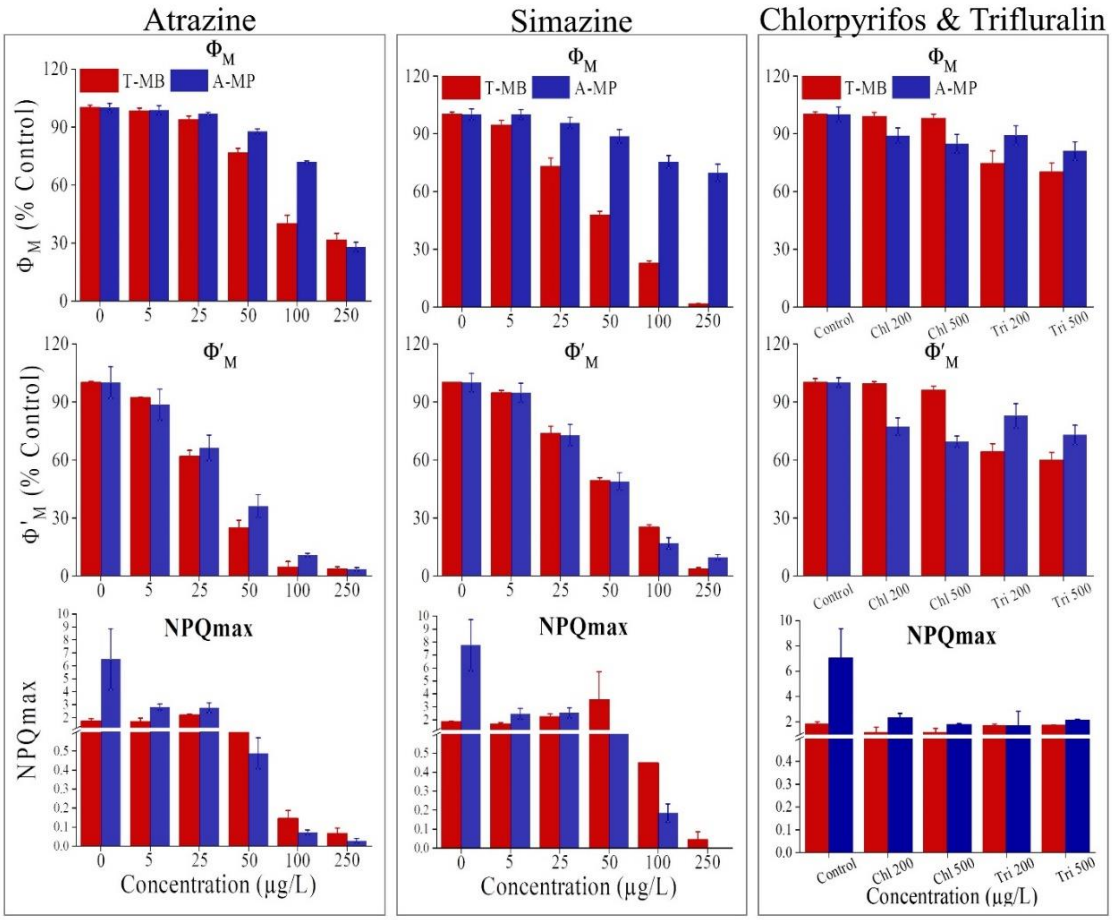

Figure 1. 4. The effects of atrazine, simazine, chlorpyrifos and trifluralin on the PSII maximal maximum quantum yield (Φ_M), the PSII operational quantum yield (Φ'_M) and non-photochemical quenching (NPQ) of temperate *M. bravo* (T-MB) and Arctic *M. polaris* (A-MP) after 72 h exposure. Data expressed as means ± SD (n = 6).

Table 1. 3. The effects of atrazine and simazine on Φ_M, Φ'_M, α, ETR_{max}, and E_k of temperate *C. neogracile* (T-CN), *M. bravo* (T-MB) and Arctic *C. neogracilis* (A-CN), Arctic *M. polaris* (A-MP) after 72 h exposure. Strains in the same column exposed to the different pesticide concentrations with different superscript letters (a-d) were significantly different (Tukey's HSD, $P < 0.05$). ND = not determined (indicating that the calculations were impeded by the strong effects of the treatments). Data expressed as means ± SD (n = 6).

Parameter	Species	T-CN	A-CN	T-MB	A-MP	T-CN	A-CN	T-MB	A-MP
	μg/L	Atrazine				Simazine			
Φ_M	0	0.65±0.00^{a}	0.58±0.00^{a}	0.65±0.01^{a}	0.62±0.04^{a}	0.66±0.01a^{b}	0.58±0.01^{a}	0.64±0.00^{a}	0.63±0.02^{a}
	5	0.63±0.00^{a}	0.58±0.01^{a}	0.63±0.00^{a}	0.61±0.00^{b}	0.64±0.01^{a}	0.58±0.00^{a}	0.64±0.00^{a}	0.63±0.00^{a}
	25	0.62±0.01^{b}	0.56±0.01^{a}	0.61±0.01^{a}	0.60±0.00^{b}	0.65±0.01^{ab}	0.57±0.00^{ab}	0.62±0.00^{b}	0.60±0.01^{a}
	50	0.63±0.01^{b}	0.53±0.01^{b}	0.50±0.02^{b}	0.54±0.00^{c}	0.65±0.01^{ab}	0.55±0.00^{b}	0.56±0.00^{c}	0.55±0.01^{b}
	100	0.64±0.00^{a}	0.51±0.00^{b}	0.26±0.03^{c}	0.44±0.01^{d}	0.65±0.01^{ab}	0.52±0.00^{c}	0.48±0.00^{d}	0.47±0.01^{c}
	250	0.66±0.00^{a}	0.46±0.01^{c}	0.21±0.02^{d}	0.17±0.02^{e}	0.67±0.00^{b}	0.50±0.01^{d}	0.21±0.01^{e}	0.42±0.01^{d}
Φ'_M	0	0.65±0.00^{a}	0.55±0.01^{a}	0.56±0.00^{a}	0.49±0.04^{a}	0.66±0.01^{a}	0.56±0.00^{a}	0.56±0.00^{a}	0.49±0.02^{a}
	5	0.59±0.00^{b}	0.48±0.01^{b}	0.52±0.01^{b}	0.43±0.00^{b}	0.57±0.06^{b}	0.50±0.01^{b}	0.53±0.01^{a}	0.46±0.01^{a}
	25	0.49±0.01^{c}	0.34±0.01^{c}	0.35±0.02^{c}	0.32±0.01^{c}	0.58±0.01^{b}	0.42±0.01^{c}	0.42±0.02^{b}	0.36±0.01^{b}
	50	0.36±0.01^{d}	0.25±0.01^{d}	0.14±0.02^{d}	0.18±0.01^{d}	0.52±0.01^{b}	0.33±0.01^{d}	0.27±0.01^{c}	0.24±0.01^{c}
	100	0.24±0.00^{e}	0.13±0.00^{e}	0.03±0.02^{e}	0.05±0.00^{e}	0.37±0.01^{c}	0.18±0.00^{e}	0.14±0.01^{d}	0.08±0.01^{d}
	250	0.15±0.00^{f}	0.08±0.01^{f}	0.02±0.01^{e}	0.02±0.01^{f}	0.23±0.00^{e}	0.12±0.01^{f}	0.02±0.01^{e}	0.05±0.01^{e}
a	0	0.68±0.01^{a}	0.55±0.02^{a}	0.60±0.02^{a}	0.52±0.05^{a}	0.69±0.01^{a}	0.53±0.01^{a}	0.59±0.02^{a}	0.52±0.05^{a}
	5	0.62±0.01^{b}	0.46±0.01^{b}	0.54±0.00^{a}	0.48±0.00^{b}	0.63±0.06^{a}	0.47±0.03^{b}	0.52±0.01^{b}	0.50±0.01^{a}
	25	0.51±0.02^{c}	0.33±0.01^{c}	0.35±0.02^{b}	0.34±0.02^{c}	0.60±0.01^{a}	0.41±0.01^{c}	0.43±0.02^{c}	0.37±0.01^{b}
	50	0.36±0.01^{d}	0.23±0.02^{d}	0.13±0.02^{c}	0.17±0.01^{d}	0.53±0.02^{b}	0.31±0.01^{d}	0.27±0.01^{d}	0.24±0.01^{c}
	100	0.22±0.01^{e}	0.11±0.00^{e}	ND	0.06±0.00^{e}	0.38±0.02^{c}	0.17±0.01^{e}	0.14±0.01^{e}	0.09±0.01^{d}
	250	0.11±0.01^{f}	0.10±0.01^{e}	ND	ND	0.20±0.00^{d}	0.11±0.01^{f}	ND	0.05±0.01^{d}
ETRmax	0	1337±439^{a}	1037±394^{a}	827±227^{a}	404±96^{a}	1366±540^{a}	1147±80^{a}	679±107^{a}	434±163^{a}
	5	1291±515^{a}	869±312^{a}	522±16^{a}	501±35^{a}	1481±833^{a}	801±332^{a}	507±10^{a}	394±40^{a}
	25	1601±804^{a}	708±35^{a}	490±249^{a}	325±70^{b}	1496±305^{a}	741±261^{a}	631±212^{a}	370±103^{a}
	50	1220±102^{a}	394±46^{b}	142±67^{b}	111±10^{c}	1550±491^{a}	595±199^{b}	368±117^{b}	234±24^{a}
	100	479±73^{b}	114±19^{bc}	2±1^{b}	28±4^{c}	938±256^{a}	219±17^{b}	131±20^{c}	44±6^{b}
	250	160±13^{b}	18±1^{c}	0±0^{b}	6±2^{C}	378±2^{b}	61±16^{c}	0±0^{c}	14±3^{b}
Ek	0	1972±663^{a}	1872±703^{a}	1389±422^{a}	764±116^{a}	1993±785^{a}	2151±128a^{b}	1141±154^{a}	820±229^{a}
	5	2065±783^{a}	1898±666^{a}	972±29^{a}	1040±70^{b}	2493±1065^{a}	1695±728a^{b}	978±1^{a}	796±66^{a}
	25	3129±1502^{a}	2138±194^{a}	1416±704^{a}	945±170^{c}	2490±550^{a}	1829±679a^{b}	1487±531^{a}	1003±258^{a}
	50	3350±183^{a}	1735±219^{a}	1036±356^{a}	654±30^{d}	2899±882^{a}	1943±657a^{b}	1347±385^{a}	980±63^{a}
	100	2185±390^{a}	1068±209^{ab}	ND	479±79^{e}	2510±790^{a}	1265±78b^{c}	974±129^{a}	512±98^{b}
	250	1476±68^{a}	184±29^{b}	ND	ND	1908±35^{a}	561±183^{c}	ND	320±104b^{c}
NPQmax	0	0.99±0.22^{a}	2.40±0.35^{a}	1.76±0.17^{a}	6.48±2.36^{a}	1.07±0.25^{a}	2.62±0.68^{a}	1.76±0.17^{a}	0.78±0.07^{a}
	5	0.84±0.14^{ab}	2.04±0.29^{a}	1.69±0.27^{a}	2.80±0.23^{b}	0.77±0.35^{a}	1.64±0.09^{a}	1.69±0.27^{a}	0.72±0.05^{b}
	25	0.76±0.09^{ab}	1.72±0.14^{a}	2.21±0.07^{a}	2.75±0.37^{bc}	0.90±0.22^{a}	1.68±0.14^{a}	2.21±0.07^{a}	0.92±0.05^{bc}
	50	0.53±0.05^{b}	1.81±0.17^{a}	0.83±0.54^{b}	0.49±0.08^{bcd}	0.90±0.24^{a}	1.32±0.18^{b}	0.83±0.54^{b}	0.70±0.08^{bd}
	100	0.25±0.12^{bc}	4.88±0.51^{b}	0.14±0.04^{c}	0.07±0.01^{e}	0.65±0.08^{b}	2.76±0.15^{c}	0.14±0.04^{c}	0.36±0.04^{dc}
	250	0.12±0.01^{d}	2.32±0.35^{c}	0.07±0.03^{c}	0.03±0.01^{e}	0.40±0.08^{c}	3.33±0.86^{c}	0.07±0.03c^{d}	0.22±0.00^{de}

Calculated parameters acquired from the rapid Chl *a* fluorescence kinetics provide useful indication on how pesticides may affect energy fluxes within PSII (Force et al., 2003). The electron transport rate per active reaction center (ET_0/RC) of all species significantly decreased under the treatment of atrazine and simazine by stopping electron flow between Q_A and Q_B (Fig. 1.5 and 1.6).

The energy conservation parameter of PI_{ABS} also declined under these treatment conditions, but to an extent that was species-dependent. We found that PI_{ABS} of the Arctic *C. neogracilis* was more affected than temperate *C. neogracile* (Fig. 1.5A-D); in contrast, the PI_{ABS} of the temperate *M. bravo* was more affected than in the Arctic *M. polaris* (Fig. 1.6A-D). The effective dissipation per reaction center (DI_0/RC) of the four species increased because of the high dissipation of the inactive RCs in the presence of atrazine and simazine. Similarly, the absorption flux per reaction center (ABS/RC), a proxy of the PSII antenna size, was increased by up to 202% in the presence of atrazine or simazine, except for the temperate *C. neogracile*. The DI_0/RC was increased in accordance with the change in ABS/RC in the presence of atrazine and simazine, and the effect of DI_0/RC on Arctic diatom *C. neogracilis* was stronger (4.5 and 1.6 times) than for the temperate *C. neogracile*; for the prasinophytes we observed the contrary (the effect on DI_0/RC of the temperate *M. bravo* was stronger than for *M. polaris*). The maximal rate at which excitons are trapped by the active reaction centers (TR_0/RC) was only altered by simazine for *M. bravo*.

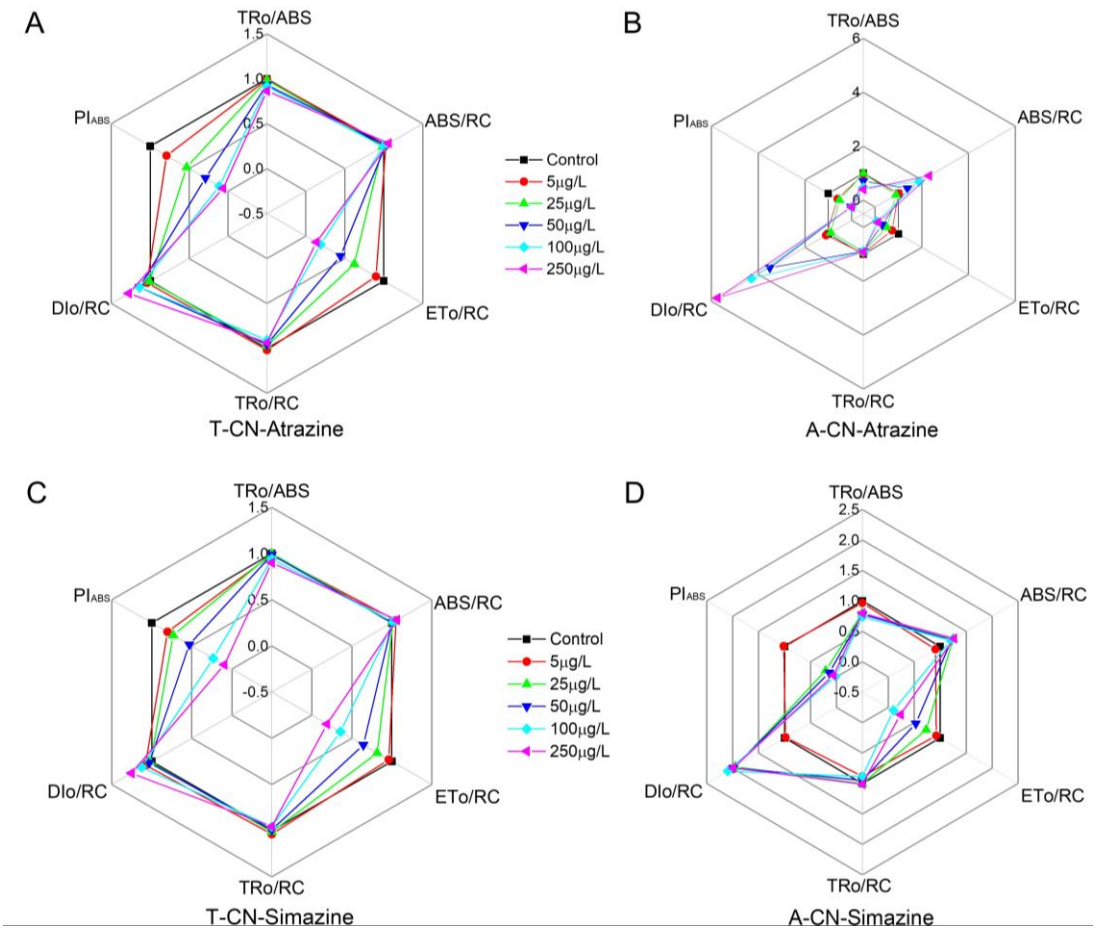

Figure 1. 5. The effects of atrazine and simazine on the chlorophyll fluorescence parameters of temperate *C. neogracile* (T-CN) and Arctic *C. neogracilis* (A-CN) after 72 h exposure.

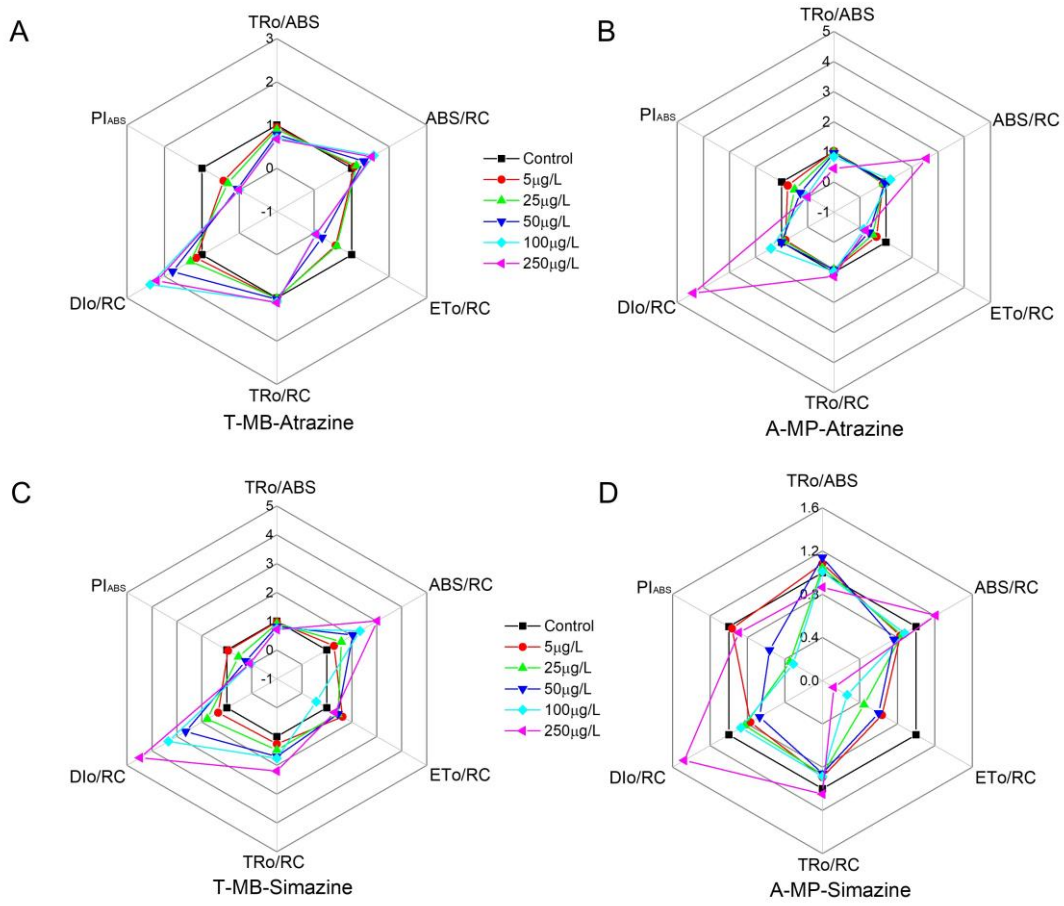

Figure 1. 6. The effects of atrazine and simazine on the chlorophyll fluorescence parameters of temperate *M. bravo* (T-MB) and Arctic *M. polaris* (A-MP) after 72 h exposure.

1.5.3.2 Effects of trifluralin and chlorpyrifos on PSII activity and the energy fluxes pathways

The Φ_M and Φ'_M of all species declined in the presence of high concentrations (200 and 500 µg/L) of chlorpyrifos and trifluralin (Fig. 1.3 and 1.4), but the declines differed between temperate (*C. neogracile* and *M. bravo*) and Arctic species (*C. neogracilis* and *M. polaris*). Indeed, both Φ_M and Φ'_M of Arctic *C. neogracilis* decreased more than temperate *C. neogracile* when exposed to chlorpyrifos, but this trend was reversed in the presence of trifluralin. For the prasinophytes, we observed similar effect where both Φ_M and Φ'_M of Arctic *M. polaris* decreased more in the presence of chlorpyrifos than ones of the temperate *M. bravo*. This trend was reversed in the presence of

trifluralin for Arctic *M. polaris* which declined less than did the temperate *M. bravo*. These results indicate greater sensitivity of Arctic species to chlorpyrifos than temperate counterparts and vice versa with trifluralin. In the presence of chlorpyrifos and trifluralin, ET_0/RC and PI_{ABS} of both *Chaetoceros* species decreased with increasing pesticide concentration concomitantly with increasing DI_0/RC (Fig. S1.1). In response to trifluralin, the Arctic and temperate *Micromonas* demonstrated opposite DI_0/RC and ABS/RC trends: these parameters increased for the temperate *M. bravo* and declined for the Arctic *M. polaris* (Fig. S1.1). Similarly, ET_0/RC and DI_0/RC responses in the two *Micromonas* species showed opposite responses to chlorpyrifos.

1.5.4 Effects of pesticides on reactive oxygen species, antioxidant enzyme activity and protein content

At the lowest concentrations of atrazine and simazine (5 and 25 µgL), the ROS content of all species was unchanged compared to control conditions (Tukey's HSD, $P>0.05$) (Fig. 1.7). However, the ROS content significantly increased (Tukey's HSD, $P<0.05$) at higher concentrations of pesticides (50, 100 and 250 µg/L). Despite similar trends, in the presence of atrazine, simazine and trifluralin, the ROS content of Arctic *C. neogracilis* increased more than for the temperate *C. neogracile*, while the opposite occurred in the presence of chlorpyrifos. We observed a less marked increase of ROS content in the presence of pesticides for the Arctic *M. polaris* compared to the temperate *M. bravo*. Moreover, under control conditions, the ROS content of Arctic microalgae was lower than its temperate counterparts (Table S1.1). The activity of SOD and CAT of all species strongly increased with atrazine, simazine and trifluralin concentrations (Fig. 1.8). Concomitantly, the protein concentration per cell significantly decreased in the presence of pesticides, indicating that pesticides induced oxidative stress and subsequent induction of mechanisms involved in the removal $O_2^{\cdot -}$ and H_2O_2. Surprisingly, for chlorpyrifos, the activity of SOD and CAT, and the total protein content of temperate *C. neogracile* and *M. bravo* decreased, suggesting that the antioxidant

enzyme system was insufficient to cope with the impact of these pesticides or at least was not the main protective measure.

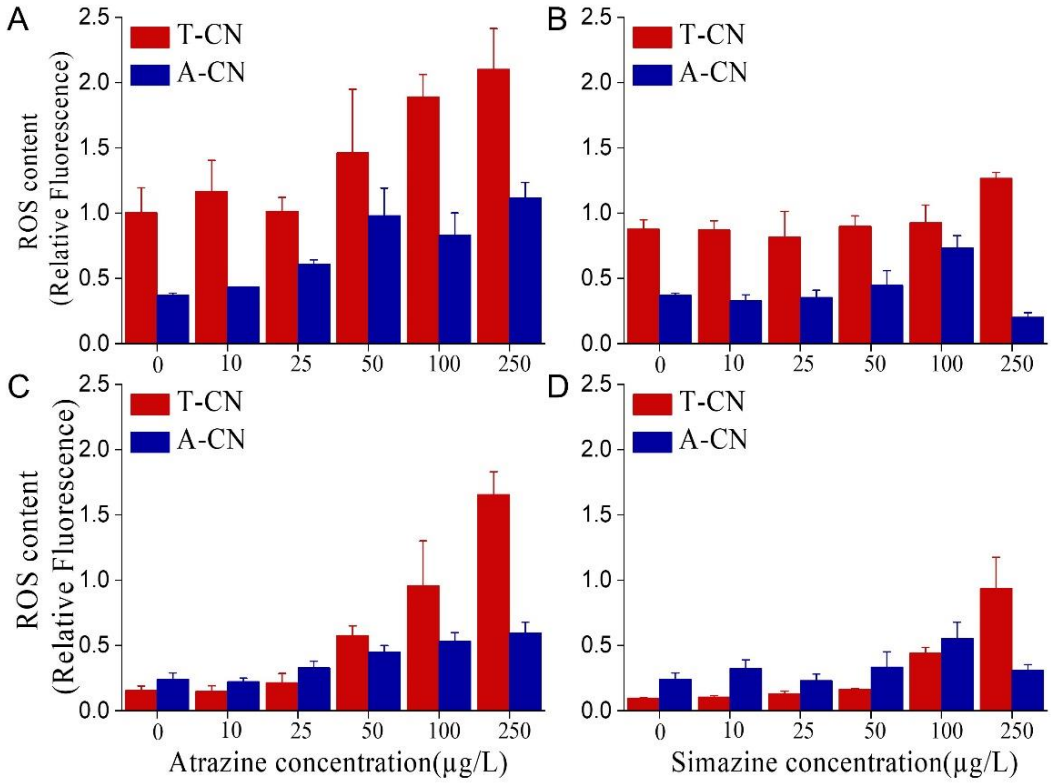

Figure 1. 7. The effects of atrazine and simazine on the reactive oxygen species (ROS) of four species, including temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN) for A and B, temperate *M. bravo* (T-MB) and Arctic *M. polaris* (A-MP) for C and D after 72 h exposure. Data expressed as means ± SD (n = 6).

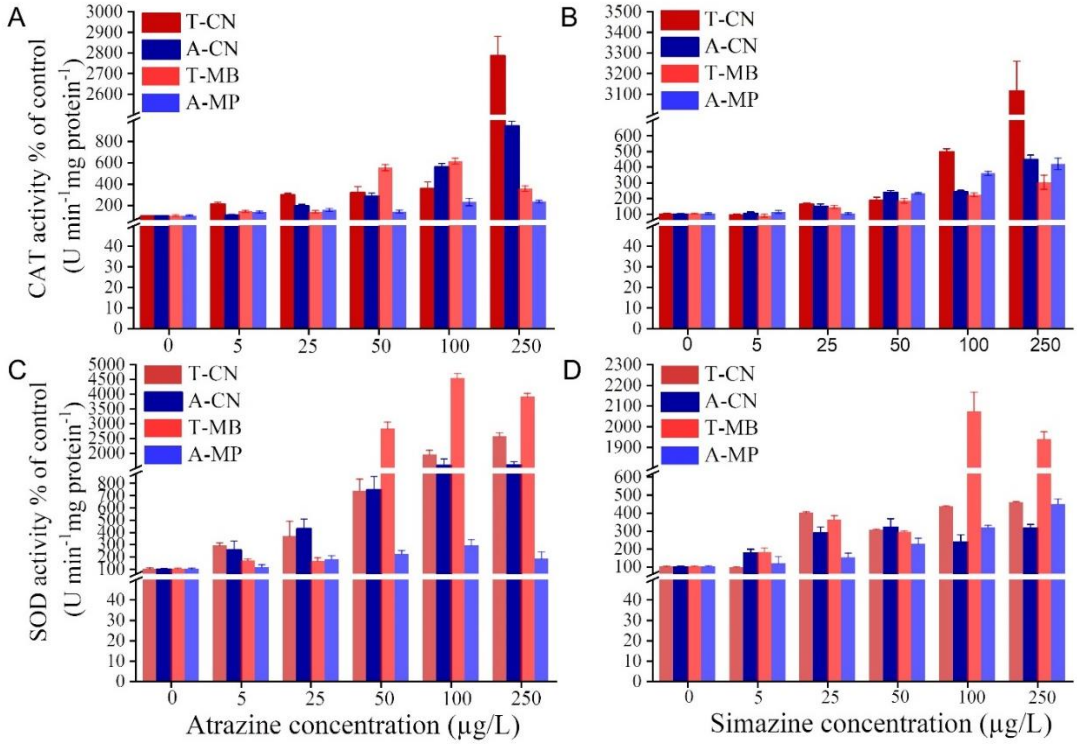

Figure 1. 8. The effects of atrazine and simazine on the catalase (CAT) and superoxide dismutase (SOD) of four species, including temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB) and Arctic *M. polaris* (A-MP) after 72 h exposure. Data expressed as means ± SD (n = 6).

1.6. Discussion

1.6.1 Different physiological characteristics between Arctic microalgae and their temperate counterparts

It is well accepted that at low temperatures Rubisco activity is the growth-limiting step (Young et al. 2015). Similarly, the cell biovolume of microalgae can be affected by growth temperature (Daufresne et al. 2009), and smaller cells have a larger surface/volume ratio compared to bigger cells, which facilitates better utilization of resources such as light and nutrients for growth

(Wirth et al. 2019). In our study, the temperate and Arctic prasinophytes have the same cell biovolume (5-8 μm^3), probably due to the fact that prasinophyte morphological and biochemical characteristics are highly conserved and may not be strongly modified by the long term growth conditions (McKie-Krisberg and Sanders 2014). On the other hand, the growth rate of temperate *M. bravo* is almost twice that of the Arctic *M. polaris*. This is consistent with previous studies indicating that growth rates of polar green algae are usually lower than for temperate one (Kottuparambil et al. 2017, Lacour et al. 2017). Thus, not surprisingly, Arctic *M. polaris* had lower ETR_{max}, E_k and α compared to temperate *M. bravo*, indicating that the utilization efficiency of light energy and photosynthetic capacity of Arctic *M. polaris* were weaker than those of temperate *M. bravo* (Table 1.3). This was also supported by the lower maximal and operational PSII quantum yields (Φ_M and Φ'_M) in *M. polaris* (Table 1.3). Interestingly, these differences were not found in the comparison between congeneric Arctic and temperate diatoms. Indeed, Arctic and temperate diatoms had similar growth rate, ETR_{max}, E_k and α (Table 1.3), indicating that Arctic *C. neogracilis*, while growing at a low temperature, has developed some strategies to improve the ability to harvest light energy and light utilization efficiency to optimize growth. However, these strategies did not prevent lower Φ_M and Φ'_M in Arctic *C. neogracilis* compared to temperate *C. neogracile* (Table 1.3), which is partly due to the presence of sustained NPQ (Lacour et al. 2018). Furthermore, Arctic *C. neogracilis* with a much lower cell biovolume compared to temperate *C. neogracile* can make better use of resources to benefit growth, which may partially explain the same growth rates of *C. neogracilis* and *C. neogracile*.

The growth rates of both diatoms were higher than for *Micromonas*, as previously shown in other species' comparisons (the diatom *Thalassiosira hyalina* with *Micromonas pusilla*) (Hoppe et al. 2018), which might due to the stronger ability of diatoms to increase the Rubisco gene expression when its activity is reduced at low temperature (Young et al. 2015). However, it is uncertain if prasinophytes also exhibit these acclimation responses (Hoppe et al. 2018). Lacour et al. (2017) observed that for a given Chl *a* content, polar diatoms grow more slowly than temperate

ones, suggesting that this difference is related to energy allocation. Interestingly, in our study, Arctic *C. neogracilis* and temperate *C. neogracile* had the same growth rate, but the Arctic diatom had a much lower Chl *a* content compared to its temperate counterpart. This indicates that different polar diatoms may have developed different adaptation strategies in concordance with their growth environment. Indeed, it has been demonstrated that diatoms have different inherent NPQ abilities in response to their respective habitats (Croteau et al. 2021, Croteau et al. 2022). Lower Chl *a* content in both Arctic microalgae helps reduce excitation pressure on the photosynthetic reaction center, particularly in situations constraining growth, such as cold environments (Halsey and Jones 2015). Recent studies have demonstrated that for the majority of polar microalgal groups, NPQ is an essential element of the species-specific photoadaptative strategies (Croteau et al. 2021, Galindo et al. 2017). Together with low Chl *a*, polar microalgae also can induce high NPQ through an efficient de-epoxidation process to jointly protect the photosynthetic apparatus against environmental stress such as sudden increase in light intensity and temperature modifications (Lacour et al. 2020, Ni et al. 2017). In comparison, both Arctic microalgae, having lower ROS content and high CAT and SOD activities, should be more likely to adapt and survive at low Arctic habitat temperatures (Blanc et 2012). In summary, Arctic species appear to have evolved different ecophysiological characteristics than their temperate counterparts: Arctic microalgae have (1) lower Chl *a* and carotenoid contents (2) a much higher intrinsic NPQ_{max}, (3) lower ROS content and (4) higher CAT and SOD activities, compared to temperate species.

1.6.2 Effects of pesticides on Arctic microalgae and their temperate counterparts

1.6.2.1 Effects of pesticides on diatoms (Arctic *Chaetoceros* neogracilis and temperate *Chaetoceros* neogracile)

PSII inhibiting herbicides (such as atrazine and simazine) that can bind to the Q_B site on the D1 protein of PSII, inhibit the PSII-PSI electron transport resulting in high excitation pressure on

PSII and ROS generation (Bai et al. 2015). If the photoprotective processes, NPQ and ROS scavenging system, are ineffective, reduced energy production and cellular damages occur, and ultimately algal growth might be reduced. Our study demonstrated that atrazine and simazine, at all tested concentrations, significantly inhibited the growth of both diatoms, as seen previously for *Navicula pelliculosa* (Chalifour and Juneau 2011). Our observations were linked to the significant reduction in Φ'_M and the electron transport rate per active RC (ET_0/RC). Concomitantly, dissipation of excess light energy (DI_0/RC) increased but was not sufficient to protect the PSII since Φ_M, a proxy of the PSII RC integrity, was affected, as previously shown when *Phaeodactylum tricornutum* was treated with PSII inhibiting herbicides (Debenest et al. 2010). This result is also supported by the significant decrease in PI_{ABS}, a sensitive indicator of plant health (Bayat et al. 2018) (Fig. 1.5A-D). The other unchanged PSII energy fluxes (ABS/RC and TR_0/RC) at low pesticide concentrations, together with the minor variations in the carotenoid contents, indicate that the protection of the photosynthetic electron transport chain from the ROS produced in the presence of atrazine and simazine, are likely due to other protective strategies (like antioxidant enzymatic systems). One could expect that, as the first line of defense against the excess of light energy under stressful conditions, NPQ would be activated (Kress and Jahns 2017, Müller et al. 2001). However, in our experiments, NPQ_{max} decreased with increasing atrazine and simazine concentrations (Fig. 1.3). As shown in previous studies, the decline of NPQ in the presence of pesticides is attributed to the low buildup of the proton gradient across the thylakoid membranes, since electron transport is decreased (Chalifour and Juneau 2011, Gomes and Juneau 2017). Therefore, we supposed that the inhibition of the ΔpH-dependant non-photochemical energy dissipation mechanism leads to a reduced ability to decrease excitation pressure at PSII RC, resulting in higher ROS production. Although SOD and CAT activities were significantly increased with increasing atrazine and simazine concentrations, it was not sufficient to cope entirely with the ROS production induced by increasing atrazine and simazine concentrations (Fig. 1.7).

Overall, as shown by the investigation of the physiological parameters in the presence of atrazine and simazine and the determined EC_{50} (Table 1.2), the Arctic *C. neogracilis* was more sensitive than the temperate *C. neogracile* to atrazine and simazine. We suspect that the more pronounced PSII RC inactivation of Arctic *C. neogracilis* results from its smaller cell biovolume, thus increasing overall contact with the pesticide molecules and resulting in an increased absorption of the contaminants (Weiner et al. 2004). However, the antioxidant enzyme system (SOD and CAT) induced in *C. neogracilis* was insufficient to cope with the ROS production in the presence of atrazine and simazine. Furthermore, as previously showed in psychrophilic diatoms, PSII repair rates are slower than the ones found in temperate diatoms (Petrou et al. 2010), since lower temperatures decreased enzyme activity and metabolism (Morgan-Kiss et al. 2006). On the contrary, compared to the Arctic diatom, the temperate diatom potentially showed lower absorption of atrazine and simazine, the capacity for an efficient antioxidant enzyme system and probably higher rate of PSII repair cycle, which ultimately lead to its lower sensitivity to these herbicides.

For trifluralin, interestingly, we observed that Arctic *C. neogracilis* was more tolerant than temperate *C. neogracile* according to the growth, and photosynthetic activity (Φ_M and Φ'_M; Fig. 1.3). According to our data (Table S1.1, Fig. S1.1), the greater decrease of PI_{ABS} and ABS/RC for *C. neogracile* compared to *C. neogracilis*, indicated that PSII RC was more damaged in *C. neogracile.* However, the Arctic diatom has more effective antioxidant capacity (SOD and CAT activities) than its temperate counterpart under the same concentration of trifluralin, indicating that its tolerance to trifluralin seems to mainly depend on the high efficiency of the antioxidant system. Although insecticides are not intended to affect plants and algae, chlorpyrifos has been shown to induce some deleterious impacts at the cellular and population levels (Asselborn et al. 2015), leading to the impairment of cell morphology and growth (Asselborn et al. 2006, Garrido et al. 2019), and the decrease in diversity of diatoms (Stratton, 1987). Similar effects were seen in our study, where chlorpyrifos not only affected the growth, and photosynthesis of both diatoms (Fig. 1.3; Arctic *C. neogracilis* was more affected than temperate *C. neogracile*), but also caused

oxidative stress. The observed difference in the sensitivity to chlorpyrifos of diatoms was mainly reflected at the electron transport level, where chlorpyrifos induced the accumulation of Q_A^- and prevented electron transfer downstream of Q_A (revealed by the more pronounced increase in O-J and J-I phases for the Arctic diatom, Fig. S1.2), also evidence in the significant decrease of Φ'_M, ETo/RC and PI_{ABS} for *C. neogracilis* (Fig. S1.1). In comparison, this impact on electron transport was accompanied by a weaker ability to dissipate excess energy (Dio/RC) in the Arctic diatom, resulting in its greater sensitivity to chlorpyrifos. In addition, some authors have suggested that insecticides disturb the cell cycle of *Selenastrum capricornutum* since they observed the inhibition of cell-separation, resulting in the intracellular accumulation of macromolecules, which are responsible for increasing the biovolume in *C. neogracilis* (Fernandez et al. 2021, Rioboo et al. 2002).

1.6.2.2 Effects of pesticides on prasinophytes (Arctic *Micromonas polaris* and temperate *Micromonas bravo*)

Prasinophytes responded to atrazine and simazine similarly to diatoms when growth, pigment composition and photosynthetic efficiency were evaluated. In contrast to diatoms, the Arctic *M. polaris* was more tolerant than temperate *M. bravo* to atrazine and simazine (Table 1.2 and Fig. 1.6). The more pronounced damage to the PSII RC (Φ_M), drastic inhibition of photosynthetic electron transport (Φ'_M and ETo/RC) and decreased light conversion efficiency (PI_{ABS}) at the PSII RC level in *M. bravo* eventually induced higher production of ROS, which further damaged these photosynthetic components. We propose several reasons why *M. polaris* was more tolerant to atrazine and simazine. First, *M. polaris* has lower Chl *a* content than *M. bravo* in the absence of pesticides, indicating that *M. polaris* may have lower PSII content and therefore fewer available molecular targets for atrazine and simazine (DeLorenzox et al. 2004). Previous studies have demonstrated that Arctic *Micromonas* have lower active PSII levels than their temperate counterparts (Ni et al. 2017). Concomitantly, the lower Chl *a* content of *M. polaris* helps to reduce

excitation pressure on the PSII RC caused by atrazine and simazine. Second, the temperate species produced more ROS in the presence of atrazine and simazine compared to the Arctic one, but the induction of CAT and SOD were insufficient to prevent oxidative damage. In comparison, the Arctic species produced lower levels of ROS and have relatively lower CAT and SOD activities compared to the temperate species, which implies that these enzymes can successfully scavenge ROS. ROS has to reach a threshold level to increase the activity of the antioxidant enzyme system (Anu et al. 2016), which may indicate why the temperate species were more affected by ROS production than the Arctic species in the presence of atrazine and simazine. Finally, *M. polaris* had higher intrinsic NPQ which was only slightly decreased in the presence of atrazine and simazine compared to *M. bravo* (Fig. 1.4), which would enhance the protection of PSII (Bai et al. 2015). In summary, the temperate *M. bravo* showed higher toxicity to atrazine and simazine than Arctic *M. polaris*, due to the non-effectiveness of its NPQ and antioxidant enzymes to cope with excess light energy and oxidative stress, resulting in photosynthetic damage. In comparison, Arctic *M. polaris* seems to mainly rely on NPQ rather than antioxidant enzymes under atrazine and simazine stress.

We observed that growth, photosynthetic efficiency, and electron transfer of the temperate *M. bravo* were more sensitive to the herbicide trifluralin than for the Arctic *M. polaris*. Furthermore, significantly increased DIo/RC, ABS/RC and decreased ETo/RC of *M. bravo* in the presence of trifluralin showed that the excess excitation energy caused by a certain number of inactivated RCs, was mostly dissipated. We propose that the different sensitivities of the prasinophytes to trifluralin are mainly determined by the antioxidant enzyme system, since the Arctic species induced five times higher SOD and CAT activities, even if it produced less ROS than its temperate counterpart (Table S1.1). The insecticide chlorpyrifos, in a way similar to what was observed for diatoms, inhibited the growth of *Micromonas* (Arctic *M. polaris* was more affected than temperate *M. bravo*) and doubled the cell biovolume of *M. polaris* (Table S1.1), for the same reasons that we proposed for diatoms. For *M. bravo*, chlorpyrifos did not affect the photosynthetic efficiency. Furthermore, the increased PI_{ABS} and ET_0/RC, and unchanged kinetics of Q_A-Q_B reduction compared to the

control (Fig. S1.1), indicate that PSII RCs and the whole photosynthetic electron transfer chain were protected under exposure to chlorpyrifos. By comparison, photosynthesis of *M. polaris* was strongly affected by this insecticide, as indicated by the strong reduction of the active PSII RC population and suppression of electron transfer between Q_A and Q_B (Fig. S1.2).

1.6.2.3 Comparative effect of pesticides on Arctic microalgae and their temperate counterparts

In our study, based on the EC_{50} value of Φ'_M and growth, the species sensitivity sequence was T-MB>A-MP>A-CN>T-CN in the presence of atrazine and simazine. For trifluralin, based on the impact on Φ_M and Φ'_M, the species sensitivity sequence was T-MB>A-MP>T-CN>A-CN and A-CN>T-CN>A-MP>T-MB for chlorpyrifos. We found that diatoms *Chaetoceros* were more tolerant to atrazine, simazine and trifluralin than the prasinophytes *Micromonas* for both temperate and Arctic strains. Diatoms are known to be dominant in most aquatic environments (Serôdio and Lavaud 2020), likely owing to their specific ecophysiological characteristics, like their high PSII/PSI ratio (Strzepek and Harrison 2004), their high non-photochemical energy dissipation potential (Lavaud and Lepetit 2013), and the presence of fucoxanthin that can prevent photooxidation (Tuchman et al. 2006). Furthermore, diatoms can more efficiently control the ATP/NADPH ratio during photosynthesis compared to other photosynthetic organisms, permitting them to optimize their carbon fixation and growth (Bailleul et al. 2015). Indeed, we found that Φ'_M, pigment, ETR_{max}, E_k and α in both diatoms were higher than in prasinophytes, suggesting that diatoms have higher intrinsic photosynthetic capacity and light utilization efficiency. Therefore, if we consider only the impact of pesticides, we propose that *Chaetoceros* would have a greater chance of survival and would become the dominant species in temperate and Arctic ecosystem contaminated with atrazine, simazine and trifluralin in relation to *Micromonas*. On the other hand, according to the EC_{50} of Φ'_M and growth (Table 1.2), atrazine induced significantly more damage than simazine to all tested algae even though both molecules have the same mode of action on the Q_B site of D1 protein of PSII. Previous studies have shown that Irgarol with high octanol/water

partition coefficient (logK_{ow}) is more toxic than diuron for *Chlamydomonas* as PSII inhibitor, and the higher logK_{ow} of Irgarol promotes its affinity for the Q_B binding site, leading to the relative higher toxicity (Kottuparambil et al. 2017). Therefore, we suggest that the higher toxicity of atrazine compared to simazine could be due to its higher logK_{ow} (Ronka 2016). Similarly, in the presence of atrazine, CAT and SOD activities were higher and the total protein content decreased more than in the presence of simazine, resulting in a decrease in the available protein for photoprotection processes, such as NPQ and the PSII repair cycle (Bai et al. 2015).

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1.7. Supplementary material

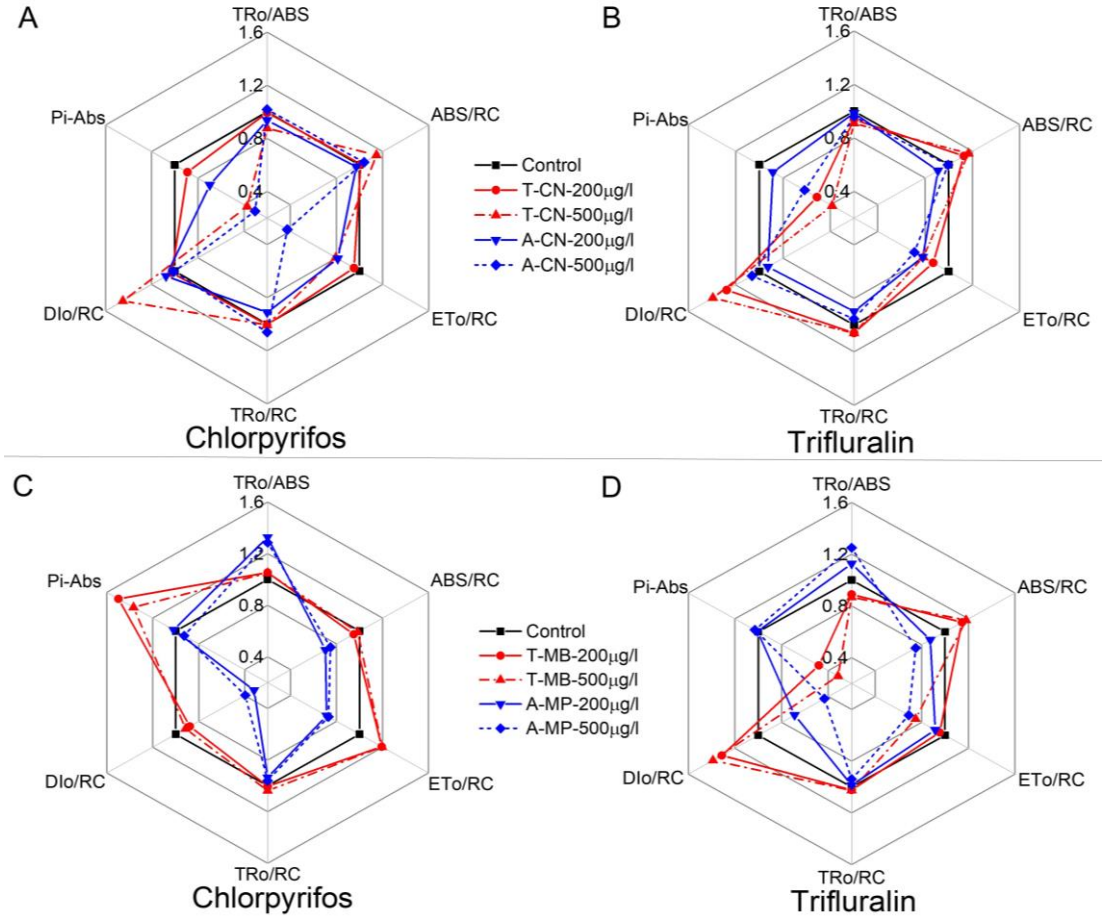

Figure S1. 1. The effects of chlorpyrifos and trifluralin on energy fluxes within PSII of four species, including temperate *C. neogracile* (T-CN), Arctic *C. neogracile* (A-CN) for A and B, temperate *M. bravo* (T-MB) and Arctic *M. polaris* (A-MP) for C and D af**ter 72 h exposure. Data expressed as means ± SD (n = 6).**

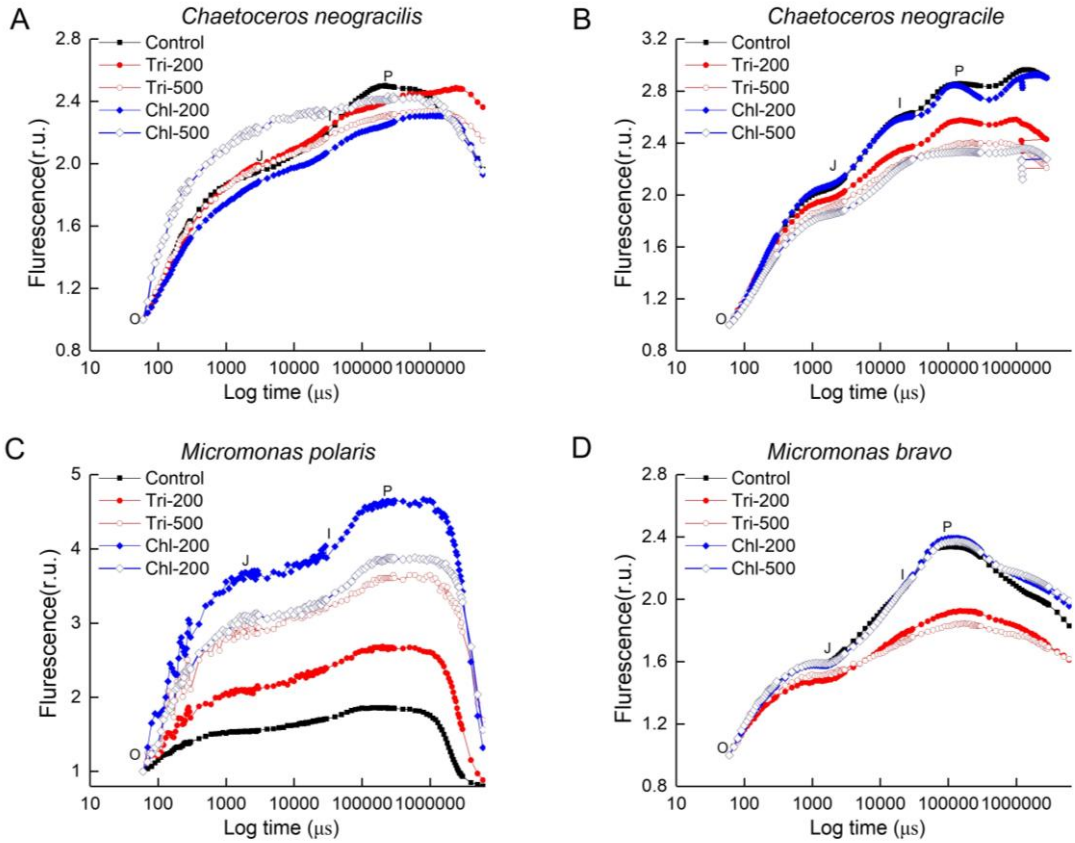

Figure S1. 2. Fast fluorescence kinetics of T-CN (A), A-CN (B), T-MB (C) and A-MP (D), exposed to chlorpyrifos and trifluralin for 72h.

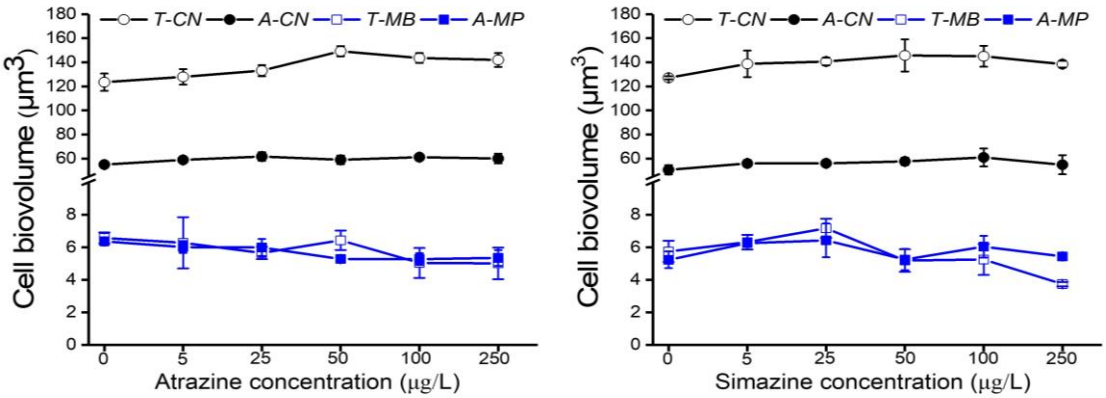

Figure S1. 3. The effects of atrazine and simazine after 72 h exposure on the cell biovolume. Data are means ± SD. for 72h.

Table S1. 1. Fluorescence parameters used in this study with their definitions.

Parameters	**Definition**
Φ_M	Maximal PSII quantum yield
Φ'_M	Operational PSII quantum yield
NPQ	Non-photochemical quenching
NPQmax	Maximum ability for dissipation of excess energy
rETRmax	Maximum relative photosynthetic electron transport rate
α	Maximum light efficiency use
E_K	Light saturation coefficient
Specific energy fluxes (per Q_A reducing PSII RC)	
ABS/RC	Absorption flux (of antenna Chls) per RC (also a measure of PSII apparent antenna size)
TRo/RC	Trapped energy flux (leading to Q_A reduction) per RC
ETo/RC	Electron transport flux (further than Q_A^-) per RC
DIo/RC	Dissipated energy flux per RC
Performance index	
PI_{ABS}=(RC/ABS)	Performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors

Table S1. 2. The effects of trifluralin and chlorpyrifos after 72 h exposure on the growth rate, cell volume, Φ_M, Φ'_M, ROS content, and Chl *a* pigment. The numbers in parentheses are the percentages of the control value. Data are means ± SD.

Parameter	Pesticide concentration (μg/L)		T-CN	A-CN	T-MB	A-MP
Growth rate		0	0.622±0.01 (100)	0.655±0.01 (100)	0.375±0.01 (100)	0.245±0.02 (100)
	Trifluralin	200	0.201±0.04 (28.5)	0.533±0.01 (69.3)	0.049±0.04 (37.6)	0.027±0.02 (52.0)
		500	0.081±0.08 (22.2)	0.346±0.15 (60.4)	-0.093±0.08 (28.2)	-0.195±0.28 (46.4)
	Chlopyrifos	200	0.608±0.00 (96.0)	0.523±0.01 (67.4)	0.382±0.02 (102)	-0.083±0.02 (37.4)
		500	0.391±0.01 (50.1)	0.102±0.03 (19.1)	0.326±0.05 (86.9)	-0.098±0.01 (35.8)
Volume (μm³)		0	144.4±0.95 (100)	45.8±2.89 (100)	9.48±0.20 (100)	6.45±0.56 (100)
	Trifluralin	200	218.9±2.51 (151)	53.1±1.28 (116)	8.43±0.77 (89.4)	7.62±0.08 (119)
		500	221.8±20.9 (154)	58.8±1.66 (129)	8.84±1.02 (93.3)	8.69±0.47 (136)
	Chlopyrifos	200	140.7±1.00 (97.2)	51.2±0.61 (115)	10.67±0.04 (112)	12.66±2.18 (199)
		500	143.8±5.32 (99.3)	91.3±9.95 (199)	10.49±0.21 (111)	12.85±0.08 (200)
Φ_M		0	0.654±0.008 (100)	0.624±0.025 (100)	0.660±0.002 (100)	0.605±0.023 (100)
	Trifluralin	200	0.574±0.004 (87.8)	0.591±0.011 (94.8)	0.492±0.057 (74.5)	0.538±0.012 (89.1)
		500	0.524±0.006 (80.2)	0.536±0.003 (86.0)	0.462±0.052 (70.0)	0.489±0.010 (80.9)
	Chlopyrifos	200	0.638±0.004 (97.6)	0.548±0.006 (87.8)	0.653±0.001 (99.0)	0.538±0.005 (89.0)
		500	0.558±0.007 (85.4)	0.344±0.045 (50.8)	0.645±0.006 (97.9)	0.512±0.013 (84.7)
Φ'_M		0	0.657±0.003 (100)	0.610±0.025 (100)	0.555±0.011 (100)	0.491±0.012 (100)
	Trifluralin	200	0.552±0.011 (84.0)	0.579±0.011 (95.1)	0.356±0.027 (64.1)	0.406±0.021 (82.8)
		500	0.483±0.009 (73.5)	0.507±0.003 (83.3)	0.332±0.024 (60.0)	0.357±0.016 (72.9)
	Chlopyrifos	200	0.636±0.002 (96.7)	0.477±0.006 (78.4)	0.551±0.007 (99.3)	0.379±0.013 (77.2)
		500	0.365±0.016 (55.6)	0.243±0.045 (38.9)	0.533±0.011 (96.0)	0.341±0.007 (69.4)
ROS content		0	0.917±0.236 (100)	0.709±0.074 (100)	0.128±0.040 (100)	0.241±0.048 (100)
	Trifluralin	200	1.07±0.052 (117)	0.870±0.064 (123)	0.508±0.030 (397)	0.947±0.197 (393)
		500	1.20±0.203 (131)	3.46±0.985 (488)	0.689±0.125 (539)	0.915±0.153 (379)
	Chlopyrifos	200	2.16±0.205 (236)	0.975±0.120 (138)	1.72±0.215 (1344)	0.662±0.151 (274)
		500	2.80±0.112 (306)	1.02±0.114 (143)	2.81±0.581 (2195)	0.492±0.153 (204)
Chla		0	3.818±0.168 (100)	1.08±0.060 (100)	0.540±0.005 (100)	0.314±0.018 (100)
(10^6 cells μg/mL)	Trifluralin	200	12.01±1.824 (330)	1.21±0.039 (109)	0.315±0.008 (58.0)	0.341±0.018 (110)
		500	6.752±0.468 (145)	1.12±0.059 (104)	0.745±0.043 (137)	0.306±0.097 (94.1)
	Chlopyrifos	200	1.16±0.053 (30.3)	1.04±0.027 (96.3)	0.497±0.059 (91.4)	0.390±0.036 (124)
		500	1.20±0.267 (31.8)	1.75±0.038 (157)	0.424±0.025 (80.0)	0.388±0.012 (123)
CAT		0	0.090±0.001 (100)	0.134±0.003 (100)	0.145±0.003 (100)	0.262±0.018 (100)
(U mg^{-1}min $protein^{-1}$)	Trifluralin	200	0.101±0.004 (105)	0.185±0.004 (138)	0.148±0.002 (102)	0.353±0.014 (161)
		500	6.752±0.003 (113)	0.290±0.002 (217)	0.281±0.003 (193)	0.400±0.087 (182)
	Chlopyrifos	200	0.087±0.001 (96.8)	0.131±0.002 (97.6)	0.112±0.059 (76.6)	0.311±0.006 (119)
		500	0.057±0.002 (63.1)	1.089±0.038 (812)	0.163±0.025 (112)	0.264±0.032 (101)

1.8. References

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CHAPTER II

RESPONSES TO HERBICIDES OF ARCTIC AND TEMPERATE MICROALGAE GROWN UNDER DIFFERENT LIGHT INTENSITIES

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Will be submitted to Environmental Pollution

2.1. Résumé

Dans les écosystèmes aquatiques, les microalgues sont exposées aux fluctuations lumineuses dues aux changements quotidiens et saisonniers. Les herbicides, tels que l'atrazine et la simazine, sont également de plus en plus présents dans ces systèmes en raison de leurs applications généralisées, et peuvent être même retrouvés dans les eaux arctiques en raison de la dispersion aérienne sur de longues distances et des biocides antisalissures utilisés sur les navires. Les effets toxiques de l'atrazine sur les microalgues tempérées sont bien documentés, mais on sait très peu de choses sur leurs effets sous des intensités lumineuses variables chez les microalgues marines tempérées et arctiques. Nous avons donc étudié les impacts de l'atrazine et de la simazine sur l'activité photosynthétique, les flux d'énergie PSII, la teneur en pigments, la capacité photoprotectrice (NPQ) et la teneur en espèces réactives de l'oxygène (ROS) sous trois conditions d'intensité lumineuse. Nous avons constaté que les microalgues arctiques et tempérées avaient des réponses physiologiques différentes lorsqu'adaptées à différentes intensités lumineuse. Simultanément, la diatomée *Chaetoceros* de la région arctique a démontré une capacité d'adaptation au changement d'intensité lumineuse plus forte que l'algue verte *Micromonas*. De plus, nous avons montré que l'atrazine et la simazine inhibaient la croissance et le transport photosynthétique des électrons, affectaient la concentration des pigments et perturbaient l'équilibre énergétique entre l'absorption et l'utilisation chez les microalgues marines. En conséquence, après une forte adaptation à la lumière, le NPQ et le pigment photoprotecteur (Car) étaient fortement activés mais pas suffisamment pour prévenir les dommages causés par les herbicides, ainsi, ces effets inhibiteurs pourraient être plus forts lorsque les microalgues rencontreraient une forte lumière l'été ou suite aux changements climatiques à venir. Par conséquent, la lumière semble être un facteur non négligeable de régulation de la toxicité des herbicides, et les différences de lumière sont susceptibles de produire des changements dans la communauté algale de l'océan tempéré.

Mots clés: Microalgues marines, Arctique, tempéré, atrazine, simazine, lumière, photoadaptation, ecophysiologie.

2.2 Abstract

In aquatic ecosystems, microalgae are exposed to light fluctuations as a result of daily and seasonal changes. Herbicides, such as atrazine and simazine, are also increasingly found in aquatic systems because of their widespread applications, and can be found in Arctic waters due to long-distance aerial dispersal and antifouling biocides used on ships. The toxic effects of atrazine on temperate microalgae are well documented, but very little is known about their effects under variable light intensities in photoadaptation temperate and Arctic marine microalgae. We therefore investigated the impacts of atrazine and simazine on photosynthetic activity, PSII energy fluxes, pigment content, photoprotective ability (NPQ), and reactive oxygen species (ROS) content under three light intensity conditions. We found that Arctic and temperate microalgae had different light adapted physiological responses to light fluctuations. The Arctic strain of the diatom *Chaetoceros* showed stronger light adaptation capacity than the Arctic green algae *Micromonas* if light intensity changes were induced by seasonal change or global warming leading to reduced ice cover and the subsequent stronger penetration of light in the water column of Arctic ocean. Furthermore, we have shown that atrazine and simazine inhibited the growth and photosynthetic electron transport, affected the concentration of pigments, and disturbed the energy balance between absorption and utilization in marine microalgae. As a result, after high light adaptation, even NPQ and photoprotective pigment (Car) were highly activated but not enough to prevent the damage caused by herbicides, thus these inhibitory effects could be stronger when microalgae encountered high light in the summer or climate change in the coming decades. Therefore, light appears to be a non-negligible factor in regulating herbicide toxicity, and differences in light are likely to produce changes in the temperate ocean's algal community.

Keywords: Marine microalgae, Arctic, temperate, atrazine, simazine, light, photoadaptation, ecophysiology.

2.3. Introduction

Light is one of the most important environmental factors influencing photosynthetic organism's growth (Edwards et al. 2015). In aquatic environments, microalgae experience intense light fluctuations due to the daily sunlight exposure and seasonal changes (Wagner et al. 2006). Meanwhile, turbidity in the water layer and refraction from the sunshine cause light intensity changes at different depths, where photon flux may be scarce in the deeper layers of the water column, or abundant at the surface (Dubinsky and Stambler 2009). To cope with the light fluctuation environment, photosynthetic organisms have evolved diverse phenotypic adjustments including photoadaptation processes (Deblois et al. 2013a, Handler 2017). Photoadaptation to low or high light environments involves the adjustments at the gene level leading to modification of their physiology, biochemistry, and morphology (Bellacicco et al. 2016, Deblois et al. 2013b). Physiological photoprotection mechanisms of photosynthetic organisms include PSII repair cycle, changes in pigment, de novo synthesis of proteins, state transitions, changes in energy efficiency transferred from the light-harvesting complex to reaction centers (RCs), and non-photochemical quenching (NPQ) induced by activation of the xanthophyll cycle (XC) (Deblois et al. 2013a, Dong et al. 2016, Hopes and Mock 2015). Among them, NPQ is the fastest and most flexible response toward light change (Goss and Lepetit 2015). In diatom, the XC is the de-epoxidation of diadinoxanthin (Ddx) to diatoxanthin (Dtx) which is activated by the light-driven acidification of the thylakoid lumen resulting in the accumulation of Dtx; and this is a prerequisite for the formation of energy-dependent quenching (qE) that is the most major component of NPQ (Lepetit et al. 2017), which is not necessarily the case in plants and green algae (Goss and Lepetit 2015, Lavaud and Goss 2014).

As primary producers, microalgae constitute the basis of aquatic ecological trophic networks, and are among the non-target aquatic organisms exposed to pesticides of land-use (Chen et al. 2016). The effect of pesticides was predominantly studied in freshwater ecosystems compared to

marine systems (Dupraz et al. 2019) and estuarine ecosystems (Dar et al. 2021). Herbicides are the most widely used among the major classes of pesticides, and their toxic effects mainly affect the growth, photosynthesis, morphology, biochemical composition, and lipid content of microalgae (DeLorenzo 2001, Sun et al. 2020). The deleterious effects of specific photosystem II (PSII) inhibitor herbicides, such as atrazine and simazine, are primarily to reduce photosynthetic efficiency by inhibiting the photosynthetic electron transfer. It induces the production of reactive oxygen species (ROS) and further damage the D1 protein of PSII and biomolecules like pigments (Chalifour and Juneau 2011, Zhao et al. 2018). Some studies have shown that short-term light changes can affect the toxicity of pesticides (Baxter et al. 2016, Dong et al. 2016). However, very little is known about the response of marine microalgae photoadapted to various light intensities and exposed to herbicides.

Most of our knowledge about the photoadaptation strategies and pesticide effects is from the temperate species (Gomes and Juneau 2017, Young and Schmidt 2020). Microalgae have a rich evolutionary history due to their widespread presence in various habitats, especially marine ecosystems, leading to a wide range of adaptations, allowing them to thrive in a variety of environmental conditions (Lacour et al. 2020). Polar microalgae adapted to permanently low temperatures and extreme variation in irradiance due to seasonally changing ice-cover and day lengths (Handler 2017). Sea-ice of Arctic Ocean is gradually melting caused by global warming, increasing light availability on the sea surface (Osborne et al. 2018). Therefore, studying the differences in the adaptation processes developed by the Arctic and temperate microalgae can provide insights into how microalgae can cope with the changing environment in the aquatic ecosystems in presence or not of herbicides. Therefore, this study aims to determine the response of the Arctic and temperate microalgae photoadapted to various light intensities and how these various photoadapted strategies affect the toxicity of herbicide. This study on the eco-physiological responses of Arctic and temperate counterparts, will also permit required to facilitate growth model development, since to date, models assessing microalgae biomass use temperate algal data.

2.4. Materials and methods

2.4.1 Algal species and growth conditions

Temperate *Chaetoceros neogracile* (T-CN-CCMP1425), temperate *Micromonas bravo* (T-MB-CCMP1646), and Arctic strain of *Micromonas polaris* (A-MP- CCMP2099) were purchased from NCMA (National Contract Management Association). Arctic strain of *Chaetoceros neogracilis* (RCC2279-A-CN) came from the culture collection in Roscoff. All species were cultivated in L1 marine medium (Guillard and Hargraves 1993) with a total volume of 100 mL medium in 250 mL Erlenmeyer flasks. The microalgae were respectively grown at three different light intensities (low light intensity-40 µmol photons m^{-2} s^{-1} (LL), medium light intensity-100 µmol photons m^{-2} s^{-1} (ML), and high light intensity-400 µmol photons m^{-2} s^{-1} (HL)) at 14:10 h (light: dark) illumination cycle and shaken moderately twice a day. Growth temperatures were 18 °C and 4 °C, respectively, for temperate and Arctic species. Cells were periodically transferred to fresh growth media to keep them in their exponential growth phase. The cells were cultured for more than ten generations in their actual growth conditions. The cell numbers were counted by using Multisizer 3 Coulter Counter (Beckman Coulter Inc., USA). The calculation of growth rate (µ) is based on the following formula: $\mu = (\ln N_n) - (\ln N_0)/(t_n - t_0)$, where µ = average specific growth rate, N_0, N_n indicate cell density (cells/mL) at respectively t_0 (beginning of the experiment) and t_n (time, in days, after the beginning of the experiment).

2.4.2 Herbicide preparation and treatment

Atrazine and simazine used in this study came from Sigma-Aldrich (PESTANAL®, analytical standard, Canada). Herbicide stock solutions were made in pure acetone (≥ 99%) and acetone concentration was 0.01% in the treatments, which was verified not to be toxic to these microalgae. Six concentrations of atrazine and simazine were used for the herbicide tests (0 µg/L,

5 μg/L, 25 μg/L, 50 μg/L, 100 μg/L, and 250 μg/L). Cells were collected during their exponential growth phase and transferred to 1 L Erlenmeyer flasks (with 350 mL growth medium) at a cell density of 2.5×10^5 (*Chaetoceros*) and 2.5×10^6 (*Micromonas*) cells/mL respectively, and then exposed to different concentrations of herbicides for 72 h under the three light conditions. All treatments were performed in triplicate. Cell density and cell biovolume were evaluated at the end of the experiment with a Multisizer 3 Coulter Counter particle analyzer (Beckman Coulter Inc., USA).

2.4.3 Pigment concentration measurements

Algal cultures (25 mL) were collected after 72 h herbicide treatment through a gentle filtration on 0.8μm filter membranes (Polytetrafluoroethylene; Xingya Purifyin Company; China). Filters were immediately submerged in liquid nitrogen and placed in 2 mL Eppendorf tubes, and then stored at -80 °C until analysis. Each sample was added to 2 mL of 90% acetone to be extracted overnight at -20 °C before pigment analysis. Cells in an ice container were broken for 20 s using an ultrasonic probe to increase extraction efficiency. The samples were centrifuged (10000×g) at 4 °C for 10 min after the extraction. The supernatants were used to quantify the content of chlorophyll (Chl *a*) and carotenoids (Car). A Cary 300 UV spectrophotometer (Varian, USA) was used to determine the absorbance spectra (400–750nm) for each extracted sample. Based on the equations from (Jeffrey and Humphrey 1975) and Seely et al. (1972), the contents of Chl *a* and Car were calculated respectively.

2.4.4 Fluorescence measurements

The samples (3 mL) were measured at their growth temperature of 4 °C and 18 °C after dark acclimation for 20 min. Fluorescence light curves performed with a fluorometer of Water-PAM (Walz, Germany) were used to evaluate the maximum (Φ_M) and operational (Φ'_M) PSII quantum yields, as well as the non-photochemical quenching (NPQ) (Du et al. 2019). The light curve was

obtained by using eight levels of actinic light intensities (0, 46, 105, 188, 276, 427, 635, 906, and 1207 µmol photons $m^{-2}s^{-1}$) with saturation pulses (3000 µmol photons m^{-2} s^{-1}, 800 ms). Φ_M, Φ'_M and NPQ were computed according to the following equations: $\Phi_M = (F_M–F_0)/F_M$; $\Phi'_M = (F'_M–F_S)/F'_M$ (Genty et al. 1989); $NPQ = (F_M–F'_M)/F'_M$ (Bilger and Björkman 1990). Maximum relative electron transport rates ($rETR_{max}$), maximum light efficiency usage (α), and light saturation coefficient (E_k) were obtained by fitting the obtained values according to (Eilers and Peeters 1988, Serodio and Lavaud 2011). To further assess the PSII energy fluxes, the polyphasic increase in fluorescence transients was also captured by a PEA fluorometer (Plant Efficiency Analyzer, UK). A 2 s red light pulse with a maximum emission at 650 nm and 3600 mol photons m^{-2} s^{-1} was used to generate OJIP transients (Jiang et al. 2008). Table S1 provides a description of each parameter.

2.4.5 Reactive oxygen species (ROS) measurement

Intracellular ROS content was determined by using a BD Accuri C6 flow cytometer (Biosciences, San Jose, CA, USA). The fluorescent dye H2DCFDA (2′,7′-dichlorodihydrofluorescein diacetate, Molecular probes, Eugene, OR, USA) was used. More information about this method was described in (Stachowski-Haberkorn et al. 2013). Samples were analyzed after incubation in complete darkness for 30 min at room temperature. To prevent possible signal variations due to herbicide influence on FL1 fluorescence, the mean FL1 values of H2DCFDA-stained samples were divided by the mean FL1 values of the same fresh samples analyzed for morphology. Results were thus expressed as FL1 ratios.

2.4.6 Atrazine determination

The concentration of atrazine removed from the growth medium was calculated by subtracting the concentration found in the sample (atrazine + growth medium + microalgae)

treated for 72 h from the abiotic control (atrazine + growth medium). To measure atrazine concentration remaining in the growth medium i.e. removal capacity of microalgae, cultures (in triplicate) were transferred to 250 mL flasks containing 100 mL of L1 growth media at cell concentrations indicated in section 2.2 for 72 h. Abiotic controls, also in triplicate, were prepared using L1 medium without cells. After inoculation of the microalgae in the medium, 2 mL aliquots of each flask were sampled and filtered on a 20 mm glass fiber filter (Type A/E, Pall Corporation, Michigan, USA) and put in 1.5 mL Eppendorf tubes (polypropylene Safe-Lock tube, Canada). The filtrate was kept at -80 °C until analysis. After 72 h, the above procedure was repeated with 2 mL of culture for herbicide analysis. Before analysis, filtered media containing atrazine were thawed and then filtered again through a 0.22 µm syringe filter (Millex-GV, Millipore, Billerica, USA).

A stock solution of atrazine at 1 µg/L was prepared in 50% methanol to perform a calibration curve ranging from 0.5-20 µg/L. An internal standard (IS) working solution of isotopically labeled atrazine-d_5 was prepared at 100 µg/L, 20 µL of which was added to each sample or standard with 180 µL. Quantitative analysis of atrazine was performed by QTRAP 5500 mass spectrometer (Sciex, Concord, ON, Canada) with a Turbo-V electrospray ionization source in positive ion multiple reaction monitoring (MRM) mode (Chalifour et al. 2016). Atrazine and IS were detected using MRM transitions *m/z* 216-174 and 221-179 for quantitation (at collision-offset voltage 30 and 25 V). Ion source and MS parameters were as follows: ESI voltage, 5000 V; source temperature, 500 °C; curtain gas, curtain gas, 35 psi; nebulizer and drying gases both at 50 psi. Peak integration was performed using Multiquant™ 3.0 (Sciex), using peak area ratios of analyte/IS and linear regression of calibration curve for quantitation.

2.4.7 Statistical analyses

Statistical analyses were performed using Origin® 7.0 (Originlab Corporation, Northampton, MA, USA). Two-way analysis of variance (ANOVA) was used to determine the effect of treatments, and Tukey's honestly significant difference (Tukey's, HSD) test was conducted to test the statistical significance of the differences between means of various treatments. The assumption of normal distribution and homogeneity of variances for all t-tests and ANOVAs presented in this paper were respectively tested using Lilliefors' and Levene's tests. Two-way analysis of variance (ANOVA) to evaluate the interactions between growth light conditions (LL, ML, and HL), and herbicide concentrations. Contrast analysis was used when there were significant differences in the studied variables between treatments. The EC_{50} (half-maximum effective concentration) values were calculated from the nonlinear least-square fits by using the inverse of the regression curve (Juneau et al. 2001).

2.5. Results

2.5.1 Effects of growth light intensity on the ecophysiological characteristics of the Arctic and temperate microalgae (in absence of herbicide)

2.5.1.1 Growth rate, cell biovolume, pigment content, and ROS content

Growth rates of Arctic and temperate microalgae (except for T-MB) grown under LL conditions were drastically lower compared to those grown under ML or HL conditions (Tukey's HSD, $P < 0.05$, Table 2.1 and Fig. 2.3). Moreover, the growth rate of Arctic microalgae (*C. neogracilis*-A-CN and *M. polaris*-A-MP) were lower than those of their temperate counterparts (*C. neogracile*-T-CN, *M. bravo*-T-MB) under three different growth light intensities; the exception being for A-CN under LL condition. In addition, the increasing trend of growth rate for Arctic

microalgae was lower than that of temperate counterparts when the growth light intensity was enhanced. Similarly, the cell biovolume of two Arctic microalgae was smaller under LL condition compared to ML and HL (Tukey's HSD, $P < 0.05$, Table 2.1 and Fig. 2.4). Cell biovolume of the two temperate microalgae was the largest under HL and the smallest under LL, with an intermediary biovolume for ML grown cultures.

The Chl *a* and Car contents of the two Arctic strains were lower than those of their temperate counterparts under LL, ML, and HL conditions. Furthermore, the Chl *a* content of the two temperate species significantly decreased when the growth light was high (ML, HL, Tukey's HSD, $P<0.05$, Fig. 2.1-atrazine concentration=0 μg/L), while both Arctic species were not changed under LL compared to ML and HL conditions. The Car content of the two temperate species increased significantly under high growth light intensity (ML and HL), while the two Arctic microalgae were not affected (Tukey's HSD, $P < 0.05$, Fig. 2.1). In addition, except for T-MB, we found higher ROS levels in microalgae grown under HL compared to the other two low light conditions (LL and ML) (Fig. 2.5-atrazine concentration=0 μg/L, Tukey's HSD, $P < 0.05$), and the ROS contents of T-CN, A-CN, and A-MP of HL grown cultures were 1.2, 2.3, and 1.4 times higher than those under grown LL, respectively.

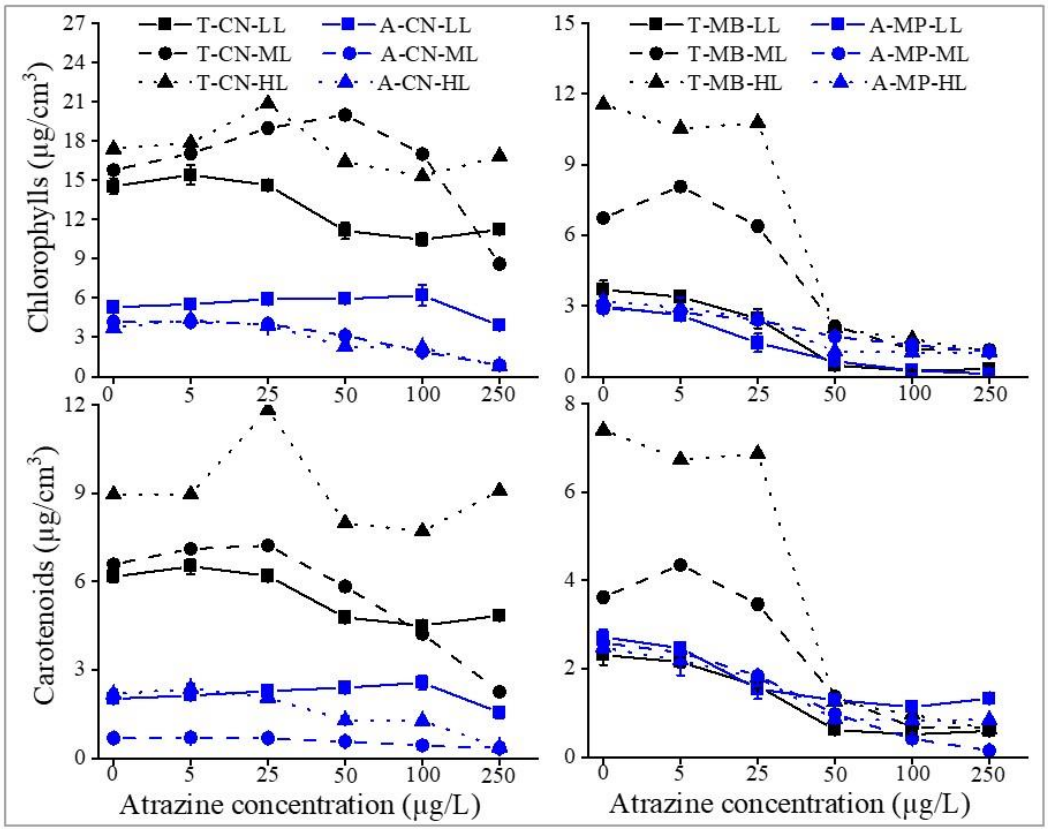

Figure 2. 1. The effects of atrazine (after 72 hours) on the pigment content of four microalgal species (temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB), and Arctic *M. polaris* (A-MP)) under LL (square), ML (circle) and HL (triangle) light intensities. Data expressed as means ± SD (n = 6).

Table 2. 1. The effects of growth light intensity (LL, ML, and HL) on the growth rate, cell biovolume, Φ_M, Φ'_M, α, ETR_{max}, E_k, and NPQ_{max} of temperate *C. neogracile* (T-CN), *M. bravo* (T-MB), Arctic *C. neogracilis* (A-CN), and Arctic *M. polaris* (A-MP). EC_{50} for the operational PSII quantum yield (Φ'_M) after 72 h exposure to atrazine and simazine under three different light intensities (LL, ML, and HL). Strains in the same column exposed to the different light intensities with different superscript letters (a-c) were significantly different (Tukey's HSD, $P < 0.05$). The numbers in parentheses are the percentages of the control values. Data expressed as means ± SD (n = 6).

Algae	Light	Growth rate (μ)	Volume (μm^3)	Φ_M	Φ'_M	α	ETRmax	Ek	NPQmax	EC_{50}-Φ'_M (Atra)	(Sim)
T-CN	LL	0.37±0.02 (100)[a]	114±8.89 (100)[a]	0.692±0.00 (100)[a]	0.682±0.01 (100)[a]	0.713±0.01 (100)[a]	333±36 (100)[a]	467±52 (100)[a]	1.5±1.24 (100)[a]	95±7.4	121±7.9
	ML	0.64±0.03 (170)[b]	148±9.59 (130)[b]	0.647±0.00 (93)[b]	0.646±0.00 (95)[b]	0.665±0.01 (93)[b]	421±17 (126)[b]	633±23 (136)[b]	1.3±0.24 (12)[b]	66±1.4	142±6.7
	HL	0.66±0.02 (177)[b]	164±4.88 (144)[c]	0.608±0.01 (88)[c]	0.510±0.03 (75)[c]	0.624±0.02 (88)[c]	253±63 (76)[c]	404±91 (87)[a]	10±0.00 (105)[a]	34±1.1	90±12
A-CN	LL	0.43±0.03 (100)[a]	45±3.06 (100)[a]	0.674±0.01 (100)[a]	0.607±0.01 (100)[a]	0.607±0.02 (100)[a]	265±34 (100)[a]	432±55 (100)[a]	1.6±0.62 (100)[a]	39±1.7	52±0.7
	ML	0.57±0.09 (131)[b]	63±3.84 (140)[b]	0.584±0.00 (87)[b]	0.553±0.01 (91)[b]	0.569±0.04 (94)[a]	370±91 (140)[b]	648±158 (150)[b]	1.8±0.54 (113)[a]	37±2.1	62±2.1
	HL	0.57±0.08 (130)[b]	61±7.57 (136)[b]	0.525±0.02 (78)[c]	0.246±0.02 (41)[c]	0.473±0.07 (78)[b]	91±13 (34)[c]	196±46 (45)[c]	3.7±0.92 (231)[b]	12±3.2	28±2.0
T-MB	LL	0.36±0.01 (100)[a]	4±0.09 (100)[a]	0.685±0.01 (100)[a]	0.678±0.02 (100)[a]	0.519±0.05 (100)[ab]	247±61 (100)[a]	369±162 (100)[a]	1.5±0.01 (100)[a]	36±5.9	61±7.0
	ML	0.58±0.03 (160)[b]	5±0.00 (134)[b]	0.647±0.00 (94)[b]	0.562±0.00 (83)[b]	0.597±0.01 (115)[a]	244±25 (99)[a]	430±48 (117)[a]	1.9±0.11 (127)[b]	31±2.3	32±1.8
	HL	0.80±0.05 (220)[c]	5±0.19 (141)[c]	0.598±0.01 (87)[c]	0.357±0.01 (53)[c]	0.499±0.07 (96)[b]	180±31 (73)[a]	368±82 (100)[a]	1.3±0.33 (87)[a]	30±4.8	49±1.4
A-MP	LL	0.27±0.01 (100)[a]	5±0.05 (100)[a]	0.675±0.02 (100)[a]	0.505±0.02 (100)[a]	0.445±0.09 (100)[ab]	114±56 (100)[a]	249±104 (100)[a]	2.2±0.71 (100)[a]	39±1.3	55±3.4
	ML	0.30±0.02 (109)[b]	6±0.26 (102)[a]	0.618±0.02 (92)[b]	0.491±0.02 (97)[a]	0.493±0.04 (111)[a]	156±21 (137)[a]	356±28 (143)[a]	9.8±0.37 (445)[b]	36±1.7	46±1.9
	HL	0.32±0.04 (118)[b]	6±0.10 (114)[b]	0.515±0.00 (76)[c]	0.222±0.01 (44)[b]	0.384±0.04 (86)[b]	106±54 (93)[a]	273±128 (110)[a]	1.6±0.20 (73)[a]	29±0.6	206±39

2.5.1.2 Photosynthesis and energy dissipation processes

The maximum PSII quantum yield (Φ_M) of all studied species significantly decreased with increasing growth light intensity and Φ_M of the two Arctic microalgae declined more than that of their temperate counterparts (Tukey's HSD, P >0.05, Table 2.1). A similar trend was observed with the operational PSII quantum yield (Φ'_M), but the amplitudes of the declines were higher than for Φ_M; except for A-MP with no alteration from LL to ML (Table 2.1). Together with the reduction of Φ'_M and Φ'_M, the maximum light efficiency usage (α) showed a decreased trend when the growth light intensity changed from LL to ML (but not necessarily significantly, depending on species) and then α of all studied microalgae decreased significantly from ML to HL conditions (Tukey's HSD, $P < 0.05$, Table 2.1). Furthermore, the maximum electron transport rates (ETR_{max}) and the light saturation coefficient (E_k) of Arctic and temperate diatoms (T-CN and A-CN) increased significantly from LL to ML (except for E_k of T-CN) and remarkably decreased from ML to HL (Tukey's HSD, $P < 0.05$, Table 2.1). Under the same condition, the E_k of Arctic and temperate green algae of *Micromonas* (T-MB and A-MP) had similar trends when growth light intensity was changed, they did not differ significantly (Tukey's HSD, P >0.05, Table 2.1). Moreover, the maximal ability for dissipation of excess energy (NPQ_{max}) of T-MB and A-MP was also significantly increased when the growth light intensity changed from LL to ML, and markedly decreased from ML to HL conditions (Tukey's HSD, $P < 0.05$, Table 2.1). However, for the NPQ_{max} of T-CN and A-CN, it increased significantly with the modification of growth light intensity (Tukey's HSD, $P < 0.05$, Table 2.1). In addition, we observed that the parameters Φ'_M, Φ'_M, α, ETR_{max}, and E_k for two Arctic species were, to different degrees, lower than those of their temperate counterparts under the different growth light intensities.

2.5.1.3 Photosystem II energy fluxes

The energy conservation parameter of PI_{ABS} was significantly decreased for all species, except

for A-CN, with increasing the growth light intensity (Tukey's HSD, $P < 0.05$, Fig. 2.2). The PI_{ABS} of the two Arctic microalgae was less affected than their temperate counterparts. The reduction in PI_{ABS} was also reflected in the decrease of electron transport per active reaction center (ET_0/RC) for all studied species when the growth light intensity was enhanced. The effective dissipation per active RC (DI_0/RC) of the two temperate microalgae (T-CN and T-MB) was increased up to 252% and 224% from LL to HL (Tukey's HSD, $P < 0.05$). This was accompanied by an increase in the absorbed flux per active reaction center (ABS/RC) as an indicator of the PSII antenna size. In contrast, DI_0/RC and ABS/RC in two Arctic microalgae did not significantly change when the algae were grown under different light intensities (Tukey's HSD, $P > 0.05$, Fig. 2.2). The maximal rate at which excitons were trapped by the active RCs (TR_0/RC) was not affected by changes in growth light intensity. Furthermore, the PQ pool size participating in the electron transport (qPQ) and non-photochemical quenching (qE_{max}) were significantly increased in the two Arctic microalgae under HL, compared to the other two low light conditions (Tukey's HSD, $P < 0.05$, Fig. 2.2). In contrast, the qPQ and qE_{max} of the two temperate species significantly decreased with increasing growth light intensity.

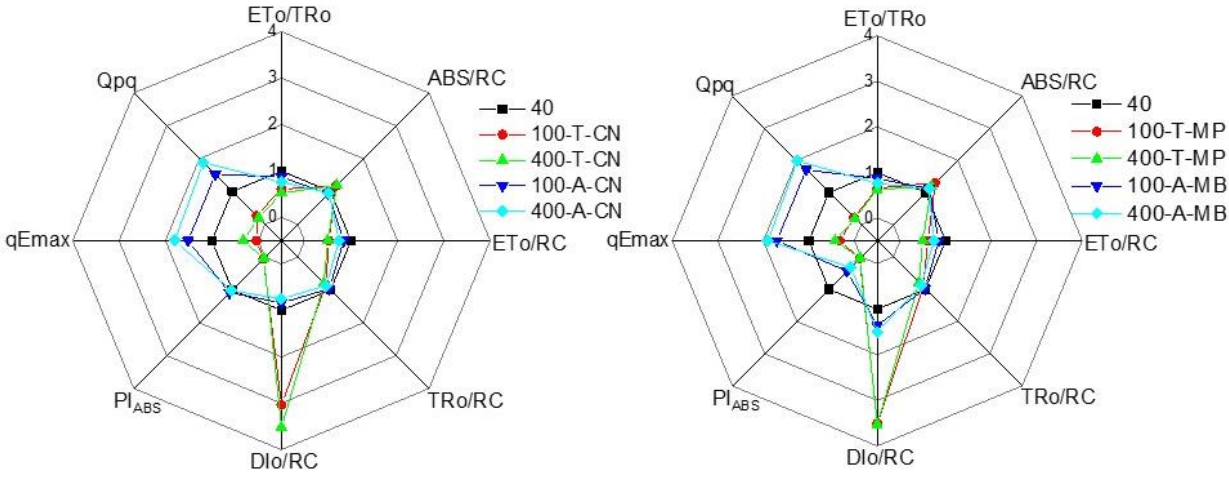

Figure 2. 2. The effects of growth light intensity (LL, ML, and HL) on the PSII energy fluxes of temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB), and Arctic *M. polaris* (A-MP). Data expressed as means ± SD (n = 6).

2.5.2 Effects of atrazine and simazine on the ecophysiological characteristics of Arctic and temperate species

2.5.2.1 Growth rate, cell biovolume, pigment content, and ROS content

The growth rates of Arctic microalgae (*C. neogracilis*-A-CN and *M. polaris*-A-MP) and their temperate counterparts (*C. neogracile*-T-CN and *M. bravo*-T-MB) significantly decreased after 72 h exposure to atrazine or simazine independently of the growth light intensity (Tukey's HSD, $P < 0.05$, Fig. 2.3). Interestingly, although that trend was similar among the various growth light intensities, the effect was stronger under the lowest light intensity. Moreover, the impact of the studied herbicides on the growth rate for Arctic microalgae was stronger than that of temperate counterparts when the growth light intensity was enhanced. Although these herbicides inhibited the growth of microalgae, cell biovolume of the four studied species did not significantly change for all tested conditions (Tukey's HSD, $P>0.05$, Fig. 2.4), except for T-MB and A-MP at high concentrations (100 and 250 µg/L) under ML and HL conditions.

Pigment contents (Chl *a* and Car) of the four studied species did not change significantly at low concentrations of atrazine and simazine (≤ 25 µg/L, Tukey's HSD, $P > 0.05$, Fig. 2.1), but were significantly decreased at high concentrations (> 25µg/L). The observed effects were enhanced under HL compared to the other two low light intensities (LL and ML). Despite a decreasing trend in Chl *a* and Car, the ratio of Car/Chl *a* remained unchanged (Tukey's HSD, $P > 0.05$). ROS levels of the four species significantly increased with increasing atrazine concentrations under each light condition (LL, ML, and HL) (Tukey's HSD, $P < 0.05$). Although this trend was similar among growth light intensities, the effect was stronger under HL (Fig. 2.5). The ROS content was also significantly increased when simazine concentrations are increased, and their effect was greater at higher light intensities (data not shown).

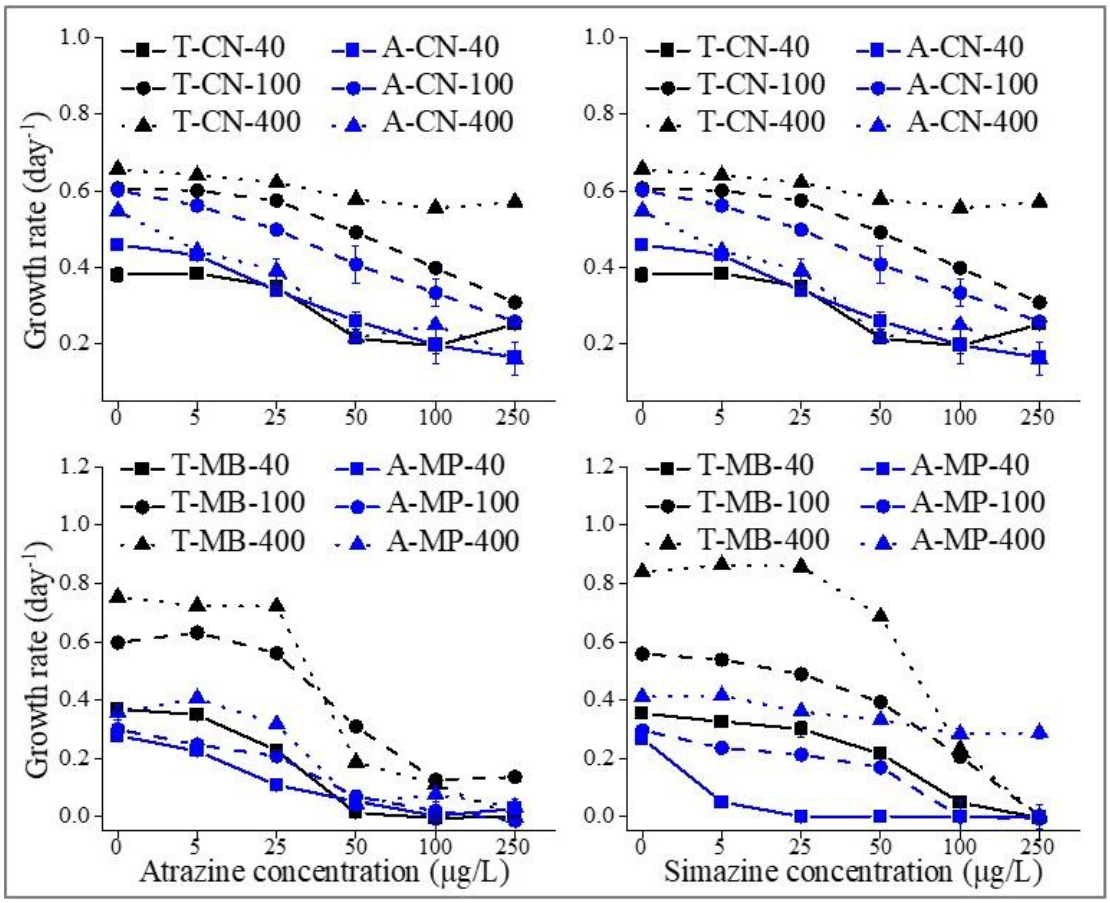

Figure 2. 3. The effects of atrazine and simazine on the growth rate of four species (temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB), and Arctic *M. polaris* (A-MP)) after 72 h exposure under LL (square), ML (circle) and HL (triangle). Data expressed as means ± SD (n = 6).

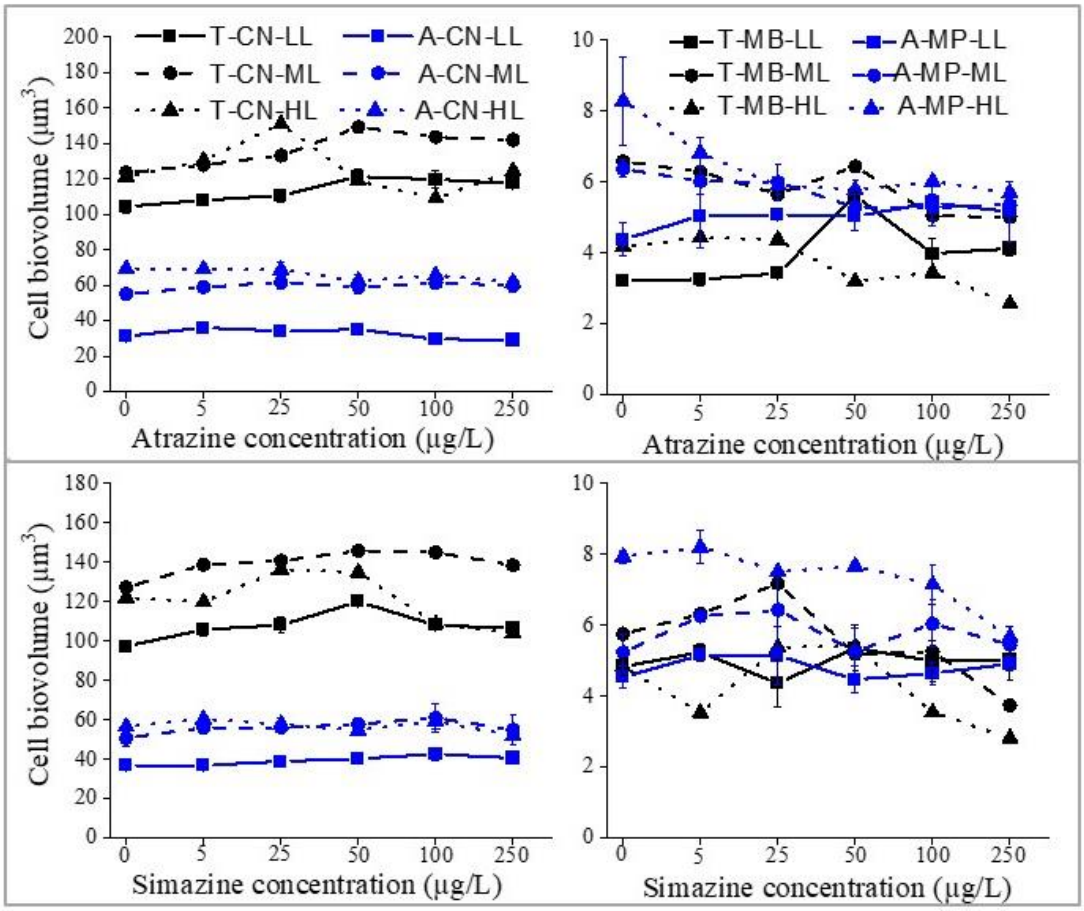

Figure 2. 4. The effects of atrazine and simazine on the cell biovolume of the temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB), and Arctic *M. polaris* (A-MP) after 72 h exposure under LL, ML, and HL. Data expressed as means ± SD (n = 6).

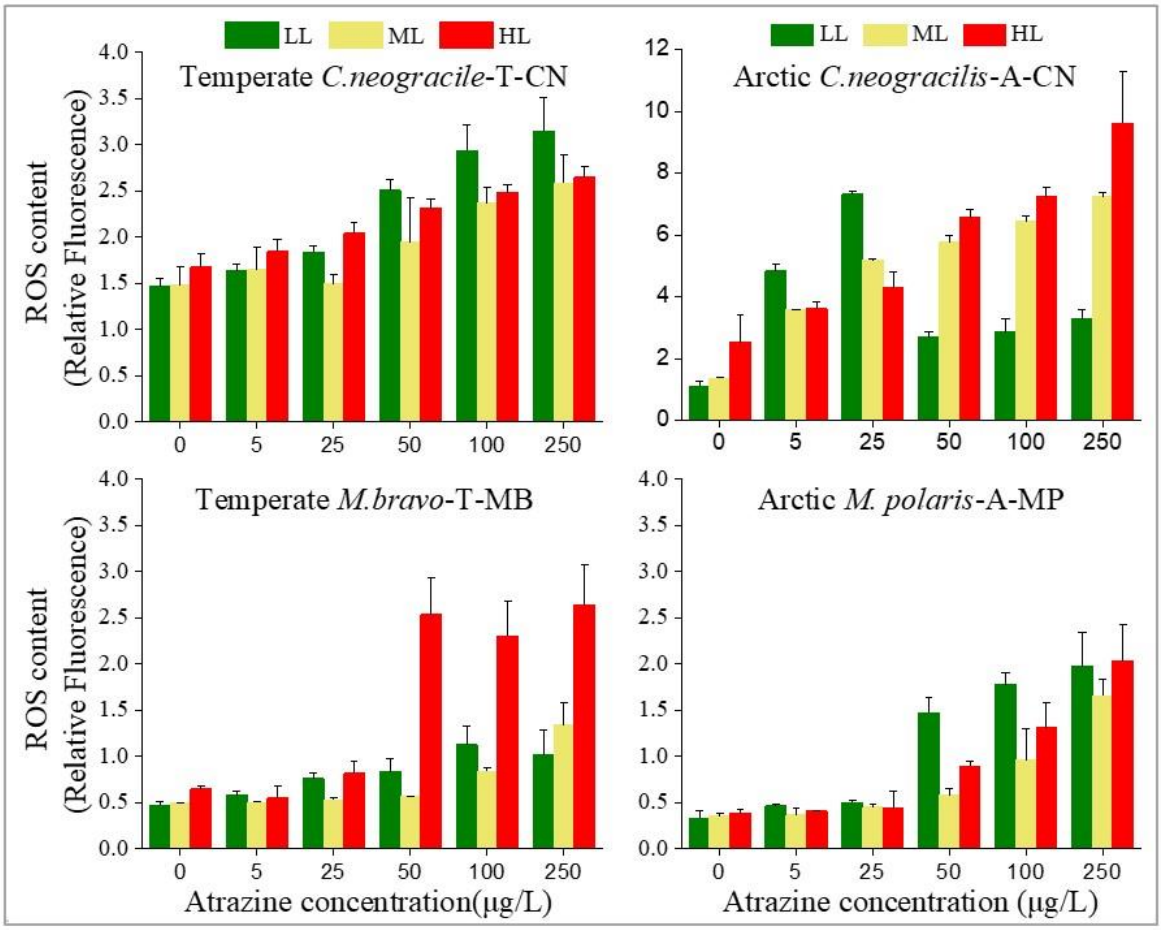

Figure 2. 5. The effects of atrazine on ROS content of the studied species (temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB) and Arctic *M. polaris* (A-MP)) after 72 h exposure under LL, ML and HL. Data expressed as means ± SD (n = 6).

2.5.2.2 Photosynthesis and energy dissipation processes

The operational PSII quantum yield (Φ'_M) of the four studied species significantly decreased (Tukey's HSD, $P < 0.05$, Fig. 2.6) with increasing atrazine and simazine concentration for each growth light condition. Moreover, both Φ_M and Φ'_M of Arctic *C. neogracilis* (A-CN) decreased more than for temperate *C. neogracile* (T-CN). In contrast, Φ_M and Φ'_M of Arctic *M. polaris* (A-MP) decreased less than for temperate *M. bravo* (T-MB) except for low atrazine concentrations under LL (< 25 μg/L, Tukey's HSD, $P < 0.05$, Fig. 2.6). The evaluated Φ'_M-EC_{50} of T-CN for

atrazine under LL, ML, and HL conditions were respectively 2.4, 1.7 and 1.8 times higher than those obtained for A-CN. Similar results were obtained for simazine. Both *Chaetoceros* species have the lowest Φ'_M-EC_{50} (respectively 90 µg/L and 28 µg/L for the temperate and Arctic species) for simazine under HL compared to VLL and LL (Table 2.1). Φ'_M-EC_{50} of T-MB and A-MP were respectively for LL, ML and HL 36 µg/L, 31 µg/L, 30 µg/L, and 39 µg/L, 36 µg/L, 29 µg/L for atrazine. The highest simazine Φ'_M-EC_{50} evaluated for A-MP under HL conditions (206 µg/L) was 4.2 times higher than that of T-MB (Table 2.1). The parameter of Φ'_M was more sensitive than Φ_M in response to the herbicides. The energy conservation parameter (PI_{ABS}) was also decreased after exposure to 50 µg/L atrazine and simazine under each light condition, but to a different extent than Φ_M and Φ'_M depending on the tested species (Table 2.2). Non-photochemical quenching (qE_{max}) of the four studied species was also significantly decreased by 50 µg/L of atrazine under LL ML, and HL conditions (Tukey's HSD, $P < 0.05$, Table 2.2).

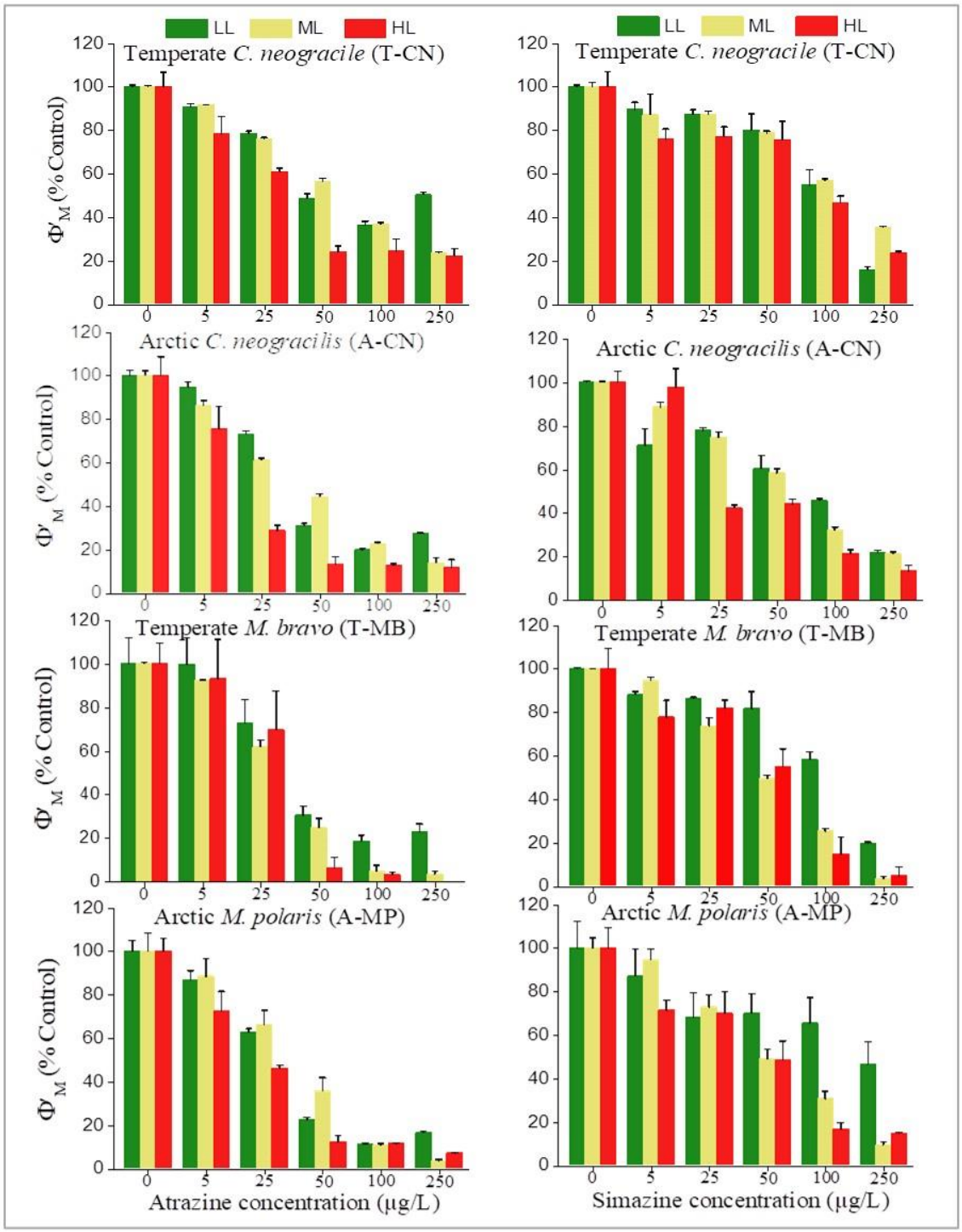

Figure 2. 6. The effects of atrazine and simazine on the PSII operational quantum yield (Φ'_M) of temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB), and

Arctic *M. polaris* (A-MP) after 72 h exposure under 40, 100 and 400 light intensities. Data expressed as means ± SD (n = 6).

Table 2. 2. Effect of atrazine (50 µg/L), ML & HL exposure and combine effect (Light and atrazine) on light energy conservation indicator (PI_{ABS}), non-photochemical quenching (qE_{max}), and ROS content, in percentage from control. Data are means ± SD of two independent experiments in triplicate. Within treatments, values followed by one asterisk are significantly different from the control, and values followed by two asterisks are significantly different from the light (ML and HL) exposure, while values followed by three asterisks are significantly different from both control and HL treatment (Tukey's HSD, $P < 0.05$). The numbers in parentheses are the percentages of the control values.

Species	Parameter	Effect of atrazine % of the control (±) LL	Effect of ML % of the control (±)	Atrazine+ ML % of the control (±)	Effect of HL % of the control (±)	Atrazine+ HL % of the control (±)
T-CN	PIabs	98* (13)	95** (16)	71*,** (4)	95** (11)	79*,** (3)
A-CN	PIabs	63* (5)	25** (1)	84*,** (5)	43** (1)	89*,** (5)
T-MB	PIabs	48* (2)	96** (12)	95*,** (14)	97** (3)	92*,** (14)
A-MP	PIabs	90* (6)	52** (9)	72*,** (8)	60** (9)	85 (15)
T-CN	qEmax	15* (2)	72** (9)	63*,** (7)	74** (6)	76*,** (4)
A-CN	qEmax	22* (4)	+18 (2)	60*,** (9)	+24** (1)	72*,** (16)
T-MB	qEmax	3 (1)	27** (3)	14 (1)	34** (1)	30*,** (2)
A-MP	qEmax	19* (1)	+83** (14)	58*,** (6)	+89** (9)	77*,** (13)
T-CN	ROS	71* (5)	1 (0)	31*,** (1)	14 (1)	38*,** (2)
A-CN	ROS	143* (15)	63** (6)	366*,** (32)	127** (8)	186*,** (11)
T-MB	ROS	344* (68)	7 (0)	62*,** (12)	16 (1)	131*,** (8)
A-MP	ROS	76* (8)	5 (0)	13*,** (1)	36** (2)	290*,** (28)

2.5.3 Effects of growth light intensity on atrazine toxicity

Because the effect of growth light intensity on simazine was similar to that of atrazine, and atrazine can be detected more frequently than simazine in water bodies, we mainly focus on atrazine for the following parts. Atrazine and ML alone significantly reduced PI_{ABS} of T-CN by 98% and 95%, respectively, while their combined action was less effective (71% inhibition) (Tukey's HSD, $P < 0.05$). Similar results were also observed for HL (Table 2.2). This trend was also observed

among the other studied species (A-CN, T-MB, and A-MP). Both Arctic species increased their non-photochemical quenching (qE_{max}) to varying degrees (18-89%) under ML and HL growth conditions compared to LL (Table 2.2). The combined effect of atrazine and ML decreased qE_{max} by 60% and 58% respectively in A-CN and A-MP (Table 2.2). Furthermore, the combined effect of atrazine and ML on the two temperate species only reduced qE_{max} by 14% for T-MB and 63% for T-CN compared to ML exposure alone (27% and 72% reduction), and the combined effect of atrazine and HL decreased more qE_{max} than the combination of atrazine and ML condition (Table 2.2). We can also notice that the treatment of the studied microalgae with atrazine alone significantly induced the production of ROS, but the combined effect of atrazine and ML or HL downregulated the production of ROS (Table 2.2). According to Fig. 2.7, the removal of atrazine was stronger under high growth light (HL) compared to the other two low light intensities (LL and ML) except the temperate *C. neogracile* (Tukey's HSD, $P < 0.05$). The removal ability of atrazine by Arctic *C. neogracilis* was higher than that of temperate *C. neogracile* under LL, ML and HL conditions. Meanwhile, the Arctic *M. polaris* also had higher removal ability of atrazine compared to temperate *M. bravo* under ML and HL conditions except for LL condition.

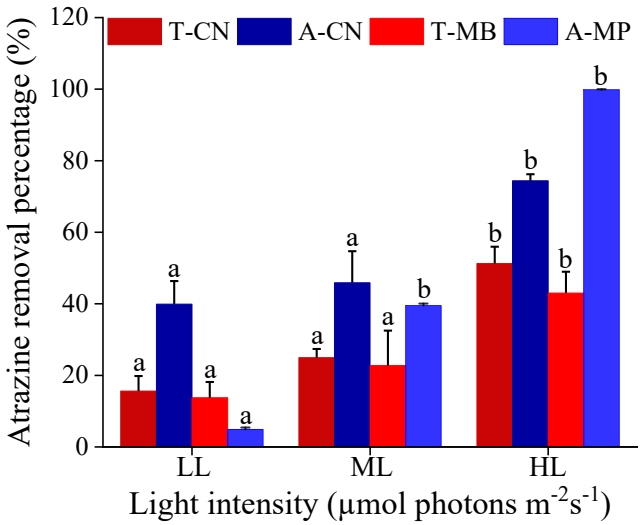

Figure 2. 7. The concentration of atrazine removed from the growth medium for temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB), and Arctic *M.*

polaris (A-MP) after 72 h exposure to 50 µg/L under LL, ML and HL conditions. Data expressed as means ± SD (n = 6). Strains in the same column exposed to the different light intensities with different superscript letters (a-b) were significantly different (Tukey's HSD, $P < 0.05$).

2.6. Discussion

2.6.1 Influence of growth light intensity on Arctic microalgae and their temperate counterparts

2.6.1.1 Photoadaptation processes

Light adaptation processes developed by microalgae allow them to thrive in low or high light environments by altering their ecophysiological properties (Agarwal et al. 2019, Young and Schmidt 2020). High light induced similar photophysiological responses among the studied species. Growth under higher light intensity (but not excessive) led to a significant increase in growth rates, which may be attributed to the Chl *a*/C evolution with changes in light intensity (Croteau et al. 2022, Lacour et al. 2018). The reduction of Chl *a* in all species after HL photoadaptation was typically seen as a result of lower light absorption since the light absorption is positively correlated to the cellular Chl *a* content (MacIntyre et al. 2002). An increase in Car may also play a role in protecting the photosynthetic apparatus when the algae grow under high light intensities. Indeed, either directly or indirectly, these pigments are involved in quenching reactive oxygen species (Sedoud et al. 2014). Furthermore, the slight but significant increase in the antenna size of all studied species following HL adaptation indicated a relatively low photochemically effective cross-section to reduce the chance of photons entering the photosynthetic electron transport chain to avoid photoinhibition (Finkel et al. 2010). These modifications observed under high light conditions minimized the excitation pressure on the photosynthetic apparatus despite the increased light availability (Agarwal et al. 2019). Non-photochemical quenching (NPQ) (the main component-qE) is known to be an efficient photoprotective mechanism in algae (Goss and Lepetit

2015). The higher intrinsic NPQ_{max} of the studied Arctic species when grown under HL, as compared to LL and ML conditions, may indicate that this process is activated to reduce PSII damage (Kirilovsky 2015). Indeed, it is well known that light intensity above the photosynthesis saturation point (E_k below 400 µmol photons m^{-2} s^{-1}, Table 2.1) can increase ROS production due to high excitation pressure on PSII caused by CO_2 fixation limitations if photoprotection mechanisms are not efficient (Metsoviti et al. 2019), which was consistent with reduced photosynthetic efficiency (Φ_M and Φ'_M) under HL. Concomitantly, the substantial photoinactivation of PSII was not enough to sustain high-speed electron transport (decrease ETR_{max}) at HL. Therefore, high ROS content and low photosynthetic efficiency (Φ_M and Φ'_M) under HL as compared to ML and LL indicated that cellular defense strategies were not sufficient to deal with photochemical damage and oxidative stress.

2.6.1.2 Difference between Arctic and temperate microalgae

The observed low Chl *a* and low performance index of photons (PI_{ABS}) could be responsible for the lower photosynthetic efficiency (Φ_M and Φ'_M) in both Arctic species compared to their temperate counterparts under all light conditions since Chl *a* is proportional to the number of photosynthetic systems (MacIntyre et al. 2002). Some authors have shown that the A-MP had only half the content of active PSII reaction centers compared to T-MB (Ni et al. 2017). Furthermore, a small amount of Chl *a* is usually present under light-limiting conditions to avoid stacking effects between chlorophylls since Arctic species long-term experience under low light intensity (Yan et al. 2018). As a result, the absence of markedly reduced Chl *a* content in high light adapted Arctic cells compared with low light adapted cells suggested that they did not possess the same light-modulating ability as temperate microalgae. Temperate microalgae usually decrease their light absorption at high irradiance levels by reducing Chl *a* associated with their light-harvesting complexes, as done in both studied temperate species (decreased Chl *a* under HL, Fig. 2.1). Interestingly, the qPQ, which reflects the plastoquinone (PQ) pool size

participating in the electron transport (Xu et al. 2019), significantly increased in two Arctic species under HL, indicating that increased electron supply to the PQ pool was satisfied by rapidly increasing the ability to transfer energy away from PSII, and accompanied by high dissipation capacity (higher qE_{max}) (Fig. 2.2). In comparison, temperate species had reduced qPQ and lower qE_{max} at HL. The redox state of the PQ pool, known to be a signal trigger, plays an important role in light adaptation of algae and regulation of gene expression in chloroplast and nucleus under light modifications (Lepetit et al. 2013, Virtanen et al. 2021). In summary, the photoadaptation strategies between temperate microalgae and Arctic microalgae are not the same. Arctic microalgae appear to mainly rely on increased PQ pool size and strong dissipation capacity (qE_{max}) to cope with higher light intensity, whereas their temperate counterparts appear to mainly rely on the reduction of pigments associated with light-harvesting complex (LHC) to regulate light absorption. In addition, we observed substantial differences in the adaptation capacity of the two microalgal classes. The diatoms had higher growth rate (μ), higher light efficiency use (α) and higher photosynthetic electron transfer rate ($rETR_{max}$) relative to the green microalgae, exhibiting their high photoadaptation capacity to change in ambient light intensity (Table 2.1). This is one of reason to explain why the dominant class of algae in the Arctic Ocean in seasonal change is diatom (Croteau et al. 2022, Wolf et al. 2018).

2.6.2 Effects of atrazine and simazine on Arctic microalgae and their temperate counterparts under each light condition

Based on the EC_{50} of Φ_M and Φ'_M (Table 2.1), temperate *C. neogracile* was more tolerant to atrazine and simazine than Arctic *C. neogracilis* under all light conditions, while Arctic *M. polaris* was less affected by these herbicides than temperate *M. bravo*, and the sensitivity sequence was T-CN<A-CN<A-MP<T-MP. We found that atrazine was more toxic than simazine in all studied species under all light conditions even though they had the same mode of action. This higher toxicity was attributed to the high log Kow of atrazine resulting in a high affinity for the herbicide

binding site (Ronka 2016). Atrazine and simazine, as photosynthetic inhibitor herbicides, can bind to the Q_B site of D1 protein on PSII (Bai et al. 2015). Therefore, the observed inhibition of photosynthetic electron transport chains (decreased Φ'_M) in the Arctic and temperate species was observed in the presence of these herbicides, which was also evidenced by lower electron transfer per active RC (ET_0/RC). The transthylakoidal proton gradient required to activate non-photochemical quenching (qE) (Lacour et al. 2018) was also reduced by inhibiting electron transport. As a result, the microalgae's reduced thermal dissipation capacity (qE_{max}) under herbicide exposure prevents the efficient dissipation of excess energy. Therefore, the studied species undergo higher excitation pressure on PSII due to blocked electron transport, resulting in the production of large concentrations of ROS (Fig. 2.5), which eventually can deactivate PSII RC (Gomes and Juneau 2017, Sun et al. 2020). This is in accordance with the increased effective antenna size of active RC (ABS/RC), showing a strong decrease in the active PSII RC population as previously demonstrated under cadmium and high light stress (Du et al. 2019). The reduction of active PSII RCs induced by the ROS produced in the presence of atrazine may explain the decreased photochemistry efficiency of PSII (Φ_M), which was ultimately reflected in the growth inhibition of all studied species under the different light conditions. This observation was consistent with previous studies showing that photosynthesis inhibitor herbicides (diuron and Irgarol) inhibit the growth of freshwater microalgae (Kottuparambil et al. 2017). In addition, the reduction of Chl *a* in all species (Fig. 2.1) was attributed to the continuous accumulation of ROS in the presence of atrazine or simazine (Almeida et al. 2017). Atrazine and simazine disturbed the balance between light absorption and energy utilization, since PI_{ABS}, as an indicator for energy conservation from photons absorbed by PSII to intersystem electron acceptors, significantly decreased no matter the growth light intensity (Sun et al. 2020).

2.6.3 Combined effects of growth light intensity and atrazine on Arctic microalgae and their temperate counterparts

Significant interactions between growth light intensity and atrazine were observed for growth, Φ_M, Φ'_M, and PI_{ABS} ($P<0.0001$). Atrazine was chosen to describe the interaction with light since the obtained results for simazine were similar due to their same mode of action. According to the EC_{50} of Φ_M and Φ'_M, all studied species were more affected by atrazine under HL compared to the other lower light intensities (LL and ML) (Table 2.1). Although atrazine and light alone respectively decreased the light energy conversion (PI_{ABS}), interestingly it appears that combination with HL and herbicides reversed this effect on PI_{ABS} (Table 2.2), indicating that photoadaptation processes, such as changes in pigments, mitigate the effects of herbicides on light energy conversation. Indeed, lower Chl *a* in both Arctic microalgae and higher Car concentration in both temperate species may decrease respectively light absorption and ROS scavenging, which help to protect the photosystem apparatus. The combined inhibitory effect was small in tolerant species (T-CN and A-MP) to atrazine compared to the sensitive species (A-CN and T-MB). Energy change in PSII photochemistry is usually associated with modifications of the energy dissipation pathway (qE_{max}) (Du et al. 2019). The combined effect of HL and atrazine (50 µg/L, concentration near the EC_{50} for most species) on the thermal dissipation ability (qE_{max}) of Arctic species was greater than the effects of atrazine alone, even though high qE_{max} was already induced after high light adaptation. While the effect of qE_{max} was small in temperate species under the combined conditions of HL and atrazine, even though HL and atrazine alone decreased qE_{max} (Table 2.2). Therefore, we can suggest that other parts of the non-photochemical energy dissipation process (qT or qI) in temperate species were highly effective under HL adaptation since some authors have shown that qE was in general much lower on green microalgae than in diatoms since qT is more important than qE in green algae of *Chlamydomonas* (Allorent et al. 2013). These results showed that qE_{max}, as an indicator of the thermal dissipation capacity, was less indicative than Φ'_M and PI_{ABS}, probably because the later integrated the effects of the herbicide and HL on photosynthetic

electron transport and related energy dissipation processes, while qE_{max} only represents one of the components of NPQ. This is in accordance with a recent study showing that the performance index PI_{ABS} was a more convincing indicator of atrazine toxicity compared to growth rate and Φ_{E0} (Sun et al. 2020). On the other hand, the combination of HL effect and 50 µg/L atrazine of microalgae mitigated the ROS burst (Table 2.2), which was attributed to the increased non-photochemical quenching (NPQ_{max}) after HL adaptation to dissipate excess energy to reduce the excitation pressure on PSII (Silva et al. 2021). Furthermore, with the exception of T-CN, the removal of atrazine by microalgae increased with increasing culture light intensity (LL-ML-HL), which can largely explain their high herbicide toxicity under high light intensity. As a result, we can assume that even the effective induction of photosynthetic defense measures (NPQ and Car content) upon high light adaptation was unable to deal with the combination of higher light intensity and herbicides. Interestingly, the growth inhibition caused by atrazine of temperate species grown under HL was alleviated more than the cells grown under LL and ML (Fig. 2.1). This can be explained that temperate microalgae can effectively utilize the absorbed light energy and perform reasonable energy allocation (high α and $PI_{ABS,}$ Table 2.1) for carbon fixation. However, Arctic microalgae grown under HL were unable to prevent growth inhibition caused by atrazine due to the observed inefficient capacity of light absorption and light energy conversion (Fig. 2.3).

In conclusion, we demonstrated that light-adaption responses between Arctic and temperate microalgae were different. These differences influence the toxicity of atrazine when the growth light intensity was increased, showing that the microalgal protection measures NPQ and Car (photoprotective pigment) were insufficient handle the combined impacts of high light intensity and atrazine stress, as atrazine removal ability was enhanced with increasing growth light intensity. As a result, light seems to play a non-negligible role in regulating the toxicity of herbicide in microalgae, suggesting that light intensity should be considered when assessing the risk assessment of herbicides in temperate ecosystems, because the photoprotective strategies and the removal ability of atrazine can be affected by the light intensity.

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CHAPTER III

HOW LIGHT INTENSITY MAY INFLUENCE THE TOXICITY OF FRESHWATER PHYTOPLANKTON OF SINGLE HERBICIDE OR IN BINARY MIXTURES

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3.1. Résumé

Certains mélanges d'herbicides inhibiteurs de la photosynthèse ont des effets synergiques sur l'inhibition de la chaîne photosynthétique de transport d'électrons. La capacité de protection contre l'excès de lumière est impliquée dans la tolérance d'un seul pesticide. Le phytoplancton de l'écosystème aquatique est simultanément stressé par les fluctuations d'intensité lumineuse et les mélanges de pesticides. À notre connaissance, la façon dont l'intensité lumineuse affecte la toxicité des pesticides mélangés est très limitée. Ainsi, nous avons évalué l'influence de la lumière (40-VLL, 100-LL, et 400-ML photons µmol m^{-2} s^{-1}) sur la toxicité de l'atrazine et de la simazine, seules et en mélanges sur trois espèces de phytoplancton d'eau douce (*Chlorella vulgaris*-CPCC90, *Microcystis aeruginosa*-CPCC299 et CPCC 632). Nous avons constaté que les trois espèces cultivées dans des conditions LL étaient plus photosynthétiquement affectées par l'atrazine et la simazine que lorsqu'elles sont cultivées dans des conditions VLL. Cependant, CPCC90 et CPCC632 adaptées à la ML étaient moins affectées par l'atrazine et la simazine que dans les conditions VLL et LL. Un mélange d'atrazine et de simazine a produit des effets synergiques (pour CPCC90), additifs (pour CPCC299) et antagonistes (pour CPCC632) sur la toxicité au niveau de la photosynthèse des trois espèces à 40 µmol de photons m^{-2} s^{-1}, et ce type d'interaction pour les deux cyanobactéries (CPCC632 et CPCC299) passe à la synergie lorsque l'intensité lumineuse augmente (40-100 µmol photons m^{-2} s^{-1}, 40-400 µmol photons m^{-2} s^{-1}). À des intensités lumineuses de 100 et 400 µmol photons m^{-2} s^{-1}, la capacité photoprotectrice (NPQ) était extrêmement sensible aux effets inhibiteurs de l'atrazine et de la simazine seules, ainsi qu'à leurs mélanges. Nos résultats démontrent que les facteurs environnementaux (non chimiques) dans les habitats d'eau douce peuvent augmenter la toxicité induite lorsque les herbicides affectant la photosynthèse sont mélangés.

Mots clés: Phytoplancton d'eau douce, atrazine, simazine, toxicité des mélanges, lumière, photosynthèse

3.2. Abstract

Some mixtures of photosynthetic inhibitor herbicides have synergistic effects on the inhibition of the photosynthetic electron transport chain. Light-sensitive photoprotective ability is involved in the tolerance of a single pesticide. Phytoplankton in the aquatic ecosystem is simultaneously stressed by light intensity fluctuations and pesticide mixtures. To our knowledge, how light intensity affects the toxicity of mixed pesticides is very limited. Thus, we assessed the influence of light (40-VLL, 100-LL, and 400-ML μmol photons m^{-2} s^{-1}) on the toxicity of atrazine and simazine, alone and their combination to three freshwater phytoplankton (*Chlorella vulgaris*-CPCC90, *Microcystis aeruginosa*-CPCC299, and *Microcystis aeruginosa* CPCC 632). We found that three species grown under LL condition were more photosynthetically toxic to atrazine and simazine on photosynthesis than cells grown under VLL condition. However, ML-adapted CPCC90 and CPCC632 were less toxic to atrazine and simazine compared to VLL and LL conditions. A mixture of atrazine and simazine produced synergistic (CPCC90), additive (CPCC299), and antagonistic (CPCC632) effects on the photosynthesis of the three species at 40 μmol photons m^{-2} s^{-1}, and this type of interaction in both cyanobacteria (CPCC632 and CPCC299) shifted to synergy when light intensity increase (40-100 μmol photons m^{-2} s^{-1}, 40-400 μmol photons m^{-2} s^{-1}). At 100 and 400 μmol photons m^{-2} s^{-1} light intensities, photoprotective ability (NPQ) was extremely sensitive to the inhibitory effects of atrazine and simazine alone, as well as their mixtures. Our results demonstrate that environmental factors (non-chemical) in freshwater habitats can enhance the toxicity induced when herbicides affecting photosynthesis are mixed.

Keywords:

Freshwater phytoplankton, atrazine, simazine, mixture toxicity, light, photosynthesis

3.3. Introduction

An important anthropogenic impact on freshwater ecosystems is the rise in pesticide usage in regions of intensive agriculture (Melero-Jimenez et al. 2021). Due to the long half-life of most herbicides, causing their high persistence in water bodies, these chemicals can exert toxic effects on numerous non-target organisms, such as phytoplankton and cyanobacteria (Arts and Hanson 2018, Melero-Jimenez et al. 2021, Smedbol et al. 2018). Atrazine and simazine are frequently detected in aquatic ecosystems since they are widely used in agriculture because of their low cost and high efficiency (Giroux 2015, 2019). Concomitantly, concentrations currently observed in some areas exceed the aquatic life protection standards of 1.8 g/L in Canada and 1.5 g/L in the USA (MDDEP 2008, US EPA 2004). The maximum residue for simazine in drinking water is limited to 4 μg/L based on the EPA directive (Callahan 1980). Atrazine levels in water bodies near agricultural areas can reach up to 30 μg/L (Sullivan et al. 2009). Simazine has been detected in surface waters with peak concentrations up to 1.2 μg/L (Li et al. 2018). Atrazine and simazine are well-known photosynthetic inhibitor herbicides that can bind to the plastoquinone B (Q_B) site of the D1 protein on PSII to block the photosynthetic electron transport chain between photosystem II (PSII) and photosystem I (PSI). This blockage results in a reduction of ATP and NADPH required for carbon fixation, which affects the growth of algae and higher plants (Bai et al. 2015, DeLorenzo 2001, Gomes and Juneau 2017). Furthermore, this inhibition of the photosynthetic electron transport chain caused by photosynthesis inhibitor herbicide also induces ROS generation, and the constant accumulation of ROS induces cellular oxidative damage, leading to the degradation of lipids, proteins, and pigments connected to the photosynthetic apparatus (Singh et al. 2016, Wang et al. 2020). Consequently, herbicides could decrease the primary productivity of phytoplankton and have significant effects on ecosystems at a variety of levels due to their toxic effects (Melero-Jimenez et al. 2021, Zhao et al. 2020).

In aquatic ecosystems impacted by human activities, phytoplankton as primary producers, are exposed to a mixture of chemicals rather than to a single chemical. Thus, studying the toxicity of

a single chemical is insufficient to evaluate the environmental risk since interactions between substances can occur (Gonzalez-Pleiter et al. 2013, Magdaleno et al. 2015). The impact of the binary combination of pesticides on phytoplankton has been previously studied and it was demonstrated that these chemicals cause greater toxicity than the sum of each one alone (Bighiu et al. 2020, Dupraz et al. 2019a, Liu et al. 2013). Pesticides and antifouling biocides mixed in binary were reported to exhibit synergistic effects in approximately 7% and 26% of cases (reviewed by Cedergreen 2014). Further to the synergistic effects, binary mixtures of pesticides may also have additive effects or antagonistic effects (lower effects than additive) on algal physiology (Crain et al. 2008). The type of interaction effects observed depends on the mode of action of the chemicals and the sensitivity of the physiological and protective mechanisms (non-photochemical quenching and antioxidant enzyme activity) of the affected organisms (Korkaric et al. 2015).

In aquatic environments, light intensity is one of the environmental factors that can alter phytoplankton photosynthesis (Virtanen et al. 2021). Therefore, in contaminated waters, the effects caused by herbicides affecting photosynthesis can be modulated by light intensity, resulting in a different response than the one expected when these stressors are present alone (Fischer et al. 2010). Some studies have clearly demonstrated the importance of considering the mechanisms involved in the interaction between light and herbicides in aquatic ecosystems (Deblois et al. 2013a, Gomes and Juneau 2017). However, most studies on phytoplankton to date have focused only on the interactions between an environmental factor and a single pesticide. Indeed, light variation and mixture of pesticides can occur simultaneously in freshwater habitats. To our knowledge, there is no information on how light intensity affects the toxicity of mixtures of herbicides. Therefore, we investigated, in the present study, the combined effects of light intensities and atrazine and simazine (alone and in binary mixtures) on the growth, and photosynthetic processes of three freshwater phytoplankton.

3.4. Materials and methods

3.4.1. Phytoplankton

Microcystis aeruginosa CPCC632 (non-toxic strain) and CPCC299 (toxic strain) and *Chlorella vulgaris* CPCC90 (green algae) were obtained from the Canadian Phycological Culture Centre (East Boothbay, USA). Each species was cultivated in 250 mL flasks with a total volume of 100 mL BG11 growth medium (Devgoswami et al. 2011). The cultures were grown (for a minimum of eight generations) under three different light intensities (40, 100, and 400 µmol photons m^{-2} s^{-1}) at 24 °C with a light: dark (14:10 h) illumination cycle and shook every day. To keep cells in their exponential growth phase they were transferred regularly into fresh growth medium. Sub-samples were collected every day to quantify biovolume and cell density by using Multisizer 3 Coulter Counter particle analyzer (Beckman Coulter Inc., USA). The following formula was used to evaluate the growth rate (µ), $\mu = (\ln N_n) - (\ln N0)/(t_n - t_0)$.

3.4.2. Herbicide and high light exposures

Atrazine and simazine were obtained from Sigma-Aldrich (PESTANAL®, analytical standard, Canada). Pure acetone (≥ 99%) was used as the solvent for dissolving pesticide stock solutions. Cultures were harvested while in their exponential growth phase and placed into sterile 24-well transparent polystyrene microplates with an initial cell density of 2.5×10^6 cells/ml and exposed to 0, 5, 25, 50, 100, and 250 µg/L atrazine or simazine for 72 h under three different light intensities. For each herbicide, concentration-response tests were carried out to determine the 72h-EC_{50}. The final percentage of acetone used in the microplate was 0.01%. Each sample has four replicates.

High light intensity treatments (HL; 1100 µmol photons m^{-2} s^{-1} for 60 min) were offered by a halogen lamp (250 W; Winchester, UK) following the 72 h exposure to growth light intensities

(with or without herbicides, alone or in mixtures). At the end of the experiment, cell density and cell biovolume were measured by using the Multisizer 3 Coulter Counter particle analyzer (Beckman Coulter Inc., USA).

3.4.3. Mixture toxicity tests

The EC_{50} of the operational PSII quantum yield (Φ'_M) from the single herbicide toxicity test was used to determine the herbicide concentration in the binary mixture experiments. Concentration-response tests were conducted on the single herbicide (considered as a mixture ratio of 0:100% and 100:0%) and mixtures at two effective concentration ratios of 75:25%, and 25:75% (atrazine: simazine), using four concentrations for two mixture ratios in four replicates. The isobole model was used to analyze the interactive effect on the mixture of herbicides. More information about this model is described in (Dupraz et al. 2018).

3.4.4. Chlorophyll fluorescence measurements

Maxi-Imaging-PAM chlorophyll fluorometer (Pulse amplitude modulation, Heinz Walz GmbH, Effeltrich, Germany) was used to determine light curves with a series of 60 s light exposures to 12 levels of irradiance (1, 21, 56, 83, 111, 186, 281, 336, 396, 461, 531 and 611 µmol photons m^{-2} s^{-1}) according to (Zhong et al. 2021), after being exposed to various herbicide concentrations/ratios for 72. The Φ_M (maximum PSII quantum yield), Φ'_M (operational PSII quantum yield), and NPQ (non-photochemical quenching) were then evaluated from the obtained light curves according to (Bilger and Björkman 1990, Genty et al. 1989).

3.4.5. Statistical analysis

By using the R opensource software 4.2.1, the isobole model curve with the 'drc' package for analyzing the concentration-response curves was obtained. More details are provided in (Dupraz

et al. 2018). The EC_{50} was obtained from the nonlinear least-square fits by using the regression curve inversely (described in Van der Heever and Grobbelaar 1996).

3.5. Results

3.5.1. Effects of growth light intensity

The growth of three phytoplankton species (both *M. aeruginosa*-CPCC632 and CPCC299, and *Chlorella vulgaris*-CPCC90) was significantly enhanced with increasing growth light intensity (VLL-LL-ML, VLL-40, LL-100, ML-400) with the exception of CPCC632 at ML compared to LL (Fig. 3.1, Tukey's HSD, $P < 0.05$). The maximal PSII quantum yield (Φ_M) of CPCC632 without atrazine and simazine treatment significantly increased with increasing growth light intensity (VLL-LL-ML, Tukey's HSD, $P < 0.05$). On the other hand, the operational PSII quantum yield (Φ'_M) of CPCC632 decreased remarkably with increasing growth light intensity (Fig. 3.2, Tukey's HSD, $P < 0.05$). For CPCC299, Φ_M and Φ'_M decreased with increasing growth light intensity (VLL-LL, Fig. 3.2). Interestingly, CPCC299 grown under the ML condition had the same growth rate as VLL and LL conditions, but the values of Φ'_M and Φ'_M were almost zero. Therefore, the results of CPCC299 grown under ML condition are no longer considered in the following discussion as there was no photosynthetic activity. Φ_M and Φ'_M of CPCC90 also declined significantly under ML compared to VLL and LL conditions and the decline amplitude of Φ'_M was greater than Φ_M (Tukey's HSD, $P < 0.05$). On the other hand, while CPCC299 did not have any non-photochemical quenching (NPQ) when grown under ML condition, the NPQ of CPCC632 and CPCC90 grown under this condition were five times higher than under the LL condition (Table 3.1). Surprisingly, NPQ of CPCC632 and CPCC299 grown under the VLL condition was absent, but CPCC90 grown under VLL condition had similar NPQ levels as in the LL condition (Table 3.1).

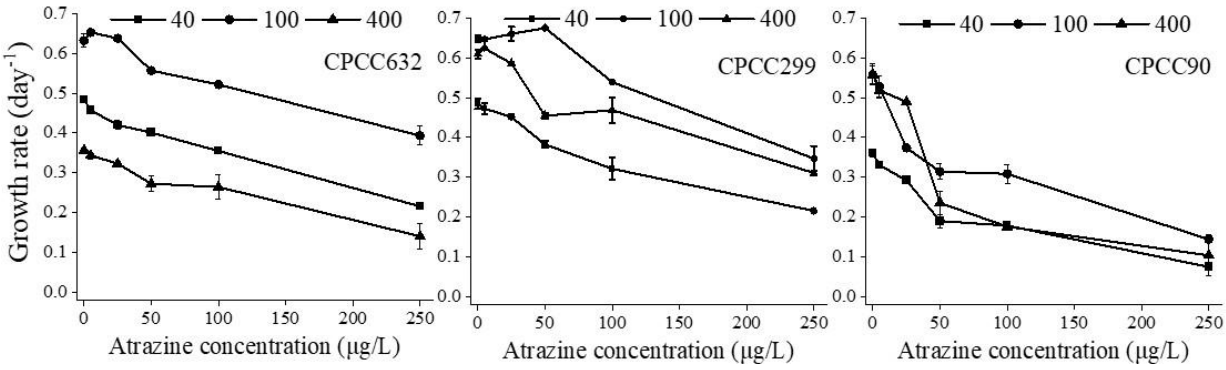

Figure 3. 1. The effect of growth light intensities (40 µmol photons m^{-2} s^{-1}: square; 100 µmol photons m^{-2} s^{-1}: circle; 400 µmol photons m^{-2} s^{-1}: triangle) on the growth rate of three phytoplankton species (CPCC632, CPCC90, and CCPC299) after exposure to various atrazine concentrations for 72 h. Data presented as means ± SD (n = 4-8).

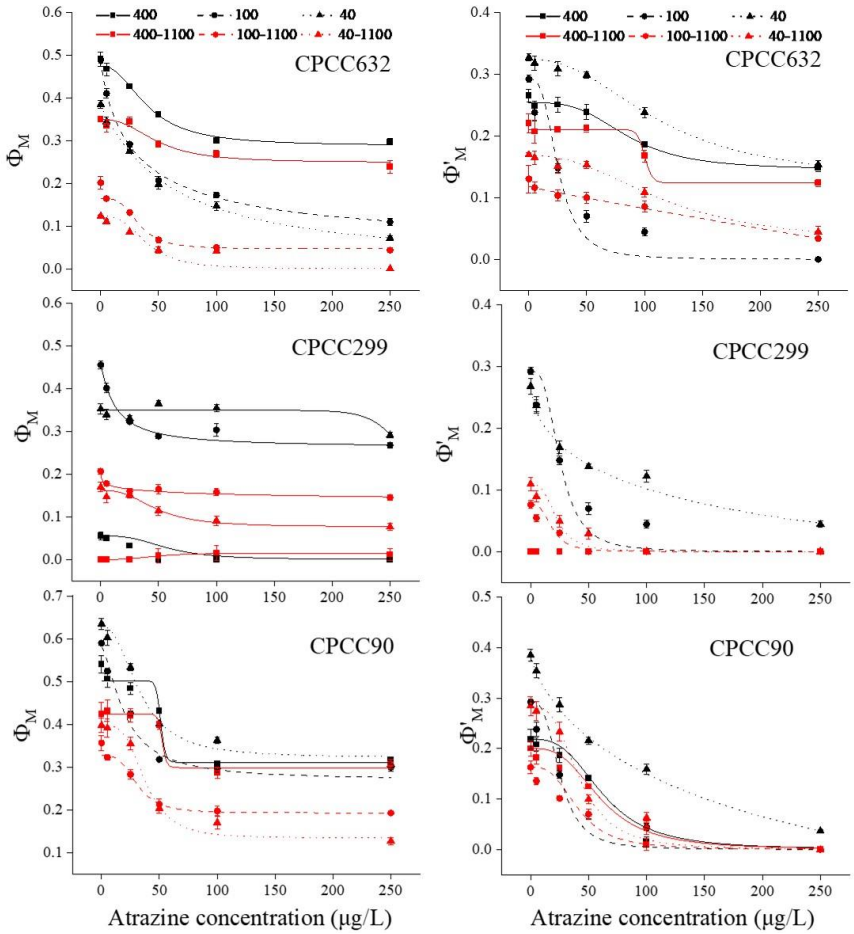

Figure 3. 2. The effect of growth light intensities (40 µmol photons m^{-2} s^{-1}: square; 100 µmol photons m^{-2} s^{-1}: circle; 400 µmol photons m^{-2} s^{-1}: triangle) on the maximum (Φ_M) and operational (Φ'_M) PSII quantum yields of three phytoplankton species (CPCC632, CPCC299, and CCPC90) after being exposed to various atrazine concentrations for 72 h, and subsequently shifted to high light condition for 60 min (red color 400-1100: straight line; 100-1100: dotted line; 40-1100: dashed line). Data presented as means ± SD (n = 4-8).

Table 3. 1. NPQ and EC_{50} values of the growth rate and operational PSII quantum yield (Φ'_M) for three phytoplankton under three different light intensities (VLL-40 µmol photons m^{-2} s^{-1}, LL-100 µmol photons m^{-2} s^{-1}, ML-400 µmol photons m^{-2} s^{-1}) after being exposed to various concentrations of atrazine and simazine for 72 h and Φ'_M-EC_{50} after being shift to HL-1100 µmol photons m^{-2} s^{-1} for 60 min. Data expressed as means ± SD (n = 4).

Species	Growth light	NPQ	μ-EC_{50} (μM)		Φ'_M-EC_{50} (μM)		Treat light	Φ'_M-EC_{50} (μM)	
			Atrazine	Simazine	Atrazine	Simazine		Atrazine	Simazine
CPCC632	40	0.00±0.01	217.1±12.7	296.4±21.1	66.4±8.5	296.4±21.1	40 -1100	20.6±11.4	142.8±12.6
	100	0.13±0.01	608.4±39.6	154.1±7.3	22.3±1.4	154.1±7.3	100-1100	29.2±0.5	142.7±23.1
	400	0.54±0.14	99.4 ±5.6	224.0±9.0	54.9±4.0	224.0±9.0	400-1100	64.5±5.8	291.9±22.7
CPCC299	40	0.00±0.01	196.9±14.5	245.8±36.3	69.1±9.5	235.8±14.8	40 -1100	21.1±2.4	92.6±17.5
	100	0.09±0.06	346.5±12.4	378.6±38.9	16.1±3.1	131.2±4.1	100-1100	16.6±8.7	108.6±10.9
	400	0.12±0.01	426.2±34.6	497.4±53.7	N.D	N.D	400-1100	N.D	N.D
CPCC90	40	0.08±0.01	38.2 ±2.1	59.3 ±9.7	75.8±1.1	539.1±49.7	40 -1100	43.2±2.1	477.6±126
	100	0.10±0.02	116.4±9.5	163.8±21.3	30.8±1.1	323.5±15.5	100-1100	44.9±12.5	339.6±77.1
	400	0.49±0.03	68.2 ±3.6	73.8±12.3	57.9±2.1	213.8±24.5	400-1100	55.2±4.0	438.9±76.1

3.5.2. Effects of growth light intensity on atrazine and simazine toxicity

The growth of both *M. aeruginosa* (CPCC632 and CPCC299) and *Chlorella vulgaris* (CPCC90) were inhibited to varying degrees after atrazine treatment for 72 h under three different light intensities (VLL, LL, and ML) (Fig. 3.1). Moreover, the three phytoplankton species grown under LL condition exhibited a lower inhibitory effect on growth than cells grown under VLL. Under ML condition, the growth of three phytoplankton was more inhibited in the presence of high concentrations of atrazine (≥ 5 μg/L) relative to the other two light intensities (VLL and LL) as also shown by the μ-EC_{50} (Table 3.1). In addition, simazine had a similar trend in the growth of three microalgal species (data not shown). Under three different growth light intensities (VLL, LL, and ML), Φ_M and Φ'_M of the three studied species showed a different extent of decline with increasing the atrazine and simazine concentrations (Fig. 3.2 and 3.3). We found a greater decline in Φ_M and Φ'_M under LL condition in the presence of atrazine and simazine compared to cells exposed under VLL condition. However, Φ_M and Φ'_M of CPCC90 and CPCC632 were less reduced for cells grown under ML compared to VLL and LL conditions, except at high herbicide concentrations of 100 and 250 μg/L. The NPQ of the three studied species under VLL, LL, and ML conditions also showed a significant downward trend when the concentration of atrazine and simazine increased (Fig. 3.4, Tukey's HSD, $P < 0.05$), and the reduction in NPQ under LL condition was stronger than in cells grown under ML conditions. Moreover, NPQ completely disappeared at high concentrations of atrazine and simazine (≥ 50 μg/L) (Fig. 3.4).

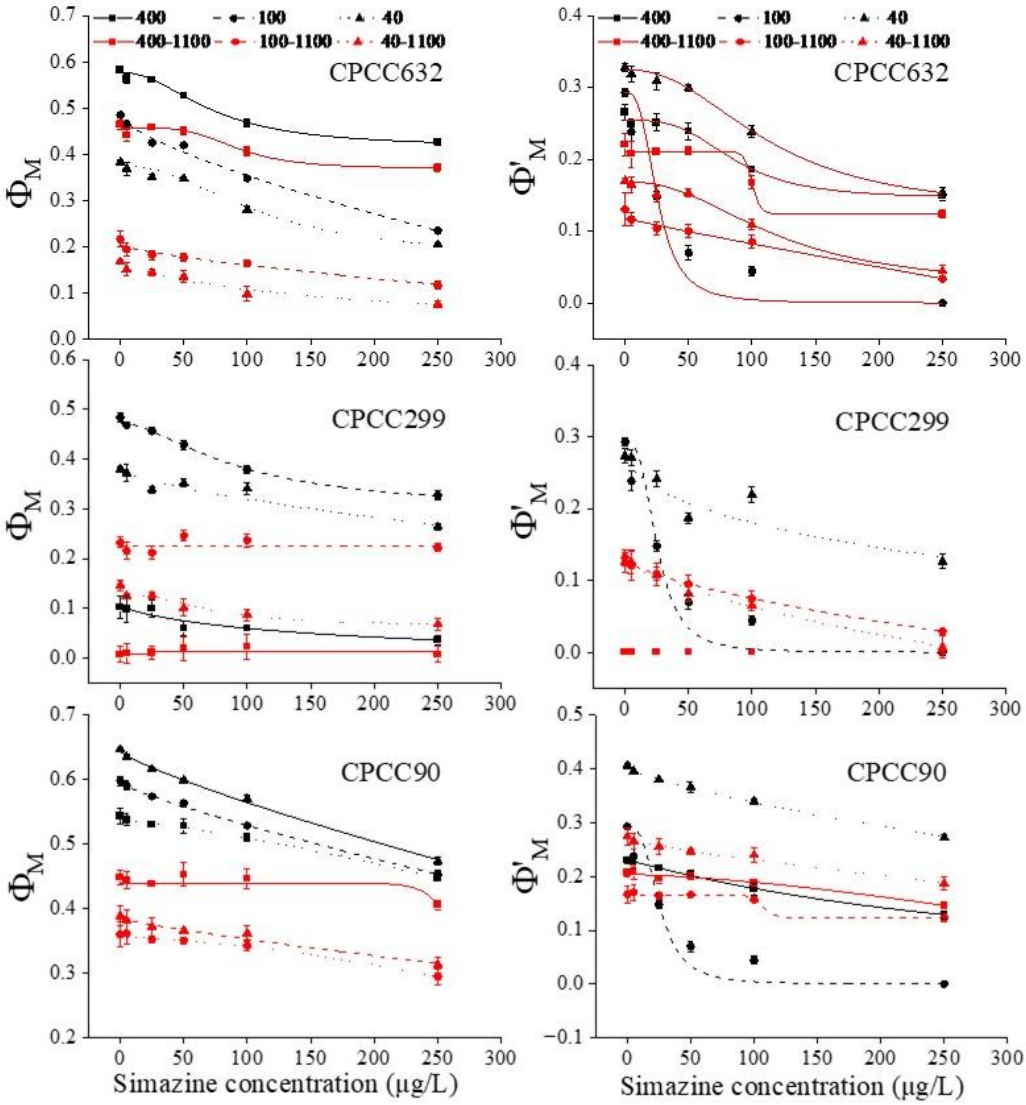

Figure 3. 3. The effect of growth light intensities (40 µmol photons m^{-2} s^{-1}: square; 100 µmol photons m^{-2} s^{-1}: circle; 400 µmol photons m^{-2} s^{-1}: triangle) on the maximum (Φ_M) and operational (Φ'_M) PSII quantum yields of three phytoplankton species (CPCC632, CPCC299, and CCPC90) after being exposed to various simazine concentrations for 72 h, and subsequently shifted to high light condition for 60 min (blue color 400-1100: straight line; 100-1100: dotted line; 40-1100: dashed line). Data presented as means ± SD (n = 4-8).

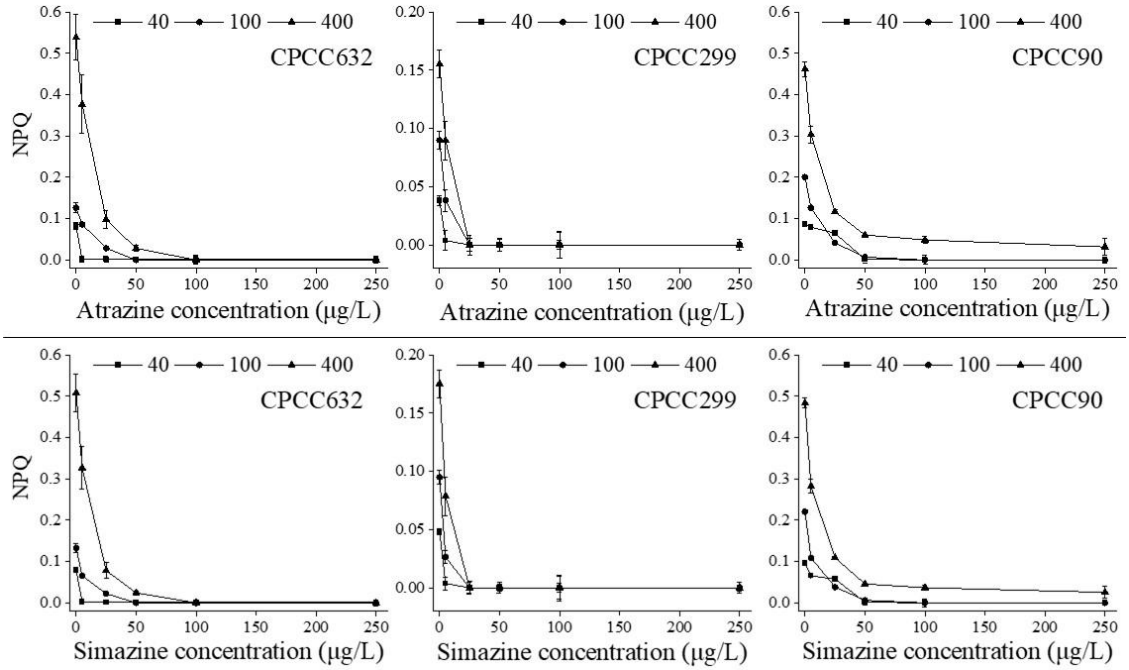

Figure 3. 4. The effects of growth light intensities (40 µmol photons m^{-2} s^{-1}: square; 100 µmol photons m^{-2} s^{-1}: circle; 400 µmol photons m^{-2} s^{-1}: triangle) on the non-photochemical quenching (NPQ) of three species (CPCC632, CPCC90, and CCPC299) after being exposed to various atrazine and simazine concentrations for 72 h. Data presented as means ± SD (n = 4-8).

3.5.3. Effects of high light intensity exposure

When the three phytoplankton species (without herbicide treatment) grown under were transferred to high light intensity (HL: 1100 µmol photons m^{-2} s^{-1}) for 60 min (VLL-HL, LL-HL, and ML-HL), their Φ_M and Φ'_M significantly decreased (Tukey's HSD, $P < 0.05$, Fig. 3.2 and 3.3). The observed decline amplitude of Φ'_M was greater than Φ_M for all species under all growth light conditions. NPQ of three species also significantly decreased when cells were transferred to HL for 60 min, CPCC90 and CPCC 632 respectively decreased 1.9 and 3.2 times from ML to HL (Tukey's HSD, $P < 0.05$, data not shown).

3.5.4. Effects of high light intensity exposure on atrazine and simazine toxicity

After 72 h of atrazine and simazine treatment under the three growth light intensities and a subsequent HL exposure for 60 min (VLL-HL, LL-HL, and ML-HL), a significant decrease in Φ_M and Φ'_M of all species was observed with increasing atrazine and simazine concentrations (Fig. 3.2 and 3.3). It appears that the decline in Φ_M and Φ'_M of CPCC90 and CPCC632 subsequently shifted to HL after grown under ML and was less than in cells shifted to HL after grown under VLL and LL (Fig. 3.2A-B, E-F and 3.3A-B, E-F). It was also observed in the EC_{50}-Φ'_M (Table 3.1), EC_{50}-Φ'_M of CPCC632 at ML to HL was 3 and 2 times more than those of in LL to HL and VLL to HL respectively in the presence of atrazine. Under the same treatment, EC_{50}-Φ'_M of CPCC90 at ML to HL was only higher than 1.3 times than those of in VLL and LL to HL in the presence of atrazine. In this case, EC_{50}-Φ'_M of CPCC90 and CPCC632 in the presence of simazine showed a similar trend to atrazine, but EC_{50}-Φ'_M of simazine was higher than those of atrazine under any treatment conditions. For CPCC299, this was observed for LL grown cells (Fig. 3.2C-D and 3.3C-D). For the studied species, in presence of atrazine or simazine, the NPQ was not present when they were exposed to the HL treatment for 60 min (data not shown).

3.5.5. Effects of the atrazine-simazine mixture

The mixture toxicity of atrazine and simazine on CPCC90 induced a synergistic effect at the three light intensities (40-VLL, 100-LL, and 400-ML, Fig. 3.5). Moreover, the synergistic effect was enhanced with increasing the growth light intensity (VLL-LL, LL-ML). Antagonism was obtained for CPCC632 under VLL condition, but the interaction shifted to synergism under LL and ML light intensities. This synergistic effect was boosted with increasing the growth light intensity (LL to ML). A slight but significant additive effect was found for CPCC299 between the two studied herbicides under VLL condition, but the interaction became synergistic under LL light intensity. On the other hand, the decreasing trend of ΦM in CPCC90 and CPCC299 under ML

condition after mixed herbicides treatment for 72h was lower than that of VLL and LL condition cells, ΦM in CPCC299 did not significantly change under the same situation (Table 3.2, Tukey's HSD, $P < 0.05$). For Φ'M, CPCC299 under the LL condition decreased less relative to the VLL condition, but CPCC90 and CPCC299 did not significantly change (Table 3.2, Tukey's HSD, $P < 0.05$). Furthermore, ΦM and Φ'M in CPCC90 and CPCC299 under ML condition after HL treatment 60 min decreased more than cells under VLL and LL conditions, and Φ'M in CPCC632 did not significantly change under the same situation (Table 3.2, Tukey's HSD, $P < 0.05$).

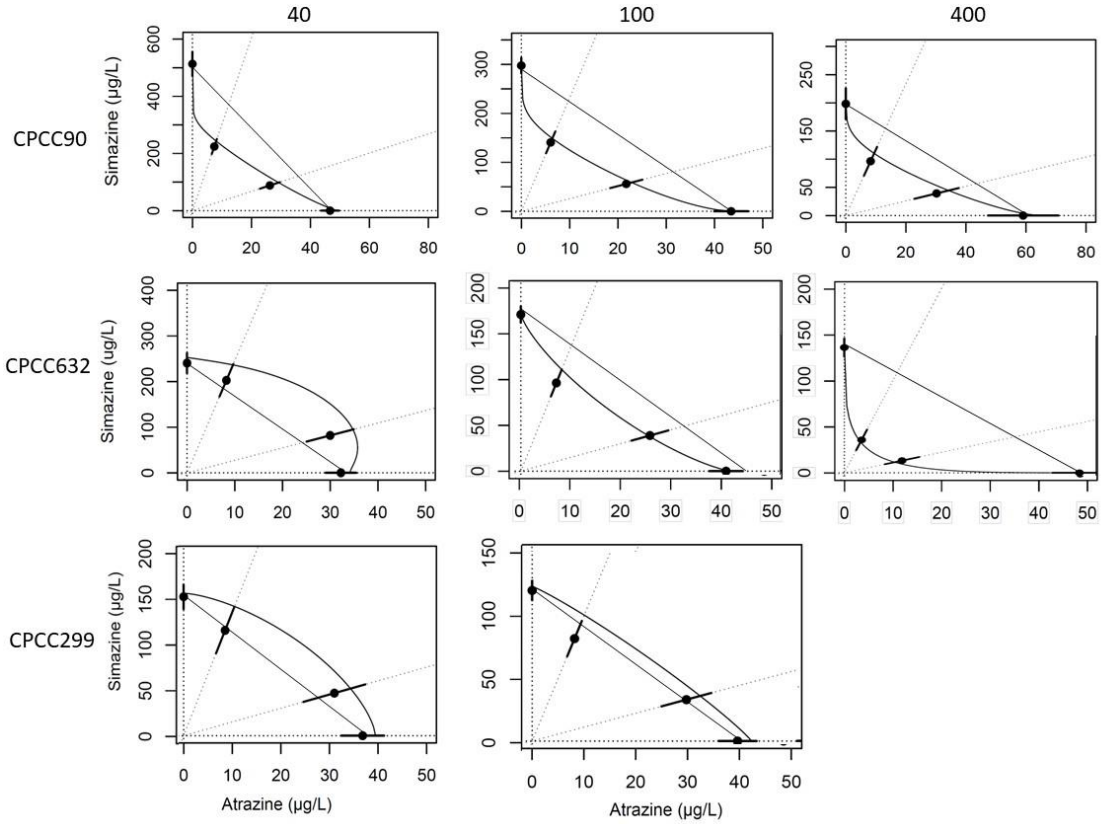

Figure 3. 5. Isobolograms of binary herbicide mixtures with the same mode of action under different light intensities for three species (CPCC632, CPCC299, and CCPC90). The dots show the $EC_{50} \pm 2$ standard error. The solid line indicates the CA isobole.

Table 3. 2. The effect of growth light intensities (40 µmol photons m^{-2} s^{-1},100 µmol photons m^{-2} s^{-1}, 400 µmol photons m^{-2} s^{-1}) on the maximum (Φ_M) and operational (Φ'_M) PSII quantum yields of three phytoplankton species (CPCC632, CPCC299, and CCPC90) after being exposed to

mixture herbicides (atrazine*simazine=EC_{25}:EC_{25}) for 72 h, and subsequently shifted to high light condition for 60 min. The numbers in parentheses are the decreased percentages relative to the control. N.D = not determined. Different superscript letters (a-b) indicate significant differences between the percentages (Tukey's HSD, $P < 0.05$). Data presented as means ± SD (n = 4-8).

Species	Growth light (μmol photons m^{-2} s^{-1})	Φ_M	Φ'_M	Treat light (μmol photons m^{-2} s^{-1})	Φ_M	Φ'_M
CPCC632	40	0.239±0.01 (39)^{a}	0.174±0.01 (48)^{a}	40 -1100	0.087±0.01 (36)^{a}	0.050±0.01 (56)^{a}
	100	0.306±0.02 (41)^{a}	0.142±0.01 (52)^{a}	100-1100	0.198±0.01 (29)^{ab}	0.080±0.01 (51)^{a}
	400	0.417±0.00 (22)^{b}	0.088±0.01 (56)^{a}	400-1100	0.394±0.01 (16)^{b}	0.089±0.01 (52)^{a}
CPCC299	40	0.200±0.01 (29)^{a}	0.092±0.01 (59)^{a}	40 -1100	0.042±0.01 (36)^{a}	0.000±0.01 (56)^{a}
	100	0.366±0.01 (27)^{a}	0.231±0.01 (40)^{b}	100-1100	0.087±0.01 (51)^{b}	0.029±0.00 (72)^{b}
	400	N.D	N.D	400-1100	N.D	N.D
CPCC90	40	0.448±0.00 (36)^{a}	0.248±0.01 (44)^{a}	40 -1100	0.284±0.01 (6)^{a}	0.134±0.01 (32)^{a}
	100	0.414±0.01 (22)^{b}	0.175±0.01 (40)^{a}	100-1100	0.247±0.01 (28)^{b}	0.072±0.02 (50)^{b}
	400	0.427±0.01 (19)^{b}	0.105±0.01 (51)^{a}	400-1100	0.377±0.03 (27)^{b}	0.100±0.03 (46)^{b}

3.6. Discussion

3.6.1. Effects of single herbicide on phytoplankton grown under different light conditions

Both cyanobacteria of *M. aeruginosa* (including non-toxic and toxic strains CPCC632 and CPCC299) and the green algae *C. vulgaris* exhibited different susceptibilities to atrazine and simazine for growth and photosynthesis under three different light intensities (VLL, LL, and ML). Both cyanobacteria strains were more sensitive to atrazine and simazine than *C. vulgaris* according to their photosynthesis-EC_{50} (Table 3.1). In contrast, μ-EC_{50} indicates that *C. vulgaris* was more sensitive to herbicides than the two *M. aeruginosa*, which agreed with a previous study showing that green algae had higher sensitivity to atrazine (100 μg/L) than cyanobacteria (Lockert et al. 2006). These results indicated that despite *M. aeruginosa* (CPCC299 and CPCC632) have a lower or equal photosynthesis sensitivity to both herbicides, *M. aeruginosa* can support a faster or similar rate of cell division than *C. vulgaris*, depending on the strain. It is well-known that photosynthesis

and respiration share the same electron transport chain in cyanobacteria (Lea-Smith et al. 2016). Therefore, the respiratory chain can also supply electrons by NADPH hydrogenase to the plastoquinone (PQ) pool associated with the photosynthetic electron transport chain (Lea-Smith et al. 2016). In presence of atrazine or simazine that binds to the PSII Q_B binding site, photosynthetic electron transport through the adjacent PQ pool is affected. Therefore, electrons from the respiratory chain may compensate by providing electrons to the PQ pool and help to form ATP and NADPH (Chalifour et al. 2016). Furthermore, cyanobacteria are known to exhibit high levels of cyclic electron flow (CEF) relative to green algae (Peltier et al. 2010), and this process is strongly induced by DCMU, another photosynthesis inhibitor (You et al. 2015). The induction of this alternative electron flow (CEF) in presence of atrazine or simazine may explain the higher growth of both *M. aeruginosa* compared to *C. vulgaris* in these conditions. This might also explain why *M. aeruginosa* CPCC299 grew better when exposed to high concentrations of atrazine and simazine than *C. vulgaris* under LL and ML conditions, as CEF may also be induced by higher growth light conditions (Du et al. 2019).

On the other hand, atrazine and simazine significantly decreased the photosynthetic efficiency (Φ_M and Φ'_M) of both *M. aeruginosa* and *C. vulgaris* and this inhibitory effect was enhanced under LL condition (VLL-LL, Fig. 3.2 and 3.3). However, CPCC632 and CPCC90 after ML adaption were less affected by atrazine and simazine. Indeed, after ML adaption and subsequent exposure to HL exposure for 60 min they were less affected by atrazine and simazine compared to the VLL and LL grown ones. Both situations can be mainly attributed to the highly activated non-photochemical quenching (NPQ) under ML condition (Table 3.1). Indeed, NPQ can dissipate the excess light energy generated by the blockage of the photosynthetic electron transport chain to reduce the overexcitation pressure of PSII (Goss and Lepetit 2015, Müller et al. 2001). Interestingly, the toxic strain CPCC299 had high growth rate but relatively low photosynthetic activity under ML condition, while the non-toxic strain CPCC 632 had a low growth rate and high photosynthetic activity. This difference between the toxic and the non-toxic strains could be linked to the role of microcystin when grown under high light (Xu et al. 2013), but further investigation is needed.

Another reason may be linked to the difference in the photoprotective ability (NPQ) between toxic and non-toxic strains to deal with photodamage under LL and ML conditions. In addition, while atrazine and simazine share the same mode of action, we showed that atrazine was 4-10 times more photosynthetically toxic than simazine for the studied species. For growth, atrazine was approximately 1.5 times more toxic than simazine. These results suggest that the degree of toxicity of the herbicides depends on the evaluated parameters, and thus choosing which parameter to assess for mixed pesticides (with known mode of action) interactions is essential for future mixture research (Moreira et al. 2020).

3.6.2. Effects of mixture herbicides on phytoplankton grown under different light conditions

The assessment of the ecological risk of chemicals using single substance may underestimate the real impact due to the interactions occurring among various chemicals in the natural environment (Bighiu et al. 2020). The concentration addition (CA) model theory is the basis for the isobole method, and it is mostly used to qualitatively analyze the effects of combined chemicals regardless of whether they exhibit synergy or antagonism (Chen et al. 2014). The basic assumption of the CA model is that mixed chemicals have the same mode of action as the individual chemical and that one chemical in the mixture can be replaced by the other and be considered as dilution of each other (Crain et al. 2008). Atrazine and simazine are the most well-known photosynthesis inhibitor herbicides, which mainly affect the photosynthetic electron transport chain (Bai et al. 2015). The different results were obtained when various parameters were used to assess the type of interaction on the mixture of pesticides by the same model (Moreira et al. 2020). Atrazine and simazine had the same mode of action on the photosynthetic electron transport chain, so the parameter Φ'_M (reflecting the efficiency of the entire chain of electron transport) was chosen rather than growth rate or other photosynthetic parameters to improve the accuracy of the interaction assessment. As we expected, Φ'_M was the most sensitive indicator in this study. We observed a stronger reduction in Φ'_M for the mixture of atrazine and simazine for all the species and light

conditions investigated, except for both *M. aeruginosa* strains under VLL condition, compared to the atrazine or simazine alone at all tested concentration, showing that the mixture herbicides produced the synergistic effect on the studied phytoplankton (Fig. 3.5). Considering that atrazine and simazine act similarly on the PSII reaction center, the observed synergy between all phytoplankton might be caused by a combined effect on the photosynthetic apparatus. Regarding the photosynthetic toxicity of mixture herbicides among the three phytoplankton species, it appears that the three species showed different sensitivities to the tested mixture herbicides under different light conditions (VLL, LL, ML). However, CPCC90 was more sensitive to the pesticide mixture than CPCC632 and CPCC299 under VLL and LL conditions, while it was the opposite response with single herbicides. This result suggests that changes in the natural phytoplankton community may occur in contaminated areas depending on the light conditions, since phytoplankton lives at different depths in water bodies. Furthermore, the mixture of pesticides also modifies the studied species' sensitivity sequence to herbicide without considering the light intensity factor. This is in accordance with what was advanced previously that herbicide mixture toxicity plays an important role in explaining phytoplankton changes (Gregorio et al. 2012).

3.6.3. Combination of light and mixture herbicides

It is generally recognized that pesticides can interact with each other in the aquatic ecosystem and light intensity as one of the environmental factors is also one stress factor, thus the combination of multiple factors may contribute to changes in the community (Fischer et al. 2010, Laetz et al. 2014). The present study demonstrated that moderate increases in ambient light boosted this synergistic phototoxicity of CPCC90 and CPCC632 at low or high herbicide concentration ranges (Fig. 3.5). Furthermore, the magnitude of the synergy observed for this mixture also depended on the species: strong for CPCC632, moderate for CPCC90, and close to additivity for CCPCC299. The effect of light as an environmental factor on the toxicity of pesticides in numerous aquatic species has been studied (Baxter et al. 2016). In general, current studies have only assessed the

interaction between light and a single chemical (first-order interactions), increasing light intensity ordinarily enhanced the toxicity (Deblois et al. 2013a, Wood et al. 2016). A few studies have reported that high light intensity can increase the uptake of pollutants, such as cadmium, zinc, and phosphorus (Du et al. 2019, Sforza et al. 2018, Xu and Juneau 2016). Furthermore, we demonstrated that the removal of atrazine from the growth medium by marine phytoplankton (green algae and diatom) was enhanced when light intensity increased (Chapter 2 of this thesis). Our current evaluation between light and mixed herbicides showed lower effect on photosynthetic efficiency (Φ'_M) for the three species when grown under LL condition (additive effect) compared to when they grow under VLL (synergistic effect). This may be attributed to the impact of light intensity on the herbicide uptake, since only light intensity did not affect photosynthetic efficiency (three species having the same Φ'_M under VLL and LL conditions). Another reason to explain the increased binary herbicide mixtures toxicity under higher light intensities was that the photoprotective ability (NPQ) of all species was not highly induced in the LL condition. However, this inhibitory effect of mixed herbicides under ML condition was further enhanced even though NPQ was highly induced. This result suggests that other protective measures, such as the antioxidant enzyme system, may play a major role against the photosynthetic damage caused by mixtures of herbicides (Lozano et al. 2014, Mofeed and Mosleh 2013). Therefore, further mechanistic studies are needed to determine how each of these processes contributes to the overall response. In addition, three species after high light adaptation (ML) decreased the toxicity of mixture herbicides compared to the low light adaptation (VLL and LL), similar to the results for single herbicide (atrazine or simazine). Moreover, this adaptation process was enough to against the dual stress of HL treatment and single herbicide, but it was not sufficient to against the damage caused by the multiple stress of HL treatment and mixed herbicides. Therefore, considering only the effects of a single pesticide and environmental factors (such as light, temperature, and nutrients) may underestimate the toxic effects of herbicides on algal communities since herbicide interaction can occur in water bodies.

As our results show, phytoplankton and other aquatic organisms are sensitive to environmental factors, including light, temperature, and other abiotic variables associated with their aquatic habitats (Baxter et al. 2016, Beyer et al. 2014). Therefore, it is necessary to pursue our quest of understanding the interactions between contaminants in aquatic environments in relation to the variable environmental factors driven by climate change. Additionally, we also identified a possible link between microalgal adverse outcomes and climate change-related environmental stress (Hooper et al. 2013).

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GENERAL DISCUSSION

This thesis has demonstrated that the ecophysiological characteristics of Arctic microalgae are different from their temperate counterparts, and these different features determined the distinct pesticide activity between the Arctic and temperate microalgae. Interestingly, this is contrary to previous prediction, showing that Arctic microalgae are always more sensitive to pollutants compared to temperate microalgae. Furthermore, we can predict, according to our results, that the inhibitory effect of pesticides will be stronger when growing light is increased in the Arctic and temperate oceans. Indeed, since the protective strategies highly activated after high light adaptation was not insufficient to cope with the dual stress of light and pesticides. In addition, light affected the type of interaction (synergy, antagonism, addition) of binary combinations of herbicides with freshwater microalgae.

Main results

In chapter I, Arctic species appear to have evolved different ecophysiological characteristics than their temperate counterparts: Arctic microalgae have (1) lower Chl *a* and carotenoid contents (2) a much higher intrinsic NPQ_{max}, (3) lower ROS content, and (4) higher CAT and SOD activities, compared to temperate species. Our results have shown that the Arctic species (*Micromonas polaris*) was more resistant to atrazine and simazine than its temperate counterpart (*Micromonas bravo*). However, the other Arctic species (*Chaetoceros neogracilis*) was more sensitive to these herbicides than its temperate counterpart (*Chaetoceros neogracile*). Concerning two other pesticide toxicity, both temperate microalgae were more sensitive to trifluralin, while Arctic microalgae were more sensitive to chlorpyrifos (insecticide). The cell size, photoprotective ability (NPQ), and antioxidant enzyme activity (SOD and CAT) can explain these different sensitivities to pesticides.

The result in Chapter II showed that the increased growth light exacerbated the toxic effects of pesticides on Arctic and temperate microalgae, and that this inhibitory effect was stronger in Arctic species than in temperate species. Interestingly, although photosynthetic efficiency decreased with increasing growth light intensity, high light significantly stimulated the growth, suggesting that microalgae can balance energy utilization between growth and photosynthesis to optimize growth under high light conditions. Furthermore, we found that the light adaption capacity of the Arctic diatom was higher than that of Arctic green algae, and thus the microalgal community in the Arctic Ocean should change when global warming causes ice melt to increase the intensity of light received by algal cells. In addition, both arctic and temperate diatoms have higher photoprotective capacity and higher resistance to pesticides than Arctic and temperate green algae, suggesting that diatoms should be the dominant species in the Arctic and temperate oceans in Summer (in presence of higher light intensity) or when exposed to pesticide pollution.

In Chapter III, our findings showed that light can affect the type of interaction (synergy, antagonism, addition) of binary combinations of herbicides with freshwater microalgae, which is highly related to the mixture of pesticide concentration. Moreover, freshwater microalgae adapated to HL reduced the toxicity of atrazine and simazine compared to cells adapated to LL, the protective strategy of NPQ plays an important role in this process. Green algae *Chlorella vulgaris* have strong light adapation ability and high resistance to herbicides compared to cynabacteria *Microcystis aeruginosa*. In addition, the toxic (CPCC299) and non-toxic (CPCC632) cyanobacteria had different toxicity to herbicides under high light conditions, suggesting the toxins maybe play an important role in the process of light adapation or the behavior of herbicides in the process of photosynthesis.

Future directions

Although most of the information on the effects of pesticides on microalgae provides a clear understanding of the mechanisms of pesticide sensitivity, our findings suggest that Arctic

microalgae have different physiological characteristics compared to temperate microalgae, leading to differences in their sensitivity to pesticides, opening a new window for researchers in this field. According to these results, we can anticipate that this knowledge, of the potential impacts of pesticides on Arctic microalgae, will have a direct implication on the use of algal bioassays to evaluate the toxicity of aquatic environments in the Northern regions. Indeed, since the algal bioassays performed nowadays use temperate species, they might be not well suited to investigate contamination of Arctic waters. These data can also be used to support the construction of the model and provide basic information for assessing the effects of light and pesticides on the physiological and photosynthetic processes of Arctic and temperate microalgae in aquatic ecosystems.

Our results suggested that Arctic and temperate microalgae have different response mechanisms to light, pesticides, and their combined effects. The main difference in light adapted responses between Arctic and temperate microalgae is that two Arctic microalgae responded to the inhibition of photosynthetic electron transport under intense light by increasing the size of the PQ pool, rather than regulating light absorption by reducing chlorophyll content (as seen for temperate microalgae). The studied microalgae showed different capacities to respond to light fluctuations from the same aquatic ecosystem. Indeed, the Arctic diatom *Chaetoceros* had a stronger adaptation capacity than the green algae *Micromonas*. Furthermore, even if the main protective mechanism (NPQ) and photoprotective pigment (Car) were highly activated after high light adaptation, it was not enough to resist the dual stress of strong light and pesticides. For instance, the effect of pesticides on microalgae would, therefore, be stronger when microalgae will be exposed to high light in the summer or after possible massive sea ice melt induced by global warming in the coming decades. But the detected pesticide concentrations in the Arctic Ocean are still too low, and future experiments should be conducted on the long-term effects of pesticides on Arctic microalgae. Therefore, these results give an opportunity to explore the entire mechanism of response to long-term exposure to low concentrations of pesticides under various light conditions. Further research

is needed to evaluate the involvement of the various NPQ components in the response of these algae to the studied factors, since they have intrinsic differences in these NPQ components.

It was interesting to find that different physiological characteristics are present in the Arctic and temperate microalgae and that should influence their responses to climate change. One should not forget that variations in light intensity is not the only impact of climate change, therefore further study should also be performed to understand the impact of other factors (such as pH and temperature) modified under climate changes on phytoplankton from the Arctic and temperate regions. Microalgae are essential for primary production in the oceans and are important bait for zooplankton in aquatic environments, and pesticides should also be considered for their deleterious effects on humans through alteration of the aquatic food chain, especially when considering the impact of light intensity on the toxicity of herbicides. Therefore, it indicates the need to find ways to consider an economical, effective, and environmental-friendly way to remove pesticides from different water bodies, or at least reduced as much as possible the input of these substances in the environment.

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