UNIVERSITÉ DU QUÉBEC À MONTRÉAL

ÉTUDE DES EFFETS DE L'INTENSITÉ LUMINEUSE ET DES HERBICIDES SUR LES ALGUES ET LES CYANOBACTÉRIES

THÈSE
PRÉSENTÉE
COMME EXIGENCE PARTIELLE
DU DOCTORAT EN BIOLOGIE

PAR
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JUILLET 2012

REMERCIEMENTS

Merci à mon directeur de recherche, Philippe Juneau, pour son soutien indéfectible tout au long de mes recherches et pour m'avoir donné la chance d'étudier la physiologie des algues dans son laboratoire. Merci aussi à mes collègues notamment Annie Chalifour et Gabrielle Vernouillet pour les discussions constructives, l'aide en laboratoire mais aussi pour leur présence pendant les moments plus festifs ou plus exigeants que nous avons partagés. Merci à Baosheng pour m'avoir accueilli en Chine pour effectuer certains travaux sur les herbicides et pour m'avoir ainsi fait découvrir ce pays et sa culture. Ces travaux n'auraient pas été possibles sans le financement par ces différents organismes: le CRSNG, le FQRNT, les bourses d'excellence de la fondation UQAM et la fondation Francine Beaudoin-Denizeau; merci pour votre support. Un merci bien spécial à Lise Blais qui a fait un travail remarquable d'édition et sans qui ce document ne serait pas aussi présentable.

Je tiens à remercier mes parents qui ont su me soutenir tout au long de mes études en m'encourageant et en m'écoutant. Finalement, un merci bien spécial à ma conjointe Maud et à ma petite fille Éloïse parce qu'elles illuminent quotidiennement ma vie.

AVANT-PROPOS

La motivation principale des travaux présentés dans cette thèse était l'approfondissement des connaissances concernant le milieu aquatique, écosystème aux interactions complexes qui me passionne depuis plusieurs années. Sur une base annuelle, ces écosystèmes sont responsables d'environ 45 % de la production primaire mondiale (Field *et al.*, 1998). Cette importante production provient des organismes photo autotrophes, c'est-à-dire des organismes qui utilisent la photosynthèse pour convertir l'énergie du soleil en énergie chimique et en matière organique. L'énergie chimique est indispensable à tous les êtres vivants et la matière organique est essentielle au maintien des formes de vie plus complexes tout comme l'est l'oxygène issu de la photosynthèse. Les organismes photo autotrophes regroupent un grand nombre d'espèces mais nous nous limiterons ici au phytoplancton qui regroupe les bactéries photosynthétiques (les cyanobactéries) et les microalgues. De nos jours, les écosystèmes aquatiques et les organismes qui y vivent subissent de fortes pressions anthropiques partout sur la planète notamment à cause des activités agricoles. Bien qu'essentielle, la production agricole contemporaine est responsable du rejet annuel de grande quantité d'engrais, de terres arables et de polluants chimiques dans les milieux aquatiques dont ils modifient les propriétés physico-chimiques.

Mes travaux de recherche avaient pour but d'étudier certaines conséquences associées à ces rejets, principalement ceux qui modifient la disponibilité en lumière et ceux qui affectent la charge en polluants chimiques afin d'en comprendre les effets au niveau de la santé et de la dynamique du phytoplancton et des cyanobactéries. Précisément, j'ai orienté mes travaux pour comprendre comment les organismes photosynthétiques s'adaptent aux changements d'intensité lumineuse mais aussi quelle est leur sensibilité face à la présence d'herbicides dans leur environnement. Dans une perspective plus large, j'ai aussi comparé la réponse de différentes espèces face à ces changements en tentant d'évaluer les effets sur l'équilibre de la communauté algale dans son ensemble.

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LISTE DES ABRÉVIATIONS

ABS RC^{-1}	Surface d'absorption effective par centre réactionnel
ADP	Adenosine diphosphate
A.I / A.L	Lumière actinique
ANOVA	Analyse de variance
ANCOVA	Analyse de covariance
APC	Allophycocyanine
ATP	Adenosine triphosphate
ATPase	Complexe de synthèse d'ATP
BBM	Milieu de culture Bold Basal
BBMsi	Milieu de culture Bold Basal enrichi en silice
BG11	Milieu de culture pour algues
Car	Caroténoide
Chl *x*	Chlorophylle (ou *x* peut correspondre à: *a, b, c* ou *d*)
CI	Intervalle de confiance
CO_2	Dioxide de carbone
Cyt b_6f	Cytochrome b_6f
DCMU	Diuron
$DI_O\ RC^{-1}$	Dissipation d'énergie par centre réactionnel
ELISA	Dosage d'immunoabsorption par enzyme liée
$ET_O\ RC^{-1}$	Transport d'électron par centre réactionnel
$ET_O\ TR_O^{-1}$	Efficacité de transfert d'électron au-delà de Q_A par le PSII
ETR	Taux de transport d'électron
FiRE	Appareil de mesure d'induction et de relaxation de la fluorescence
F'_M	Niveau maximal de fluorescence en présence de lumière actinique
F_M	Niveau maximal de fluorescence
F_MDCMU	Niveau maximal de fluorescence en présence de DCMU

F'_O	Niveau minimal de fluorescence en présence de lumière infrarouge
F_O	Niveau minimal de fluorescence à l'obscurité
FR.L	Lumière infrarouge
F_S	Niveau minimal de fluorescence en présence de lumière actinique
F_V/F_M	Rendement quantique maximal des PSII
GP	Taux de production d'oxygène brute
HL	Intensité lumineuse élevé
HL_{AI}	Lumière actinique d'intensité lumineuse élevé
HL_C	Cellule acclimatée à une intensité lumineuse élevée
IC	Courbe d'induction de fluorescence chlorophylienne
IC_{10} / EC_{10}	Concentration inhibant 10 % de l'activité d'une variable donnée
IC_{50} / EC_{50}	Concentration inhibant 50 % de l'activité d'une variable donnée
LED	Diode électroluminescente
LHC	Antenne collectrice de lumière / Complexe collecteur de lumière
LL	Intensité lumineuse faible
LL_C	Cellule acclimatée à une intensité lumineuse faible
MAXI-PAM	Appareil de mesure de fluorescence chlorophylienne par image
MCYST	Microcystine
MeOH	Méthanol
$NADP^+$	Nicotinamide adénine dinucléotide phosphate
NADPH	Forme réduite de $NAPD^+$
NP	Taux de production d'oxygène net
NPQ	Dissipation non-photochimique d'énergie
NPQ_{HL}	Dissipation non-photochimique d'énergie induite par une intensité lumineuse élevée
O_2	Dioxygène
O.D.	Densité optique
PAM	Fluorescence de type « Pulse Amplitude modulated »
PAR	Radiation photosynthétiquement active (entre 400 et 700 nm)

PBS	Phycobilisome
PC	Phycocyanine
PE	Phycoerytrine
PEA	Appareil d'analyse d'efficacité photochimique des plantes
PE curve	Courbe de photosynthèse en fonction de l'intensité lumineuse (*idem* PI curve)
PFD	Densité du flux de photon / intensité lumineuse de croissance
PNP	Pigment non-photosynthétique
PP	Pigment photosynthétique
PPI	Test d'inhibition de la protéine phosphatase 2A
PQ	Plastoquinone
PQH_2	Hydroplastoquinone
PS (I ou II)	Photosystème, I ou II
Q, Q_A & Q_B	Quinone, quinone a et quinone b
qN_{REL}	Dissipation relative d'énergie de façon non-photochimique
qP_{REL}	Dissipation relative d'énergie par la photochimie
QR	Nombre d'électrons requis pour former 1 molécule de dioxygène
R	Consommation d'oxygène
RC, I ou II	Centre réactionnel, associé au photosystème I ou II aussi appelé respectivement P700 et P680 ou P700* et P680* dans l'état oxydé
rETR	Taux relatif de transport d'électrons
RLC	Courbe rapide d'induction de fluorescence par la lumière
ROS	Espèce réactive oxygénée
RUBISCO	Ribulose-1,5-diphosphate carboxylase/oxygénase
SEM	Erreur type de la moyenne
S.L	Lumière saturante
S.P	Flash de lumière saturante
$TR_O\ ABS^{-1}$	Probabilité de transfert d'un électron (correspond à F_V/F_M)
$TR_O\ RC^{-1}$	Taux maximal de transfert d'énergie par centre réactionnel

UQF_{REL}	Dissipation relative d'énergie sous forme de fluorescence
UV	Lumière ultraviolette
Vis	Lumière visible

LISTE DES SYMBOLES

α	Pente initiale de la relation photosynthèse lumière (PE curve)
a^*_{φ}	Moyenne entre 400 et 700 nm de $a^*_{\varphi}(\lambda)$
$\bar{a}^*_{\varphi}$	Moyenne entre 400 et 700 nm de $\bar{a}^*_{\varphi}(\lambda)$
$a^*_{\varphi}(\lambda)$	Coefficient d'absorption *in vivo* de la Chl *a*
$\bar{a}^*_{\varphi}(\lambda)$	$a^*_{\varphi}(\lambda)$ normalisé au spectre d'intensité lumineuse (E (λ)) de la chambre de croissance
a^*_{φ}(red)	Moyenne entre 670 et 680 nm de $a^*_{\varphi}(\lambda)$
$a^{*\,\mu m}_{\varphi}$	a^*_{φ} normalisé en fonction du biovolume (μm^3)
a^*_{PSII}	Moyenne entre 400 et 700 nm de $a^*_{PSII}(\lambda)$
$\bar{a}^*_{PSII}$	Moyenne entre 400 et 700 nm de $\bar{a}^*_{PSII}(\lambda)$
$a^*_{PSII}(\lambda)$	Coefficient d'absorption *in vivo* de la Chl *a* spécifique au PSII
$\bar{a}^*_{PSII}(\lambda)$	a^*_{PSII} normalisé au spectre d'intensité lumineuse (E (λ)) de la chambre de croissance
a^*_{PSII}(red)	Moyenne entre 670 et 680 nm de $a^*_{PSII}(\lambda)$
β	Constante d'inhibition dépendant de la lumière
E (λ)	Spectre d'intensité lumineuse (entre 400 et 700 nm)
E_K	Intensité lumineuse à saturation de la photosynthèse ($E_K^{Chl,\,\mu m}$) ou de la croissance ($E_K^{\mu d}$)
E_M	Intensité lumineuse requise pour atteindre la photosynthèse ($E_M^{Chl,\,\mu m}$) ou la croissance maximale ($E_M^{\mu d}$)
F_{II}	Fraction de l'énergie associée au PSII par rapport au PSI
fAQ_{PSII}	Fraction de l'énergie totale absorbée par le PSII et les LHCII
Γ	Nombre théorique de photons pour former une molécule de dioxygène (0.25 O_2 électron^{-1})
Φ'_M	Rendement quantique opérationnel des PSII
Φ_M	Rendement quantique maximal des PSII

P_M	Taux de photosynthèse maximal
P_{O2}	Taux de production d'oxygène
P_{O2}^{Chl}	Taux de production d'oxygène par mg de Chl *a*
$P_{O2}^{\mu m}$	Taux de production d'oxygène par μm^3
P_{SAT}	Taux de photosynthèse à saturation
Ψ_o	Efficacité de transfert d'électron au-delà de Qa par le PSII
ρ	Facteur de connectivité
μ_c	Taux de croissance cellulaire
μ_d	Taux de croissance cellulaire
μm^3	Volume cellulaire, biovolume
μ_M	Taux de croissance maximal théorique
μ_{MAX}	Taux de croissance maximal réel

RÉSUMÉ GÉNÉRAL

Dans les milieux dulcicoles, le phytoplancton (algues et cyanobactéries) est très diversifié et plusieurs espèces cohabitent grâce à l'établissement d'une saine compétition pour les ressources limitantes dans leur habitat. Cependant, cet équilibre fragile est souvent perturbé par les activités agricoles qui modifient les conditions physico-chimiques des milieux aquatiques environnants. Une des conséquences fréquemment observée est l'apparition de fleurs d'eau de cyanobactéries souvent toxiques. En plus d'ajouter des éléments nutritifs requis pour soutenir une forte production de biomasse, les conséquences de l'agriculture incluent aussi un changement de la disponibilité en lumière à cause de l'ajout de matière en suspension dans l'eau ainsi que des effets toxiques sur les organismes aquatiques à cause de l'ajout d'herbicides capables d'affecter différents aspects de la photosynthèse des organismes photoautotrophes.

Les résultats de cette thèse ont été obtenus à partir de cultures *in vitro* représentant les principaux groupes phytoplanctoniques. Lorsqu'exposées à un gradient de lumière de 14 à 1079 µmol de photons m^{-2} s^{-1}, les différentes espèces ont démontré une étonnante capacité d'acclimatation possible grâce à un ensemble de modifications phénotypiques. Ces modifications ont permis de diminuer l'efficacité d'absorption de la lumière tout en stimulant les mécanismes de photoprotection en réponse à une exposition à des intensités lumineuses saturantes pour la photosynthèse ($E : E_k > 1$) tandis que l'inverse a été observé aux intensités de lumière limitant la photosynthèse ($E : E_k < 1$). L'ensemble des modifications observées a permis aux différentes espèces de maximiser leur taux de croissance en fonction de la lumière conformément à la théorie de la photoacclimatation.

Selon les résultats des chapitres II et III, la toxicité des fleurs d'eau de cyanobactéries peut aussi être modifiée par la lumière et ce en modulant la toxicité des cellules elles-mêmes mais aussi en favorisant ou non les souches toxiques relativement aux souches non-toxiques. Ainsi, en combinant ces différents effets de la lumière, il est possible de mieux comprendre les changements de toxicité des fleurs d'eau. De plus, les résultats montrent que le contenu en microcystines, dont le rôle physiologique est inconnu, varie avec le contenu en chlorophylle *a* et le transport d'électrons. Cela suggère que ces molécules sont peut-être impliquées dans certains processus photosynthétiques ou du moins, que la régulation de leurs synthèse dépend de facteurs liés à la photosynthèse.

Bien que les différents herbicides testés aient tous causé des effets significatifs sur l'activité photosynthétique des 17 espèces comparées, aucune espèce n'a démontré une résistance accrue à tous les herbicides et il n'a pas été possible d'établir le rôle des herbicides et de l'agriculture dans l'instauration des fleurs d'eau de cyanobactéries. Cependant, ces résultats ont permis de démontrer qu'il faut aussi tenir compte des facteurs environnementaux notamment la lumière comme modulateurs de la toxicité des herbicides.

Finalement, en mettant en évidence l'importance de la lumière et des herbicides comme facteurs pouvant moduler la compétition entre les différentes espèces phytoplanctoniques, l'ensemble des travaux présentés dans cette thèse souligne l'importance de mieux encadrer les activités agricoles pour en contrôler les impacts sur les milieux aquatiques et les organismes qui y vivent.

INTRODUCTION GÉNÉRALE

Mise en contexte

Les milieux aquatiques renferment une grande diversité de microorganismes photosynthétiques, le phytoplancton. Le phytoplancton est divisé en deux groupes important, celui des micro-algues et celui des cyanobactéries (Wetzel, 2001). Les cyanobactéries sont des procaryotes et elles sont les seules bactéries capables de faire la photosynthèse en présence d'oxygène, l'oxyphotosynthèse. Elles sont ubiquistes et regroupent plus de deux mille espèces (Chorus et Bartram, 1999). Leur présence remonte à plus de trois milliards d'années et elles sont les précurseurs de la photosynthèse chez les eucaryotes incluant les végétaux supérieurs (Blankenship, 2001). En outre, le phytoplancton est composé d'organismes eucaryotes très diversifiés, avec de nombreuses espèces séparées en taxons incluant notamment les chlorophytes, les bacillariophytes, les cryptophytes et les chrysophytes (Reynolds, 1998; Wetzel, 2001). Lorsque l'on compare tous ces organismes, on observe une grande hétérogénéité. On note entre autre que leur taille est très variable puisque certaines espèces ne font pas plus d'un micron tandis que d'autres sont visibles à l'œil nu (Beardall *et al.*, 2009). Certaines espèces forment des colonies, d'autres sont dotées de flagelles et peuvent se déplacer dans la colonne d'eau. D'un point de vue physiologique, les différents groupes se distinguent également par la présence ou non de certains pigments spécifiques associés à la photosynthèse. D'abord, tous ces organismes synthétisent de la chlorophylle *a* (Chl *a*), un pigment essentiel à la photosynthèse comme nous le verrons plus loin; cependant, seuls certains groupes synthétisent d'autres formes de chlorophylle (*b*, *c* ou *d*) (Wetzel, 2001). Chez les cryptophytes et les cyanobactéries, ces pigments accessoires sont remplacés par des phycobiliprotéines et chez les cyanobactéries ces pigments sont même associés en une structure protéique complexe appelée phycobilisome (PBS) (Grossman *et al.*, 1993; Schagerl et Donabaum, 2003; Stomp *et al.*, 2007). Comme nous le verrons plus

loin, ces différents pigments ainsi que certaines adaptations morphologiques confèrent des avantages compétitifs non négligeables aux espèces les possédant. Malgré cette grande hétérogénéité, un point commun à tous ces organismes est leur capacité à faire la photosynthèse en utilisant l'eau comme source d'électrons; l'oxyphotosynthèse (Wetzel, 2001). L'oxygène qui en découle est essentiel à la vie sur terre tout comme la production de matière organique issue de ces organismes représentant 45 % de la production primaire mondiale (Field *et al.*, 1998).

La photosynthèse joue un rôle central dans le contrôle de la croissance du phytoplancton et des cyanobactéries (Herzig et Dubinsky, 1992; Beardall *et al.*, 2001). La croissance dépend aussi de plusieurs autres facteurs dont les plus déterminants sont la lumière, la température, la disponibilité en carbone inorganique (CO_2) et la concentration d'éléments nutritifs, notamment l'azote (N) et le phosphore (P) (Phlips *et al.*, 1997; Reynold, 1998; Levine et Schindler, 1999; Wetzel, 2001). Ces facteurs varient naturellement entre les milieux selon la situation géographique, les caractéristiques hydrologiques ou le type de sol du bassin versant. Par exemple, les lacs situés sur le sol rocheux du bouclier Canadien sont souvent pauvres en éléments nutritifs et plus acides comparativement aux lacs situés sur les terres argileuses des basses terres du St-Laurent dont les eaux sont alcalines et riches en nutriments (Schetagne *et al.*, 2005; Prairie et Parkes, 2006).

L'importance qu'un facteur donné (aussi bien physique que chimique) représente pour le contrôle de la croissance des algues dépend de la loi de Liebig ou loi du minimum. Cette loi stipule que la croissance algale n'est pas contrôlée par la quantité totale de ressources dans l'environnement mais plutôt par la disponibilité du facteur le moins abondant donc limitant (Reynolds, 1998). Dans les lacs boréaux et tempérés, le facteur limitant la croissance du phytoplancton est généralement le phosphore tandis que l'azote ou le fer sont limitants dans les environnements marins (Wetzel, 2001). Le facteur limitant peut changer dans un milieu donné selon la période de l'année ou à cause de certaines conditions externes. Un exemple concret:

la baisse de température que subissent les lacs boréaux à l'automne et la forte diminution du taux de croissance algale qui s'en suit (Wetzel, 2001). Parallèlement aux variations géographiques et physico-chimiques, on sait aussi que la capacité d'assimilation, l'efficacité de conversion et la dépendance des espèces face à certaines ressources sont variables (Tilman *et al.*, 1986; Herzig et Falkowski, 1989; Reynolds, 1998). On peut penser aux baccilariophytes dont la croissance requiert impérativement la présence de silice. Ainsi, les espèces qui utilisent plus efficacement une ressource donnée ou qui en dépendent peu sont théoriquement moins affectées par une limitation de celle-ci (Reynolds, 1998; Scheffer *et al.*, 2003; Descamps-Julien et Gonzalez, 2005). D'autre part, des caractéristiques spécifiques à certaines espèces permettent de modifier leur dépendance vis-à-vis d'une ressource. Par exemple, les algues flagellées peuvent se diriger activement vers une strate de la colonne d'eau pour laquelle l'intensité lumineuse ou la concentration en nutriments est optimale (Beardall *et al.*, 2009). Il en va de même pour certaines cyanobactéries dotées de vacuoles de flottaison (Visser *et al.*, 2005). L'hétérogénéité spatiale et temporelle dans la répartition des facteurs limitants et les différentes aptitudes des algues confrontées à ces variations permettent à de nombreuses espèces de performer à un moment ou l'autre de la saison de croissance (Lafforgue et al 1995; Hyenstrand *et al.*, 1998; Interlandi et Kilham, 2001). En conséquence, on trouve des communautés algales très diversifiées et il y a un nombre important d'espèces dans les milieux aquatiques malgré un nombre limité de facteurs déterminants, comme le décrit Hutchison dans son concept du Paradoxe du plancton (Hutchinson, 1961).

À l'instar des variations naturelles, plusieurs études ont montré que certaines activités anthropiques, notamment l'agriculture, engendrent des variations importantes des conditions physico-chimiques des lacs et rivières environnantes (Codd, 2000; Tilman *et al.*, 2001; Smith *et al.*, 2003; Søndergaard et Jeppesen, 2007). Ces variations consistent en un enrichissement en éléments nutritifs par rapport aux conditions naturelles, ce qui accélère le processus naturel d'eutrophisation. De plus,

les rejets agricoles contiennent souvent des substances toxiques comme des herbicides et autres pesticides et ceux-ci sont nuisibles pour plusieurs organismes aquatiques (Tomlin, 2000; Delorenzo *et al.*, 2001; Shaner and Henry 2007, Giroux, 2010). Aussi, l'eau des rejets agricoles et l'érosion accélérée des terres bordant les cours d'eau augmentent la quantité de matière en suspension dans l'eau et cette matière diminue la transparence de l'eau et donc la quantité de lumière disponible pour la photosynthèse (Tilman *et al.*, 2001). Tous ces aspects agissent directement ou indirectement sur plusieurs des facteurs habituellement limitants pour la croissance algale et permettant le maintien d'un équilibre qui favorise la diversité dans l'écosystème. En conséquence, il n'est pas étonnant que le changement de l'abondance et de la distribution des microorganismes photosynthétiques de la communauté constitue un des problèmes fréquemment rencontrés dans les milieux dont le bassin versant est dominé par l'agriculture (Reynolds, 1987; Codd 2000; Oliver et Ganff, 2000).

Au niveau mondial, une des tendances fréquemment observées dans ces conditions est une transition d'une communauté algale diversifiée et équilibrée vers une communauté principalement composée de cyanobactéries qui culmine par la formation de fleurs d'eau (Pearl 1996; Watson *et al.*, 1997; Reynolds, 1998; Codd, 2000; Dokulil et Teubner, 2000). Les fleurs d'eau de cyanobactéries sont un symptôme visible du déséquilibre de la compétition qui a lieu dans la colonne d'eau. Ce changement d'état est rendu possible par la modification de certaines caractéristiques clées qui semble avantager les cyanobactéries au détriment de leurs compétiteurs (Hyenstrand *et al.*, 1998). Au niveau de l'écosystème, l'apparition d'une fleur d'eau est problématique pour de nombreuses raisons. La dense couche de cellules des floraisons monopolise la majorité de la lumière disponible ce qui augmente le stress sur les compétiteurs et diminue leur capacité d'acquisition des éléments nutritifs essentiels (Paerl, 1996). À ce stade d'une floraison, le phytoplancton a déjà perdu l'avantage et ce monopole de la lumière rend le retour de

la communauté vers son état initial encore plus improbable. De plus, la dégradation bactérienne de la biomasse accumulée pendant une fleur d'eau, additionnée à la respiration normale de cette dense couche de cellules, augmente le risque d'anoxie durant la nuit. Ce phénomène est responsable des épisodes de mortalité de masse des poissons (Chorus et Bartram, 1999; Havens 2008). Finalement, plusieurs cyanobactéries qu'on retrouve dans les fleurs d'eau sont capables de synthétiser des cyanotoxines (Codd, 1995; Carmichael 2001). Ces molécules dont l'utilité physiologique est encore inconnue sont très toxiques pour les organismes aquatiques et les humains (Codd, 1995; Chorus et Bartram, 1999; Carmichael, 2001; Codd *et al.*, 2005). En outre, la présence de cyanotoxines nuit aux activités récréo-touristiques associées au milieu dulcicole en plus de causer des problèmes de santé publique au niveau des prises d'eau potable (Metcalf et Codd, 2004).

Comme nous l'avons vu, il existe des différences entre les organismes photosynthétiques au niveau de l'utilisation et de la dépendance vis à vis des facteurs limitant la croissance, notamment au niveau des éléments nutritifs et de la lumière (Reynolds, 1998; Beardall *et al.*, 2001). Nous connaissons aussi la variabilité de leur sensibilité en présence de stress environnementaux comme ceux potentiellement induits par les herbicides. (Delorenzo *et al.*, 2001; Juneau *et al.*, 2007). Conséquemment, on peut se demander pourquoi les activités agricoles affectent à ce point l'abondance et la distribution des cyanobactéries? Plus précisément, quelles sont les caractéristiques physiologiques qui permettent aux cyanobactéries d'instaurer de telle dominance sur leurs compétiteurs et y a-t-il un facteur clé responsable de ce phénomène? On peut aussi se demander si la dominance des cyanobactéries est le résultat non pas d'un avantage compétitif propre à ce groupe phytoplanctonique mais plutôt le résultat d'une défaillance des compétiteurs ?

Ainsi cette thèse suivra deux grands axes, le premier traitant des processus de photoacclimatation, photorégulation et réponse au stress lumineux de différentes espèces et souches de cyanobactéries et d'algues (voir les Chapitres I à III). Le

deuxième axe vise à explorer la réponse de certains de ces organismes face à l'ajout d'herbicides dans leur milieu (voir les Chapitre IV et V).

Les sections suivantes font la synthèse des connaissances reliées à ces sujets afin de mieux appréhender les différents chapitres de la thèse. Nous commencerons par un sommaire des connaissances sur les cyanobactéries dont la dominance dans certains écosystèmes a engendré la problématique de cette thèse. Puis nous présenterons les mécanismes de photoacclimatation et photorégulation pour terminer avec l'effet connu des herbicides sur les communautés aquatiques qui nous intéressent.

Notons qu'il est indispensable de comprendre les mécanismes de la photosynthèse pour appréhender les mécanismes de photoacclimatation et de photorégulation ainsi que les différentes méthodes employées dans mes travaux notamment celles ayant trait à la fluorescence chlorophyllienne. La photosynthèse en elle-même n'étant pas le sujet de cette thèse, ses mécanismes (constituants, fonctionnement) sont présentés en annexe à cette introduction (annexe 1).

Aperçu sur les communautés de cyanobactéries

Plusieurs études ont tenté de mettre en évidence le ou les facteurs responsables de la dominance des cyanobactéries (Hyenstrand *et al.*, 1998; Dokulil et Teubner, 2000; Downing *et al.*, 2001; Havens, 2008). Parmi ces facteurs, l'azote et le phosphore sont souvent corrélés avec leur biomasse (Kardinaal et Visser, 2005). Ces relations ne sont pas étonnantes puisque ces éléments contrôlent la croissance des producteurs primaires dont font partie les cyanobactéries (Schindler, 1978; Wetzel, 2001). Cependant, ce qui est étonnant vient du fait que les cyanobactéries ne sont pas de bonnes compétitrices pour l'azote ou le phosphore (Tilman *et al.*, 1986; Chorus et Bartram, 1999; Visser *et al.*, 2005) et on peut se questionner sur les raisons qui font que ce ne sont pas les autres groupes d'algues qui profitent de l'abondance d'éléments nutritifs des milieux eutrophes et hypereutrophes (Pearl 1988). D'autres

facteurs sont aussi corrélés avec la dominance des cyanobactéries comme l'augmentation de la température de l'eau ou un pH élevé jumelé à de faibles concentrations de dioxyde de carbone (CO_2) (Robart and Zohary, 1987; Shapiro 1990). Dans certains cas, la turbidité et les précipitations permettent aussi d'expliquer leur présence (Kardinaal et Visser, 2005). Cependant, l'importance de ces facteurs varie selon chaque milieu étudié et peut aussi changer pour un même milieu dans le temps (Dokulil et Teubner, 2000). La complexité des processus écologiques impliqués rend donc ce phénomène de dominance difficile à prédire et à contrôler (Havens *et al.*, 1998; Reynolds, 1998; Levine et Schindler, 1999; Dokulil et Teubner, 2000; Ferber *et al.*, 2004; Kardinaal et Visser, 2005; Visser et *al.*, 2005).

Le succès évolutif des cyanobactéries repose sur l'utilisation d'une combinaison de stratégies qui peuvent varier entre les espèces (Hyenstrand *et al.*, 1998; Downing *et al.*, 2001). Parmi les plus importantes, mentionnons d'abord la capacité de fixer l'azote atmosphérique, un élément nutritif essentiel pour la croissance pouvant être limitant en milieu aquatique (Visser *et al.*, 2005). Les cyanobactéries ont aussi la capacité de s'associer en colonies de forme et de taille variées, ce qui les rend plus difficiles à manipuler et leur offre une certaine protection contre des prédateurs communs comme *Daphnia sp.* (Visser *et al.*, 2005). Mentionnons finalement la capacité de certaines espèces à former des vacuoles gazeuses qui leur permettent de réguler leur position dans la colonne d'eau et de se positionner là où les conditions de lumière et d'éléments nutritifs sont optimales pour leurs besoins (Oliver, 1994; Visser *et al.*, 2005). De plus, les cyanobactéries peuvent synthétiser des pigments accessoires, les phycobiliprotéines, formant les phycobilisomes (Grossman *et al.*, 1993). Les propriétés optiques des phycobiliprotéines comme la phycocyanine, l'allophycocyanine et la phycoerythrine permettent d'intercepter la lumière à des longueurs d'ondes différentes des algues eucaryotes, un avantage pour les cyanobactéries dans la compétition pour la lumière (Campbell *et al.*, 1998; Falkowski et Raven 2007; Stomp *et al.*, 2007). Malgré ces

stratégies variées, les cyanobactéries sont considérées comme des espèces opportunistes plutôt que compétitrices (Tilman *et al.*, 1986). En fait, les coûts énergétiques requis pour maintenir des stratégies comme la fixation de l'azote ou la flottaison sont élevés et pour cette raison, les cyanobactéries ont des besoins métaboliques exigeants et un taux de croissance faible (Reuter et Petersen, 1987). Elles sont d'ailleurs considérées comme des espèces de fin de succession puisqu'on les retrouve généralement vers la fin de l'été et durant l'automne dans les lacs tempérés non perturbés comme ceux du Québec (Lafforgue *et al.*, 1995; de Figueiredo *et al.*, 2006). En plus de ces stratégies, la présence ou l'absence de certaines espèces jumelées aux conditions à un moment précis semblent jouer un rôle déterminant pour expliquer le succès des cyanobactéries (Hyenstrand *et al.*, 1998). Par exemple, lorsqu'un milieu est limité par l'azote, les cyanobactéries fixatrices d'azote pourraient devenir dominantes à condition que d'autres facteurs ne nuisent pas à leur croissance voire à leur présence. Les éléments nutritifs ne sont donc pas nécessairement les seuls responsables de la dominance des cyanobactéries et d'autres facteurs doivent être étudiés pour mieux comprendre leur succès. D'autre part, aucune information ne permet à ce jour d'expliquer pourquoi dans certaines conditions, ce sont les souches toxiques qui sont dominantes tandis que dans d'autres cas aux conditions similaires, ce sont les souches non-toxiques de la même espèce qui deviennent dominantes (Kardinaal *et al.*, 2007). Une meilleure compréhension des différences existant entre les souches permettrait de prédire plus efficacement la toxicité d'une fleur d'eau et de prévenir les risques de santé humaine.

Photoacclimatation et photorégulation

La lumière est indispensable au bon fonctionnement de la photosynthèse et, à son tour, l'énergie issue de la photosynthèse est déterminante pour la croissance des photoautothrophes. La lumière est donc un facteur essentiel pour la croissance des algues et des cyanobactéries (Beardall *et al.*, 2001). En milieu aquatique, la

disponibilité en lumière est variable aussi bien à l'échelle temporelle (variations journalière et saisonnière) que spatiale (en fonction de la latitude) (Wetzel, 2001). De plus, la lumière est graduellement absorbée en pénétrant dans l'eau et son intensité diminue exponentiellement en fonction de la profondeur (Wetzel, 2001). En conséquence, dans les lacs dont les eaux sont limpides, le coefficient d'atténuation de la lumière est faible et les photons pénètrent plus profondément dans la colonne d'eau comparativement aux lacs de forte turbidité (Schanz *et al.*, 1997). Bien qu'essentielle, la lumière peut aussi avoir des effets négatifs sur la croissance des organismes photosynthétiques (Barber et Anderson, 1992). Lorsque l'intensité lumineuse est trop faible, l'organisme ne réussit pas à produire suffisamment d'énergie par photosynthèse, ce qui induit une « crise énergétique » et une baisse de croissance (Falkowski et La Roche, 1991; Quigg *et al.*, 2006). Lorsque l'intensité lumineuse est trop élevée par rapport à la capacité d'absorption de l'appareil photosynthétique, il y a une photoinhibition, caractérisée par un excès d'énergie acheminé aux centres réactionnels (RC) des photosystèmes (PS) (Tytler *et al.*, 1984; MacIntyre *et al.*, 2002; Murata *et al.*, 2007). Lorsque cet excès énergétique n'est pas contrôlé, il entraine la formation de radicaux libres endommageant l'appareil photosynthétique par stress oxydatif, ce qui affecte aussi la croissance (Bartosz, 1997; Mittler, 2002; Song *et al.*, 2006).

Pour survivre aux variations d'intensité lumineuse, les organismes photosynthétiques doivent donc modifier la quantité de photons qu'ils interceptent ainsi que l'utilisation qu'ils en font. Les modifications que subit alors l'organisme sont regroupées en mécanismes de photorégulation et de photoacclimatation et se distinguent par l'échelle de temps requis pour leur mise en place (Richardson *et al.*, 1983; MacIntyre *et al.*, 2002; Dubinsky et Stambler, 2009). En effet, toutes les modifications associées à la photoacclimatation requièrent un certain temps avant d'être activées: c'est pourquoi on parle d'acclimatation. Cependant, à cause des conditions météorologiques ou du mouvement du phytoplancton dans la colonne

d'eau, la lumière varie aussi de façon journalière. Face à ces variations, il existe des mécanismes plus rapides qui sont activés à l'échelle de la minute et même de la seconde : ce sont les mécanismes de photorégulation (Masojidek *et al.*, 1999; Steiger *et al*, 1999; Niyogi, 2000; Müller *et al.*, 2001; Wilson *et al.*, 2006; Karapetyan, 2007). La photoacclimatation et la photorégulation ont des buts semblables: la protection de l'appareil photosynthétique en situation d'excès de lumière et l'optimisation de l'utilisation des photons vis-à-vis des besoins pour la croissance (Falkowsky et LaRoche, 1991; Dubinsky et Stambler, 2009). Cependant, l'utilisation de ces mécanismes dépend avant tout de leur présence dans le bagage génétique de l'individu et est déterminée par le degré de photoadaptation propre à chaque espèce (MacIntyre *et al.*, 2002).

Les mécanismes de photoacclimatation

En théorie, le rôle des mécanismes de photoacclimatation est d'ajuster la quantité des divers constituants de l'appareil photosynthétique afin d'obtenir un équilibre entre l'énergie lumineuse interceptée par l'organisme, l'énergie utilisée pour la photosynthèse et l'énergie nécessaire pour la fixation du carbone, l'assimilation des nutriments et les autres processus biochimiques (Anderson *et al.*, 1995; Sonoïke *et al.*, 2001; Dubinsky et Stambler, 2009). De plus, la photoacclimatation tend à maximiser la production d'ATP et de NADPH selon les besoins de la cellule, ce qui lui permet d'atteindre un taux de croissance maximale en fonction des limites imposées par les conditions environnementales.

C'est en 1946 que les premières études, par Myers *et al.* (1946 a et b), menant au concept de photoacclimatation ont été publiées. En diminuant l'intensité lumineuse de croissance d'une algue verte, le groupe de Myers *et al.* (1946a) a observé une augmentation du contenu cellulaire en chlorophylle *a* chez *Chlorella pyrenoidosa*. De plus, leurs travaux ont montré que la taille ainsi que le contenu en carbone de *C. pyrenoidosa* diminue à faible intensité lumineuse (Myers *et al.*, 1946a).

Au niveau de l'activité photosynthétique, ces travaux ont montré que le taux de production d'O_2 atteint un niveau maximal à des intensités intermédiaires de lumière. Ce résultat indiquait clairement que l'appareil photosynthétique peut être saturé par trop de lumière (Myers *et al.*, 1946b). De nos jours, les grandes lignes de la photoacclimatation et les caractéristiques typiques d'une plante ou d'une algue placée à faible ou à forte intensité lumineuse sont mieux connues et confirment pour la plupart la justesse des travaux du groupe de Myers (MacIntyre *et al.*, 2002).

Nous savons qu'il existe plusieurs mécanismes associés à la photo-acclimatation notamment des modifications 1) de la taille des antennes collectrices de lumière (LHC), 2) de la concentration des pigments chlorophylliens, 3) de la concentration et de la composition des pigments accessoires et de photoprotection et 4) de la stœchiométrie entre les PSI et PSII ainsi qu'avec les autres éléments de la chaine de transport d'électrons (Kawamura *et al.*, 1979; Anderson *et al.*, 1995; Huner *et al.*, 1998; MacIntyre *et al.*, 2002). En augmentant ou en diminuant la taille des LHC, l'énergie acheminée vers les PS est régulée à la hausse ou à la baisse, ce qui permet de capter plus d'énergie lorsque la lumière est limitante et inversement lorsqu'elle est saturante (Dubinsky *et al.*, 1986; Behrenfeld *et al.*, 2004). Avec une augmentation graduelle de l'intensité lumineuse, on observe généralement une diminution du contenu en pigment photosynthétique (PP) comme la chlorophylle *a* (Falkowski et Owen 1980; Prézelin, 1981; Richardson *et al.*, 1983; Huner *et al.*, 2003). Ce changement permet de diminuer le nombre de photons interceptés par l'organisme et minimise les excès d'énergie dans l'appareil photosynthétique (Richardson *et al.*, 1983; Herzig and Falkowski, 1989; Anderson *et al.*, 1995; Huner *et al.*, 1998; Falkowsky and Chen, 2003). On observe également des changements de la quantité de pigments non-photosynthétiques (PNP) comme par exemple les pigments de photoprotection de type caroténoïdes (Lichtentaler 1987; Steiger *et al.*, 1999; Schagerl and Müller, 2006; Lichtentaler, 2007). Ces changements des quantités de PP et de PNP entraînent habituellement l'augmentation du ratio PNP:PP. Ainsi la

probabilité que l'énergie d'un photon capturé soit acheminée jusqu'aux RC diminue puisque les PNP, comme les β-carotènes, dissipent l'énergie qu'ils interceptent sous forme de chaleur (Herzig and Falkowski, 1989; Falkowsky and Chen, 2003; Kirilovsky, 2007). Finalement, un autre effet induit par l'augmentation de l'intensité lumineuse est une diminution du nombre total de RC qui participent à la photosynthèse et un changement dans la stœchiométrie RCII:RCI (Kawamura *et al.*, 1978; Falkowski and Owens, 1980; Falkowski *et al.*, 1981; Mauzerall and Greenbaum, 1989). Le changement stœchiométrique des photosystèmes permet, par une augmentation ou une diminution du rapport PSI : PSII, d'équilibrer le drainage de l'énergie associée aux PSII vers les PSI et de diminuer la pression exercée sur le PSII et sur l'appareil photosynthétique par un excès d'énergie lumineuse (Sonoïke *et al.*, 2001). A forte lumière, tous ces ajustements physiologiques ont pour effet de diminuer la pression lumineuse sur l'appareil photosynthétique et donc de le protéger contre la photoinhibition et les dommages causés par les espèces réactives oxygénées (ROS) ou radicaux libres qui résultent de cet excès de lumière. (Huner *et al.*, 1998; Wilson *et al.*, 2006). A faible lumière, ils permettent de minimiser le déficit énergétique associé à la rareté des photons en interceptant une plus grande proportion d'entre eux, ce qui permet à l'organisme d'atteindre un taux de croissance plus élevé qu'en absence de photoacclimatation (Berhenfeld *et al.*, 2004; Dubinsky et Stambler, 2009).

L'efficacité des mécanismes de photoacclimatation et la capacité de régulation de l'énergie excédentaire peuvent être outrepassées lorsque l'intensité lumineuse dépasse la capacité de photoadaptation de l'espèce ou que l'organisme n'a pas eu de temps de s'acclimater aux nouvelles conditions. De plus, l'importance et l'efficacité de ces mécanismes diffèrent entre les groupes de microorganismes photosynthétiques. Par exemple, en comparant la taille et le nombre de PS chez une chlorophyte (*Dunaniella tertricola*) et une diatomée (*Phaeodactylum tricornutum*) exposées à un gradient d'intensité lumineuse, *Dunaniella tertricola* modifie la taille

et le nombre de PS actifs tandis que *Phaeodactylum tricornutum* modifie préférentiellement le nombre de PS (Quigg *et al.*, 2006). Un autre aspect variable au niveau des aptitudes à gérer les modifications de l'intensité lumineuse vient des différences fondamentales existant entre les groupes taxonomiques au niveau de l'appareil photosynthétique. Parmi ces différences, on peut mentionner le ratio de PSI : PSII qui est généralement supérieur à 1 chez les cyanobactéries, égal à 1 chez les chlorophytes et inférieur à 1 chez les diatomées (Herzig et Dubinsky, 1993). De plus, chez les cyanobactéries et les cryptophytes, l'énergie lumineuse est principalement interceptée par les phycobiliprotéines plutôt que par des LHC (Grossman *et al.*, 1993; Wetzel, 2001). Finalement, les espèces appartenant à certains groupes comme celui des bacillariophytes, contiennent beaucoup de caroténoides qui permettent de diminuer l'énergie acheminée vers les PS en plus de participer à la neutralisation des radicaux libres (Krinsky 1989; Demmig-Adams et Adams, 1996; Steiger *et al.*, 1999; Latowski *et al.*, 2004; Lavaud *et al.*, 2004). Ces organismes sont donc mieux protégés face aux fluctuations de l'intensité lumineuse.

Les mécanismes de photorégulation

Lors d'un changement d'intensité lumineuse sur une courte échelle temporelle, ce sont les mécanismes de photorégulation qui entrent en jeu et les objectifs sous jacents à ces mécanismes sont semblables aux objectifs de la photoacclimatation (Niyogi, 2000). Par exemple, les PBS et les LHC peuvent se déplacer entre les PSI et PSII (état de transition), ce qui permet de modifier l'activité des PSI relativement à celle des PSII (Williams et Allen, 1987; Papageorgiou *et al.*, 2007; Zhang *et al.*, 2007). L'effet de ce genre de réallocation de l'énergie est semblable aux changements du rapport PSI : PSII induits par la photoacclimatation mais n'implique pas la synthèse de nouveaux PS. Le cycle des xanthophylles, un autre mécanisme de photorégulation important (Masojidek *et al.*, 1999; Müller *et al.*, 2001; Dimier *et al.*, 2007), répond activement au gradient de protons (ΔpH) issu du

transport d'électrons entre les PS. Lorsqu'il y a une altération de l'activité photosynthétique et un surplus d'énergie issu des PS, la configuration des pigments associés au cycle des xanthophylles change et l'énergie lumineuse qu'ils absorbent est dissipée sous forme de chaleur (Latowski *et al.*, 2004). Lorsque le gradient de protons diminue, les pigments retrouvent leur configuration initiale et le cycle peut recommencer. En comparant le cycle des xanthophylles entre les grands groupes phytoplanctoniques, on trouve des différences importantes. Par exemple, chez les chlorophytes, c'est la violaxantine qui est impliquée dans ce cycle tandis que l'on retrouve plutôt la diadinoxantine chez les bacillariophytes (Wetzel, 2001; Lavaud *et al.*, 2004). D'ailleurs, les bacillariophytes possèdent 4 à 5 fois plus de xanthophylles que de chlorophylle si on les compare aux chlorophytes ou aux plantes supérieures, ce qui fait des diatomées un groupe très efficace pour dissiper sans dommage les surplus d'énergie (Lavaud *et al.*, 2004). Chez les cyanobactéries, il n'y a pas de cycle des xanthophylles. Toutefois, elles peuvent synthétiser certains caroténoides qui permettent aussi la dissipation de l'énergie sous forme de chaleur ainsi que des phycourobilines qui dissipent l'excédent d'énergie sous forme de fluorescence (Subramaniam *et al.*, 1999, Schagerl et Müller, 2006, Kirilovsky, 2007). À l'instar de ces mécanismes, certaines algues possèdent des flagelles et certaines cyanobactéries comme *Microcystis sp.* utilisent des vacuoles gazeuses ce qui leur permet de se déplacer dans la colonne d'eau et ainsi de diminuer les stress causés par de fortes intensités lumineuses (Oliver 1994; Visser *et al.*, 2005; Beardall *et al.*, 2009).

Effets connus des herbicides sur les communautés aquatiques à l'étude

En milieu agricole, un facteur potentiellement important pour expliquer le succès des cyanobactéries a été négligé jusqu'à présent. Comme mentionné précédemment, en plus de modifier la transparence de l'eau, les activités agricoles sont responsables d'un ajout d'herbicides dans les milieux aquatiques et ceux-ci peuvent affecter les communautés algales (Herman *et al.*, 1986; Peterson *et al.*, 1994;

Caux et Kent, 1995). Il en existe une grande diversité et ceux-ci sont conçus pour affecter directement ou indirectement le fonctionnement de l'appareil photosynthétique (Tomlin 2000; Juneau *et al.*, 2007). Au Québec, la culture du maïs et du soja requiert l'utilisation de plus de trente pesticides différents (Giroux, 2010). Selon le ministère de l'environnement du Québec, ce sont les herbicides qui sont généralement favorisés puisqu'ils représentent 58,8 % des épandages (Ministère de l'environnement, 2003). Toujours selon le ministère de l'environnement, l'utilisation généralisée et les épandages multiples durant la saison de croissance font que les herbicides présentent plus de risque d'atteindre les cours d'eau. En outre, nombre d'entre eux ont des temps de demi-vie relativement longs et ils peuvent donc s'accumuler dans les écosystèmes et agir durant tout l'été (Shaner et Henry, 2007). Parmis les herbicides utilisés, l'atrazine est un des plus efficace et des moins couteux, il est donc fréquemment utilisé dans le monde (Graymore *et al.*, 2001). Il est habituellement appliqué avant l'émergence des plantules et peut donc facilement être entraîné dans les milieux aquatiques par l'irrigation et la pluie (Solomon *et al.*, 1996; Delorenzo *et al.*, 2001; Konstantinou *et al.*, 2006). Au Québec, cet herbicide est fréquemment rencontré dans les cours d'eau en plus du métolachlore, du dicamba, du bentazone et du glyphosate (Giroux, 2010). Dans l'environnement, l'atrazine peut affecter les différents organismes photosynthétiques puisque c'est un inhibiteur du PSII qui agit en bloquant les électrons entre les quinones a et b au niveau du PSII (Jursinic et Stemler, 1983; Tomlin, 2000). À cause de son mode d'action, l'atrazine engendre des effets qui se rapprochent de ceux causés par un excès de lumière puisque l'énergie reste bloquée au niveau du PSII à cause de l'arrêt du transport d'électrons et la photoinhibition qui en résulte engendre un stress cellulaire en augmentant la quantité de radicaux libres et réduit la croissance (Rutherford et Krieger-Liszkay, 2001). Plusieurs études ont déjà montré les effets néfastes de l'atrazine sur la croissance du phytoplancton incluant des diatomés, des cyanobactéries et des chlorophytes (voir synthèse de DeLorenzo *et al.*, 2001). La

majorité de ces études sont des bioessais de courtes durées au cours de laquelle la sensibilité des espèces est mesurée par inhibition de la croissance ou de la biomasse via une réduction de la chlorophylle. Selon ces études, aucun groupe taxonomique ne semble être systématiquement plus sensible qu'un autre à tous les herbicides (Huber, 1993; DeLorenzo *et al.*, 2001). De plus, dans une revue de littérature de Solomon *et al.*, (1996) ainsi que dans une étude récente de Baxter *et al.*, (2011), les auteurs concluent que des concentrations d'atrazine < 50 µg par litre ne devraient pas engendrer d'effets sur les organismes photosynthétiques dans l'environnement. À l'opposé, d'autres études ont montré qu'aux concentrations fréquemment mesurées dans l'environnement (< 5 µg litre^{-1}), la communauté phytoplanctonique peut être affectée par la présence d'atrazine (Peterson *et al.*, 1994; Caux et Kent, 1995). Malgré ces études, on ne sait toujours pas s'il existe un lien entre la présence d'atrazine ou autres herbicides et la dominance des cyanobactéries.

Finalement, puisque les herbicides notamment l'atrazine s'attaquent aux processus photosynthétiques et que ces derniers sont principalement régulés par la disponibilité en lumière, il ne faut pas négliger l'intéraction entre ces deux facteurs. A cet effet, une interaction entre l'intensité lumineuse et l'atrazine a déjà été montrée pour deux espèces d'algues, *Nannochloris occulata* et *Phaeodactylum tricornutum* (Mayasich *et al.*, 1986) ainsi que chez des communautés de périphyton acclimatés à forte ou faible intensité lumineuse (Guaush et Sabater, 1998). Les résultats de ces études montrent qu'une augmentation de l'intensité lumineuse augmente la sensibilité à l'atrazine. Comme nous l'avons vu précédemment, la lumière est très variable en milieu naturel et le phytoplancton survit à ces fluctuations grâce à la photorégulation et à la photoacclimatation. On peut donc se demander si une espèce qui a des mécanismes de photoprotection efficace peut être moins affectée par les herbicides qui inhibent le transport d'électrons et induisent la photoinhibition? Conséquemment, on peut se demander si les espèces ayant des mécanismes de photoprotection efficaces deviendront dominantes dans leur environnement?

Structure de la thèse

À cause du lien étroit entre photosynthèse et croissance, toute modification de l'habitat qui entraîne un changement de l'activité photosynthétique est susceptible de modifier la croissance du phytoplancton et donc leur distribution. Comme mentionné précédemment, les rejets agricoles modifient la transparence de l'eau et donc la disponibilité en lumière pour la photosynthèse. L'agriculture est aussi responsable du rejet d'herbicides dont le mode d'action vise directement l'inhibition des processus photosynthétiques. Dans ces conditions, la capacité de réponse de l'appareil photosynthétique des organismes devient déterminante pour la croissance et donc le succès écologique de l'organisme (Grossman *et al.*, 1993; MacIntyre *et al.*, 2002). Les premiers chapitres de cette thèse ont comme objectif de comparer la réponse physiologique de différents organismes photosynthétiques exposés à un gradient d'intensités lumineuses représentatif des milieux dulcicoles tandis que les derniers chapitres visent à comparer la sensibilité des algues et des cyanobactéries en présence de certains herbicides couramment utilisés en agriculture. L'objectif principal étant de déterminer si la lumière ou les herbicides représentent des facteurs importants pour l'établissement de la dominance des cyanobactéries

CHAPITRE I

COMPARISON OF PHOTOACCLIMATION IN TWELVE FRESHWATER PHOTOAUTOTROPHS (CHLOROPHYTES, BACCILARIOPHYTES, CRYPTOPHYTE AND CYANOPHYTES), ISOLATED FROM A NATURAL COMMUNITY

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CONTEXTE

Dans ce chapitre, nous comparons la capacité de photoacclimatation de plusieurs espèces de phytoplancton pré-sélectionnées pour leurs caractéristiques morphologiques et physiologiques variées. L'objectif principal est de comprendre si les mécanismes de photoacclimatation sont identiques entre tous les groupes et espèces.

* Tel que publié: Deblois, C.P., Marchand, A., Juneau, P. 2013. Comparison of photoacclimation in twelves freshwater photoautotrophs (Chlrophytes, Baccilariophytes, Cryptophytes and Cyanophytes) isolated from a natural community. PlosOne. 8(3). Art. No. e57139.

1.1 ABSTRACT / RÉSUMÉ

Different representative of algae and cyanobacteria were isolated from a freshwater habitat and cultivated in laboratory to compare their photoacclimation capacity when exposed to a wide range of light intensity and to understand if this factor may modify natural community dominance. All species successfully acclimated to all light intensities and the response of phytoplankton to increased light intensity was similar and included a decrease of most photosynthetic pigments accompanied by an increase in photoprotective pigment content relative to Chl *a*. Most species also decreased their light absorption efficiency on a biovolume basis. This decrease not only resulted in a lower fraction of energy absorbed by the cell, but also to a lower transfer of energy to PSII and PSI. Furthermore, energy funnelled to PSII or PSI was also rearranged in favour of PSII. High light acclimated organisms also corresponded to high non-photochemical quenching and photosynthetic electron transport reduction state and to a low Φ'_M. Thus photoacclimation processes work toward reducing the excitation pressure in high light environment through a reduction of light absorption efficiency, but also by lowering conversion efficiency. Interestingly, all species of our study followed that tendency despite being of different functional groups (colonial, flagellated, different sizes) and of different phylogeny demonstrating the great plasticity and adaptation ability of freshwater phytoplankton to their light environment. These adjustments may explain the decoupling between growth rate and photosynthesis observed above photosynthesis light saturation point for all species. Even if some species did reach higher growth rate in our conditions and thus, should dominate in natural environment with respect to light intensity, we cannot exclude that other environmental factors also influence the population dynamic and make the outcome harder to predict.

Key index words: Algae, Biooptic, Chlorophyll fluorescence, Cyanobacteria, Growth rate, Oxygen production, Pigments, Photoacclimation, Photosynthesis cyanobacteria.

RÉSUMÉ

Différents représentants des algues et des cyanobactéries ont été isolés d'un milieu aquatique dulcicole et cultivées en laboratoire pour comparer leur capacité de photoacclimatation lorsqu'exposées à un large éventail d'intensités lumineuses. L'objectif étant de comprendre si la photoacclimatation peut modifier la dominance phytoplanctonique dans les communautés naturelles. Pour ce faire, nous avons comparé la croissance, la teneur en pigment, les caractéristiques biooptiques et la physiologie associée à la photosynthèse par des mesures d'absorption et de fluorescence chlorophyllienne. Premièrement, toutes les espèces se sont acclimatées à toutes les intensités lumineuses avec succès. La réponse des différentes espèces était similaire et comprenaient une diminution de la plupart des pigments photosynthétiques accompagnée par une augmentation de la teneur en pigment de photoprotection par rapport à la Chl *a*. La plupart des espèces ont aussi diminué leur efficacité d'absorption de la lumière par unité de biovolume. Cette efficacité réduite entraînant une diminution marquée de la fraction de l'énergie absorbée par la cellule à efficacement réduit la quantité d'énergie transférée vers les PSI et PSI. En outre, l'énergie acheminée vers les PSI ou PSII a également été réorganisés en faveur des PSII. Les cellules acclimatées à forte intensité lumineuse avaient un NPQ plus élevé, un plus grand état d'oxydoréduction de la chaine de transport d'électron et un $\Phi`_M$ plus petit. Ainsi, les processus de photoacclimatation ont induit une réduction de la pression d'excitation dans les environnements de forte intensité lumineuse en réduisant l'efficacité d'absorption de la lumière, mais aussi en abaissant le rendement de conversion photosynthétique de l'énergie absorbée. Il est intéressant de noter que toutes les espèces de notre étude ont suivi cette tendance en dépit de leur appartenance à différents groupes fonctionnels (coloniale, flagellé, de taille différente) et de leur phylogénie, démontrant donc la grande capacité d'adaptation et la plasticité du phytoplancton à leur environnement lumineux. De plus ces ajustements, issus de la photoacclimatation, peuvent expliquer le découplage entre le taux de croissance et la photosynthèse observée pour toutes les espèces au dessus du point de saturation de la photosynthèse. Même si certaines espèces ont atteint un taux de croissance plus élevé dans nos conditions et devraient donc dominer dans l'environnement naturel par rapport à l'intensité lumineuse, il ne faut pas exclure l'influence et l'importance des autres facteurs environnementaux sur la dynamique des populations et donc, prédire la dominance des communautés reste difficile.

Mots clés: Algues, cyanobactéries, biooptique, fluorescence chlorophylienne, photacclimatation, production d'oxygène.

1.2 INTRODUCTION

In aquatic environment, success of microalgae and cyanobacteria depends on their individual capacity to convert light into biochemical energy through photosynthetic light reactions and to transform carbon and nutrients into biomass. Because of the physical properties of water and the presence of suspended particles, available light intensity for photosynthesis is highly variable in freshwater habitat [1, 2]. During sunny days, light intensity at the surface of waterbodies can be high enough to induce photoinhibition and cellular damage to exposed photosynthetic organisms [3, 4, 5]. Simultaneously, only few meters below the surface (sometime less), light intensity become limiting for photosynthesis and may represent less than 1 % of surface irradiance [2, 6]. In order to cope with such variability, photosynthetic organisms have developed an array of phenotypic adjustments including photoacclimation processes [2, 7, 8, 9]. Photoacclimation to low or high light environments involves mid to long term adjustments of the photosynthetic apparatus and includes down regulation and de novo synthesis of cell constituents such has photosynthetic and non-photosynthetic pigments, photosystems I and II (PSI and PSII), RUBISCO, as well as changes in cell ultrastructure [8, 10, 11, 12, 13]. When exposed to high light environments, photoacclimation responses of most algae and cyanobacteria include a decrease of photosynthetic pigments (chlorophylls and phycobiliproteins) combined with an increase in photoprotective carotenoids [14, 15, 16]. The decrease in chlorophyll a (Chl a) is normally associated to a decrease in the number of photosystems, while a decrease in accessory pigments (Chl b, c, d, and phycobiliproteins) is associated to a decrease in the size of the light harvesting complexes (LHC) [9]. Combined to an increase in carotenoid (Car) content relative to Chl a, these adjustments allow a reduction of the excitation pressure on the photosynthetic apparatus and protect the organism against light induced reactive oxygen species damages [17, 18, 19]. On the other hand, under light limiting

conditions, algae and cyanobacteria adjust their cellular constituents to increase light absorption efficiency [7, 9, 20].

The wide diversity observed in phytoplankton is impressive, and may influence their light utilization efficiency. Interspecific variation of size between individual cells can reach many orders of magnitude from sub-micrometric picoplankton up to microplanktonic species [21, 22]. While many species remain single cells (e.g. Chlamydomonas sp., Cryptomonas sp., Navicula sp.), others grow into structured colonies (e.g. Pediastrum sp., Pandorina sp., Volvox sp., Merismopedia sp.), filaments (e.g. Aphanizomenon sp., Anabaena sp.) or more or less defined clusters of cells (e.g. Microcystis sp., Sphaerocystis sp., Ankistrodesmus sp.) [23, 24]. Colonial organization provides some benefits such as protection against grazing, but this characteristic also comes with inconvenience such as increased density inducing stronger sinking rate and increased self-shading leading to lower light availability [21, 25, 26]. Some of these species may also have flagella or vacuoles permitting them to move in the water column in order to optimize light harvesting [2]. This broad diversity influencing their light utilization is not limited to the morphological properties of phytoplankton, but can also be seen at the photosynthetic, biochemical or physiological levels such as distinct pigmentation [27]. Chlorophyll a has a crucial role in the photosystem reaction center (RC) core and in the light harvesting complexes of oxygenic phytoplankton, thus, this pigment is common to all species. Nevertheless, light harvesting capacity also differs between species because of variability in composition of pigments such as chlorophyll b, c, d, carotenoïds and phycobiliproteins [20, 28]. Thus the great diversity of phytoplankton characteristics influencing photosynthesis and light harvesting may influence the efficiency of photoacclimation processes.

Many lakes from the eastern Townships in Québec (Canada) are impacted on a periodic basis by cyanobacterial bloom apparitions [29]. This phenomenon is a visible consequence of changes in algal community equilibrium, but the factors influencing this dynamic are not fully understood. Since light is a factor that can

modify algal community [1, 30, 31], comparing photoacclimation responses of various species may help to estimate if this factor contribute to the periodic community imbalance observed in these aquatic ecosystems. In this study, we isolated 12 species of phytoplankton belonging to different algal groups from a single algal assemblage of a temperate dimictic eutrophic lake. Species were selected for their different sizes and strategies to harvest light (pigments, movement) in order to compare their photoacclimation responses and to determine their active light range and photoacclimation capacity. We showed that the general photoacclimation processes among the studied species were similar, but the extent of the responses varies providing possible selective advantages to some species.

1.3 MATERIAL AND METHODS

1.3.1 Sampling and cell culture

In mid-July of 2008, water from the euphotic zone of the Réservoir Choinière, (Eastern Townships, Québec, Canada) was collected and inoculated into bold basal medium (BBM) enriched with carbonate (25 mg L^{-1}) and silicate (80 mg L^{-1}) (BBMsi). Species that successfully grew in that medium were isolated and cultivated in laboratory. From the species initially isolated, 12 species were selected in order to have a diversity of algal groups (Chlorophyte, Bacillariophyte, Cryptophyte and Cyanophyte) and traits: colonial, unicellular, flagellates, buoyant and different cell sizes (see Table 1.1 for details). Throughout the experiment all species were grown in 125 ml of fresh BBMsi in 250 ml flasks and periodically (frequency depending on growth rate) transferred into fresh medium to maintain the cells in exponential growth phase which provides reproducible physiological characteristics. Periodic inoculum transfers also minimized dead cell accumulation which in any cases remained negligible as confirmed by monitoring, on a daily basis, the stability of the maximum PSII quantum yield (F_V/F_M) and by microscopic observations.

Each species was acclimated for several weeks in an environmental growth chamber (MTR30, Conviron, Manitoba, Canada) with a light:dark cycle of 16: 8 at 21 °C to seven light intensities:14, 43, 76, 191, 341, 583 and 1079 µmol photons (PAR) m^{-2} s^{-1} (measured with a US-SQS/L Micro quantum sensor, Heinz Walz GmbH, Effeltrich, Germany, in the center of the culture flask containing 125 mL BBMsi). Both fluorescent (cool white fluorescent tubes Philips F72T8/TL841/HO) and incandescent bulbs (Philips 60W) were used and in our conditions (one growth chamber containing all cultures simultaneously) light quality was similar for all light intensities (confirmed by spectroradiometric measurements; HR2000 UV+Vis, Ocean Optics Inc, USA). All measurements were done on independent triplicates of fresh and healthy cultures. For each trial, a new culture was prepared from the previous one

and was allowed to grow until maximum PSII quantum yield and similar cell conditions (F_0, F_M and F_V/F_M) were attained. Moreover, using fixed signal gain and dark acclimation period during chlorophyll fluorescence measurements allowed to use the F_0 as a proxy of cell concentration from day to day [32] and this method was used to estimate the growth rate for each trial.

Table 1.1

Group, species names and relevant morphological and physiological characteristics including pigments for each of the 12 species selected for this study. The code is the abbreviation associated to the group and used in the figures.

Groups	Species names	Codes	Characteristics	Major pigments
Chlorophyte	*Ankistrodesmus falcatus*	CHL1	Colonial (2-6 cells) non-motile elongate cells,	Chl *a*, *b*, *c*
	Pandorina morum	CHL2	Colonial (16 cells), flagelate, big size	Chl *a*, *b*, *c*
	Oocystis lacustris	CHL3	Single non-motile ovoid cell or small colony (2-3 cells).	Chl *a*, *b*, *c*
	Pediastrum boryanum	CHL4	Colonial, planar, non-motil	Chl *a*, *b*, *c*
	Chlamydomonas snowii	CHL5	Unicellular, flagelates	Chl *a*, *b*, *c*
Baccilariophyte	*Aulacoseira granulata* var. *angustissima*	BAC2	Elongate curvated filament of 2-3 cells, non-motil, very low pigmentation	Chl *a*, *b*
	Fragilaria crotonensis	BAC3	Colonial, non-motil	Chl *a*, *b*
Cryptophyte	*Cryptomonas obovata*	CRY1	Unicellular, flagelates	PE / Chl *a*, *d*
Cyanophyte	*Phormidium mucicola*	CYA1	Small 0.8 µm non-buoyant rod-like colony (up to 4 cells), toxic.	PC / APC / Chl *a*
	Microcystis flos-aquae	CYA2	Colony (non-mucilaginous), small spherical cells 2 µm diameter, buoyant, toxic.	PC / APC / Chl *a*
	Aphanizomenon flos-aquae	CYA3	Association of numerous buoyant filamentous colony, toxic.	PC / APC / Chl *a*
	Anabaena spiroïdes	CYA4	Filamentous colony, buoyant, toxic.	PC / APC / Chl *a*

1.3.2 Chlorophyll fluorescence measurement

Induction curves (IC) were measured using WATER-Pulse-Amplitude-Modulated fluorometer (WATER-PAM) (Heinz Walz GmbH, Effeltrich, Germany) on 15 min dark adapted algal suspensions (3 mL) using standard IC protocol with the actinic light carefully matched to growth photon flux density (PFD). The PSII maximum and operational quantum yields (F_V/F_M and Φ'_M), the non-photochemical quenching (NPQ) and the unquenched fluorescence level (UQF_{rel}) were calculated from each IC [33, 34]. For cyanobacteria, F_M was estimated at the end of each IC using 50 μM Diuron (DCMU) in presence of actinic light [35].

1.3.3 Pigments determination

At each sampling, known volumes of culture were filtered under dime green light on GF/F filters (Whatman, USA) and kept frozen at -80 °C until pigment and phycobiliprotein extraction and determination. Chlorophylls (Chls) and carotenoids (Car) were extracted 5 min in 4 mL of boiling methanol, rigorously vortexed 1 min and kept at -80 °C for overnight extraction. Prior to measurement, the extract was filtered on GF/F and the optical density was read between 350 and 800 nm with a Cary 300 WinUV spectrophotometer (Varian, USA). The average OD from 750 to 800 nm was used to correct for sample turbidity. The concentrations of Chl a, b, c and d were estimated according to [36], while carotenoid concentration was estimated following [37]. Phycobilioproteins: phycocyanin (PC), allophycocyanin (APC), and phycoerythrine (PE), were extracted using 4 freeze-thaw cycles in 0.1 M potassium phosphate buffer (pH 6.8), sonicated on ice between the second and third cycle (2 watts for 1 min., sonic dismembrator model 100-Fisher Scientific, USA), and finally centrifuged at 5000 x g for 15 min. The absorbance spectra of the supernatant was recorded between 500 and 700 nm using a Cary 300 WinUV spectrophotometer

(Varian, USA) and pigment concentrations were calculated according to the equation given in [38].

1.3.4 Cell division rate

The cell concentration and size were measured using a Multisizer III Coulter counter (Beckman Coulter Inc, Fullerton, USA) when cell morphology allowed it, while for the other species (colony, filament or non-spherical cells) a sample was fixed with Lugol solution for measurement and counting under a microscope. Species specific growth rate (μ_d) was calculated from these data and fitted to growing light intensity (PFD) with a classic 4 parameter PE curve model with photoinhibition [39]:

$$\mu_d = \mu_M \cdot (1 - e^{\frac{-\alpha \cdot PFD}{\mu_M}}) \cdot e^{\frac{-\beta \cdot PFD}{\mu_M}} \quad \text{eq. 1,}$$

where α represents the initial slope of the curve, β is a light dependent inhibition constant and μ_M is the theoretical maximum growth rate. From these coefficients, the secondary parameters: achieved maximal growth rate (μ_{MAX}) and its corresponding light intensity ($E_M{}^{\mu d}$) were calculated [40].

1.3.5 Biooptical measurements

All measurements were carried under dime green light. Sample were concentrated (5x) by gentle filtration on polycarbonate membrane filter 0.8 µm pore size (Millipore, USA) and resuspended in 3 mL of BBMsi directly in the measuring quartz cuvette. Cell lost on the filter was negligible. The *in vivo* light absorption spectrum of the concentrated solution (O.D. m^{-1}) was measured (between 350 and 800 nm) with a Cary winUV spectrophotometer (Varian, USA) using the integrating sphere attachment. Immediately after this measurement, 10 µL of DCMU was added to the sample at a final concentration of 50 µM and the sample was maintained 1 minute under white light (500 µmol photons m^{-2} s^{-1}) to eliminate any variable

fluorescence. Following this treatment, the sample was immediately transferred in a CaryEclipse spectrofluorometer (Varian, USA) and the *in vivo* fluorescence excitation spectrum (400-700 nm) of the cell suspension was monitored at 730 nm. To avoid light scattering from the apparatus and cell sample, a long pass glass filter (RG695, Schott, AG, Mainz, Germany) was placed in front of the emission beam [20].

1.3.6 Chl a specific absorption coefficient

The in vivo light absorption spectrum (O.D. m^{-1}) was corrected for sample turbidity (average OD750_800) and converted to Chl a specific absorption coefficient $a^*_{\varphi}(\lambda)$ (m^2 mg Chl a^{-1}) according to eq. 2:

$$a^*_{\varphi}(\lambda) = \frac{2.3 \cdot (O.D.)}{(d \cdot Chl)} \qquad \text{eq. 2,}$$

where 2.3 is the Log to Ln conversion factor, d is the cuvette path length (m^{-1}) and Chl is the corresponding Chl a concentration (mg m^{-3}). The fluorescence excitation spectra was quantum corrected using the dye Basic Blue 3 (4.1 g L^{-1}) which corrects for instrument specific wavelength variation of the excitation beam intensity [41]. The corrected spectra was then scaled to $a^*_{\varphi}(\lambda)$ using the no-overshoot procedure to obtained the PSII Chl a specific absorption coefficient: $a^*_{PSII}(\lambda)$ [20]. We averaged $a^*_{\varphi}(\lambda)$ and $a^*_{PSII}(\lambda)$ between 400 and 700 nm or in the red band, between 670 and 680 nm, to obtain the light absorption coefficients specific to the whole cell (a^*_{φ}), to PSII and associated LHCII (a^*_{PSII}), to Chl a in whole cell (a^*_{φ} (red)) or to Chl a associated to PSII ($a^*_{PSII}(\lambda)$). High wavelength absorption efficiency (high $a^*_{\varphi}(\lambda)$ or $a^*_{PSII}(\lambda)$) is only significant when photons of that energy are available, hence, both coefficients were spectrally weighted according to the light spectrum E(λ) of the growth chamber. This correction was done by normalizing the E(λ) area to unity and by multiplying this dimensionless spectrum with $a^*_{\varphi}(\lambda)$ or $a^*_{PSII}(\lambda)$ which yield the

spectrally weighted ($\bar{a}^*_{\varphi}(\lambda)$ and $\bar{a}^*_{PSII}(\lambda)$) coefficient to be utilized in oxygen production estimates (see below).

1.3.7 Oxygen production estimate

The best method to estimate oxygen production rate per chlorophyll unit (PO2Chl) using biooptical approach and chlorophyll fluorescence measurement was showed to be the method relying on ā*PSII to calculate light available to PSII photochemistry [42]. Therefore, we calculated the PO2Chl data accordingly:

$$P_{O2}^{Chl} = \Phi'_M \cdot PFD \cdot \Gamma \cdot \bar{a}^*_{PSII} \cdot 3.6 \qquad \text{eq. 3,}$$

where $\bar{a}^*_{PSII}$ represent light absorption specific to PSII (m^2 mg chl a^{-1}) spectrally weighted to available light intensity and spectrum over the PAR range, PFD is the light intensity in the growth chamber (μmol photons (PAR) m^{-2} s^{-1}), Φ'_M is the PSII operational quantum yield, Γ is the minimum theoretical quantum requirement of PSII in order to evolve one O_2 molecule (0.25 O_2 per electron) [43], and 3.6 convert second to hour (3600) and μmol to mmol giving the final dimension for P_{O2}^{Chl} of mmol O_2 mg chl a^{-1} hr^{-1}. For some analysis, P_{O2}^{Chl} was converted to oxygen production rate per biovolume unit ($P_{O2}^{\mu m}$) express in fmol O_2 μm^{-3} hr^{-1}. Each rate obtained for individual species at their specific growth light intensity was plotted against acclimation PFD (PE curve) and fitted to the waiting in-line function using eq. 4 [44]:

$$P_{O2} = A \cdot PFD \cdot Kw \cdot e^{-Kw \cdot PFD} \qquad \text{eq. 4,}$$

where, P_{O2} is oxygen production rate normalized to Chl a (P_{O2}^{Chl}) or biovolume ($P_{O2}^{\mu m}$), A and Kw are scaling factors for the height of the curve and X-axis respectively and PFD was the light intensity (μmol photons (PAR) m^{-2} s^{-1}) in the growth chamber. From this function, we estimated the saturation (P_{SAT}^{Chl} / $P_{SAT}^{\mu m}$) and maximum (P_M^{Chl} / $P_M^{\mu m}$) rates of oxygen production and their corresponding light intensity (E_K^{Chl} / $E_K^{\mu m}$ and E_M^{Chl} / $E_M^{\mu m}$) following the equation presented in [44].

1.3.8 Statistical analysis

All analysis were made in JMP 6.0 (SAS institute, USA) or GraphPad Prism software version 5.00 for Windows (GraphPad Software, San Diego California USA). The confidence interval (CI) at 95% was calculated for each coefficient in eq. 1 and eq. 4 using matrice inversion [44]. These coefficients value ± CI were compared by ANOVA and post Hoc Tukey Kramer mean comparison tests. Comparison between light limited and light saturated conditions was done for each species independently with student t-test, while two-way ANOVA was used to compare light limitation and light saturation responses between phylogenic groups [45]. Achieved maximal growth rate (μ_{MAX}) obtained for individual species was compared to the fit obtained for all species grouped (All species) using Dunnett's test ($p<0.05$) [46]. Subsequent comparison was done with Tukey Kramer test ($p<0.05$) to rank species in each subgroup (higher, equal or lower than All species μ_{MAX}).

1.4 RESULTS

1.4.1 Cell division rate and primary production

Our results showed that all species successfully acclimated to all growth light conditions and that their specific cell division rates (μ_d day^{-1}) varied between 0.024 and 1.12 (Fig 1.1a). Specific maximal growth rates (μ_{MAX}) of *Ankistrodesmus falcatus*, *Pandorina morum*, *Chlamydomonas snowii* and *Phormidium mucicola* were significantly higher compared to the overall averaged μ_{MAX} of 0.54 (± 0.05) using Dunnett's mean comparison (Table 1.2). In this group of fast growing species, mean comparison (Tukey HSD) showed that *C. snowii* reached the highest μ_{MAX}, while there was no significant difference between *P. mucicola*, *A. falcatus* and *P. morum*. Above growth light saturation (between 200 and 400 µmol photons PAR m^{-2} s^{-1}: data not shown), these fast growing species attained μ_d values that allowed population to double in a day or less (μ_d > 0.693). Such level was not reached for any light conditions in other species of the present study (Fig 1.1a). It is worth to notice that although *C. snowii* has the highest μ_d values under saturating irradiance, this species also has the lowest μ_d (0.024 ± 0.002) when grown at 14 µmol photons (PAR) m^{-2} s^{-1}, indicating that this light intensity was very close to its compensation point. Among the remaining species, *Aulacoseira granulata* var. *angustissima*, *Fragilaria crotonensis* and *Aphanizomenon flos-aquae* had significantly lower μ_{MAX} compared to the overall value and formed the group of slow growing species (Table 1.2). In this group, *F. crotonensis* had the lowest μ_{MAX} and was therefore the slowest growing species of this study.

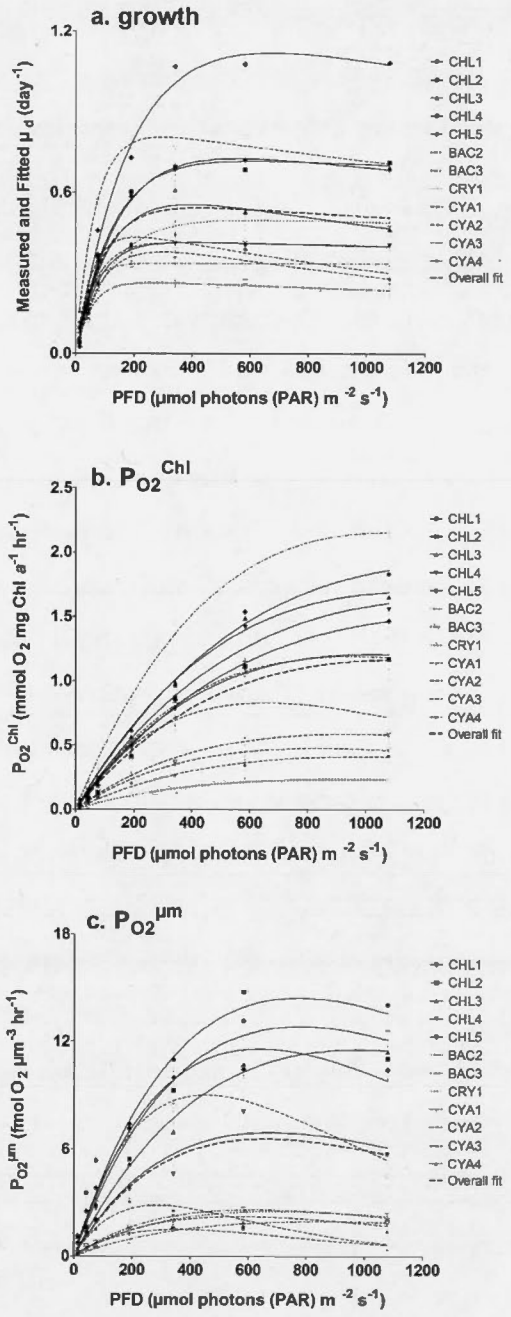

Figure 1.1 a. Cell division rate (μ_d). b. Oxygen production normalized to Chl *a* (P_{O2}^{Chl}) or c. to biovolume ($P_{O2}^{\mu m}$) obtained at each growing light intensities of photoacclimated phytoplankton (see Table 1.1 for the species list). The corresponding fits for growth or photosynthesis versus irradiance curve (PE curve) were obtained using eq. 1 (for μ_d) or eq. 4 (for P_{O2}). Overall fit represents the result obtained for the whole data set.

Light intensity required to reach μ_{MAX} ($E_M^{\mu d}$) varied between 187 (± 6) and 605 (± 39) μmol photons m^{-2} s^{-1} for all species with an average of 401 (± 29) μmol photons m^{-2} s^{-1} (Table 1.2). The species showing the lowest $E_M^{\mu d}$ were cyanophytes (except *Microcystis flos-aquae*) and the bacillariophyte *F. crotonensis*. Although they reached μ_{MAX} at similar light intensity, *F. crotonensis* was able to achieve constant growth rate above this point, while up to 35% growth inhibition was observed for the cyanophytes (except *M. flos-aquae*) (Fig 1.1a). Growth inhibition was also observed for the cholorophyte *Oocystis lacustris* despite its high $E_M^{\mu d}$. From all species, the cyanophyte *P. mucicola* was the best low light adapted organism since it achieved the highest μ_d at PFD below 191 μmol photons (PAR) m^{-2} s^{-1} (Fig 1.1a). On the other hand, the best high light adapted organism was *C. snowii* because of its high μ_{MAX} and μ_d at light above 191 μmol photons (PAR) m^{-2} s^{-1}.

Table 1.2

Achieved maximum growth rate (μ_{MAX}) and light intensity required to reach that rate ($E_M^{\mu d}$) estimated from growth versus irradiance fit (R^2 of each fit are presented) using eq. 1. The achieved maximal growth rate (μ_{MAX}) obtained for individual species was compared to the fit obtained for all species grouped (All species) using Dunnett's test ($p<0.05$). Subsequent comparisons with Tukey test ($p<0.05$) were done to rank species in each subgroup (higher, equal or lower than All species μ_{MAX}). Presented error corresponds to the 95% confidence interval.

Species	$E_M^{\mu d}$ (µmol photons m^{-2} s^{-1})	Error	μ_{MAX} (day^{-1})	Error	R^2 of Fit	p-value Dunnett's (for μ_{MAX})	Tukey HSD (for μ_{MAX})
C. snowii (CHL5)	585	25	1.12	0.14	0.98	<.0001	a
P. mucicola (CYA1)	279	5	0.80	0.03	0.98	0.0002	b
A. falcatus (CHL1)	503	30	0.73	0.08	0.96	0.0095	b
P. morum (CHL2)	588	39	0.72	0.08	0.95	0.0151	b
O. lacustris (CHL3)	340	2	0.55	0.06	0.91	1	a
C. obovata (CRY1)	605	39	0.50	0.05	0.94	0.9688	ab
A. spiroïdes (CYA4)	187	6	0.43	0.03	0.87	0.2497	ab
M. flos-aquae (CYA2)	369	64	0.41	0.06	0.73	0.1189	b
P. boryanum (CHL4)	403	19	0.41	0.02	0.97	0.1189	b
A. flos-aquae (CYA3)	252	6	0.38	0.05	0.81	0.0257	a
A. granulata (BAC2)	375	18	0.34	0.01	0.95	0.0032	a
F. crotonensis (BAC3)	255	20	0.26	0.02	0.90	<.0001	b
All species	401	29	0.54	0.05	0.48	1	-

Oxygen production (P_{O2}^{Chl}) estimates for all species varied between 0.008 and 2.239 mmol O_2 mg Chl a^{-1} hr^{-1} and the resulting PE curves depicted typical increases of photosynthetic activity in function of acclimation light intensity (Fig 1.1b). When comparing the maximal oxygen production rate (P_M^{Chl}), our data showed significant differences between algal groups (Table 1.3). It was higher for the bacillariophytes (1.67 ± SD of 0.54 mmol O_2 mg chl a^{-1} hr^{-1}) and chlorophytes (1.59 ± SD of 0.27 mmol O_2 mg chl a^{-1} hr^{-1}), while it was lower for the cyanophytes with 0.58 (±0.19) mmol O_2 mg chl a^{-1} hr^{-1} and the cryptophyte with 0.23 (±0.02) mmol O_2 mg chl a^{-1} hr^{-1} (Table 1.3). Oxygen production was also normalized to biovolume ($P_{O2}^{\mu m}$ unit: fmol O_2 μm^{-3} hr^{-1}) and allowed to compare species with respect to biomass, provided that cellular volume (μm^3) was a good proxy of species biomass [47]. Fitting $P_{O2}^{\mu m}$ with eq. 4, yielded different parameter estimates ($P_M^{\mu m}$ and $E_M^{\mu m}$) compared to the result obtained from P_{O2}^{chl} data (Fig 1.1b and 1.1c). When comparing the maximal oxygen production rate per biovolume ($P_M^{\mu m}$), our data showed that chlorophytes reached the highest value with an average of 11.46 (± 2.82) fmol O_2 μm^{-3} hr^{-1} (Table 1.3). Comparatively, it was up to 10 times lower for bacillariophytes, cryptophytes and cyanophytes (except *M. flos-aquae*) and varied between 1.50 and 2.84 fmol O_2 μm^{-3} hr^{-1} (Table 1.3). The light intensities at which P_M^{Chl} and $P_M^{\mu m}$ were achieved (E_M^{Chl} and $E_M^{\mu m}$) were also different and our data showed that $E_M^{\mu m}$ was significantly lower for all species (Table 1.3). For E_M^{Chl}, it varied between 660 and 1376 µmol photons m^{-2} s^{-1}, while for $E_M^{\mu m}$ it varied between 287 and 979 µmol photons m^{-2} s^{-1} (Table 1.3).

Table 1.3

Calculated parameters and associated errors (95% interval) of PE curves fitted (see also Fig 1.1b and c) using waiting in line function (eq. 4) for each species or combined all data (All species). Presented error corresponds to the 95 % confidence interval.

	P_M^{Chl} (mmol O^2 mg Chl s^{-1} hr^{-1})	error	E_M^{Chl} (µmol photons m^{-2} s^{-1})	error	$P_M^{\mu m}$ (fmol O^2 μm^{-3} hr^{-1})	error	$E_M^{\mu m}$ (µmol photons m^{-2} s^{-1})	error
A. falcatus (CHL1)	1.91	0.09	1376	112	14.44	1.29	774	74
P. morum (CHL2)	1.21	0.16	1022	174	12.82	2.02	780	133
O. lacustris (CHL3)	1.69	0.13	1169	131	11.54	0.94	979	101
P. boryanum (CHL4)	1.64	0.17	1335	225	6.92	1.15	678	113
C. snowii (CHL5)	1.50	0.09	1361	131	11.58	0.81	639	43
A. granulata (BAC2)	2.15	0.15	1061	98	1.95	0.42	851	211
F. crotonensis (BAC3)	1.19	0.12	961	152	2.61	0.27	622	62
C. obovata (CRY1)	0.23	0.02	926	77	2.52	0.32	697	89
P. mucicola (CYA1)	0.48	0.07	843	148	2.22	0.32	570	76
M. flos-aquae (CYA2)	0.83	0.15	660	119	9.95	2.14	480	89
A. flos-aquae (CYA3)	0.59	0.12	951	232	1.50	0.29	363	57
A. spiroïdes (CYA4)	0.41	0.06	1002	176	2.84	0.42	287	33
All species	1.17	0.18	1129	243	6.56	1.24	660	123

Species differences in PE curve inevitably introduce noise when comparing light dependent variables such as pigments [9]. This problem was accounted for by dividing growth PFD (E) to the photosynthesis light saturation point per biovolume ($E_K^{\mu m}$) obtained for each species. This variable ($E:E_K^{\mu m}$) was utilised to compare light dependent variables between species and allowed to form 2 groups corresponding to light limitation ($E:E_K^{\mu m} < 1$) or saturation ($E:E_K^{\mu m} > 1$) [9]. We also extended this approach to other variables: $P_{O2}^{\mu m}$ using the rate of photosynthesis at saturation $P_{SAT}^{\mu m}$ and μ_d using μ_{MAX} (Fig 1.2a and 1.2b). Comparing these variables showed that most species, regardless of their phylogeny, followed very similar trend with respect to saturation of photosynthesis and cell division rate (Fig 1.2a). The relationships between $\mu_d:\mu_{MAX}$ and $E:E_K^{\mu m}$ and between $P_{O2}^{\mu m}:P_{SAT}^{\mu m}$ and $E:E_K^{\mu m}$ showed for all species that cell division reached μ_{MAX} ($\mu_d:\mu_{MAX} = 1$) and that photosynthesis reached saturation ($P_{O2}^{\mu m}:P_{SAT}^{\mu m} = 1$) at $E_K^{\mu m}$ (Fig 1.2a). Similar results were obtained when comparing the relationship between $P_{O2}^{\mu m}:P_{SAT}^{\mu m}$ and $\mu_d:\mu_{MAX}$ (Fig 1.2b) and we observed that below saturation of photosynthesis, growth rate linearly increased, while it stabilized to μ_{MAX} above photosynthetic saturation (Fig 1.2b).

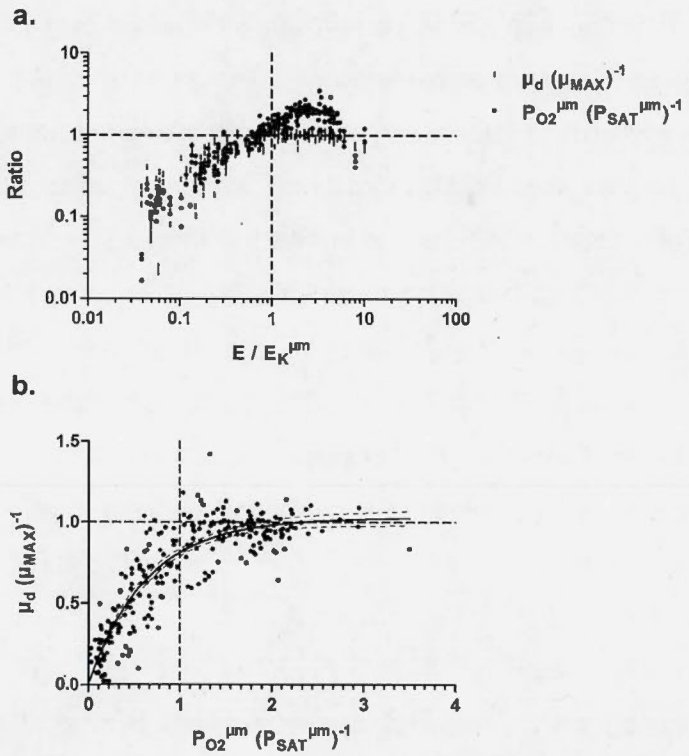

Figure 1.2 a. Oxygen production per biovolume ($P_{O2}^{\mu m}$) relative to oxygen production at saturation ($P_{SAT}^{\mu m}$) and achieved growth rate (μ_d) relative to maximal growth rate (μ_{MAX}) obtained for a gradient of growing light intensity (E) normalized to saturating light intensity of oxygen production ($E_K^{\mu m}$); b. relationship between obtained growth rate μ_d normalized to μ_{MAX} and oxygen production per biovolume ($P_{O2}^{\mu m}$) normalized to oxygen production at light saturation ($P_{SAT}^{\mu m}$). For both panels, the dashed line was set to 1 for all ratios and by definition corresponds to the point where the achieved value (μ_d, $P_{O2}^{\mu m}$ or E) equals the normalized coefficient value: μ_{MAX}, $P_{SAT}^{\mu m}$ or $E_K^{\mu m}$.

1.4.2 Pigment content

For all species, Chl *a* content was higher in phytoplankton exposed to light limiting conditions (PFD < $E_K^{\mu m}$) compared to saturating conditions (PFD > $E_K^{\mu m}$), although it was not significant for *O. lacustris* (Fig 1.3a). Our data also showed that bacillariophytes have less Chl *a* compared to most species. There was great variability in photoprotective carotenoid response since it tended to increase above light saturation for most cyanophytes but it decreased for most chlorophytes and bacillariophytes or remained unchanged in cryptophyte and one cyanophyte (Fig 1.3b). Despite these different responses, the Car to Chl *a* ratio followed a similar trend for all species (except *O. lacustris*) and was significantly higher above light saturation (Fig 1.3c). The sum of accessory pigments (PC, APC, PE, Chl *b*, Chl *c* and Chl *d*) relative to Chl *a*, reflecting the size of the light harvesting antennae, also varied in function of light intensity. This ratio was higher under light limiting conditions, while it was lower when phytoplankton grew under light saturating conditions (it was the opposite for cyanophytes) (Fig 1.3d). In cyanophytes and cryptophyte this ratio was generally higher compared to the other species because of phycobiliproteins, important for light harvesting in these species [48]. Our results showed that phycobiliproteins content decreased above light saturation for *M. flos-aquae*, *Anabaena spiroïdes* and *Cryptomonas obovata* and that the ratio of PC to APC also decreased for both filamentous species, *A. flos-aquae* and *A. spiroïdes* (Fig 1.3e and 1.3f). Finally, we observed that phycobiliproteins (PC and APC or PE) decreased on average by 30 % from low to high light, while Chl *a* decreased by 40 % for the same conditions.

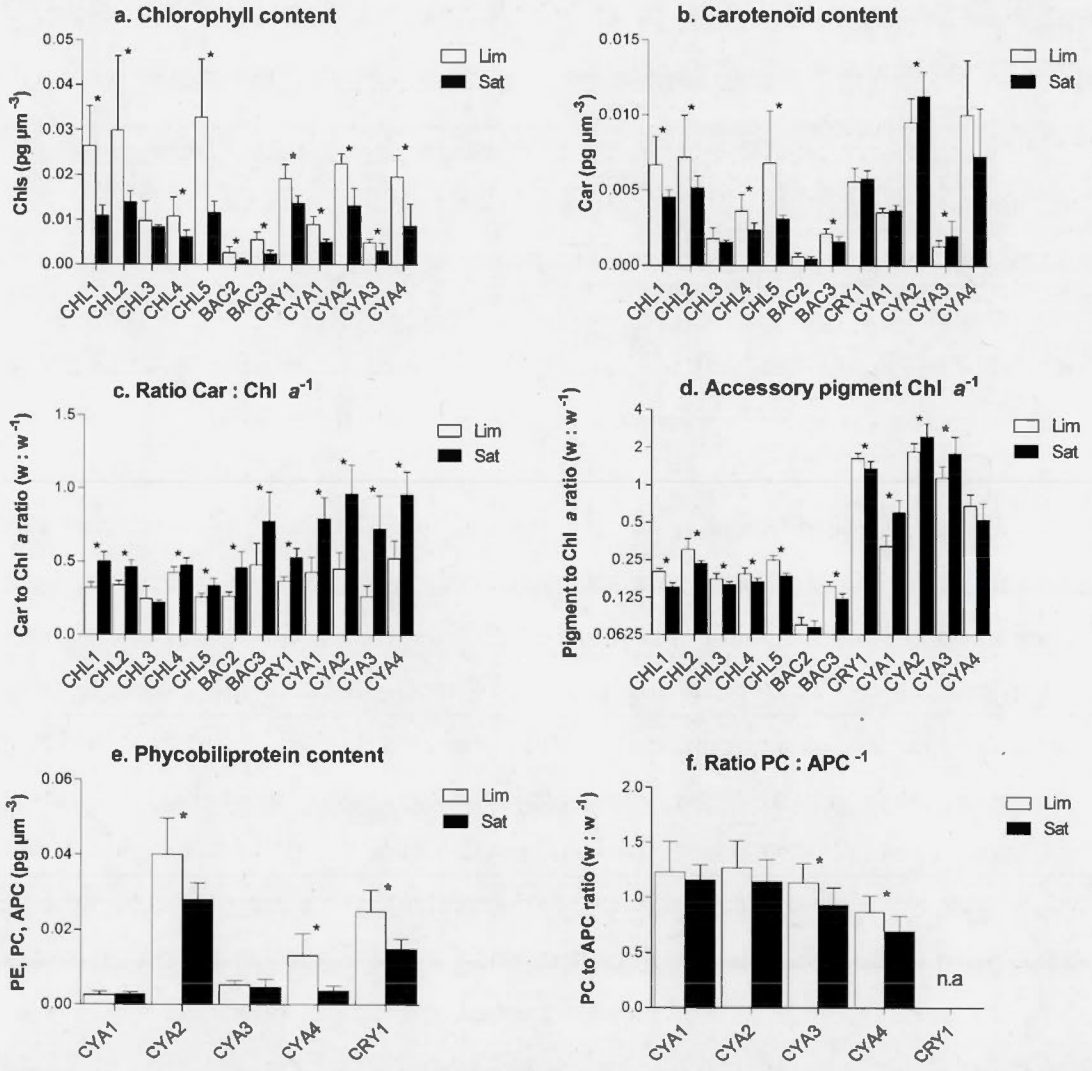

Figure 1.3 Comparison of the average pigment content normalized to biovolume (pg μm^{-3}) or the average pigment ratios obtained for each species grown under photosynthetic light limiting (Lim) or light saturating (Sat) conditions; a. total chlorophyll content, b. carotenoid content, c. Car to Chl *a* ratio, d. sum of accessory pigments (Chl *b*, *c*, d and phycobiliproteins), e. phycobiliprotein content for species having these pigments and f. phycocyanin (PC) to allophycocyanin (APC) ratio in cyanophytes. * Significant difference between treatment obtained for each species using t-test ($p<0.05$). See table 1.1 for species list.

1.4.3 Biooptical characteristic

Modifications of pigments content induced by photoacclimation processes also modified the biooptical properties of individual cells. For all conditions and studied species, the *in vivo* Chl *a*-specific light absorption coefficient in the red (a^*_φ (red)) varied between 0.002 and 0.025 m^2 mg chl a^{-1} with the lowest value (< 0.005) obtained for *C. obovata* (Table 1.4). When averaged over the whole spectrum, the corresponding light absorption coefficient reflecting total light absorption by pigments (a^*_φ) or specific to PSII (a^*_{PSII}), varied between 0.001 and 0.032 m^2 mg Chl a^{-1} and 0.001 and 0.013 respectively (Table 1.4 and e.g. Fig 1.4a). For most species, these coefficients were higher under photosynthetic light saturation conditions, except for *C. obovata* and *O. lacustris* (for a^*_φ, a^*_{PSII} and a^*_φ (red)) and *A. granulata* (for a^*_φ (red)) (Table 1.4). Increased light absorption efficiency in high light, as presented here, was counterbalanced by the lower Chl *a* content above light saturation (Fig 1.3a). In fact, when normalizing light absorption coefficients to the Chl *a* content per biovolume, correcting for changes in Chl *a* quotas due to photoacclimation processes, our data showed a decrease of all coefficients (a^*_φ, a^*_{PSII} and a^*_φ (red)) above light saturation (e.g. $a^*_\varphi{}^{\mu m}$ in Fig 4b; data not shown for other coefficients). The fraction of light absorption associated to PSII and LHCII relative to light absorption by the whole cell (fAQ$_{PSII}$) decreased by 8.9 to 26.1 % for most species following high light acclimation (Fig 1.4c). On the other hand, the cellular fraction of Chl *a* associated to PSII relative to that associated to PSI (F_{II}) was found to increase by 5.7 to 76.9 % depending on species (Fig 1.4d). Regardless of the light conditions, we also found that a significantly higher fraction of light absorption was directed toward PSII (fAQ$_{PSII}$) in chlorophytes (0.73 ± 0.05) and bacillariophytes (0.72 ± 0.05) compared to cryptophyte (0.63 ± 0.08), while it was much lower (0.33 ± 0.06) in cyanophytes (Fig 1.4c). For all light conditions, cyanophytes were also characterized by very low F_{II} (0.14 ± 0.07) compared to cryptophyte (0.35 ± 0.01), chlorophytes (0.56 ± 0.04) and bacillariophytes (0.61 ± 0.10).

Table 1.4

Averaged data (% CV) obtained and compared between light limiting (Lim) and light saturating (Sat) intensity for photosynthesis of the 12 studied species. Pigment absorption was averaged over the whole light absorption spectrum (400 to 700 nm) for whole cell (a^*_{φ}) or specific to PSII (a^*_{PSII}), or was averaged in the red band (670 to 680 nm) for Chl *a* specific absorption (a^*_{φ} (red)).

	a^*_{φ} Lim	Sat	a^*_{PSII} Lim	Sat	a^*_{φ} (red) Lim	Sat
A. falcatus (CHL1)	0.008 (19)	0.011 (8)*	0.005 (16)	0.007 (3)*	0.012 (19)	0.015 (4)*
P. morum (CHL2)	0.004 (17)	0.006 (14)*	0.003 (16)	0.004 (15)*	0.006 (13)	0.008 (14)*
O. lacustris (CHL3)	0.011 (24)	0.011 (4)	0.008 (27)	0.008 (7)	0.016 (20)	0.017 (4)
P. boryanum (CHL4)	0.009 (16)	0.012 (15)*	0.007 (16)	0.008 (10)*	0.012 (15)	0.014 (10)*
C. snowii (CHL5)	0.006 (17)	0.009 (12)*	0.004 (16)	0.006 (9)*	0.009 (17)	0.013 (9)*
A. granulata (BAC2)	0.014 (7)	0.016 (8)*	0.010 (6)	0.012 (9)*	0.021 (8)	0.022 (10)
F. crotonensis (BAC3)	0.007 (15)	0.012 (33)*	0.005 (12)	0.007 (20)*	0.009 (12)	0.012 (13)*
C. obovata (CRY1)	0.002 (18)	0.002 (10)	0.001 (18)	0.001 (17)	0.002 (18)	0.002 (14)
P. mucicola (CYA1)	0.010 (16)	0.016 (17)*	0.003 (13)	0.004 (8)*	0.012 (13)	0.014 (8)*
M. flos-aquae (CYA2)	0.014 (9)	0.025 (20)*	0.004 (6)	0.006 (17)*	0.015 (6)	0.017 (12)*
A. flos-aquae (CYA3)	0.014 (19)	0.023 (33)*	0.003 (15)	0.004 (26)*	0.014 (13)	0.017 (17)*
A. spiroïdes (CYA4)	0.010 (14)	0.013 (16)*	0.003 (10)	0.003 (13)*	0.011 (8)	0.013 (8)*

* Significantly different by t-test ($p<0.05$), unequal variance assumed.

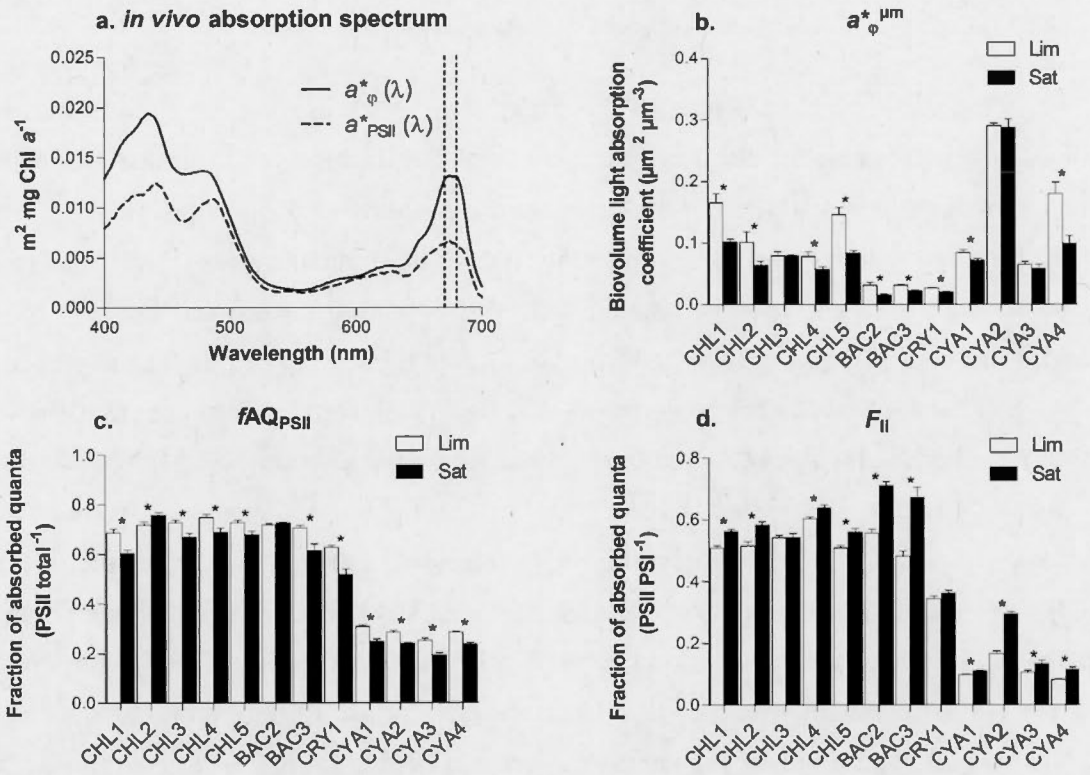

Figure 1.4 a. example of *in vivo* Chl *a* absorption spectrum ($a^*_\varphi(\lambda)$ and $a^*_{PSII}(\lambda)$) obtained for *A. falcatus* acclimated to 76 µmol photons m^{-2} s^{-1}. Averaging $a^*_\varphi(\lambda)$ or $a^*_{PSII}(\lambda)$ over the whole spectrum (400 to 700 nm) yielded to a^*_φ and a^*_{PSII} respectively, while averaging the coefficient in the red band (670 to 680 nm) yielded to a^*_φ (red) and a^*_{PSII} (red) respectively. Other panels, comparison of averaged biooptical data obtained for each species grown under photosynthetic light limiting (Lim) or light saturating (Sat) conditions where b. is the averaged light absorption coefficient normalized to biovolume, c. the fraction of absorbed quanta to PSII ($fAQ_{PSII} = a^*_{PSII} / a^*_\varphi$) and d. the fraction of absorbed quanta associated to PSII relative to PSI ($F_{II} = a^*_{PSII}$ (red) / a^*_φ (red)). * Significant difference between treatment obtained for each species using t-test ($p<0.05$). See table 1.1 for species list.

1.4.4 Photosynthetic electron transport and quantum requirement

In this section, we compared the effect of photoacclimation on photosynthesis through photosynthetic electron transport, light utilisation and dissipation and quantum requirement (QR). Decrease in the PSII operational quantum yield (Φ'_M) was observed with increasing light intensity for the 12 studied species (Fig 1.5a). Our data showed that Φ'_M remained stable under light limiting conditions ($E:E_K^{\mu m} < 1$) and decreased by 54 to 85% at light intensities above $E_K^{\mu m}$ (Fig 1.5a). The averaged Φ'_M was the highest for chlorophytes (0.19 – 0.75) followed by cryptophyte (0.28 – 0.66) and bacillariophytes (0.15 to 0.63), while it was the lowest in cyanophytes (0.06 – 0.51) (Fig 1.5a). The decrease of Φ'_M under high light conditions also affected the number of photons required to evolve 1 O_2 molecule (oxygen quantum requirement: QR). As seen, QR increased (from 149 up to 279 %) for all species when light intensity increased above saturation level (Fig 1.5b). Under light limiting conditions, where Φ'_M was the highest, the QR was on average 13.2 ± 1.4 mol e mol O_2^{-1} for all algal species, while it was higher in cyanophytes with 19.4 ± 2.0 mol e mol O_2^{-1}. Furthermore, we noticed that the decrease of Φ'_M and the concomitant increase in QR were accompanied by an increase in non-photochemical quenching (NPQ) and in the level of unquenched fluorescence (UQF_{rel}) (Fig 1.5c and 1.5d). Group comparison showed that NPQ was higher in cyanobacteria (0.38 ± SE 0.02) and chlorophytes (0.33 ± SE 0.02) compared to cryptophyte (0.19 ± SE 0.02) and bacillariophytes (0.13 ± SE 0.01) and was surprisingly low in the latter group. We also demonstrated that NPQ tended to increase from limiting to saturating light conditions (average 220 %) although that tendency was not significant for two of the cyanophytes studied in which NPQ was constant (Fig 1.5c). Similarly, the unquenched fluorescence level (UQF_{rel}), which is proportional to the redox state of the photosynthetic electron transport chain [34], increased (average 255 %) for all species between limiting and saturating light conditions (Fig 1.5d). When averaged over all light conditions, the highest UQF_{rel} value was measured in bacillariophytes (0.21 ± SE 0.01) followed by cyanophytes (0.17 ± SE 0.01) and cryptophyte (0.15 ± SE 0.02), while for chlorophytes the value (0.12 ± SE 0.01) was significantly lower.

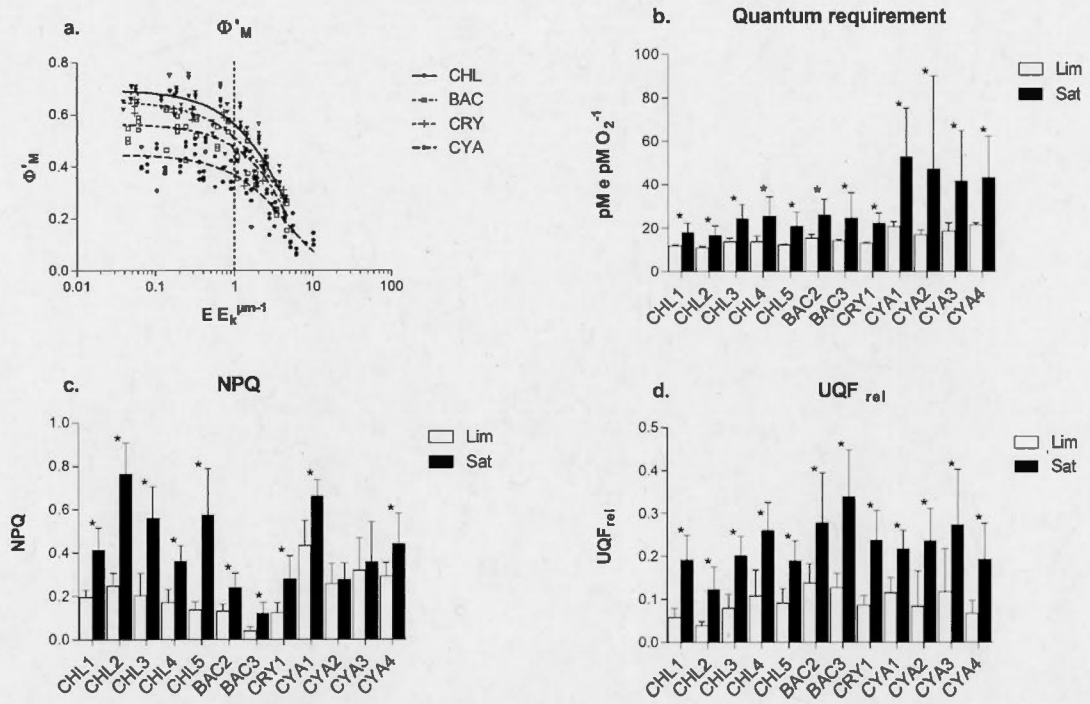

Figure 1.5 a. Group specific relationship between PSII operational quantum yield (Φ'_M) and growth light intensity normalized to photosynthetic light saturation point ($E_K^{\mu m}$). Other panels, comparison of averaged chlorophyll fluorescence paramaters obtained for each species grown under photosynthetic light limiting (Lim) or light saturating (Sat) conditions where b. is the quantum requirement, c. the non-photochemical quenching (NPQ) and d. the relative unquenched fluorescence parameter (UQF_{Rel}). * Significant difference between treatment obtained for each species using t-test ($p<0.05$). See table 1.1 for species list.

1.5 DISCUSSION

1.5.1 Effect on growth

In this study, we showed that chlorophytes, bacillariophytes, cryptophyte and cyanophytes successfully acclimate and grow under a wide range of light conditions (from 14 to 1079 µmol photons m^{-2} s^{-1}) when given proper acclimation period. As expected, the achieved growth rate increased with light intensity and reached its maximal value between 187 and 605 µmol photons m^{-2} s^{-1} depending on the species. Above that point, growth inhibition was observed for most cyanophytes, corresponding to the response of low light adapted organisms [7, 30], but also for *O. lacustris*, while growth of other species remained unaffected by high light up to 1079 µmol photons m^{-2} s^{-1}. Photoinhibition observed for *O. lacustris* was not surprising since we have shown low pigment plasticity and overall low photoacclimation driven responses (pigments, biooptic, photosystem ratio) for that species. As expected, this lack of response under high light conditions resulted in suboptimal growth [7, 17]. Although not mandatory, our growth data suggested that being chlorophyte, flagellate and/or small organism are characteristics allowing higher than average growth rate (Table 1.2). Conversely, most of the species presenting low growth rates (*A. granulata*, *F. crotonensis* or *A. flos-aquae*) were colonial or filamentous and had large cell as seen by their high averaged biovolume of 484 to 3593 μm^3. It is well admitted that usually larger organisms have slower growth rates compared to smaller organisms due to their cell metabolism and higher package effect [21, 49]. However, we noticed some exceptions to that trend as seen with *M. flos-aquae* and *P. morum*. For *M. flos-aquae*, we have measured low growth rate despite its small size (± 27 μm^3), but this species also formed colonies. Another exception was observed for the colonial species *P. morum* for which high growth rate was measured despite its large size. For that species, individual cells are flagellated and we observed that their motion can actively position the colony in relation to available light and this may

contribute to optimize its growth [27, 50]. Knowing that capacity to acclimate to light and morphological characteristics are important to determine growth [24, 31], our results tend to demonstrate that size, which affects the light absorption efficiency (package effect), and aptitude to movement (to optimize light harvesting) were relevant factors.

1.5.2 Pigment acclimation

Because light can be damaging for photosystems, by causing oxydative stress to individual cell, and is indispensable as a source of energy, phytoplankton capacity to acclimate to a limitation or excess in photon flux is critical [12]. In this study, we showed that most species presented similar response to light acclimation, but to varying degrees. We found a decrease of photosynthetic pigment content (chlorophylls and phycobiliproteins) in all species following high light acclimation confirming previous observations [2, 8, 9, 13]. In most species, this decrease was accompanied by a decrease in carotenoid content and in the size of the light harvesting antennae as seen by lower accessory pigments to Chl *a* ratio (Fig 1.3b and 1.3d). In cyanophytes, we observed a small reduction in the size of PBS under light saturation, but the proportion of PBS relative to Chl *a* increased suggesting an increase of LHC antenna size in that group (see below for more details). Lowering pigment content and antenna size is a typical response of high light acclimated cells [51]. These responses directly decrease the number of photons absorbed by the LHCs and decrease energy transfer to PSII and PSI RCs [51]. This adjustment results in a lower excitation pressure on the photosynthetic apparatus and is essential to minimize photoinhibition and cell damage induced by oxidative stress [52, 53]. In low light environment, and as observed in this study, these pigment modifications also worked in the opposite direction. In fact, increased pigmentation and antenna size allowed to maximize light harvesting and thus, alleviated the energy deficit caused by surrounding light scarcity [7, 9]. The light dependent variation in photosynthetic

pigment content described here, was accompanied by modifications of the proportion of photoprotective pigments (Car) with respect to Chl *a* (Fig 1.3c). This ratio was the highest under light saturation condition for all species and corresponded to previous finding showing that high Car to Chl *a* ratio increases protection against excess photon flux by allowing light energy dissipation through NPQ processes [13, 54, 55].

For all light conditions, cyanobacteria and cryptophyte had significantly more accessory pigments relative to Chl *a* and that was attributed to the presence of phycobiliproteins reflecting the dominance of these pigments for light harvesting in these species [2, 47, 56]. Surprisingly, this ratio was significantly higher under saturating light condition for three cyanobacteria, while it was lower in the other tested species of this study and others [9, 57, 58]. Since this increase was accompanied by a decrease of Chl *a* and PBS individually, it indicates that when acclimated to high light, these species favour light harvesting through PBS relative to Chl *a*. Phycobilisomes are highly mobile pigment complexes that can unbind from the RC core when exposed to high irradiance and thus prevent energy funnelling under excess light condition [59, 60]. Furthermore, previous studies have shown that in some cyanophytes, orange carotenoids interact with PBS when exposed to high light intensity in order to induce dissipation of excess energy [61, 62, 63]. Thus, the observed increase of PBS relative to Chl *a* may help to protect against high light as a complementary mechanism to energy dissipation through carotenoids. We can also hypothesis that favouring PBS over carotenoids is a strategy allowing higher light harvesting flexibility for organisms suddenly exposed to a lower light environment.

1.5.3 Biooptical acclimation

Analysis of the biooptical data showed that changes in pigment content and ratio reported here successfully modified light harvesting efficiency and energy allocation between PSI and PSII (Fig 1.4 and Table 1.4). For most species, the Chl *a* specific light absorption coefficients (a^*_{φ}, a^*_{φ} (red) and a^*_{PSII}) significantly increased

with light intensity. This increase was important (reaching up to 169 %) for all species with minor exceptions (*O. lacustris*, *A. granulata* and *C. obovata*) and as was found previously, this was related to an increase in light absorption efficiency for high light acclimated cells [20]. This counterintuitive result may be attributed to an increased light absorption of Car (relative to Chl *a*) and assigned to Chl *a* in a^* calculation, but also to a lower pigment packaging (reduced self-shading) in high light acclimated cells [2, 20, 64, 65, 66]. According to our results, both phenomena occurred in our conditions since we observed an increase in the Car to Chl *a* ratio and the a^*_{φ} (red) values never reached 0.033 m^2 mg Chl a^{-1} (max value obtained was 0.025 m^2 mg Chl a^{-1}, see also Table 1.4) and this value is expected to be close to the absorption coefficient of Chl *a* embedded in thylakoid membrane without any package effect [64]. Nevertheless, the higher light absorption efficiency observed for high light cells was mitigated by a lower content in Chl *a* per biovolume (Fig 1.3a). When taking that variable into account, we observed that the biovolume specific absorption cross section coefficient ($a^*_{\varphi}{}^{\mu m}$) decreased or remained stable as can be expected following acclimation to high light [2, 20, 48]. Very similar results and conclusions were drawn for PSII specific absorption coefficient (a^*_{PSII}) and Chl *a* absorption in the red (a^*_{φ} (red)) indicating that following high light acclimation, energy directly associated to Chl *a* and PSII tended to decrease (in most cases) or remained stable on a biovolume basis. These modifications observed under high light conditions minimized the excitation pressure on the photosynthetic apparatus despite the increased light availability [18].

Comparison in the partition of harvested energy showed that above light saturation of photosynthesis, a lower proportion of intercepted photon was directed toward PSII in almost all species (Fig 1.4c). This can be explained by the increase of Car to Chl *a* ratio and associated increased proportion of energy dissipation through heat by carotenoids and/or PBS uncoupling in cyanophytes. Our data also showed that energy balance between PSII and PSI was modified under high light, since a higher fraction of the energy was associated to PSII compared to PSI (Fig 1.4d).

Thus, it indicates that photoacclimation process did not only decrease PSII and PSI excitation pressure under higher light intensity, but it also redirected light absorption toward PSII. This rebalance of energy between the photosystems is necessary to prevent any excess energy to one of the photosystems (minimize excitation pressure) and to optimize electron flow between photosystems [11, 17, 18, 67].

1.5.4 Photoacclimation and photosynthesis

Our data clearly showed that the photosynthetic activity of PSII was also affected by photoacclimation processes. As seen, the PSII operational quantum yield (Φ'_M) changed in close relationship with light limitation to light saturation gradient (Fig 1.5a). It remained high and stable under light limited conditions and it decreased when light intensity was above the saturation point. Interestingly, that tendency was similar for all studied species regardless of their taxonomic groups and despite different average Φ'_M or pigment composition. It also indicates that under light limitation, phytoplankton optimized light utilisation through high PSII quantum yield, while other biochemical or physiological factors became limiting in draining electrons under high light [9, 10]. Concomitantly to these changes, we observed an increase of NPQ and UQF_{REL} above light saturation. Non-photochemical quenching and associated processes allowed the dissipation of excess energy and alleviated the excitation pressure on PSI and PSII [54]. The unquenched fluorescence reflects the redox state of the electron transport chain [34] and the high values obtained above light saturation indicates that the PSI and/or other electron sinks were less efficient to drain electrons under high light compared to low light conditions. This lower capacity to drain electrons from PSII may be induced by PSII:PSI energy imbalance or by a lack of available reductants (NADP+ and ADP) [10, 18, 52]. Finally, the variations observed for Φ'_M were also reflected in the quantum requirement (QR) which remained close to the theoretical value of 8 photons per O_2 molecule evolved with 13.4 ($\pm$ 1.4 mol e mol O_2^{-1}) for chlorophytes, bacillariophytes and cryptophyte under

light limiting conditions. However, for the cyanophytes the average QR was higher (19.4 ± 2.0 mol e mol O_2^{-1}) indicating that this group was less efficient to convert light energy into chemical energy. Under saturating conditions, QR increased for all species to more than 30 (> 50 in cyanophytes) indicating a lower photosynthetic efficiency compared to low light conditions. Despite the lower conversion efficiency under light saturation, phytoplankton cells were able to maintain high growth rates indicating that adjustments to their energy dissipation processes (high NPQ and UQF_{rel} and low Φ'_M) under high light conditions were not disadvantageous. These differences between phytoplankton groups clearly indicate variations in photoacclimation processes, as was also observed for pigments and optical properties.

1.5.5 Primary production and growth uncoupling

The oxygen production estimates calculated from a combination of chlorophyll fluorescence and biooptical method [42] and normalized to Chl *a* (P_{O2}^{Chl}) varied between 0 and 2.2 mmol O_2 mg Chl a^{-1} hr^{-1} for all species (Fig 1b). This was comparable to the range reported previously for different phytoplankton species [9, 28, 42, 44, 68]. Oxygen production was lower when normalized to biovolume ($P_{O2}^{\mu m}$) and this difference can be attributed to variation in the ratio of Chl *a* per biovolume specific to individual species following photoacclimation. When normalized to Chl *a*, oxygen production was informative of the photosynthetic apparatus efficiency where high values correspond to high photosynthetic efficiency. Oxygen production normalized to biovolume allows to relate photosynthetic efficiency to biomass, and therefore to the achieved growth rate. In our study, PE curves were reconstructed similarly to growth versus light curves since they were based on photosynthetic activity obtained at different growth light intensities. This method is slightly different to PE curves obtained by short term exposure to different light intensities of pre-acclimated phytoplankton [9]. Thus, our approach permits to directly estimate if there is a relationship between growth and photosynthesis when phytoplankton is

acclimated to specific light conditions. Interestingly, when comparing the light intensity required to reach maximal photosynthesis for both variables (P_M^{Chl} and $P_M^{\mu m}$), we found that $E_M^{\mu m}$ (287-979 µmol photons m^{-2} s^{-1}) was always lower than E_M^{Chl} (660-1376 µmol photons m^{-2} s^{-1}) for all studied species (Table 1.2). This difference reflects a decoupling between photosynthesis and cellular investment in chlorophyll (see below). We may therefore advance that cellular investment in the photosynthetic components and Chl *a* relative to the other cellular constituents was sub optimal for the studied species and this suggests that phytoplankton cells, in our growth conditions, did not try to maximize their photosynthetic activity, otherwise $E_M^{\mu m}$ should tend toward E_M^{Chl}. A good example of that phenomenon was observed for *A. granulata* since this diatom has the highest P_M^{Chl} of all tested species (Fig 1.1b) and thus high photosynthetic efficiency relative to cellular Chl *a* investment. However, this high photosynthetic efficiency was not reflected into a better growth rate. In fact, we found very low oxygen production on a biomass basis ($P_M^{\mu m}$) for this species, indicating that its strategy was not to invest in photosynthetic apparatus and Chl *a* (Fig 1.1b and 1.1c). Consequently, this species presented one of the lowest μ_d and μ_{MAX} values of this study despite a potential of high photosynthetic efficiency. Our findings that growth rate approached its maximal value when oxygen production per biovolume reached saturation and the absence of change of μ_d above photosynthesic saturation and up to maximal photosynthesis was another indication of the decoupling between cell division and photosynthesis. This can be attributed to lower Chl *a* content in high light acclimated cells and can also be caused by an increase in the respiration processes relative to photosynthesis or by accumulation of compound that were not included in our growth rate estimates such as lipids.

1.5.6 Conclusions

The general response of phytoplankton to increased light intensity worked toward reducing the excitation pressure on the photosynthetic apparatus and also toward reducing their efficiency to utilize the absorbed energy. According to our results, these mechanisms induced a decoupling between photosynthesis and growth rate when light intensity was above photosynthetic saturation level indicating that photoacclimation processes do not necessarily optimize photosynthesis to maximize growth. Interestingly, all species of our study followed that tendency despite being of different functional groups (colonial, flagellated, different size) and of different phylogeny. Even if some species did reach higher growth rates in our conditions and thus, should dominate in natural environment with respect to light intensity, we cannot exclude that other environmental factors also influence the population dynamic making the outcome difficult to predict. Finally, the fact that morphologically distinct species isolated from the same community, but belonging to different phylogenic groups, were able to adjust to a wide range of light intensities (from 14 to 1079 µmol photons m^{-2} s^{-1}) demonstrates the great plasticity and adaptation ability of freshwater phytoplankton to their light environment and help to understand their ubiquity in natural environment.

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CHAPITRE II

RELATIONSHIP BETWEEN PHOTOSYNTHETIC PROCESSES AND MICROCYSTIN IN *MICROCYSTIS AERUGINOSA* GROWN UNDER DIFFERENT PHOTON IRRADIANCE

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CONTEXTE:

Dans le chapitre précédent, nous avons montré que les processus de photoacclimatation engendrent des modifications physiologiques importantes chez les algues et les cyanobactéries. Dans ce chapitre, nous étudions s'il existe un lien entre les modifications issues de la photoacclimatation et la toxicité de *Microcystis aeruginosa*, une cyanobactérie réputée pour sa capacité à former des floraisons toxiques dans plusieurs écosystèmes dulcicoles. Le second objectif est l'amélioration de notre compréhension concerant le rôle physiologique des microcystines, encore inconnu chez *M. aeruginosa*.

* Tel que publié: Deblois, C.P., Juneau, P. 2010. Relationship between photosynthetic processes and microcystin in *Microcystis aeruginosa* grown under different photon irradiances. *Harmful Algae*, 9: 18-24.

2.1 ABSTRACT (RÉSUMÉ)

Microcystis aeruginosa is well known for its ability to produce the group of hepatotoxic microcystins (MCYST) but the mechanisms that control their synthesis are not clearly understood. Possible effect of light and nutrients on MCYST content was already proposed, but contradictory results were reported. In this study, we have grown *M. aeruginosa* (UTCC299) under a wide range of light conditions (24 to 820 µmol photons (PAR) m^{-2} s^{-1}) and showed that MCYST content decreased (106 (SE ±7) to 47 (SE ±3) fg MCYST per cell) with increasing photon irradiance. Our data showed that MCYST content was positively linked to Chl *a* content and inversely correlated to cell specific division rate. Chlorophyll *a* fluorescence kinetics was used to assess photosynthetic activity and the relative electron transport rate (rETR). The latter was negatively correlated with MCYST content and was the best predictor of MCYST content compared to light and growth. Moreover, the redox state of the photosynthetic apparatus estimated by the unquenched fluorescence parameter (UQF_{REL}) was also negatively correlated to MCYST content per cell. Therefore, our data demonstrated that photosynthetic light reactions, through electron transport rate (rETR) and PSII redox state, modulate MCYST content at the cellular level. Knowing that environmental conditions affect photosynthetic light reactions, our results indicated that future work should also pay closer attention to the involvement of photosynthesis in the modulation of MCYST.

Key index words: Microcystin, Photosynthesis, Relative electron transport rate, Photosystem II redox state, Oxygen production.

RÉSUMÉ

Microcystis aeruginosa est bien connu pour sa capacité à produire des microcystines (MCYST) hépatotoxiques, mais les mécanismes qui contrôlent cette production ne sont pas clairement compris. Certaines études ont montré des effets de la lumière et des éléments nutritifs mais des résultats contradictoires ont été publiés. Dans cette étude, nous avons cultivé *M. aeruginosa* (UTCC299) dans une grande gamme d'intensité lumineuse (24 à 820 µmol photons (PAR) m^{-2} s^{-1}) et avons montré que le contenu en MCYST diminue de 106 (SE ± 7) à 47 (SE ± 3) fg par cellule en fonction de l'augmentation de l'intensité lumineuse de croissance. Nos données ont montré que le contenu de MCYST était positivement lié au contenu en Chl *a* et inversement corrélée au taux de croissance. Des cinétiques de fluorescence chlorophyllienne ont été utilisées pour évaluer l'activité photosynthétique et le taux relatif de transport d'électrons (rETR). Ce dernier était négativement corrélé avec le contenu en MCYST en plus d'être la meilleure variable permettant de prédire la teneur en MCYST comparativement à l'intensité lumineuse ou au taux de croissance. En outre, l'état d'oxydoréduction de l'appareil photosynthétique estimée par le paramètre de fluorescence non-absorbé (UQF_{rel}) était également corrélée au MCYST et ce négativement. Par conséquent, nos données montrent que certains aspects de la photosynthèse, notamment le taux de transport des électrons (rETR) et l'état redox du PSII, peuvent moduler le contenu en MCYST au niveau cellulaire. Sachant que les conditions environnementales comme la lumière affectent les réactions photo-synthétiques, nos résultats indiquent que les travaux futurs devraient également porter une attention particulière au rôle de la photosynthèse dans la modulation du contenu en MCYST.

Mots clé: Microcystine, Photosynthèse, Transport relative d'électron, Photosystème II état d'oxydo-réduction, Production d'oxygène.

2.2 INTRODUCTION

Because of their ability to produce toxic compounds, widespread occurrences of cyanobacterial blooms are of increasing concern in regard to aquatic organisms and public safety. In freshwater, toxic species of the genera *Microcystis* are the most studied bloom forming cyanobacteria because of the ability of some strains to produce microcystins (MCYST), a group of potent hepatotoxic peptides (Carmichael, 2001; Chorus and Bartram, 1999; Codd *et al.*, 2005a). Microcystins are heptapeptides, and are strong inhibitors of the serine threonine protein phosphatase 1 and 2A in both mammals and higher plants (Mackintosh *et al.*, 1990). They have been linked to illness and death of cattle, dogs, and in extreme cases humans (Codd *et al.*, 2005b).

Although the toxicological effects of MCYST are well known (Dawson, 1998), the factors regulating its content at the cellular level are not fully understood (Kaebernick and Neilan, 2001; Kardinaal and Visser, 2005; Schatz *et al.*, 2007). Several studies on the regulation of MCYST content in *M. aeruginosa* have been conducted in the past 20 years, showing links between MCYST content and a variety of environmental factors including temperature (van der Westhuizen and Eloff, 1985), nutrients such has phosphorus, nitrogen or iron (Utkilen and Gjølme, 1995; Bickel *et al.,* 2000; Lee *et al.*, 2000; Oh *et al.*, 2000; Long *et al.*, 2001; Downing *et al.*, 2005a, b) and light (Utkilen and Gjølme, 1992; Wiedner *et al.,* 2003), but contrasting results were obtained. As seen from these studies, it is difficult to find a general mechanism that regulates MCYST cell quota in *M. aeruginosa*. However, in a literature review by Sivonen and Jones (1999), it was concluded that cyanobacteria seem to produce more toxin when the conditions were the most favourable for growth. This possible link between MCYST production and growth rate was also reported by Orr and Jones (1998) and Long *et al.* (2001) for nitrogen limited

M. aeruginosa cultures and also confirmed in a study under phosphorus limitation (Oh *et al.*, 2000).

One other key factor that regulates growth of phytoplankton and cyanobacteria is light (Wetzel, 2001), but as for nutrients, studies on the effect of photon irradiance on microcystin content had not led to a unique conclusion (Wiedner *et al.*, 2003; Kardinaal and Visser, 2005). These contradictions in the influence of photon irradiance were mainly attributed to different growth conditions, experimental setups and inter-strain variability (Kardinaal and Visser, 2005; Sivonen and Jones, 1999). In a study by Wiedner *et al.* (2003), MCYST content was found to linearly increase when *M. aeruginosa* was grown under light limiting condition, while under saturating light intensities, MCYST content tended to decrease with increasing photon irradiance. The results obtained in this study suggested that photon irradiance is involved in the regulation of MCYST biosynthesis but the mechanisms remained to be elucidated (Wiedner *et al.*, 2003).

A common effect of a change in photon irradiance and nutrient availability is the induction of physiological changes associated to alteration of photosynthesis and energy production (Herzig and Falkowski, 1989; Li *et al.*, 2003; Lippemeier *et al.*, 2003). Therefore, variations of toxin cell quota in *M. aeruginosa* reported to be related to growth rate (Orr and Jones, 1998; Long *et al.*, 2001), nutrient limitation (Lee *et al.*, 2000; Oh *et al.*, 2000) or photon irradiance (Utkilen and Gjølme, 1992; Wiedner *et al.*, 2003) may in fact be the result of physiological changes related to light reactions of photosynthesis, and not a direct effect of nutrient or light stress. Whether the alteration of light reactions of photosynthesis can also lead to change in MCYST content was not investigated directly, although some reports speculated on this possibility (Hesse *et al.*, 2001; Kaebernick and Neilan, 2001; Long *et al.*, 2001; Jähnichen *et al.*, 2007).

In order to better understand the possible role of photosynthesis as a MCYST cellular content modulator, we investigated here the response of photosystem II – photosystem I (PSII – PSI) electron transport, growth and MCYST cell quota in *M.*

aeruginosa cultivated under different photon irradiances (from 24 to 820 μmol photons (PAR) m^{-2} s^{-1}). Therefore, our results allowed a direct comparison of changes in light reactions of photosynthesis and MCYST cell quota.

2.3 MATERIAL AND METHODS

2.3.1 Cell culture

The toxic strain *Microcystis aeruginosa* (UTCC299) was grown at 24°C in 500 ml flask in batch culture supplied with 200 ml BBM medium and maintained in exponential phase by periodic (3-5 days) inoculum (10 ml) transfer into fresh culture medium during the light acclimation phase. This light acclimation phase was done prior to the measurements, and the cultures were acclimated for at least three weeks to the five photon irradiances (24, 56, 136, 272 and 820 µmol photons (PAR) m^{-2} s^{-1}) with a light:dark cycle of 16:8 hr (incandescent bulbs and fluorescent tubes-Philips F72T8/TL841/HO).

Keeping exponentially growing cultures and avoiding dead cell accumulation prevented any significant extracellular released of MCYST (Rohrlack and Hyenstrand, 2007). After light acclimation, an inoculum (5 ml) of exponentially growing cells were transferred into fresh medium under the same growing conditions described previously and after 3-5 days, cells were harvested in their exponential phase (cell density of approximately 500,000 cells/ml) and used to obtain the presented data. Sampling was done at the same time of the day for the different photon irradiances. Cell number and biovolume were measured for the entire growing period prior and during the experiment with a Multisizer III Coulter counter (Beckman Coulter Inc, Fullerton, USA) and since cells were not in colonies no disaggregation was necessary before cell counting. Cell specific division rates (μ_c) were calculated from plots of the log of cell number against incubation time in day.

2.3.2 Fluorescence measurements

Chlorophyll *a* fluorescence kinetics were obtained with a WATER-Pulse-Amplitude-Modulated (WATER-PAM) (Heinz Walz GmbH, Effeltrich, Germany). Prior to the measurements, the samples were maintained in the dark for 15 minutes to

completely oxidize the PSII electron transport chain. Constant fluorescence level of dark adapted sample (F_O) and maximum fluorescence level (F_M) were assessed respectively before and after the addition of diuron (10 μM) and illumination to actinic light (Campbell *et al.*, 1998). Diuron concentration used was high enough to permit the reduction of all PSII reaction centers, since the use of a saturating flash did not permit further increase of the fluorescence yield. Actinic light was adjusted to match photon irradiance found in the growth chamber for each treatment. Saturating light (800 ms, 3000 μmol m^{-2} s^{-1}) given periodically (every 60 s) provided the maximum fluorescence yield for a light-adapted sample (F'_M). At steady state fluorescence yield (F_S), actinic light was turned off and, following a far red illumination, the light-adapted constant fluorescence yield (F'_O) was obtained. From the PAM induction curve, fluorescence parameters: Φ_M (maximum PSII quantum yield), Φ'_M (operational PSII quantum yield), $UQF_{(REL)}$ (relative unquenched fluorescence) and ETR (relative photosynthetic electron transport rate) were calculated according to Juneau and Harrison (2005).

2.3.3 Oxygen evolution (GP)

O_2-evolution was measured on each sample using a Clark type electrode (Oxy-lab DW2 system, Hansatech, UK) under dark condition to measure the respiration rate (R) and light condition at similar photon irradiance as the growth chamber to measure the net rate of oxygen production (NP). The sum of both NP and the absolute value of R gave the gross rate of oxygen production (GP). The GP rates obtained with this method were normalized to Chl a^{-1}.

2.3.4 Pigment determination

For each treatment, 25 ml of culture was filtered through a 0.8 µm pore size membrane filter to minimize the sticking of cells to the filter and stored in Eppendorf tubes at -20°C until analysis. Chlorophyll *a* and carotenoids were extracted in boiling MeOH at 78°C according to Lorenzen (1967). The extract was then centrifuged at 4°C and the supernatant was measured with a spectrophotometer Cary300WINUV (Varian, USA) at wavelengths of 480 and 665 nm prior and after acidification with HCl. A measurement at 750 nm was used to correct for the sample turbidity. Calculation of carotenoid content was done according to Davies (1976) and Chl *a* was done according to Lorenzen (1967).

2.3.5 Microcystin extraction and PPI measurement

For each light treatment, 25 ml of culture was filtered on a 0.8 µm pore size membrane filter to minimize adsorption and store in Eppendorf tubes at -20°C until analysis. Microcystin was extracted with 1.5 ml of 80% v:v MeOH:H_2O, sonicated (5-10 Watts for 30 seconds with a Sonic dismembrator model 100-Fisher Scientific, USA) and centrifuged (15 minutes at 22,000 x g). The supernatant was kept in a glass tube at -20°C while the pellet was reextracted twice using the same technique. The final volume of extract (4.5 ml) was then evaporated under a stream of air and resuspended in milliQ water. Microcystin was then measured using a fluorometric protein phosphatase inhibition (PPI) assay according to Deblois *et al.*, (2008). In this study, all the results obtained with PPI are expressed as MCYST-LR equivalents because the standard curve in the PPI assay is based on pure MCYST-LR.

2.3.6 Statistical analysis

The difference between each photon irradiance treatment was assessed using the Tukey HSD mean comparison test ($p < 0.05$), and regression was used to assess the relationship between the different variables (Quinn and Keough, 2003). A stepwise analysis was used to find the best predictive variables of microcystin cell quota with a probability of $p < 0.05$ to keep the variable in the model. Data of the relative electron transport rate (ETR) and gross rate of oxygen production per Chl *a* were fit against photon irradiance through a classic P/E curve according to Jassby and Platt (1976) and the fitted data were plotted to compare both methods. All statistical analysis and mathematical modeling were achieved with Jump 5.1 statistical software (SAS institute, USA).

2.4 RESULTS

At low photon irradiances (below 136 µmol photons (PAR) m^{-2} s^{-1}), the cell specific division rate of *M. aeruginosa*, was positively affected by the photon irradiance, therefore growth was light-limited under these conditions (Table 1). Under higher photon irradiance levels, (272 and 820 µmol photons (PAR) m^{-2} s^{-1}), cell specific division rate was not significantly affected and therefore light was saturating under these conditions (Table 2.1). When *M. aeruginosa* was grown under high photon irradiance (820 µmol photons (PAR) m^{-2} s^{-1}), the cellular Chl *a* content was 2.4-fold lower than when it was grown under low photon irradiance (128 fg Chl *a* $cell^{-1}$ compared to 312 fg Chl *a* $cell^{-1}$) (Fig. 2.1A). Similar result was obtained when Chl *a* cell quota was expressed on a biovolume basis (Fig. 2.1B). As was the case for Chl *a*, the cellular MCYST content was lower at higher photon irradiances by up to 2.2 fold (106 (SE ±7) to 47 (SE ±3) fg MCYST $cell^{-1}$), and by up to 2.7 fold (1.71 (SE ±0.08) to 0.63 (SE ±0.07) fg MCYST μm^{-3}) when expressed on a biovolume basis (Fig. 2.1C and 2.1D). Since the cell biovolume was significantly lower at 24 µmol photons m^{-2} s^{-1} (40 μm^3 $cell^{-1}$) compared to the four other photon irradiances (79 μm^3 $cell^{-1}$), it explained the discrepancy observed at the lowest photon irradiance between MCYST or Chl *a* content expressed per cell quota and biovolume (Table 2.1). For all treatments we observed that MCYST cell quota was positively correlated with Chl *a* cell quota (Fig. 2.2A) and negatively correlated with cell specific division rate (Fig. 2.2B). The difference observed in Chl *a* cell quota between the different treatments (Fig. 2.1A and 2.1B) was accompanied by a 2.2 fold difference in the carotenoid (Car) to Chl *a* ratio (Table 1). Under all photon irradiances, it was found that MCYST cell quota was three time lower than the Chl *a* cell quota (Table 2.1).

Table 2.1

Average cell specific division rate (μ_c day^{-1}) and cell biovolume (µm^3 cell^{-1}), carotenoid (pg Car) and microcystin (fg MCYST) to chlorophyll *a* (fg Chl *a*) ratio for each photon irradiance (from 24 to 820 µmol photons (PAR) m^{-2} s^{-1}).

Light condition (PAR) µmol photons m^{-2} s^{-1}	Cell specific division rate μ_c day^{-1}	Cell volume µm^3 cell^{-1}	Cell ratio	
			Car : Chl *a* (pg Car fg Chl *a*$^{-1}$)	MCYST : Chl *a* (fg MCYST fg Chl *a*$^{-1}$)
24	0.33 (±0.01) ^{A}	49 (±2) ^{A}	757 (±81) ^{A}	0.32 (±0.06) ^{AB}
56	0.37 (±0.01) ^{B}	80 (±16) ^{B}	932 (±133) ^{AB}	0.34 (±0.05) ^{AB}
136	0.41 (±0.02) ^{C}	75 (±12) ^{B}	949 (±89) ^{AB}	0.37 (±0.04) ^{AB}
272	0.42 (±0.01) ^{CD}	84 (±12) ^{B}	1150 (±132) ^{B}	0.28 (±0.03) ^{B}
820	0.43 (±0.02) ^{D}	77 (±12) ^{B}	1638 (±189) ^{C}	0.37 (±0.08) ^{A}

* For each mean presented, N = 6 ± SE. Mean connected with different letter are different by Tuckey HSD ($p < 0.05$)

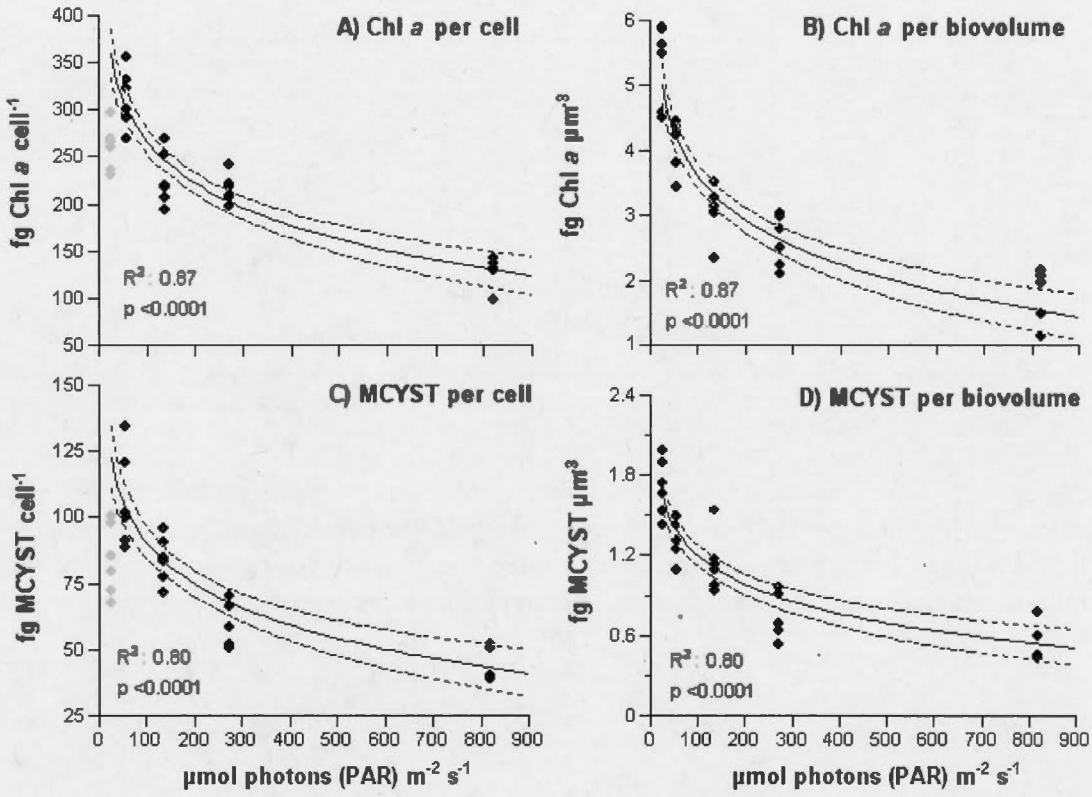

Figure 2.1 Variation in the content of Chlorophyll *a* (Chl *a*) A) per cell B) per biovolume and variation in the content of microcystin (MCYST) C) per cell and D) per biovolume, in relation to the growth light condition (PAR from 24 to 820 µmol photons (PAR) m^{-2} s^{-1}). The R^2 of the relationship and associated probability are presented for each regression. For both MC and Chl *a* expressed per cell, the data from the lowest photon irradiance (in grey) were removed from the regression.

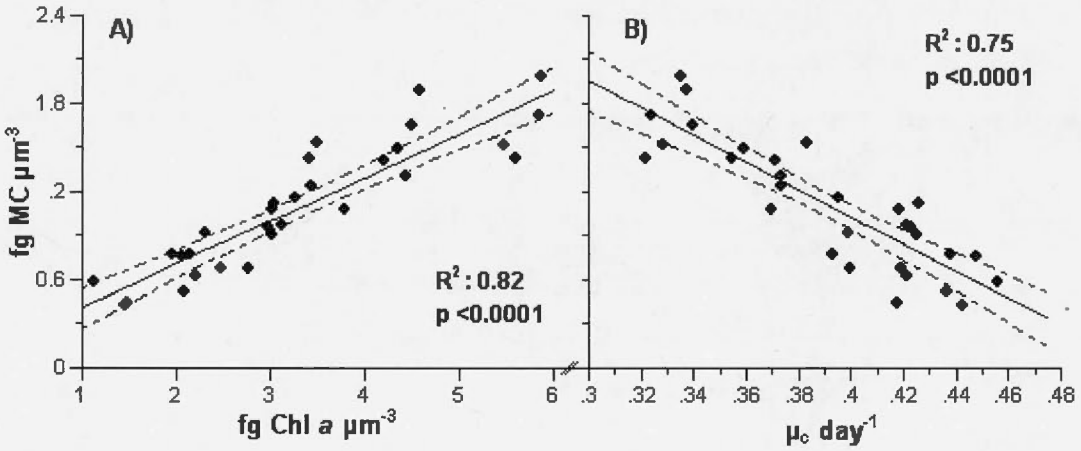

Figure 2.2 Relationship between A) microcystin content (MCYST) and chlorophyll *a* content (Chl *a*) per biovolume, and B) between microcystin content (MCYST) per biovolume and the cell specific division rate (μ_c day^{-1}). The R^2 of the relation and associated probability are presented for each regression.

When analysing the kinetics of chlorophyll *a* fluorescence, normalized to F_O, (see a subset of the data representing the lowest (Fig. 2.3A) and the highest photon irradiance treatments (Fig. 2.3B)), we observed that high photon irradiance had no significant effect on the maximum fluorescence value F_M and the maximum PSII quantum yield (Φ_M) (Fig. 2.4A). On the other hand, the light adapted maximum fluorescence value (F'_M) was significantly lower under high photon irradiance compared to low photon irradiance. This indicated modification of energy dissipation processes linked to photosynthesis. Moreover, the fluorescence value at steady state electron transport (F_S) was higher under high photon irradiance, resulting in the rise of the unquenched fluorescence parameter ($UQF_{(REL)}$) (Fig 2.3A & 2.3B). The unquenched fluorescence parameter ($UQF_{(REL)}$), which is related to the redox state of PSII (Juneau *et al.*, 2005), was negatively correlated to the cellular MCYST cell quota (Fig. 2.4B) and suggested that high PSII redox state is associated to low MCYST cell quota. The photosynthetic activity, estimated using the relative electron transport rate (ETR) or the gross rate of oxygen production (GP), was positively affected by increase in photon irradiance level (Fig. 2.5) and both methods allowed a similar estimation of photosynthetic light reaction activity since the ETR and GP P/E curves were strongly correlated (R^2 0.99; $p < 0.0001$). When looking at the link between photosynthetic activity and MCYST cell quota, our data showed a strong relationship between MCYST and ETR (R^2 0.83; $p < 0.0001$), which suggested that high steady state electron transport rate is associated with low MCYST cell quota (Fig. 2.6).

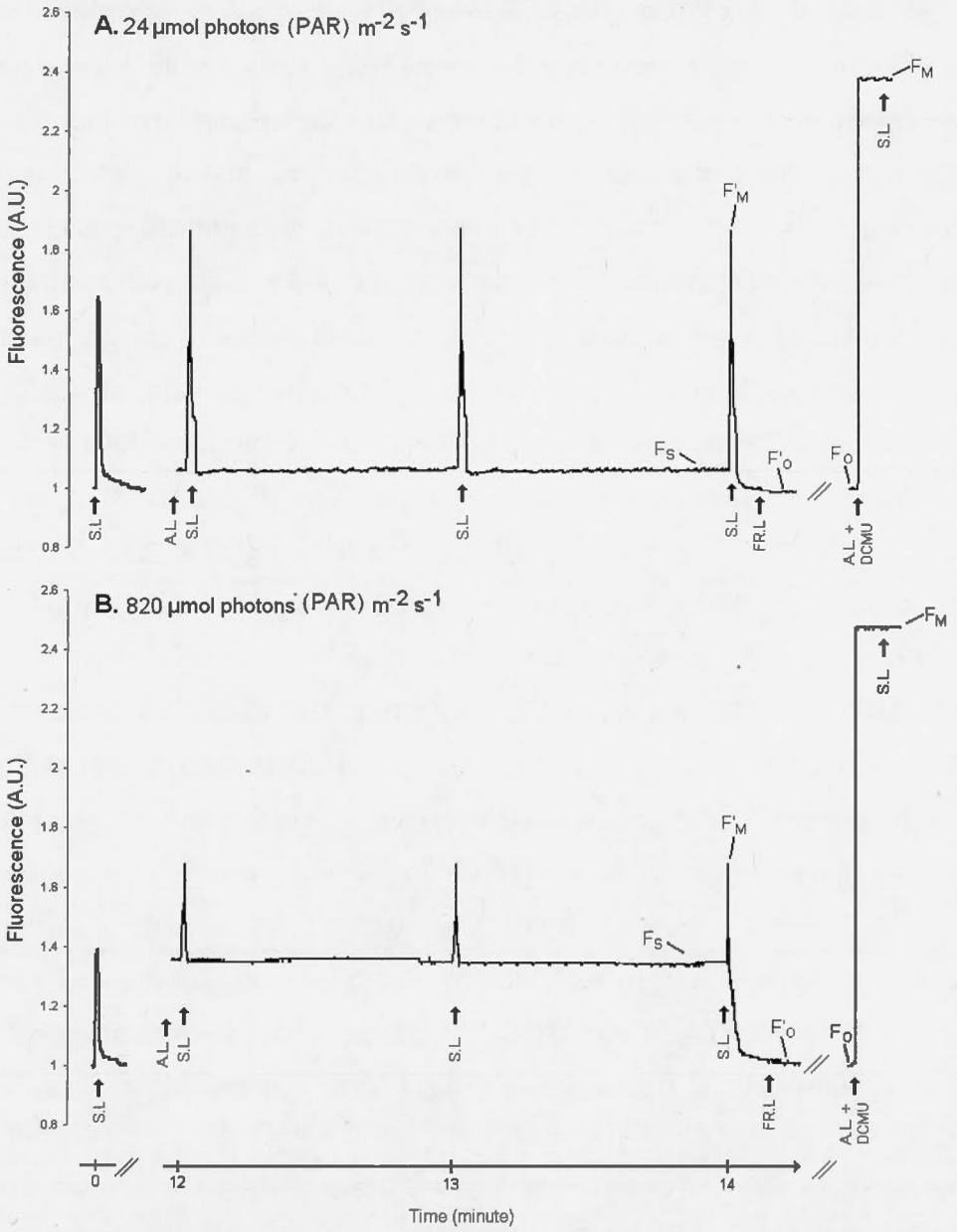

Figure 2.3 Kinetics of the induction curve of the chlorophyll *a* fluorescence for *M. aeruginosa* growth under A) low photon irradiance and B) high photon irradiance where S.L., A.L and FR.L are saturating, actinic and far red light respectively and F_M, F'_M, F_S and F'_O represented the fluorescence under each light condition of the induction curve. For comparison, all fluorescence data where normalized to F_O. For cyanobacteria, the addition of 10 µmol of diuron (DCMU) is necessary to obtain the F_M value.

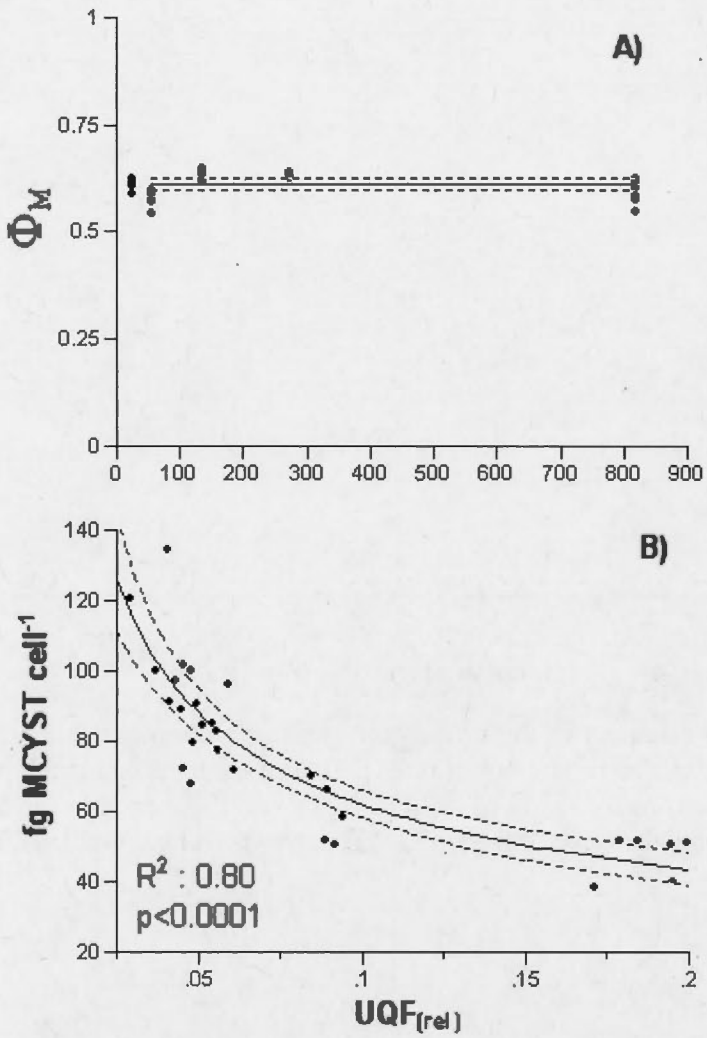

Figure 2.4 Relationship between A) the maximum PSII quantum yield (Φ_M) and PAR intensity (µmol photons (PAR) m^{-2} s^{-1}) and B) relationship between the microcystin cell quota (MCYST) and the unquenched fluorescence parameter ($UQF_{(REL)}$). The R^2 of the relation and associated probability are presented when appropriate. The bar in Fig 3A represents the general average and it standard error (N = 30).

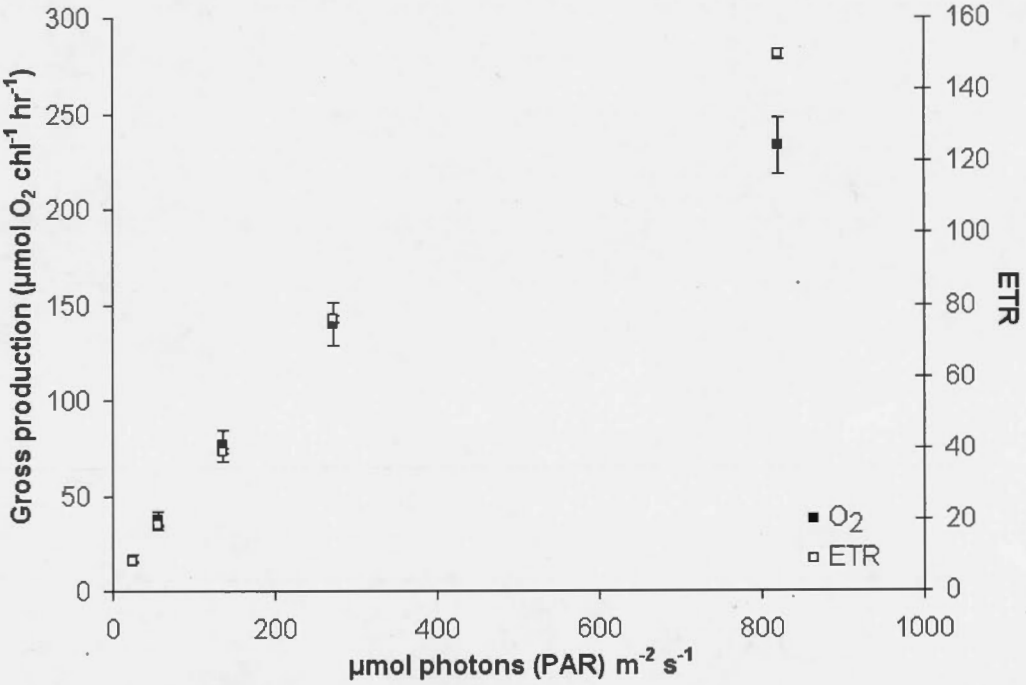

Figure 2.5 Variations in the rate of oxygen production per unit of chlorophyll *a* and in the relative electron transport rate (ETR) for each photon irradiance. Both ETR and GP estimates are the result of a P/E curve fit on the measured ETR and GP data. The bar represents the error of the fitted P/E curve for each variable (ETR and GP).

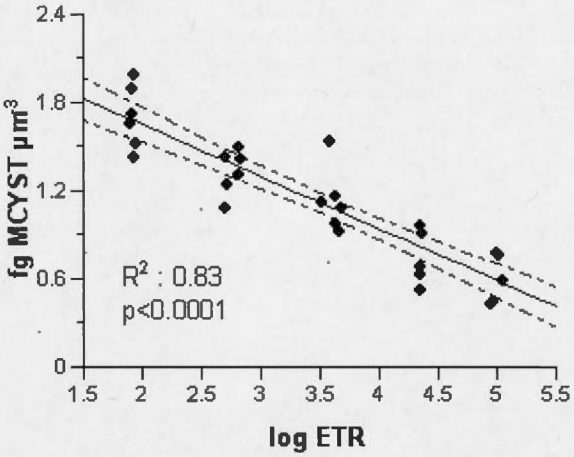

Figure 2.6 Relationship between the microcystin concentration (MCYST) and the relative electron transport rate (ETR) expressed in logarithm. The R^2 of the relation and associated probability are presented.

2.5 DISCUSSION

It is known that changing photon irradiance induced changes of MCYST cell quota (van der Westhuizen and Eloff, 1985; Sivonen, 1990; Utkilen and Gjølme, 1992; Rapala *et al.*, 1997; Wiedner *et al.*, 2003), but also of photosynthetic activity (MacIntyre *et al.*, 2002; Seaton and Walker 1992; Falkowsky and Chen, 2003), and the latter is known to be associated to photoacclimation processes. Under increasing photon irradiance, these processes allow the protection of the photosynthetic apparatus from excess energy and photodamage through the decrease in pigment content (chlorophyll *a* and phycobilin) (Raps *et al.*, 1983; Tytler *et al.*, 1984; Raps *et al.*, 1985; Huner *et al.*, 2003), modification of the carotenoid content (Steiger *et al.*, 1999; Schagerl and Müller, 2006) and change in the PSII / PSI stoichiometry (Kawamura *et al.*, 1979; Hihara *et al.*, 1998; Sonoike *et al.*, 2001; MacIntyre *et al.*, 2002).

In the present study, photoacclimation of *M. aeruginosa* occurred since a higher carotenoid to chlorophyll *a* ratio was observed at high photon irradiance compared to low photon irradiance. This was mainly due to a lower cellular chlorophyll *a* content since no modification was observed for carotenoid cell quota. Similar results were reported previously in *M. aeruginosa* and attributed to photoacclimation (Raps *et al.*, 1983). Another indication that photoacclimation occurred was reflected by the maximum fluorescence value (F_M) and maximum PSII quantum yield (Φ_M) which remained unchanged for all light treatment conditions (Fig. 2.3 and 2.4A). In fact, it is known that when photon irradiance induces damage to PSII photosynthetic unit due to a lack of photoacclimation, the maximum PSII quantum yield (Φ_M) declined (Björkman, 1987; Külheim *et al.*, 2002). Furthermore, our results showed an increase of the non-photochemical energy dissipation processes under high photon irradiance, which is another indication of photoacclimation (Campbell *et al.*, 1998; Müller *et al.* 2001; MacIntyre *et al.*, 2002).

Concomitantly to these photoacclimative responses, we have shown clear differences in the MCYST cell quota for *M. aeruginosa* UTCC299 grown under different photon irradiance (See Fig. 2.1C and 2.1D). Although these results are in MCYST-LR equivalents and reflect toxicity, they were comparable to what was found previously where MCYST cell quota increased under light limiting conditions and then decreased at saturating photon irradiances (Wiedner *et al.*, 2003). In our study MCYST concentration on a biovolume basis gradually decreased from low to high photon irradiances. Furthermore, we have found that MCYST production was related to cell specific division rate (data not shown), as was also demonstrated previously (Orr and Jones, 1998; Oh *et al.*, 2000; Wiedner *et al.*, 2003). However, at saturating photon irradiance, MCYST cell quota decreased without any change in cell specific division rate (this study and Wiedner *et al.* 2003) indicating that photoacclimative responses may influence the MCYST content.

The effect of photon irradiance on chlorophyll *a* was very similar to changes observed for MCYST. Indeed, as for MCYST, chlorophyll *a* cell quota was maximal at a PAR of 56 µmol photons (PAR) m^{-2} s^{-1} and lower under higher photon irradiance (Fig. 2.1A and 2.1B). These two variables (MCYST and Chl) were strongly correlated, on both cell quota and biovolume basis, and this finding was similar to the relationship reported in previous studies on the effect of nitrogen and phosphorus (Lee *et al.*, 2000; Long *et al.*, 2001; Downing *et al.*, 2005b). Since it is known that Chl content is modulated by photosynthetic processes and related to photoacclimation (Geider *et al.*, 1997; Kana *et al.*, 1997), the relationship between MCYST and Chl suggests that photosynthetic activity can also modulate MCYST cell quota. This idea was also suggested by Long and co-workers (2001) and recently it was shown that MCYST to Chl ratio decreased when *M. aeruginosa* photosynthetic activity was inhibited by ultraviolet radiation (Gouvêa *et al.*, 2008).

Due to the adjustment in pigment content and energy dissipation processes, occurring during photoacclimation, *M. aeruginosa* was able to maintain efficient utilization of energy under the photon irradiance gradient from 24 to 820 µmol

photons (PAR) m^{-2} s^{-1}, as seen by the positive effect of light on the relative electron transport rate (ETR), gross oxygen production (GP), and growth rate. A good correlation was found between ETR and GP showing that photosynthetic activity assessed by the chlorophyll *a* fluorescence technique is reliable and directly proportional to more direct methods such as oxygen evolution measurement. Concerning toxin content, our data showed a strong negative relationship between steady state photosynthesis (ETR) and MCYST concentration (see Fig. 2.6). To our knowledge, this is the first direct indication that light reactions of photosynthesis are related to MCYST concentration in *M. aeruginosa*. The involvement of photosynthetic processes in MCYST regulation was proposed previously although not demonstrated directly (Hesse *et al.* 2001; Kaebernick and Neilan, 2001; Long *et al.*, 2001; Jähnichen *et al.*, 2007). The link presented in our study between light reactions of photosynthesis and MCYST cell quota is in good agreement with previous results showing an interaction between MCYST and primary production determined by carbon fixation (Wicks and Thiel, 1990; Oh *et al.*, 2000; Downing *et al.*, 2005a; Jähnichen *et al.*, 2007). Another consequence of photoacclimation was reflected in the unquenched fluorescence (UQF_{REL}), which is related to PSII redox state (Juneau *et al.*, 2005). Higher $UQF_{(REL)}$ value observed under higher photon irradiance (low Chl *a* cell quota) indicated that PSII reaction centers stayed in a more reduced state and suggested a lower capacity of PSI to drain electrons. This is probably due to a low PSI:PSII ratio as reported previously for cyanobacteria grown under high light condition (Riethman *et al.*, 1988) and/or to a decrease of Chl and phycobilisomes associated to PSI reaction center when *M. aeruginosa* was exposed to high photon irradiance (Raps *et al.*, 1983; Raps *et al.*, 1985). Change in PSII redox state is known to modulate energy sensors participating in regulation of gene expression and biochemical processes related to plant growth and development (Huner *et al.*, 1998; Wilson *et al.*, 2006). It is known that a gene cluster (*mcy* genes) is responsible for MCYST biosynthesis through the MCYST synthetase complex (Nishizawa *et al.*, 2000; Tillett *et al.*, 2000). Consequently, the negative relationship

observed between PSII redox state ($UQF_{(REL)}$) and MCYST cell quota (see Fig. 2.4B) is a possible explanation of how light reactions of photosynthesis may control toxin content. Therefore, we hypothesize that ETR and PSII redox state, working as environmental sensor mechanisms, participate in the modulation of *mcy* genes expression and ultimately MCYST cell quota.

We cannot exclude an alternative explanation that changes of MCYST concentration were related to changes in thylakoid membrane content. Indeed, MCYST was shown to be closely associated to thylakoid membranes (Young *et al.*, 2005), and the concentration of these membranes in cyanobacteria are known to change with photon irradiance (Kana and Glibert 1987a, b). The good correlation found between MCYST and Chl *a* cell quota (Fig. 2.2A) supports this hypothesis since Chl is known to follow the changes in thylakoid content in cyanobacteria (Kana *et al.*, 1988).

It is widely accepted that photosynthesis, and ultimately growth, are controlled by environmental conditions such as nutrient limitation, temperature and light (Herzig and Falkowski, 1989; Huner *et al.*, 1998; Li *et al.*, 2003; Lippemeier *et al.*, 2003), and all these factors were previously related, positively or negatively to MCYST (reviewed in Sivonen and Jones, 1999; discussed in Kardinaal and Visser, 2005). The findings reported in our study, where a link was found between MCYST and photosynthetic light reactions, through electron transport rate (ETR) and PSII redox state, may reconcile the results published previously.

In conclusion, our data demonstrated that light reactions of photosynthesis and chlorophyll *a* content are closely linked to MCYST content and that this relationship may be responsible of the observed changes previously linked to nutrient availability. Therefore, we propose that future work on the modulation of MCYST content by various environmental factors should also pay closer attention to the involvement of photosynthetic light reactions.

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CHAPITRE III

COMPARISON OF RESISTANCE TO LIGHT STRESS IN TOXIC AND NON-TOXIC STRAINS OF *Microcystis aeruginosa* (CYANOPHYTA)

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CONTEXTE

Dans les chapitres précédents nous avons montré que la photoacclimatation engendre des ajustements au niveau de l'appareil photosynthétique mais aussi à d'autre niveau de la physiologie des algues et des cyanobactéries, notamment la croissance. Nous avons aussi établi qu'au cours de ce processus, le contenu en microcystine de *M. aeruginosa*, une cyanobactérie toxique, est aussi modifié. Dans ce chapitre nous comparerons la résistance de souche toxique et non-toxique de *M. aeruginosa* lorsque celle-ci sont exposées à des changements brusques de leur environnement lumineux via un stress de forte intensité lumineuse. L'objectif est de déterminer si la lumière peut affecter la compétition entre les souches toxiques et non-toxiques et ainsi influencer la toxicité globale des floraisons de *M. aeruginosa* et autres cyanobactéries toxiques.

* Tel que publié: Deblois, C.P., Juneau, P. (2012). Comparison to light stress in toxic and non-toxic strains of *Microcystis aeruginosa* (Cyanophyta). *J. of Phycology*. 48 (4): 1002-1011.

3.1 ABSTRACT (RÉSUMÉ)

Blooms of *Microcystis aeruginosa* occur frequently in many freshwater ecosystems around the world, but the role of environmental factors in promoting the growth and determining the proportion of toxic and non-toxic strains still require more investigation. In this study, four strains (toxic CPCC299 & FACHB905 and non-toxic CPCC632 & FACHB315) were exposed to high light (HL) condition, similar to light intensity found at the surface of a bloom, in order to evaluate their sensitivity to photoinhibition. We also estimated their capacity to recover from this HL stress. For all strains, our results showed an increased inhibition of the photosynthetic activity with HL treatment time. When comparing the extent of photoinhibition between strains, both toxic strains were more resistant to the treatment and recovered completely their photosynthetic capacity after 3 hours, while non-toxic strains needed more time to recover. For toxic strains, the rETR under HL was higher compared to the rETR under LL control condition despite 50% photoinhibition. This suggests that the detrimental effect of HL (up to 2hr) is outweighed by their higher photosynthetic potential. This conclusion did not stand for non-toxic strains and indicates their preference for LL environment. We also demonstrated that a LL/HL cycle induced a 259 % increase in cell yield for a toxic strain and a decrease by 22% for a non-toxic strain. This also indicates that toxic strains have higher tolerance to HL in a fluctuating light environment. Our data demonstrated that difference of sensitivity to HL between strains can modify the competitive outcome between toxic and non-toxic strains and may affect bloom toxicity.

Key index words: Cyanobacteria, *Microcystis aeruginosa*, toxic strain, non-toxic strain, photoinhibition, chlorophyll *a* fluorescence, PSII quantum yields.

RÉSUMÉ

Les épisodes de fleurs d'eau de Microcystis aeruginosa sont fréquents dans de nombreux écosystèmes dulcicoles à travers le monde. Le rôle des facteurs environnementaux dans le contrôle de la croissance et de la proportion de souches toxiques et non toxiques n'est pas encore résolu. Dans cette étude, quatre souches (toxiques CPCC299 & FACHB905 et non-toxiques CPCC632 & FACHB315) ont été exposées a de forte intensité lumineuse (HL), semblable à l'intensité lumineuse mesurée à la surface d'une floraison, et de durées différentes afin d'en évaluer les effets. Nous avons également estimé la capacité des souches à récupérer suite au stress HL. Pour toutes les souches, nos résultats ont montré une inhibition de l'activité photosynthétique s'intensifiant en fonction de la durée d'exposition au traitement HL. En comparant la photoinhibition entre les souches, nos résultats ont montré que les deux souches toxiques étaient plus résistantes au traitement HL que les souhces non-toxiques. De plus, elles avaient récupéré leur capacité photosynthétique initiale après 3 heures de repos tandis que la même période de repos n'était pas suffisante pour une récupération complète chez les souches non-toxiques. Pour les souches toxiques, le taux relatif de transport d'électrons (rETR) sous HL était plus élevé par rapport à celui sous faible intensité lumineuse (témoin) malgré 50 % de photoinhibition. Ce résultat suggère que l'effet néfaste du traitement HL (allant jusqu'à 2 hr) est compensé par un plus fort potentiel photosynthétique des souches toxiques. Cet effet de compensation était absent chez les souches non-toxiques ce qui correspond à leur préférence pour l'environnement de faible luminosité. Nous avons également démontré qu'une alternance cyclique LL / HL induit une augmentation de 259% du rendement des cellules d'une souche toxique tandis qu'il est responsable d'une diminution de 22% chez une souche non-toxique. Comme les précédents, ce résultat indique que les souches toxiques ont une plus grande tolérance aux intensités lumineuses fortes même dans un environnement lumineux variable. En affectant différemment la photosynthèse des différentes souches, nos données démontrent que la lumière peut modifier le résultat de la compétition entre les souches toxiques et non-toxiques et peut influer sur la toxicité globale des floraisons.

Mots clés: Cyanobactérie, *Microcystis aeruginosa*, souche toxique et non toxique, Photoinhibition, Fluorescence chlorophyllienne, rendement quantique du PSII.

3.2 INTRODUCTION

Blooms of toxic *Microcystis aeruginosa* are problematic in many freshwater environments worldwide. During these events, the high phytoplankton biomass is not only a nuisance for recreational activities and wildlife but can also impair human health through microcystin contamination of drinking water supplies (Christoffersen 1996, Chorus and Bartram 1999, Codd et al. 2005a). Microcystins (MCYSTs) are a group of potent phosphatase inhibitor and are toxic for many organisms (Mackintosh et al. 1990, Codd et al. 2005b). Although extensively studied, the physiological functions and factors that regulate production and content of MCYSTs as yet to be elucidated (Kaebernick and Neilan 2001, Kaardinal and Visser 2005, Schatz et al. 2007, Deblois and Juneau 2010). At the molecular level, a group of genes responsible for the biosynthesis of MCYST was found in many but not all *Microcystis* strains (Dittmann et al. 1997, Tillett et al. 2000). It was also shown that both genotypes can be found in the environment (Janse et al. 2004, Kardinaal et al. 2007a). Several studies reported also the coexistence of toxic and non-toxic *Microcystis* strains in blooms (Via-ordorika et al. 2004, Ouellette et al. 2006, Rantala et al. 2006, Davis et al. 2009, Krüger et al. 2010, Joung et al. 2011).

Since the proportion of toxic to non-toxic *Microcystis* sp. strains is a major determinant for global bloom toxicity, it is essential to understand the factors that promote or decrease growth efficiency of these strains (Chorus et al. 2001, Kardinaal et al. 2007b). Light was demonstrated to be an important factor in species selection of algae and cyanobacteria (Huisman et al. 1999, Litchman 2003). It was also shown, in a laboratory study, that two non-toxic strains of *M. aeruginosa* were able to out-compete two toxic strains under low light condition, even when important numerical advantage was initially provided to the toxic strains (Kardinaal et al. 2007b). In this case, low light intensity was a key factor of the competitive outcome. During a bloom, light penetration in the water column decreases as a consequence of higher

cell concentration and therefore can limit the amount of light available for photosynthesis in the deeper water layers (Tomioka et al. 2011). To mitigate such limitation, *Microcystis* cells are able to move toward the surface due to their gas vacuoles but, although this mechanism provide a great advantage for nutrient and light competition it is not fully controllable and therefore not always efficient (Oliver and Ganf 2000, Brookes et al. 2003). In fact, cell position in the water column also depends on water turbulences and mixing (Visser et al. 1996) and to some extent on cell crowding at the surface. Buoyant cells reaching the surface may be subject to photoinhibition due to the high light intensity (Brookes et al. 2003). In such condition, the extent of photoinhibition will depend on the time spent at the surface and on the cell resistance to high light condition. At the surface, photoinhibited cells can remain trapped because of persistent positive buoyancy. When photosynthetic activity is inhibited, it slowed down the carbohydrate accumulation required to increase cellular density and allowed cells sinking in the water column (Brookes and Ganf 2001). In extreme situation persistent over buoyancy and the photoinhibition can induce cellular death because of light induced oxidative damage (Abeliovich and Shilo 1972, Eloff et al. 1976, Walsby et al. 1991).

Photosynthetic organisms have developed photoprotection mechanisms to cope with excess light intensity for short period of time, but these mechanisms and there efficiency varied among algal and cyanobacterial species (Müller et al. 2001, Horton and Ruban 2005, Karapetyan 2007). Cyanobacteria are typically low light adapted organisms (Zevenboom and Mur 1984, Wetzel 2001), and measurable photoinhibition and photoinactivation of PSII were reported for *Microcystis* cells exposed for up to 4 hours to light intensity of 1000 µmol photons (PAR) m^{-2} s^{-1} (Whitelam and Codd 1983, Tytler et al. 1984). Since such level of light intensity can be frequently encountered at the surface of lakes, we decided to explore how light stress affects photosynthetic tolerance and resilience of toxic and non-toxic strains of *Microcystis aeruginosa*.

3.3 MATERIAL AND METHODS

3.3.1 Strains characteristics

Four strains of *Microcystis aeruginosa* from different origins (North America – CPCC and China – FACHB) were utilised in this study. Two strains produce microcystins (CPCC299 and FACHB905) while the other two are non-toxic (CPCC632 and FACHB315). The toxicity of each strain was confirmed by ELISA (according to the method detailed in Deblois and Juneau 2010). Under our growth conditions, the $MCYST_{quotas}$ was higher for CPCC299 compared to FACHB905 with 115 fg · $cell^{-1}$ and 51 fg · $cell^{-1}$ respectively.

3.3.2 Cell culture

Each strain was grown in 600 ml of fresh Bold Basal Media (BBM) in 1 litter flask. The growing light intensity was 40 µmol photons (PAR) · m^{-2} · s^{-1} (cool white fluorescent tube TWS2000) with a light:dark cycle of 16:8 h at 24°C. The cells were acclimated to these conditions for one month prior to the treatment and meticulously maintained in exponential growing phase through daily monitoring of cell concentration with a Multisizer III Coulter counter (Beckman Coulter Inc, Fullerton, USA) and weekly periodic inoculums transfer into fresh culture medium. The inoculums were transferred before cells reached the late-exponential phase of the growth curve in order to maintain cells in healthy physiological state.

3.3.3 Photoinhibitory treatment

Once the culture reach the mid-exponential phase of the growth curve, it was separated into three equal volumes (200 mL) in bakers aligned in front of two halogen light bulbs and shook with an orbital shaker (50 RPM) for the whole treatment duration. The light intensity for the high light (HL) treatment was adjusted

to 1,200 µmol photons (PAR) · m^{-2} · s^{-1} in the center of the cell suspension using a QSL scalar PAR irradiance sensor (Biospherical Instruments Inc). Each algal suspension was exposed for 20, 65 and 120 minutes to this light condition and sampled at each interval for pigments determination, cell counting, chlorophyll fluorescence kinetic measurements and rapid rise fluorescence measurements. Meanwhile, a sub sample was placed for 3 hours under dime light (20 µmol photons (PAR) · m^{-2} · s^{-1}) to allow recovery from the HL treatment. After this period, each recovered sub sample was processed through the analysis mentioned above for the HL treatment.

3.3.4 Pigment determination

A known volume (20 to 30 mL) of the cyanobacterial suspension was filtered on 0.8 µm Nucleopore membrane filter (Millipore, USA) under dimmed green light and kept frozen until quantification of chlorophyll a (Chl *a*) and carotenoid (Car). A second filter was prepared and kept frozen for phycobilisome (PBS) determination. Chl a and Car were extracted in 4 mL of boiling methanol for 5 minutes, the extract was then stored overnight at -80°C and filtered with GFF filter (Whatman, USA) prior to spectrophotometric measurements done with a Cary 300 WinUV spectrophotometer (Varian, USA). Determination of Chl *a* and Car concentrations were done according to Ritchie (2008). The phycobilins phycocyanin (PC) and allophycocyanin (APC) were extracted using 4 freeze-thaw cycles in 0.1 M potassium phosphate buffer (pH 6.8) and centrifuged at 5,000 x *g* for 15 min. The absorbance spectra of the supernatant (500–700 nm) was recorded using a Cary 300 WinUV spectrophotometer (Varian, USA) and the concentration of PC and APC was calculated according to the equation given in Bennett and Bogorad (1973).

3.3.5 Chlorophyll fluorescence measurements

At each sampling interval, 3 mL of HL exposed cells were immediately transferred (< 30 sec) into a WATER-Pulse-Amplitude-Modulated fluorometer (WATER-PAM) (Heinz Walz GmbH, Effeltrich, Germany) to assess the steady state fluorescence value (F_S) under actinic light intensity. The actinic light intensity was set to 800 µmol photons (PAR) · m^{-2} · s^{-1}. This intensity was chosen in order to obtain steady state fluorescence signal without being to low and permit recovery of the treated cells nor being to high to induce more photoinhibitory effect preventing steady state to be reached. Maximal fluorescence under actinic light (F'_M) value was obtained using flashes of saturating light (800 ms, 3,000 µmol · m^{-2} · s^{-1}) applied periodically (every 60 sec.) until these two conditions were reached: 1) a stable F_S value and 2) reproducible (> 3 flashes) maximal fluorescence yield (F'_M). After this step, actinic light was turned off and far red illumination was turned on to measured the light-adapted constant fluorescence yield (F'_O). Finally, actinic light was turned on again and 10 µM of Diuron (DCMU) was added to the cell suspension to measure the maximum fluorescence level (F_MDCMU) according to Campbell et al. (1998). The concentration of DCMU used was high enough to permit the reduction of all PSII reaction centers, since saturating flash did not further increase the maximal fluorescence level F_M (data not shown). Meanwhile, a sample of the cell suspension was kept in the dark for 15 minutes to completely oxidize the PSII electron transport chain. From this sample, the fluorescence level of dark adapted sample (F_O) was measured.

The chlorophyll fluorescence induction curve after recovery was measured on a 15 min dark adapted sample with actinic light set to normal growth condition of 40 µmol photons (PAR) · m^{-2} · s^{-1}. At the end of the kinetic, DCMU was added to the cell suspension with actinic light to asses the maximal fluorescence level F_MDCMU. From these chlorophyll fluorescence measurements, the following fluorescence parameters were calculated: Φ_M (maximum PSII quantum yield with F_MDCMU), Φ'_M (operational PSII quantum yield), $qP_{(REL)}$ (relative photochemical quenching), $qN_{(REL)}$ (relative non-photochemical quenching) and $UQF_{(REL)}$ (relative unquenched fluorescence) according to Schreiber et al. (1986) and Juneau et al. (2005). The rETR (Φ'_M x PAR = rETR) was calculated according to Juneau and Harrison (2005) were PAR was the actinic light intensity applied in the Water-PAM (LL and HL; respectively 40 and 800 µmol photons · m^{-2} · s^{-1}).

3.3.6 Rapid rise fluorescence induction

For all conditions, a sample of the algal suspension (2ml) was kept in the dark for 15 minutes and used to assess the fast polyphasic chlorophyll fluorescence kinetic, namely, the OJIP-test using a Plant Efficiency Analyser fluorometer (PEA, Hansatech Ltd., UK) with the liquid compartment attachment. All parameters (ABS, TR_0, ET_0 and DI_0 per reaction center (RC), F_V/F_M, Vj and the trapping probabilities $TR_0 \cdot ABS^{-1}$ and $ET_0 \cdot TRo^{-1}$) were calculated according to Force et al. (2003).

3.3.7 Light variation experiment

Exponentially healthy growing cultures of *M. aeruginosa* CPCC632 and CPCC299, previously acclimated to light condition of 40 µmol photons (PAR) · m^{-2} · s^{-1}, were exposed for a period of 5 days to fluctuating LL/HL cycles (from 75 to 900 µmol photons (PAR) · m^{-2} · s^{-1}) according to Fig. 3.1. Photosynthesis versus irradiances curve (P/I curve) was performed at each sampling interval (Morning, Midday and Evening, see Fig 3.1) at day 1, 3 and 5 with 15 min dark adapted samples. DCMU was added at the very end of the kinetic to obtain the maximal fluorescence value (F_MDCMU). Initial cell concentration was similar for both strain (5 x 10^6 cell · ml^{-1}) and monitored at each sampling interval with a Multisizer III coulter counter (Beckman Coulter Inc., USA). The P/I curve was calculated as the product of the operational quantum yield (Φ'_M) and PAR intensity for each of the 8 actinic light intensities selected (84, 123, 190, 280, 428, 639, 907, 1,256 µmol photons (PAR) · m^{-2} · s^{-1}). To estimate the P/I curve light-saturated photosynthetic activity (P_M) and the light limited initial slope (α), the obtained rETR data were fit to a hyperbolic tangent model according to Jassby and Platt (1976).

3.3.8 Statistical analysis

Comparison of photosynthetic parameters with respect to treatment time were done with ANOVA and Tukey-Kramer analysis in JMP 6.0 software (SAS Institute) for each strain separately while inter-strain comparison was done for predetermined treatment time individually (Quinn and Keough 2003).

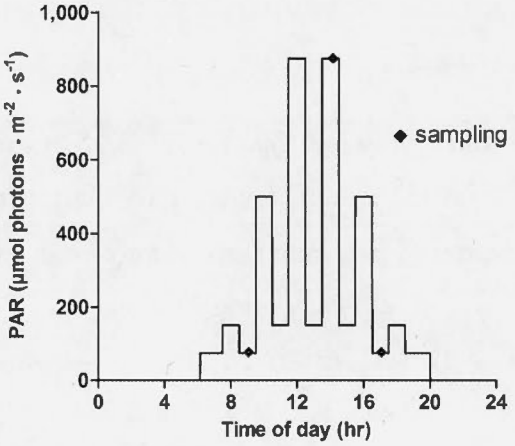

Figure 3.1 Light intensity (µmol photons (PAR) m^{-2} s^{-1}) pattern during the Low light/High light cycle of the fluctuating light experiment. Sampling periods are indicated by the black diamonds.

3.4 RESULTS

Under our control condition, the specific growth rate and photosynthetic performance of all strains of *Microcystis aeruginosa* present some differences (Table 3.1). The strain with the lowest growth rate was CPCC299, while growth was intermediate for FACHB315 and higher for CPCC632 and FACHB905. Cell volume of CPCC299 was more than two time the size of the three other strains (69 vs 30 μm^3). Both toxic strains had less Chl *a* per biovolume unit when compared to non-toxic strains, but the Car content was similar. Consequently, higher (for CPCC299) or slightly higher (FACHB905) Car to Chl *a* ratio were obtained for the toxic strains compared to the non-toxic strains. As for Chl *a* the phycobilins (PC and APC) content was lower for toxic strains compared to non-toxic strains and the ratio of PC:APC was similar for all strains but slightly higher for FACHB315 (Table 3.1). The PSII maximum quantum yield (Φ_M) varied between 0.56 and 0.65 and was comparable to what can be found in the literature for laboratory cultures of *M. aeruginosa* (Brookes et al. 2003, Zhang et al. 2007, Wang et al. 2010, Chalifour and Juneau 2011). The PSII operational quantum yield (Φ'_M) was between 9 and 23% higher for FACHB315 (0.49 ± 0.01) compared to the other strains (Table 3.1).

Changes in the photosynthetic activity of each strain exposed for 10 minutes to actinic light intensity of 40 and 800 µmol photons (PAR) $\cdot$ m^{-2} $\cdot$ s^{-1} are shown in Figures 3.2a and 3.2b. At 800 µmol photons (PAR) $\cdot$ m^{-2} $\cdot$ s^{-1} a strong decrease in photochemical quenching (qP_{REL}) and Φ'_M was induced for all strains and was accompanied by an increase in non-photochemical quenching (qN_{REL}) and unquenched fluorescence (UQF_{REL}) levels (Fig 3.2a & 3.2b). The decrease in the photochemical activity is a well known physiological response of photosynthetic organisms to high light due to limited biochemical reaction activity (Barber and Anderson 1992, Gerber and Häder 1995, MacIntyre et al. 2002). Interestingly, qN_{REL}

Table 3.1

Average specific growth rate, cell biovolume, pigment content (Chl *a,* Car, PC and APC) normalized to cell volume, ratio of Car to Chl *a* and PC to APC, maximum and operational PSII quantum yields (Φ_M and Φ'_M) for the four strains of *M. aeruginosa* grown under normal growth condition (for each level n = 3). Levels connected by different letters are significantly different by Tukey-Kramer (see Materials and methods for details).

	FACHB315		CPCC632		CPCC299		FACHB905	
μ_{spec}	**0.40** ^{ab}	*±0.03*	**0.44** ^{a}	*±0.03*	**0.32** ^{b}	*±0.02*	**0.45** ^{a}	*±0.03*
Biovolume (μm^3 $cell^{-1}$)	**29.7** ^{b}	*±0.8*	**29.1** ^{b}	*±0.6*	**68.9** ^{a}	*±0.6*	**30.4** ^{b}	*±1.4*
Chl *a* μm^{-3}	**11.53** ^{a}	*±0.61*	**11.79** ^{a}	*±0.37*	**9.03** ^{b}	*±0.28*	**9.20** ^{b}	*±0.27*
Car μm^{-3}	**3.99** ^{a}	*±0.34*	**3.87** ^{a}	*±0.40*	**4.55** ^{a}	*±0.68*	**3.67** ^{a}	*±0.37*
Car : Chl a^{-1}	**0.35** ^{b}	*±0.01*	**0.33** ^{b}	*±0.03*	**0.50** ^{a}	*±0.06*	**0.40** ^{b}	*±0.04*
PC μm^{-3}	**18.06** ^{a}	*±0.90*	**14.69** ^{b}	*±1.30*	**5.69** ^{d}	*±1.52*	**10.76** ^{c}	*±2.14*
APC μm^{-3}	**3.65** ^{a}	*±1.09*	**8.02** ^{b}	*±0.95*	**2.19** ^{c}	*±0.64*	**3.38** ^{bc}	*±0.99*
PC:APC^{-1}	**5.55** ^{a}	*±2.44*	**1.91** ^{b}	*±0.34*	**2.60** ^{b}	*±0.08*	**3.40** ^{b}	*±1.25*
Φ_M	**0.58** ^{b}	*±0,01*	**0.58** ^{bc}	*±0,01*	**0.65** ^{a}	*±0,01*	**0.56** ^{c}	*±0.02*
Φ'_M	**0.49** ^{a}	*±0,01*	**0.45** ^{ab}	*±0,01*	**0.40** ^{b}	*±0.02*	**0.44** ^{b}	*±0.03*

of CPCC299 did not increase with higher light intensity as it was observed for the other strains. In cyanobacteria, respiration and photosynthetic processes share some of the electron carriers and therefore induction of pH gradient across the thylakoid membranes and non-photochemical quenching may occur in the dark (Campbell et al. 1998). This gradient can be eliminated by the addition of DCMU (Campbell et al. 1998) and, in the present study, DCMU addition resulted in a high increase of the F_M level (by 91.4%) for *M. aeruginosa* CPCC299 suggesting that non-photochemical quenching (caused by respiratory processes) was important for this strain. For the other *Microcystis* strains, these processes seem to be less important since DCMU addition only increased the F_M value by 10.4% (FACHB315), 16.7% (FACHB905) and 22.5% (CPCC632). A consequence of the high F_MDCMU level in CPCC299 under low light condition is the apparent strong qN_{REL} observed (Fig. 3.2a). The experimental conditions presented in Figures 3.2a and 3.2b were taken as our LL and HL controls, respectively. Therefore, the estimation of the physiological effect caused by the HL treatment (see below) was compared to the control under HL condition while the extent of recovery was compared to the control under LL condition.

3.4.1 High Light Treatment

The high light treatment induced a strong photoinhibitory response for each studied strains. The PSII operational quantum yield (Φ'_M) was strongly affected and the extent of photoinhibition increased with treatment duration (Fig 3.3). For all strains, the first 20 minutes of exposure to HL resulted in a comparable decrease in Φ'_M of 31%, 28% 26% and 27% for FACHB315, CPCC632, FACHB905 and CPCC299 respectively. After 65 min of treatment, photoinhibion further increased for all strains and Φ'_M inhibition for FACHB315 and CPCC632 reached 84% and 78%, while it reached 41% and 48% for CPCC299 and FACHB905 respectively. Inter-strain variations were significantly different by ANOVA and Tukey-Kramer mean

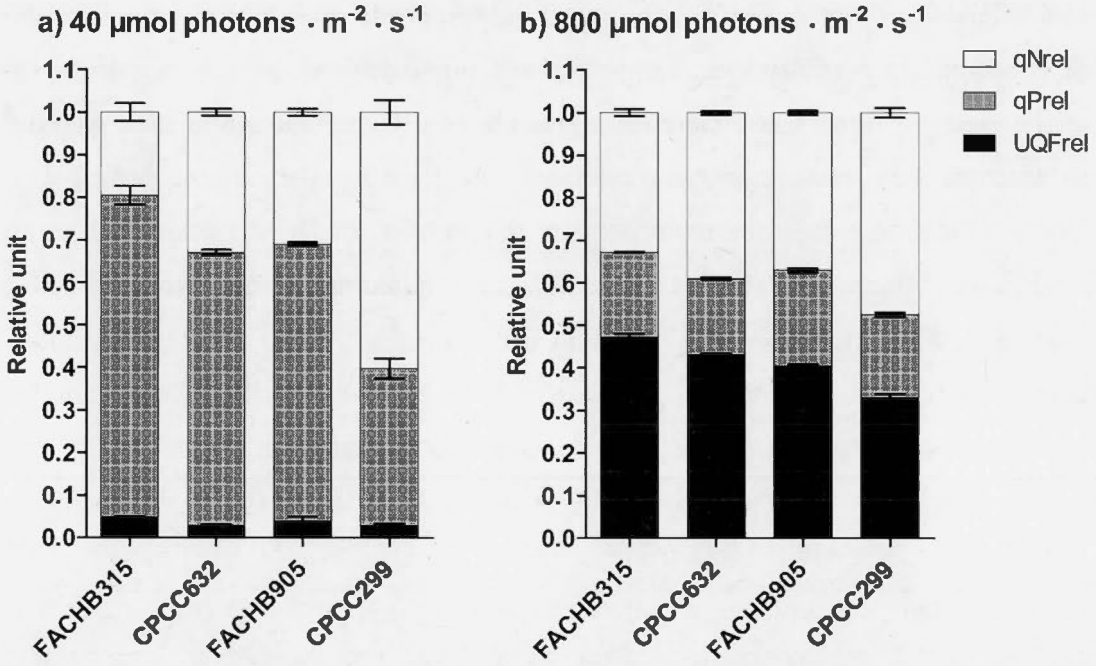

Figure 3.2 Relative non-photochemical quenching (qNrel), relative photochemical quenching (qPrel) and relative unquenched fluorescence (UQFrel) of each *Microcystis aeruginosa* strain (FACHB315, CPCC632, FACHB905 and CPCC299) exposed to a) low light actinic intensity (same as growth light intensity) and, b) high light actinic intensity.

comparison analysis indicating that the photosynthetic activity of the toxic strains was less affected by 65 minutes of HL compared to the non-toxic strains (Fig. 3.3a). The inhibition of Φ'_M after 120 minutes of HL exposure was similar to the inhibition observed after 65 minutes except for the non toxic strain FACHB315 for which Φ'_M further decreased and reached more than 95% of inhibition (Φ'_M at 0.02). The stabilisation of the photoinhibitory effect between 65 and 120 minutes of treatment suggests that most of the strains can actively protect and adjust their photosynthetic apparatus in response to the light stress. Meanwhile, our results also indicated that the physiological responses of the non-toxic strains were not efficient or fast enough to prevent an almost complete inhibition of their photosynthetic activity since Φ'_M reached very low value (<0.05) in both cases (Fig. 3.3a). Photosynthetic adjustment was apparently better for the toxic strains since their photosynthetic activity (Φ'_M) remained close to 0.1 which represents 50% of their initial photosynthetic capacity under HL even after 120 minutes of treatment. Measured operational quantum yield can be converted in rETR to take into account the different photon flux available for photosynthesis. In this study, the conversion of Φ'_M into rETR yielded to inhibition level similar to that observed for Φ'_M with respect to treatment time, but allowed to compare the relative electron transport achieved under the HL condition with the rETR achieved under LL condition (Fig 3b). For toxic strains, the rETR (between 65 and 150) under HL condition was higher than the rETR (around 16) at LL condition for all treatment times (Fig. 3.3b). In the case of the non-toxic strains, the HL rETR was higher after 20 minutes of treatment but was similar or lower after prolonged HL exposure. This seems to indicate that photosynthesis of toxic strains would be increased under HL, while it would tend to decrease for non-toxic strains (Fig. 3.3b). Moreover, the rETR of FACHB905 and CPCC299 was up to 3 times higher than the rETR of the non-toxic strains after the 65 and 120 min treatment. It is interesting to notice that the strain having the highest Φ'_M level under low light intensity (See Table 3.1; FACHB315) was the most sensitive strain to HL treatment, while CPCC299 which had the lowest low light Φ'_M was the most HL resistant strain.

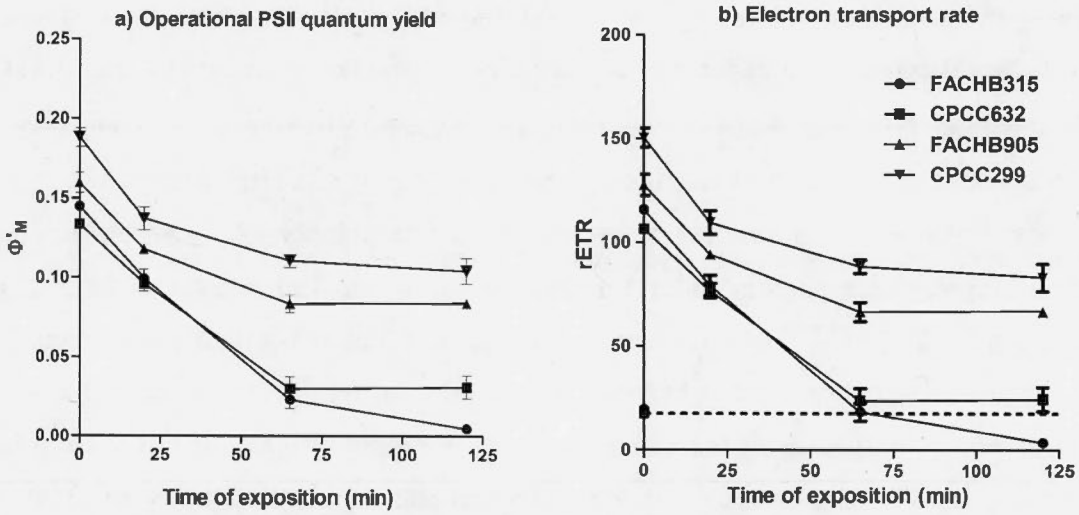

Figure 3.3 Changes in a) the operational PSII quantum yield (Φ'_M) and b) the relative electron transport rate (rETR) of *Microcystis aeruginosa* strains (FACHB315, CPCC632, FACHB905 and CPCC299) exposed 0, 20, 65 and 120 min to High Light (1,200 µmol photons (PAR) · m^{-2} · s^{-1}). The dashed line in the Fig 3b) represents the averaged rETR of all four strains under low light intensity.

Variation in pigment content (Chl *a* and Car) were also monitored during the experiment (data not shown). Our data revealed that Chl *a* decreased with increased treatment time. It was not statistically different from the control after 20 minutes of treatment, but started to decrease after 65 minutes for both non-toxic strains. After 120 minutes of treatment Chl *a* content was decreased by 16% (±4%) for both FACHB315 and CPCC632. Since Chl *a* did not vary significantly for the toxic strains this is another indication of the higher sensitivity of non-toxic strain to HL treatment. These changes in Chl *a* were not accompanied by changes in Car content or phycobilins.

Rapid rise fluorescence parameters of *M. aeruginosa* strains during the HL treatment showed many significant modifications in PSII energy fluxes, but only few differences in the response between strains were observed (Table 3.2). Our data showed that maximal efficiency of PSII photochemistry (F_V/F_M) or the trapping probability ($TR_0 \cdot ABS^{-1}$) were greatly reduced after 120 minutes of HL treatment as seen by the decrease by more than 50 % compared to the control values. This decrease was accompanied by an increase in the proportion of closed RCs (higher Vj), a decrease in the electron transport rate per active RC ($ET_0 \cdot RC^{-1}$) and a decrease probability that an electron enters in the electron transport chain (low $ET_0 \cdot TR_0^{-1}$). The decreased PSII capacity to funnel the energy in the photosynthetic transport chain also affected the light absorption process since our result indicated a 2 to 5 fold increase in the size of the cross section of the light absorbing antennae ($ABS \cdot RC^{-1}$) and a concomitant increase in the amount of energy dissipated by antenna ($DI_0 \cdot RC^{-1}$). These changes clearly showed that the energy was absorbed by the antenna but was not effectively transferred to PSII RC. This can be explained by the fact that PSII RCs are present in a relatively small quantity or more likely inactivated due to photoinhibition. Although all strains reacted to the HL treatment in a very similar fashion, the changes in some parameter values were bigger for non toxic strains (F_V/F_M, $TR_0 \cdot ABS^{-1}$, $ABS \cdot RC^{-1}$ and $DI_0 \cdot RC^{-1}$) suggesting a higher sensitivity.

Table 3.2
Percent of changes relative to the control and associated error for photosynthetic parameter following 120 min treatment to HL (1,200 µmol photons (PAR) · m^{-2} · s^{-1}) for each strain. Levels connected by different letters are significantly different by Tukey-Kramer HSD (n = 3).

	FACHB315		CPCC632		FACHB905		CPCC299	
Fv/Fm ou $TR_0 \cdot ABS^{-1}$	**29** ^{c}	*± 2*	**41** ^{b}	*± 2*	**50** ^{a}	*± 2*	**57** ^{a}	*± 1*
$ABS \cdot RC^{-1}$	**503** ^{a}	*± 6*	**350** ^{b}	*± 13*	**287** ^{c}	*± 7*	**189** ^{d}	*± 8*
$DI_0 \cdot RC^{-1}$	**942** ^{a}	*± 21*	**548** ^{b}	*± 28*	**451** ^{c}	*± 16*	**232** ^{d}	*± 12*
$ET_0 \cdot RC^{-1}$	**51** ^{a}	*± 13*	**64** ^{a}	*± 3*	**58** ^{a}	*± 2*	**83** ^{a}	*± 6*
$ET_0 \cdot TR_0^{-1}$	**36** ^{b}	*± 11*	**45** ^{b}	*± 3*	**41** ^{b}	*± 1*	**78** ^{a}	*± 8*
Vj	**146** ^{a}	*± 8*	**142** ^{a}	*± 2*	**143** ^{a}	*± 1*	**107** ^{b}	*± 2*

3.4.2 Recovery

The extent of recovery of the PSII operational quantum yield (Φ'_M) after HL treatment is shown in Fig. 3.4. After 20 minutes of HL exposure followed by 3 hours under dime light, all strains had recovered their full photosynthetic activity. However, that was not the case for the 65 minute HL treatment, since Tukey-Kramer analysis revealed that only the toxic strains fully recovered from the light stress, while the non-toxic strains FACHB315 and CPCC632 recovered respectively up to 88% (±3%) and 91% (±2%) of the pre-treatment condition (LL control). This discrepancy between toxic and non-toxic strains was more pronounced after 120 minutes of treatment since Φ'_M recovery for the non-toxic strains only reach 73% (±5%) and 81% (±5%) of the control for FACHB315 and CPCC632 respectively, while for the toxic strains Φ'_M was fully restored. Similar conclusion could be drawn from the rapid rise fluorescence result. Non-toxic strains recovered less compared to toxic strain (for the 65 and 120 min treatment), but the recovery was sufficient to regain values close to pre-treatment condition for all parameters tested (data not shown).

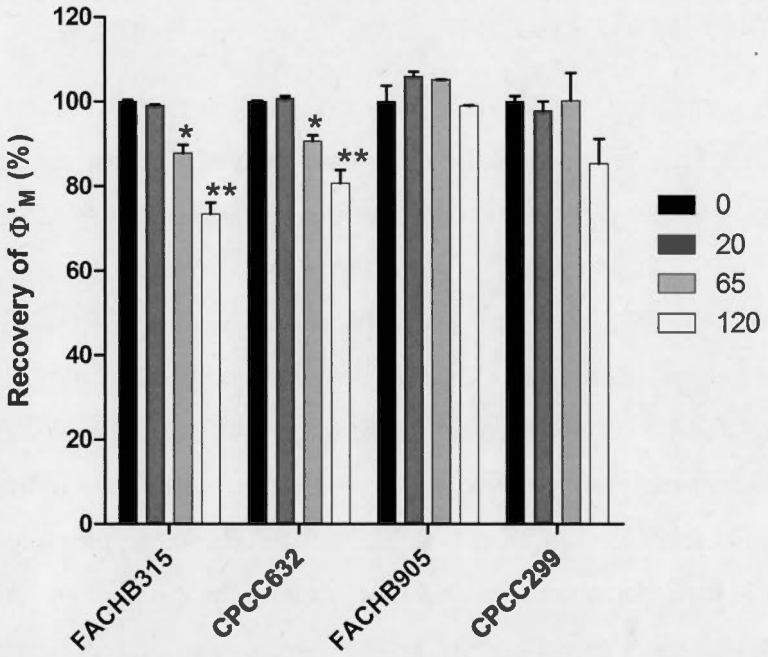

Figure 3.4 Percentage of recovery of the operational PSII quantum yield (Φ'_M) following different High Light treatment time of 0, 20, 65 and 120 minutes. Recovery was done under dime light (20 µmol photons (PAR) · m^{-2} · s^{-1}) for 200 minutes for each treatment time and was calculated as the percent recovered from the operational PSII quantum yield obtained under normal growth condition. Levels connected by different symbols are statistically different by ANCOVA and Tukey-Kramer HSD means comparison. For each condition n = 3.

3.4.3 Daily light variation experiment

When comparing the Morning, Midday and Evening P/I curve coefficients averaged on the three days of sampling, our data showed a significant decrease in P_M and α values at Midday compared to the Morning sampling for CPCC632, while only α decreased for CPCC299 (Table 3.3). The Evening data revealed that, for CPCC632, recovery of these two parameters was not completed and required more time (Table 3.3). However, we can assume that the recovery was completed during the night since the Morning P_M and α values were similar throughout the experiment. The change in the rETR between sampling periods was compared for each strain individually using ANCOVA. This analyse showed that CPCC299 rETRs in the morning and evening were similar and higher compared with the Midday sampling (Fig 3.5b), while for CPCC632, this analysis reveal that the rETRs were highest in the morning, lowest at Midday and intermediary in the evening sampling (Fig 3.5a). Since there was more variation of the rETR between sampling periods for CPCC632, it suggests that this strain has a lower photoprotection capacity in response to fluctuating light intensity compared to CPCC299. This result was also supported by our growth rate measurements as we noticed a strong difference between CPCC632 and CPCC299 when comparing their growth rates during the 5 days of LL/HL cycle against the growth rates achieved under non fluctuating LL condition (Tables 3.1 and Table 3.3). For CPCC632, the impact of fluctuating light intensity decreased its growth rate from 0.44 to 0.39 while for CPCC299 the growth rate increased from 0.32 to 0.51 when grown under fluctuating light conditions. From these data, we calculated the theoretical cell yield after 5 days under both light scenarios and found that for CPCC299, the cell concentration should increase by 259% in fluctuating light environment, while it should diminish by 22% for CPCC632. It is important to

Table 3.3

P/I curve coefficient (α and P_M) and associated growth rate estimated for each strain at each sampling period. For individual strain, levels connected by different letters are significantly different by Tukey-Kramer HSD (n = 3).

		P_M	error	α	error	Growth rate[1] ($\mu \cdot day^{-1}$)
CPCC632	Morning	**173.2** [a]	*±8.2*	**0.37** [a]	*±0.03*	0.39 ± 0.01
	Midday	**117.4** [b]	*±10.5*	**0.22** [b]	*±0.03*	
	Evening	**147.4** [ab]	*±9.6*	**0.29** [ab]	*±0.03*	
CPCC299	Morning	**215.4** [a]	*±9.9*	**0.47** [a]	*±0.04*	0.51 ± 0.03
	Midday	**197.9** [a]	*±12.7*	**0.37** [b]	*±0.03*	
	Evening	**227.7** [a]	*±10.6*	**0.41** [ab]	*±0.03*	

1 Specific growth rate calculated for the whole experiment.

mention that the average light intensity experienced under LL scenario was 40 μmol photons (PAR) · m^{-2} · s^{-1} compared to 275 μmol photons (PAR) · m^{-2} · s^{-1} in the fluctuating light scenario of Fig 3.1. Because CPCC299 growth rate was greatly increased in the fluctuating light scenario, we can conclude that the detrimental effect of the HL stress was smaller than the beneficial increase in overall light availability and, therefore, this light regime was clearly advantageous for the toxic strain CPCC299.

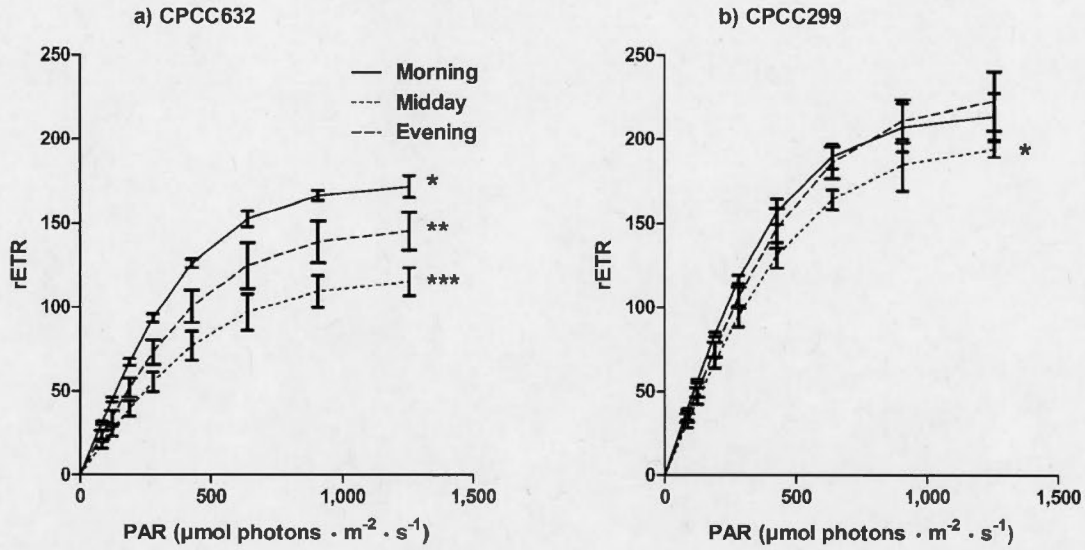

Figure 3.5 Average of the P/I curves obtained at day 1, 3 and 5 for each sampling period for a) CPCC632 and b) CPCC299. Levels connected by different symbols are statistically different by ANCOVA and Tukey-Kramer HSD means comparison. For each condition n = 3.

3.5 DISCUSSION

3.5.1 Effect of high light intensity

The data presented in this study demonstrated that the sensitivity to high light is different between *M. aeruginosa* strains and clearly showed that the toxic strains were less affected by HL exposure compared to non-toxic strains. After 20 minutes of high irradiance level, all strains were photoinhibited to a similar level, but a difference between toxic and non-toxic strains was apparent after 65 and 120 minutes. For these treatment times we have demonstrated that both toxic strains had retained at least 50 % of their PSII operational quantum yield (Φ'_M), while their non-toxic relatives had lost more than 80% of it. This decrease in PSII operational quantum yield was also reflected in the rETR since it was more than 3 times higher for the toxic strains after 65 min and 120 minutes of HL treatment compared to non-toxic strains (Fig 3.3b). These results suggest that if these strains were mixed in a community and exposed to high light intensity (as it occurs at the surface of a bloom), the toxic strains would tend to accumulate more energy at the surface of the water column through photosynthesis compared to their non-toxic relatives.

This tendency was also demonstrated by our daily light fluctuation experiment in which the non-toxic strain CPCC632 was more photoinhibited (lower P_M) by successive LL/HL cycle and need more time to recover compared to CPCC299 (Table 3.3 and Fig. 3.5). The higher sensitivity of CPCC632 to high light also decreased its cell yield after 5 day of HL/LL fluctuation when compared to the cell yield obtained under fixed LL condition. Interestingly, this was the opposite for CPCC299 which really took advantage of the higher light level available in the fluctuating light regime and increased its cellular yield by more than 200% despite being photoinhibited by HL at midday.

It appears that although toxic and non-toxic strains were affected at different extents by the high light treatment, their PSII energy dissipation pathway responses

were similar. Following the HL treatment, we noticed an important increase in the $ABS \cdot RC^{-1}$ and $DI_0 \cdot RC^{-1}$ and a decrease in the maximal efficiency of PSII photochemistry (F_V/F_M) and in the trapping probability ($TR_0 \cdot ABS^{-1}$). The decrease in F_V/F_M is often used to evaluate PSII damage caused by light or pollutants (Juneau et al. 2007). When this decrease is accompanied by a significant increase in the PSII antenna size ($ABS \cdot RC^{-1}$), it can be due to an increase in the number of light harvesting complexes per PSII reaction center and/or to an inactivation of the reaction centers (Krüger et al. 1997, Force et al. 2003, Eullaffroy et al. 2009). According to our pigments data, cellular chlorophyll content was changed by only 16% after 120 minutes HL treatment, while PC and APC remained stable. This suggests that the decrease in F_V/F_M cannot be mainly explained by modification of light harvesting complexes, but is mostly attributed to the degradation of the PSII RCs, as it was previously found for algae and cyanobacteria under light stress conditions (Tytler et al. 1984, Gerber and Häder 1995). We also noticed that the effective dissipation of energy in active reaction centers ($DI_0 \cdot RC^{-1}$) increased drastically during high light treatment (between 230% and 942%). This dissipation process is known to be influenced by the ratio of active/inactive reaction centers and its increase indicates that the absorption of photons was in excess of the RC trapping capacity (Force et al. 2003). Therefore, the observed concomitant increase in the effective dissipation ($DI_0 \cdot RC^{-1}$) and decrease in the electron transport probability ($ET_0 \cdot TR_0^{-1}$) indicate that inactivation of PSII RC and photoinhibitory damages occurred as can be expected under these conditions (Barber and Anderson 1992, Gerber and Häder 1995). These results help also to understand the observed decrease in the operational PSII quantum yield (Φ'_M) following the HL stress. Decreased energy transfer per RC and excess energy trapped in the antenna resulted in a lower Φ'_M and a slower electron transport rate. Moreover, the observed overexcitation of the PSII antenna tends to demonstrate that PSI or other electron sinks were not able to drain efficiently electrons from PSII.

One possible explanation of the higher resistance of the toxic strains in these different experiments is their lower photosynthetic pigment content and high Carotenoid to Chl *a* ratio. High pigment content may have contributed to the higher photoinhibition observed for non-toxic strains since more pigments favoured more energy funnelled to PSII RCs. The observed lower resistance of non-toxic strains to HL seems to corroborate that non-toxic strains tend to be better competitor in low light environment (Kardinaal et al. 2007b).

3.5.2 Possible implication at the ecosystem level

The movement of *M. aeruginosa* in the water column is mainly controlled by their gas vacuoles (Reynolds 1987, Wallace and Hamilton 1999). This ability for buoyancy can be clearly seen during intense blooms of *M. aeruginosa* by the accumulation of dense mats of cells at the surface of calm water bodies. This active mechanism allows cells to take advantage of the nutrient rich water layer (depth layer) without losing the high photon level found near the surface. Buoyancy is not perfect and in some cases, studies have suggested that during bloom events, individual cells or colonies can be trapped at the surface because of over buoyancy and turbulences (Eloff et al. 1976, Oliver and Ganf 2000, Brookes et al. 2003). Under such circumstances individual cells can be exposed to high light intensity for prolonged period of time. As for most cyanobacteria, *M. aeruginosa* is generally a low light adapted organism (Wiedner et al. 2003, Kardinaal et al. 2007b) and could consequently be very sensitive to high light intensity experienced at the surface of the water column. As discussed above, for all strains studied here we observed a clear decrease in the photosynthetically active RCs resulting in a decrease in the PSII operational quantum yield (Φ'_M) when exposed to HL. The observed decrease in Φ'_M did not necessarily result in a decrease of the energy stored through photosynthetic process. Indeed, the conversion of Φ'_M into relative electron transport rate showed

that the high density of photon experienced during HL condition mitigates the observed decrease in Φ'_M (Fig 3.3b). Therefore, a lower Φ'_M can result in a higher rETR when compared to the rETR estimates under LL condition or by analogy, when cyanobacteria remained in deeper water layers. When comparing our rETR data between HL and LL conditions, we demonstrated that a cell of the studied strains going at the surface of the water column will store more energy through photosynthesis compared to a cell remaining in deeper waters and that, even if light condition at the surface may induce adverse effect on the photosynthetic apparatus. In the case of non-toxic strains, this affirmation was only true for a HL treatment of 20 minutes, since the balance between high photon density and lower photosynthetic yield was lost for longer exposure times (65 and 120 minutes) leading to a rETR that was similar (CPCC632) or below (FACHB315) the rETR calculated under LL condition. In the case of the toxic strains, the rETR remained higher under HL condition independently of the treatment time and suggested that these strains cannot only tolerate HL, but will also have a higher capacity for energy storage compared to their non-toxic relatives in HL environment. High rETR observed for the toxic strains may also explain the increased cellular yield measured under the light fluctuating experiment and confirm that toxic cells will accumulate more energy under HL condition despite PSII photoinhibition and that they can effectively convert this energy into biomass.

The strong photosynthetic activity recovery after HL treatment indicates that *M. aeruginosa* cells possess very efficient mechanisms to recover from light stress similar to what they can experience in natural environment. Recovery was also found in the daily light fluctuation experiment and took place even when light remained high (between 75 and 500 µmol photons (PAR) $\cdot$ m^{-2} $\cdot$ s^{-1}) prior to the evening sampling. In both experiments, our data showed that the non-toxic strains were more affected by HL and required more time to fully recover from light stress compared to the toxic strains. Longer recovery time together with their lower rETR indicate that

these strains should be less competitive in a high light environment compared to their toxic relatives. Indeed, under HL, our data suggested that the toxic strains would not only continue to be photosynthetically active at the surface and store energy for carbon fixation (higher rETR), but that they will also be faster to convert this energy into organic matter in the deeper nutrient rich water layers because of the lower time lost for photoinhibition recovery.

3.5.3 Conclusion

It was previously shown that non-toxic *M. aeruginosa* strains dominated toxic ones under low light condition (Kardinaal et al. 2007b). According to our study, we can advance that an increase in toxic strains proportion will be observed in condition where high light intensity is experienced by *M. aeruginosa* cells (condition that may occur at the onset of a bloom or also at the surface of a dense bloom). Indeed, our results clearly showed that non-toxic strains were more sensitive to high light exposure and need longer time to recover from this photoinhibitory stress compared to toxic strains. It is also known that MCYST quotas in toxic *Microcystis* change by a factor of up to 3 times depending on their photoacllimation status (Wiedner et al. 2003, Deblois and Juneau 2010). Therefore, in blooms where low light condition is dominant (weak buoyancy and high biomass), non-toxic strains should dominate, but the remaining toxic strains should be more toxic while, if high light prevail (such as in an over buoyancy situation), toxic strains should dominate but with a lower cellular toxicity. Because of this complex interaction, we propose that the global toxicity of a bloom depends not only on the impact that light has on the competitive outcome of toxic versus non-toxic *Microcystis*, but also depends on the impact of light intensity on cells toxicity through modification of cyanobacterial physiology.

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CHAPITRE IV

EFFECT OF HERBICIDES (DIURON AND OXADIAZON) ON PHOTOSYNTHETIC ENERGY DISSIPATION PROCESSES OF DIFFERENT SPECIES OF CYANOBACTERIA AND TWO GREEN ALGAE

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CONTEXTE

Dans les premiers chapitres, nous avons montré l'importance de la lumière comme facteur modifiant la physiologie chez les algues et les cyanobactéries. De plus nos résultats ont montré que les espèces répondent différemment tant d'un point de vue de l'acclimatation à la lumière que face à un stress lumineux. Dans ce chapitre, nous comparons la sensibilité d'une sélection d'algues (cyanobactéries et chlorophytes) lorsqu'exposé à des herbicides couramment utilisés et se retrouvant en milieu aquatique soit l'oxadiazon et le diuron.

* Tel que publié: Deblois, C.P., Qiu, B., Juneau, P. Effect of herbicides (diuron and oxadiazon) on photosynthetic energy dissipation processes of different species of cyanobacteria and two green algae. *in* Photosynthesis, Energy from the Sun (Edited by Allen, J.F., E. Gant, J.H. Golbeck and B. Osmond), Springer, The Netherlands. 1435-1438.

4.1 ABSTRACT / RÉSUMÉ

Blooms of cyanobacteria are a major concern for freshwater ecosystems because of cyanotoxin production and economic impact of these blooms. Despite a better understanding of environmental factors such as nutrient and light availability on cyanobacteria proliferation, little is known about the contribution of herbicides in these events. By using fluorometric methods, we have investigated the energy dissipation processes of photosynthesis in species from three genera of cyanobacteria (*Synechococcus sp., Synechocystis sp.,* and *Microcystis aeruginosa*) and two green algae (*Raphidocelis subcapitata* and *Chlorella sp.*) when exposed to two herbicides having different modes of action (diuron and oxadiazon). For all cyanobacteria, the maximal photosystem II quantum yield (Φ_M) was not affected by diuron, but the operational photosystem II quantum yield (Φ'_M) was decreased by 50% at 30, 9.4 and 5.1nM of diuron for *M. aeruginosa, Synechocystis sp.* and *Synechococcus sp.* respectively. In presence of 2.89µM oxadiazon, the only affected cyanobacteria were *Synechocystis sp.* and *Synechococcus sp.* (5.4% and 40% decrease of Φ'_M respectively). For green algae, 0.3µM of oxadiazon decreased Φ'_M by 98.1% (*Raphidocelis subcapitata*) and 0.6% (*Chlorella sp.*). These data showed that cyanobacteria have different sensitivity to herbicide and are more resistant than green algae. These results may help to explain why *M. aeruginosa* is a frequently reported species in bloom events.

Key index words: Herbicide, photosynthesis, cyanobacteria, oxadiazon, diuron

RÉSUMÉ

Les épisodes de floraison de cyanobactéries en milieu dulcicole sont préoccupants en raison de la production de cyanotoxines et de l'impact économique de ces fleurs d'eau. Malgré une meilleure compréhension du rôle des facteurs environnementaux tels que la disponibilité des nutriments et la lumière sur la prolifération des cyanobactéries, on ne sait pas si les herbicides jouent aussi un rôle dans ces événements. En utilisant des méthodes basées sur la fluorescence chlorophyllienne, nous avons étudié les processus de dissipation d'énergie au cours de la photosynthèse chez les espèces de trois genres de cyanobactéries (*Synechococcus* sp., *Synechocystis* sp. et *Microcystis aeruginosa*) et deux algues vertes (*Raphidocelis subcapitata* et *Chlorella* sp.) exposés à deux herbicides (diuron et oxadiazon) ayant des modes d'action différents (direct ou indirect). Pour toutes les cyanobactéries, le rendement quantique maximal du PSII (Φ'_M) n'a pas été affectée par le diuron tandis que le rendement quantique operationnel du PSII (Φ'_M) a diminué de 50% en présence de 30, 9,4 et 5,1 nM de diuron chez *M. aeruginosa*, *Synechocystis* sp. et *Synechococcus* sp. respectivement. En présence d'oxadiazon (2.89 µM) seul *Synechocystis* sp. et *Synechococcus* sp. avec une baisse respective de 5,4 % et 40 % du Φ'_M ont été affectés chez les cyanobactéries. Pour les algues vertes, 0,3µM d'oxadiazon a diminué Φ'_M par 98,1% (*Raphidocelis subcapitata*) et 0,6% (*Chlorella* sp.). Ces données montrent que les cyanobactéries n'ont pas la même sensibilité aux herbicides que les certaines algues vertes et sont plus résistantes lorsqu'elles sont exposées au diuron ou à l'oxadiazon. Ces résultats peuvent aider à expliquer pourquoi *M. aeruginosa* est fréquemment détectée dans les épisodes de floraison en milieu agricole.

Mots clés: Herbicide, photosynthèse, cyanobactérie, oxadiazon, diuron.

4.2 INTRODUCTION

Freshwater blooms of cyanobacteria and cyanotoxin production have important ecological and economical impact worldwide. Despite a better understanding on the role of environmental factors such as nutrient and light availability (Chorus and Bartram, 1999), little is known about the possible effect of herbicides on cyanobacterial blooms proliferation. Herbicides are widely used and reached lakes and river by leaching and run off waters. The majority of these compounds act directly on the photosynthetic processes or indirectly through inhibition of photosynthetic pigments and lipid/protein synthesis (Tomlin 2000), therefore, affecting the physiological state of photosynthetic organisms. Furthermore, the difference in sensitivity of phytoplankton species to these herbicides may change the algal and cyanobacterial community. Among herbicides, diuron is well known to block the electron transport between photosystem II and I preventing ATP production and NADPH synthesis (Samuelsson and Öquist 1977). A study by Haynes et al (2000) has demonstrated different sensitivities of phytoplankton taxa to diuron. Another herbicide oxadiazon, an inhibitor of chlorophyll *a* synthesis (Sandmann et al 1984) is widely used in rice fields of Asia and Europe (Comoretto et al 2007). Despite the widespread use of this herbicide, little is known about its effect on photosynthesis and electron transport in phytoplankton. In this study, the effect of diuron and oxadiazon on photosynthetic activity of three genera of cyanobacteria and two green algae was investigated in order to establish if they can play a significant role in cyanobacterial bloom proliferation.

4.3 MATERIAL AND METHODS

Five strains of cyanobacteria: *Synechococcus sp.* (PCC7942), *Synechocystis sp.* (FACHB898), *Microcystis aeruginosa* (FACHB 315, 469 and 905) and two green algae *Chlorella sp.* (FACHB271) and *Raphidocelis subcapitata* (FACHB1068) were grown at 24°C in BG11 media in semi-batch culture and maintained in exponential growing phase during all the experiments. Growing light intensity was 40 µmol photon m^{-2} s^{-1} with a light: dark cycle of 16: 8hr. The five strains of cyanobacteria were exposed in triplicate for 72hr to diuron concentrations up to 757nM and the amount of herbicide per cell was kept constant during the whole experiment by addition of diuron every morning to account for growth in the culture. Oxadiazon was added at the beginning of the 72 hr long experiment for all 7 species at concentrations up to 2.89µM (maximum solubility in water). The effect of herbicides on photosynthesis and energy dissipation processes was measured using Pulse Amplitude Modulated (PAM) fluorometer, rapid rise fluorescence (PEA) and Fluoresence Induction and Relaxation (FIRe) systems after 15 min dark adaptation to ensure total oxidation of Q_A-Q_B-PQ pool. Calculation of the different parameters was done according to Juneau et al (2005). Chlorophyll *a* was measured according to Sartory and Grobelaar (1984) and cell numbers were measured using a Beckman Coulter Counter system. The concentration inhibiting the activity of the operational photosystem II quantum yield (Φ'_M) and the connectivity factor (ρ) by 50 % (IC_{50}) were calculated using jump 5.1 statistical software.

4.4 RESULTS AND DISCUSSION

4.4.1 Effect of diuron

The effect of diuron on cyanobacterial strains, compared by the IC_{50} values of ρ, showed that *M. aeruginosa* (FACHB 315, 469 and 905) were the most resistant (455.4, 508.3 and 530.0nM respectively) followed by *Synechocystis sp.* (130.4nM) and *Synechococcus sp.*(38.6nM) (Table 4.1). The Φ'_M-IC_{50} responded similarly to ρ-IC_{50} with *M. aeruginosa* (FACHB 315, 469 and 905) being less sensitive (30.6, 31.3 and 30.0nM respectively) then *Synechocystis sp.* (9.37nM) and *Synechococcus sp.* (5.1nM) (Table 4.1). These data showed that Φ'_M was more sensitive than ρ by a factor of 8 to 18. Our Φ'_M-IC_{50} values (5.1 to 31.3nM) showing a difference in sensitivity of cyanobacteria to diuron are in the same range than those (9 to 25nM) reported in studies on phytoplankton reviewed in Juneau et al (2007).

The relative absorption cross section per reaction center (ABS RC^{-1}) was differently affected for the three genera studied. The ABS RC^{-1} for *Synechocystis sp.* was not affected for all concentrations used in this experiment (0 to 80nM). For *M. aeruginosa*, the only observed effect was obtained with concentration 10 times higher (757nM) (Table 4.1). On the other hand, *Synechococcus sp.* was the most affected species since ABS RC^{-1} decreased up to 69% with increasing concentration of diuron.

The parameter UQF_{REL} (unquenched fluorescence) reflecting the PSII reduction state under continuous illumination, increased with diuron concentration for every species (data not shown). According to Table 4.1, diuron concentration between 1.4nM and 1.8nM was needed to double the UQF_{REL} parameter for all *M. aeruginosa* strains, while only 0.4nM and 0.6nM produced this effect for *Synechocystis sp.* and *Synechococcus sp.* respectively. We have shown that UQF_{REL} was much more sensitive than the other parameters used in this study (Φ'_M, ρ, ABS

Table 4.1

Concentration of diuron required for the inhibition of 50 % of the parameters values (PHI'm and p) for each strains of cyanobacteria. The unquenched fluorescence (UQF_{REL}) and the absorption cross section per reaction center ($ABS\ RC^{-1}$) are also presented.

Method	Parameter	*M. aeruginosa* FACHB			*Synechocystis sp.*	*Synechococcus sp.*
		315	469	905	FACHB 898	PCC 7942
PAM	Φ'_M	30.6nM	31.3nM	30.0nM	9.37nM	5.1nM
	SD	±2.8	±2.2	±4.4	±2.3	±0.8
	UQF_{REL} *	1.8nM	1.4nM	1.7nM	0.4nM	0.6nM
FIRe	ρ	455.4nM	508.3nM	530.0nM	130.4nM	38.6nM
	SD	±19.9	±57.7	±72.2	±34.4	±11.5
PEA	$ABS\ RC^{-1}$ **	120.1%	149.5%	77.4%	98.0%	31.5%
	SD	±4.4	±11.4	±7.9	±26.1	±8.2

* DCMU concentration required to double UQF_{REL}

** Effect at highest concentration of DCMU (757, 80 & 65nM for *Microcystis*, *Synechocystis* & *Synechococcus* respectively)

RC^{-1}), but give the same result with respect to sensitivity between species. Our results regarding diuron are in agreement with its expected effect, the inhibition of electron transport at the Q_B level.

4.4.2 Effect of oxadiazon

The effect induced by 2.89µM of oxadiazon varied greatly between species ranging from no effect for *M. aeruginosa* to complete inhibition of the cell growth for *Raphidocelis subcapitata* (Table 4.2). For *Synechocystis sp.*, Φ'_M decreased by 5.6% compared to the control and no effect was observed at 0.3µM. For this specie, the chlorophyll *a* content per cell decreased by 17.9% at 2.89µM and did not change at 0.3µM. Similar results were found with *Synechococcus sp.* for chlorophyll *a* per cell but Φ'_M was more affected and decreased by 40.1%. *Chlorella sp.* was less affected than *Raphidocelis subcapitata* by a factor of 10 and 100 at 2.89 and 0.3µM respectively (Table 4.2). Because, for *Raphidocelis subcapitata*, Φ'_M was not affected at 0.03µM and completely inhibited at 0.3µM, the IC_{50} should be between those concentrations. As seen in Table 4.2, the strong effect on chlorophyll *a* concentration was not reflected in the operational PSII quantum yield except for *Synechococcus sp.* This suggests the involvement of a protective mechanism, such as carotenoid synthesis, to maintain photosynthetic activity unaffected, which might not be present in *Synechococcus sp.* In comparison, the chlorophyll *a* content in cyanobacteria was less affected than for green algae. These results on the effect of oxadiazon on chlorophyll *a* inhibition in green algae are in accordance with previous study on *Scenedesmus sp.* (Sandmann et al 1984)

Table 4.2

Effect of oxadiazon on the relative activity of Φ'_M parameter and the relative chlorophyll *a* content per cell for each genera of cyanobacteria (*Microcystis*, *Synechocystis* and *Synechococcus*) and both species of green algae (*Raphidocelis subcapitata* and *Chlorella sp.*) compared to the control.

Parameters	Oxadiazon concentration	*Microcystis sp.* 3 strains	*Synechocystis sp.* FACHB 898	*Synechococcus sp.* PCC 7942	*Raphidocelis subcapitata*	*Chlorella sp.*
Φ'_M	0.03µM	--	--	--	Not affected	--
	0.3µM	--	Not affected	Not affected	-98.1 %	-0.6 %
	2.89µM	Not affected	-5.6 %	-40.1 %	-100 %	-8.2 %
Chlorophyll *a*	0.03µM	--	--	--	-23.7 %	--
	0.3µM	--	Not affected	Not affected	-71.8 %	-57.7 %
	2.89µM	Not affected	-17.9 %	-13.4 %	-100 %	-60.3 %

4.4.3 Conclusion

This study clearly showed that diuron and oxadiazon have different effects on cyanobacterial cells with diuron being more toxic. Moreover, we have demonstrated that cyanobacterial species had large variation in photosynthetic activity when exposed to these herbicides. To our knowledge, this is the first study showing a difference in the sensitivity of cyanobacteria and green algae when exposed to diuron and oxadiazon. Our result concerning higher tolerance of *M. aeruginosa* to these herbicides may explain why it is one of the most frequently reported species in cyanobacterial bloom events. Therefore, we recommend including herbicide as a relevant factor in the study of cyanobacterial blooms.

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CHAPITRE V

RESPONSE TO INCREASED LIGHT INTENSITY IN PHOTOACCLIMATED ALGAE AND CYANOBACTERIA EXPOSED TO ATRAZINE

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CONTEXTE

Dans le chapitre précédent, nous avons montré que la sensibilité de certaines espèces de phytoplancton en présence d'herbicide varie. Dans les premiers chapitres, nous avons aussi montré l'importance de la lumière comme facteur modifiant la physiologie chez les algues et les cyanobactéries. Dans ce chapitre, nous comparons la sensibilité à l'atrazine chez 10 espèces de phytoplancton. Un second objectif est d'étudier l'interaction entre la lumière et la présence d'atrazine en comparant la sensibilité à l'atrazine chez des algues acclimatées à faible et à forte intensité lumineuse.

* Tel que publié : Deblois, C.P., Dufresne, K., Juneau, P. 2013. Response to variable light intensity in photoacclimated algae and cyanobacteria exposed to atrazine. *Aquatic Toxicology*. 126: 77-84.

5.1 ABSTRACT / RÉSUMÉ

Atrazine is frequently detected in freshwater ecosystems exposed to agricultural waste waters and runoffs worldwide and it can affect non-target organisms (mainly photoautotrophic) and modify community structure. Meanwhile, light environment is known to vary between aquatic ecosystems, but also before and during the exposure to atrazine and these variations may modify the sensitivity to atrazine of photoautotroph organisms. In this study, 10 species of phytoplankton (chlorophytes, baccilariophytes and cyanophytes) acclimated to low or high light intensities were exposed to atrazine and light of different intensities to compare their combined effect. Our data showed that chlorophytes and baccilariophytes were more resistant to atrazine compared to cyanophytes for all light conditions. Atrazine was found to inhibit Φ'_M, Ψ_0, P_M and non-photochemical quenching for all species indicating an effect on electron transport, primary production and photoregulation processes. These data also indicate a higher sensitivity of Ψ_0 (average Ψ_0-EC_{50} of 91 nM ± 11 or 19.6 $\mu g\ L^{-1}$ ± 0.9) compared to Φ'_M (average Φ'_M-EC_{50} of 217 nM ± 19 or 46.8 $\mu g\ L^{-1}$ ± 4.1) and suggest that photoregulation processes activated in presence of light decrease the effect of atrazine. We also showed that increasing light intensity decreased Φ'_M-EC_{50} in both low (except baccilariophytes) and high light acclimated conditions. Despite this similarity, most species acclimated to high light were found to have higher or similar Φ'_M-EC_{50} compared to low light acclimated cells and thus, were less sensitive to atrazine in low light and high light environments. We concluded that an increase in the plastoquinone pool induced by acclimation to high light decreased the sensitivity to atrazine in phytoplankton and we hypothesized that the effect observed was the result of a dilution of atrazine toxicity through increased binding site availability (quinones) combined with increased photoregulation processes capacity.

Key index words: Phytoplankton, atrazine, photoacclimation, photoregulation, chlorophyll fluorescence, light.

RÉSUMÉ

L'atrazine est souvent détecté dans les écosystèmes d'eau douce exposés aux eaux usées et aux écoulements agricoles à travers le monde et sa présence peut affecter des organismes non ciblés (principalement photoautotrophes) et modifier la structure de la communauté algale. Dans cette étude, 10 espèces de phytoplancton (chlorophytes, baccilariophytes et cyanophytes) acclimatées à des intensités lumineuses faibles ou élevées ont été exposées à l'atrazine ainsi qu'à de rapides augmentations de l'intensité lumineuse pour comparer l'effet de la lumière sur la sensibilité à l'atrazine. Nos données ont montré que les chlorophytes et les baccilariophytes étaient plus résistantes à l'atrazine par rapport aux cyanophytes pour toutes les conditions de lumière. L'atrazine a eu des effets inhibiteurs observables sur Φ'_M, Ψ_0, P_M et NPQ pour toutes les espèces indiquant un effet sur le transport des électrons, la production primaire et les processus de photorégulation. Les données indiquent également que le paramètre Ψ_0 (moyenne Ψ_0-EC_{50} de 91 nM ± 11) était plus sensible par rapport à Φ'_M (moyenne Φ'_M-EC_{50} de 217 nM ± 19) et suggèrent que les processus de photorégulation activés en présence de lumière diminuent les effets de l'atrazine. L'augmentation de l'intensité lumineuse a eu pour effet de diminuer Φ'_M-EC_{50} de façon similaire chez les algues acclimatées à forte ou faible intensité lumineuse (sauf les baccilariophytes). En dépit de cette similitude, la plupart des espèces acclimatées à fortes intensités lumineuses ont atteint des Φ'_M-EC_{50} similaires ou plus élevés relativement aux même espèces acclimatées à de faible intensité lumineuse. Les algues acclimatées à forte lumière étaient donc moins sensibles à l'atrazine par rapport aux cellules acclimatées à de faible luminosité. En conclusion, la disponibilité accrue des PQ chez les algues acclimatées à forte intensité lumineuse, diminue la sensibilité à l'atrazine et nous soulevons l'hypothèse que cette diminution est principalement attribuable à une dilution de la toxicité de l'atrazine par une augmentation de la disponibilité du nombre de site de liaison (quinones) combinée à une modulation par les processus de photorégulation.

Mots clés: Phytoplancton, atrazine, photoacclimatation, photorégulation, fluorescence chlorophylienne, lumière.

5.2 INTRODUCTION

Atrazine is widely used in agriculture because of its low cost and high effectiveness (Tomlin, 2000; Graymore et al., 2001; Ramakrihnan et al., 2010). This herbicide is directly applied on pre-emergent crops (and occasionally post-emergent) to control broadleaf weeds and grass plants, principally corn but also sorghum and sugar cane (Solomon et al., 1996; Giddings et al., 2002; Giroux, 2010). Atrazine is considered to have moderate water solubility (33 mg L^{-1} at 20 °C) and soil mobility facilitating its transfer from the treated soil to surface and subsurface waters following irrigation and rain events (Solomon et al., 1996; Giddings et al., 2005). In the aquatic environment, atrazine is problematic because of its toxic effect on non-target organisms (mainly photoautrophic) and because of its high persistence in water (half-life of up to 400 days) due to its resistance to chemical and bacterial degradation (Howard et al., 1991; DeLorenzo et al., 2001). Environmental monitoring and water quality surveys, frequently report the presence of atrazine in water samples (Konstantinou et al., 2006; Sullivan et al., 2009; Giroux, 2010) and in some areas, observed concentrations recurrently exceeded the aquatic life protection criteria of 1.5 µg L^{-1} (6.9 nM) in USA and 1.8 µg L^{-1} (8.3 nM) in Canada (US EPA, 2004; MDDEP, 2008). High concentrations of atrazine can also be found following its application and after heavy rain events but usually, the levels rapidly (day to weeks) decrease to lower concentrations (Solomon et al., 1996; Graymore et al., 2001).

Atrazine is a known inhibitor of photosynthetic activity of algae and higher plants which blocks the Q_B binding site on the D1 protein and therefore impedes electron flow toward PSI (Jursinic and Stemler, 1983). Consequently, atrazine normally decreases primary production in phytoplankton (Graymore et al., 2001; Solomon et al., 1996). The biological effect of this herbicide on microalgae and cyanobacteria was shown to vary greatly between phytoplankton species and groups, but also between studies (Huber, 1993; Fairchild et al., 1997; Fairchild et al., 1998;

Delorenzo et al., 2001). Exposure to atrazine was shown to reduce phytoplankton growth at concentrations ranging from 1 $\mu g\ L^{-1}$ to 430 $\mu g\ L^{-1}$ (Torres and O'Flaherty, 1976; Mayasich et al., 1986; Tang et al., 1997). Different sensitivity between algal groups was found to modify the community structure, but there was not a systematically more sensitive group (Herman et al., 1986; Hamilton et al., 1988; Caux et Kent, 1995; Guaush and Sabater, 1998). Furthermore, because high concentrations are generally required to induce significant effect, some studies concluded that persistent impacts of atrazine on aquatic ecosystems should not be expected at environmentally relevant concentrations (Solomon et al., 1996; Baxter et al., 2011). Nevertheless, some studies have also reported interactions between atrazine and environmental factors (such as light and temperature) and these interactions may modify the sensitivity to atrazine of natural algal assemblages (Mayashi et al., 1986; Chalifour and Juneau, 2011).

In aquatic ecosystems, photoacclimation processes allow phytoplankton to adjust their photosynthetic apparatus in relation to their surrounding light environment, thus helping phytoplankton to survive under low or high ambient light intensity (MacIntyre et al., 2002). Movement of organisms in the water column also modifies available light intensity on a short period of time, where photon flux may be scarce deep in the water column, or abundant at the surface (Dubinsky and Stambler 2009). In the latter case, prolonged exposure of phytoplankton to high light intensity can induce photoinhibition and photodamage (Huner et al 1998, Abeliovich and Shilo 1972). One important impact of agriculture for aquatic ecosystems is an increase of water turbidity and thus, a decrease in available light intensity for phytoplankton (Tilman et al. 2001). High turbidity also increases the variations associated to the movement in the water column and the organisms are thus exposed to stronger short term variations of light intensity (Dubinsky and Stambler 2009). Moreover, studies have shown that suspended and dissolved matters which increase turbidity generally co-vary with the presence of atrazine (Sullivan et al., 2009; Giblin et al., 2010; Brain et al., 2012). Although this clearly demonstrates the importance to consider the

interaction between light and atrazine in aquatic ecosystems, only a few studies compared sensitivity of phytoplankton to atrazine with respect to light intensity (Mayasich et al., 1986; Guasch and Sabater, 1998; Mayer et al., 1998). A study by Mayasich et al. (1986) showed that higher light intensity increased the effect of atrazine on growth of *Nanochloris oculata* and *Phaeodactylum tricornutum*. This result suggests that phytoplankton would be less sensitive to atrazine in highly turbid environments. Furthermore, light acclimation was also found to change atrazine sensitivity of periphytic communities (Guasch and Sabater, 1998) and *Selenastrum capricornutum* (Mayer et al., 1998), but contrasting results were obtained and acclimation to low light resulted in a lower (Guasch and Sabater, 1998) or higher (Mayer et al., 1998) sensitivity to atrazine. The results obtained in the previous studies demonstrated that light acclimation and also exposure to low or high light may modify algal sensitivity to atrazine, but the involvement of photoacclimation and photoregulation mechanisms in the observed sensitivity to atrazine is still unclear.

In this study, we compared the effect of atrazine on photosynthetic electron transport of algal and cyanobacterial species isolated from Réservoir Choinière (Québec, Canada) where low (< 1 $\mu g\ L^{-1}$) but recurrent concentrations of atrazine are found (Trudeau et al., 2010). Our goal was to evaluate if acclimation to different light intensities, similar to those found in natural environment (from turbid to clear waters), and if differences in photoregulation capacity following exposure to various light intensities, modified the sensitivity to atrazine of various phytoplankton species.

5.3 MATERIAL AND METHODS

5.3.1 Cultures

Phytoplankton species compared in this study were isolated in 2008 from a single water sample of the Réservoir Choinière (Eastern Township, Québec, Canada). The selected species included four chlorophytes (*Ankistrodesmus falcatus*, *Pandorina morum*, *Pediastrum boryanum* and *Chlamydomonas snowii*), two baccilariophytes (*Fragilaria crotonensis* and *Aulacoseira granulata* var. *angustissima*) and four cyanophytes (*Phormidium muscicola*, *Microcystis flos-aquae*, *Aphanizomenon flos-aquae* and *Anabaena spiroïdes*), which were grown in 125 mL of fresh bold basal media enriched with 80 mg L^{-1} of silicate (BBMsi) at pH 7.4. Before the experiment, cell material was acclimated for many weeks to low and high light intensity (LL and HL) of 76 and 583 µmol photons (PAR) m^{-2} s^{-1}. All experiments were conducted in an environmental growth chamber (MTR30, Conviron, Manitoba, Canada) with a light dark cycle of 16: 8 at 21°C. The irradiance was provided by fluorescent (cool white fluorescent tube TWS2000) and incandescent bulbs.

5.3.2 Atrazine treatments and chlorophyll fluorescence measurements

An aliquot from a healthy exponential culture (5 mL) was transferred into 50 mL assay tubes prefilled with 20 mL of fresh culture media containing different nominal atrazine concentrations (0, 25, 65, 125, 250, 500, 1000, 2000 and 10000 nM) in triplicates (M.W. of atrazine 215.7 g Mol^{-1}). Stock atrazine solution was made from a dilution (in miliQ water) of the commercial Aatrex 480 liquid herbicide (Syngenta, Plattsville, Canada). Concentration of atrazine of the stock solution was verified by ELISA (Abraxis, Warminster, USA) and as we demonstrated in a previous study done under similar conditions no degradation of atrazine occurred over the 72 hr duration of the treatment (Chalifour and Juneau, 2011). Each inoculum was grown for 72 hr at their respective light intensity and agitated twice per day. At

sampling time, a determined volume of cell suspension (see below) was filtered on glass fiber filter pre-wetted with BBMsi for sample homogeneity and placed on flat layer of wet kimwipes in a plastic microplate cover. The manipulation for all the samples from one species were completed within 10 min and preliminary tests showed that photosynthetic activity and fluorescence signal were not affected (data not shown). The volume filtered was calculated from chlorophyll fluorescence measurement following 15 min dark adaptation (F_0) using a WATER-Pulse-Amplitude-Modulated fluorometer (WATER-PAM) (Heinz Walz GmbH, Effeltrich, Germany). The F_0 obtained for each sample was used to calculate the volume of culture required to reach a predetermined fluorescence level per filter and was maintained constant between atrazine treatments and between species when possible. For some species, high atrazine concentration (10 µM) which caused reductions in algal density required the pooling of the triplicate samples to obtain a detectable signal in the MAXI-PAM (Heinz Walz GmbH, Effeltrich, Germany). Algal samples (filters) were placed in the MAXI-PAM chamber (equipped with Red-LED head) and kept in the dark for 15 min, then the measuring light was activated until signal stabilization (< 5 sec). After the signal stabilization, a 11 step Rapid Light Curve (RLC) routine was initiated where a series of saturating pulses (SP) were triggered at 1 min interval with actinic light intensity (AI) varying for each step (0, 88, 182, 248, 361, 510, 668, 889, 1118, 1454 and 2000 µmol photons m^{-2} s^{-1}). This routine permitted us to determine F_0, F_M, F_S and F'_M needed to calculate the PSII maximal and operational quantum yields (Φ_M and Φ'_M) and the non-photochemical quenching (NPQ) following the equations described in Schreiber et al. (1986). Because of the difficulty to add diuron on the filtered samples to estimate F_M (as proposed by Campbell et al. 1998), NPQ was not calculated for that group. The relative electron transport rate (rETR) was calculated according to Juneau and Harrison (2005) and was used to produce photosynthesis versus irradiance curves (PE curve).

5.3.3 Fast polyphasic chlorophyll fluorescence kinetic

Each replicate was kept in darkness for 15 min before assessing the fast polyphasic chlorophyll fluorescence kinetic, (OJIP-test) using a Plant Efficiency Analyser fluorometer (PEA, Hansatech Ltd., UK) with the liquid compartment attachment. From this measurement, PSII transfer efficiency beyond Q_A ($ET_0 \cdot TR_0^{-1}$ or Ψ_0) was calculated according to Force et al. (2003).

5.3.4 Statistical analysis

Concentration-response curves were fitted using non-linear equation (three parameters) in GraphPad Prism software version 5.00 for Windows (GraphPad Software, San Diego California, USA) using least square method. This equation allowed us to estimate the effective concentration of atrazine required to inhibit half of the selected variable (Φ'_M-EC_{50} or Ψ_0-EC_{50}). Comparison of EC_{50} values between species was done on the averaged values for LL and HL adapted cells with one-way ANOVA ($p<0.05$) and post Hoc Tukey-Kramer HSD mean comparison test ($p<0.05$) using JMP 6.1 statistical software (SAS institute, USA). Differences between LL and HL adapted cells for Φ'_M-EC_{50}, Ψ_0-EC50, P_M and NPQ_{HL} were tested using a t-test for each species individually. Photosynthesis versus irradiance curves were fitted in GraphPad Prism using the waiting in-line model (Ritchie, 2008). Comparison of NPQ and P_M between atrazine treatments was made by t-test for each species independently. To compare the effect of photoacclimation, Φ'_M-EC_{50} data were normalized to the Φ'_M-EC_{50} value obtained at 88 µmol photons m^{-2} s^{-1} (the lowest light intensity available in the RLC routine) and the relationship between normalized Φ'_M-EC_{50} and actinic intensity in low and high light adapted cells was compared by covariance analysis using JMP 6.1 statistical software. Linearity required for proper covariance analysis was achieved by transforming the light intensity to $\log_{10}$ (Quinn and Keough, 2003). Unless specified, all error presented are standard error of the mean (SEM).

5.4 RESULTS

5.4.1 Effect of atrazine on photosynthesis

Exposure to atrazine for 72 hr yielded measurable inhibition effect of photosynthetic activity in all phytoplankton species acclimated to low or high light conditions (LL and HL). Inhibition of the PSII operational quantum yield ($\Phi`_M$) was different between phytoplankton groups and our data showed that group averaged-Φ'_M-EC_{50} (LL and HL included) was similar for baccilariophytes and chlorophytes with 315 nM (± 186) and 282 nM (± 109) respectively, but was significantly lower for cyanophytes with 102 nM (± 82). The largest Φ'_M-EC_{50} value was obtained for the baccilariophyte *F. crotonensis* with 546 nM (± 42) adapted to LL. This result was 30 times higher than the lowest observed Φ'_M-EC_{50} (18 nM ± 6) of the cyanophyte *A. spiroïdes* adapted to HL (Fig 5.1a). The chlorophytes, *A. falcatus*, *P. morum* and *C. snowii* reached similar Φ'_M-EC_{50} values while *P. boryanum* had a significantly lower Φ'_M-EC_{50} comparable to those obtained for the cyanophytes (Fig 1a). In cyanophytes, the species with the highest Φ'_M-EC_{50} value was *M. flos-aquae* and *A. flos-aquae* (Fig 1a). These data also showed that Φ'_M-EC_{50} values were significantly lower for LL compared to HL adapted cyanophytes and *F. crotonensis*, but no difference was observed for the other species (Fig 5.1a).

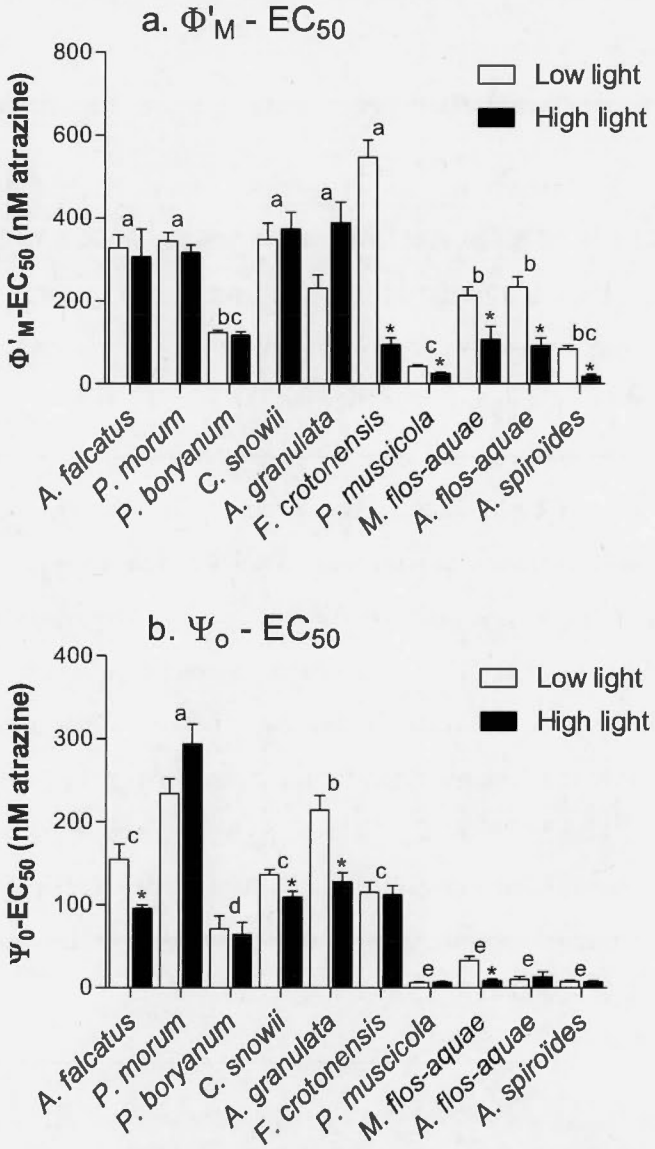

Figure 5.1 Atrazine effective concentration (nM) required for 50 % inhibition of a. PSII operational quantum yield (Φ'_M) and b. electron transport beyond Q_A (Ψ_o) presented for each species in low and high light acclimated cells (LL and HL). Levels connected by different letters were different by oneway ANOVA and post Hoc Tukey-Kramer mean comparison ($p<0.05$) when species average values (LL and HL included) were compared. * Significant difference by t-test ($p<0.05$) between LL and HL for individual species. The errors represent the standard error of the mean (SEM).

Inhibition of PSII electron transfer was also estimated by PSII electron transfer efficiency beyond Q_A^- (Ψ_0). Comparison of Ψ_0-EC_{50} between groups showed that it was higher in baccilariophytes and chlorophytes compared to cyanophytes with average values of 142 nM (± 48), 145 nM (± 80) and 11 nM (± 10) respectively. Comparison of LL adapted species showed that all cyanophytes as noticeably low Ψ_0-EC_{50} values representing up to 37 times less atrazine compared to the highest concentration observed for *P. morum* (Fig 5.1b). In comparison, the concentration of atrazine required to induce half inhibition of Ψ_0 in the other species occurred at concentrations close to 100 nM (Fig 5.1b). It was significantly lower for HL adapted *A. falcatus*, *C. snowii*, *A. granulata* var. *angustissima* and *M. flos-aquae.* Interestingly, when comparing $\Phi`_M$ and Ψ_0 sensitivity, including LL and HL conditions, with match paired model, our data showed that Ψ_0-EC_{50} was significantly lower (2.7 times) compared to Φ'_M-EC_{50} with respective overall mean of responses of 91 nM (± 11) and 217 nM (± 19).

As a consequence of the inhibition of $\Phi`_M$ by 250 nM atrazine, the relative electron transport rate (rETR) (data not shown) and thus the maximal rate of photosynthesis (P_M) were also affected (Fig 5.2a). The atrazine treatment of 250 nM was selected because this concentration was close to the average Φ'_M-EC_{50} obtained for all species (217 nM (± 19)). When exposed to 250 nM, our results showed that P_M for LL and HL adapted species were significantly lower than their respective control for all tested species (Fig 5.2a). Inhibition of P_M was less than 50 % (between 24 and 44 %) for *A. granulata* var. *angustissima*, *F. crotonensis*, *A. falcatus* and *P. morum* adapted to LL while it was higher than 50 % for all cyanophytes and some chlorophytes (Fig 5.2a). For two chlorophytes and most cyanophytes, inhibition of P_M was greater for HL compared to LL adapted cells. It reached up to 98 % for *A. spiroïdes,* while it was lower or similar for the other species.

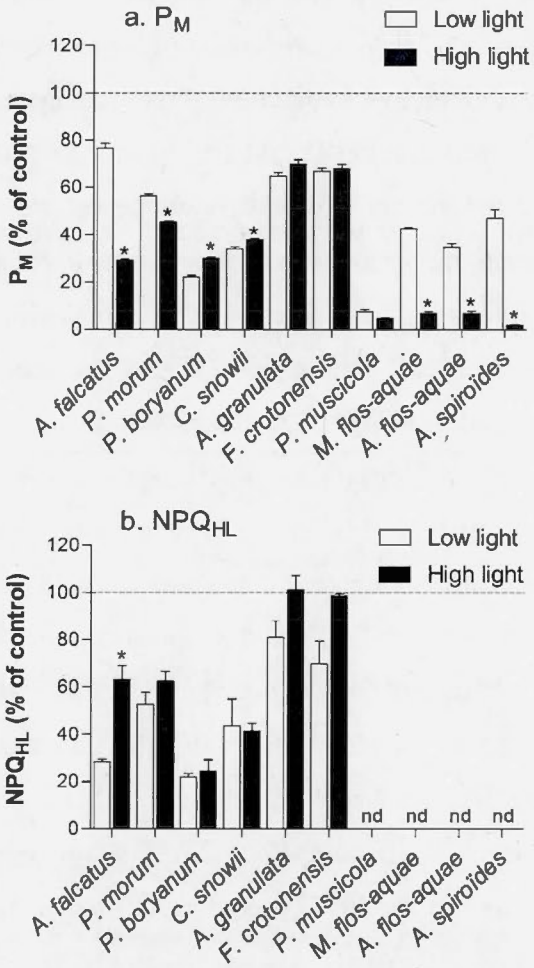

Figure 5.2 Effect of 250 nM atrazine treatment on a. the maximal photosynthetic rate (P_M) and b. the non-photochemical quenching induced under HL (NPQ_{HL}) relative to the control condition in low and high light adapted cells (LL and HL). The dash lines represent the control values (100%). All levels were significantly different relative to the control when individually compared by t-test ($p<0.05$) except for NPQ_{HL} in HL of *A. granulata* and *F. crotonensis*. * Significant difference by t-test ($p<0.05$) between LL and HL for individual species. The errors represent the standard error of the mean (SEM).

In the presence of 250 nM of atrazine, inhibition of NPQ_{HL} was significant for all species and light conditions (except for the baccilariophytes adapted to HL), and NPQ_{HL} was 20 to 82 % and 33 to 100% relative to the controls for LL and HL adapted respectively (Fig 5.2b). Inhibition of NPQ_{HL} was lower in *A. falcatus* acclimated to HL while this variable was not significantly modified for the other species.

5.4.2 Algal response to light in presence of atrazine

When studying the effect of atrazine on photosynthetic activity (Φ'_M-EC_{50}) of LL and HL adapted phytoplankton, after 1 minute exposure to selected light intensities (88, 182, 361, 668 and 1118 µmol photons m^{-2} s^{-1}during the rapid light curve measurements), our data showed strong variations with respect to increasing light intensity (Fig 5.3). For all chlorophytes and cyanophytes adapted to LL or HL, Φ'_M-EC_{50} significantly decreased when actinic light intensity increased (Fig 5.3). For baccilariophytes, light had a contrasting effect on Φ'_M-EC_{50} and was positive for LL adapted and negative for HL adapted (Fig 5.3c and Fig 5.3d). Variation of Φ'_M-EC_{50} with respect to light followed a semi-logarithmic relationship for most species grown under LL and also under HL for cyanophytes. This tendency indicates that changes of Φ'_M-EC_{50} was more pronounced under low light intensity and stabilized at higher light level (Fig 5.3). For chlorophytes and *A. granulata* var. *Angustissima* acclimated to HL, the relationship was linear and indicated that the change of Φ'_M-EC_{50} was proportional to change in light intensity (Fig 5.3).

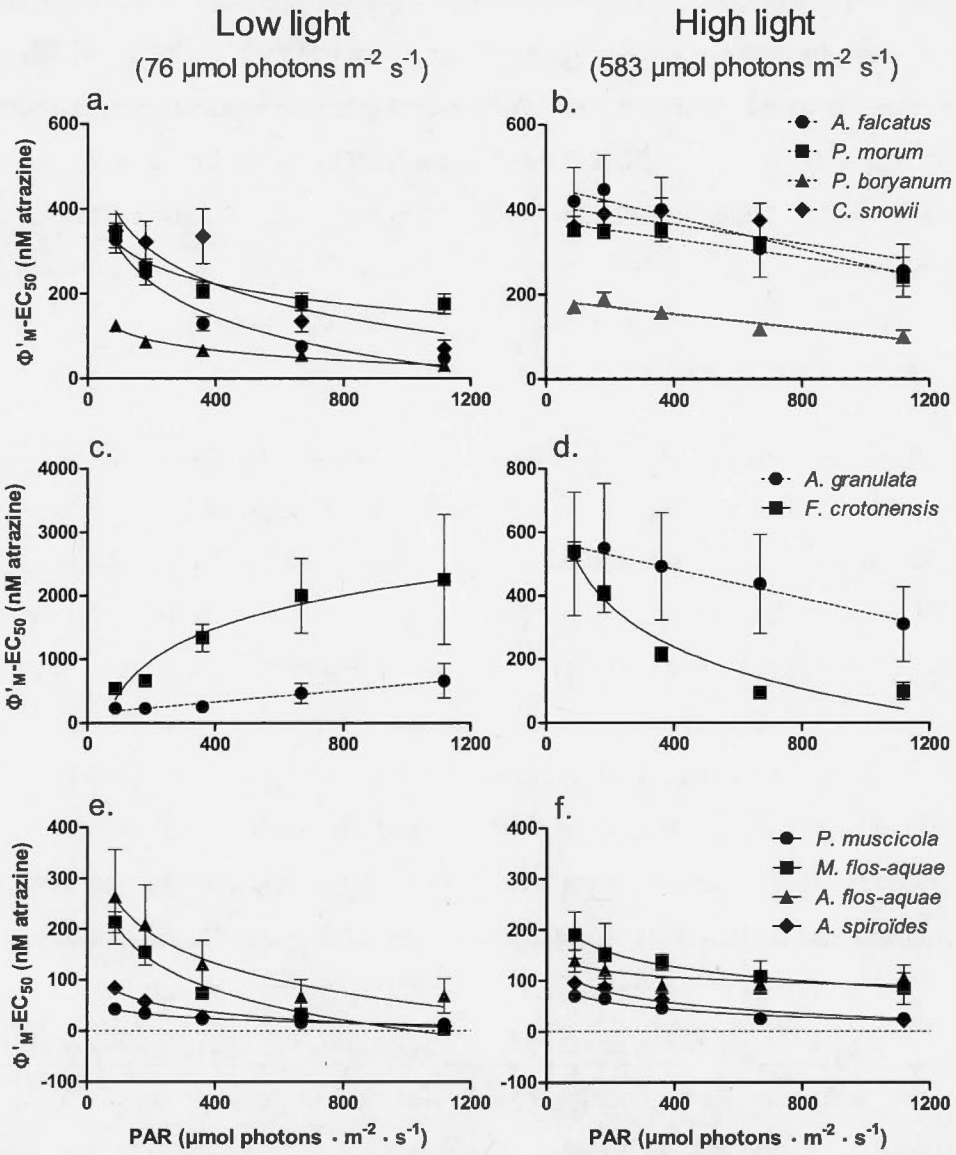

Figure 5.3 Effect of light on the effective atrazine concentration (EC_{50} in nM) required to inhibit PSII operational quantum yield (Φ'_M) in low and high light acclimated cells (LL and HL) exposed to increasing light intensity of short duration in a rapid light curve measurement. Response of chlorophytes (panels a and b), baccilariophytes (panels c and d) and cyanophytes (panels e and f) are presented. Semi-logarithmic (fill line) or linear curve (dash line) were selected by comparing the goodness of fit using both models using least square method. The errors represent the standard error of the mean (SEM).

Normalized Φ'_M-EC_{50} (to the value obtained at 88 µmol photons m^{-2} s^{-1}) were compared for each species (Fig 5.4) using covariance analysis to evaluate if acclimation to high light may protect against atrazine effect under light stress. As seen, when normalized, the data followed a semi-logarithmic curve for all species. Covariance analysis showed that the slope of the relationship between actinic light intensity and Φ'_M-EC_{50} was significantly different between LL and HL adapted for *A. falcatus, C. snowii, A. granulata* var. *angustissima* and *F. crotonensis* (Table 5.1). For these species, the slopes were smaller in HL adapted and this indicates a lower inhibiting effect of light in the presence of atrazine compared to LL adapted. Furthermore, independently of light intensity, the averaged normalized Φ'_M-EC_{50}, was lower in LL compared to HL adapted for all chlorophytes and cyanophytes, although in the latter group the difference was only significant for *M. flos-aquae* (Table 5.1). Contrasting results were obtained for *A. granulata* var. *angustissima* and *F. crotonensis* since Φ'_M-EC_{50} in HL adapted was inhibited by 13 and 50 % while in LL adapted it was enhanced to 160 and 249 % of the control respectively. Similar results were obtained by comparing inhibition of photosynthetic activity between LL and HL adapted exposed to high actinic light intensity (HL_{AI}) of 668 µmol photons m^{-2} s^{-1} (Table 5.2). For LL adapted cells of chlorophytes and *M. flos-aquae* exposed to HL_{AI}, the estimated Φ'_M-EC_{50} was significantly lower compared to Φ'_M-EC_{50} obtained for the same species but adapted to HL (Table 5.2). The estimated Φ'_M-EC_{50} was not different for the other species, except for *F. crotonensis* for which it was lower for HL adapted cells. These results demonstrated that photoacclimation to HL helps to protect some species of phytoplankton against deleterious effects of atrazine.

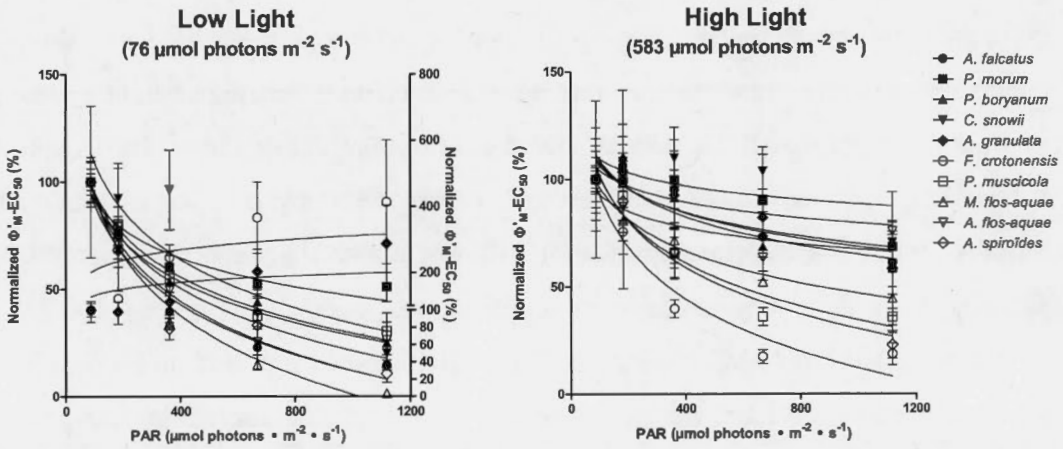

Figure 5.4 Effect of light intensity on Φ'_M-EC_{50} (%) normalized to the value obtained at low actinic light intensity of 88 µmol photons m^{-2} s^{-1} for each species individually acclimated to low or high light condition (LL or HL). These data were compared by ANCOVA (see table 5.1). The errors represent the standard error of the mean (SEM).

Table 5.1

Results obtained for the ANCOVA analysis comparing the effect of light intensity (PFD) on low light (88 µmol photons m^{-2} s^{-1}) normalized Φ'_M-EC_{50} value between low and high light acclimated cells (LL_c and HL_c). For each species, n = 30.

	Model R^2	Log PFD	Slope interaction	Low light normalized Φ'_M-EC_{50} (% of control)			
				LL_c	SE	HL_c	SE
A. falcatus	0,71	**	*	50,6	± *3,7*	87,1 ***	± *4,0*
P. morum	0,77	**	ns	67,9	± *5,0*	91,3 ***	± *4,7*
P. boryanum	0,84	**	ns	58,3	± *5,9*	85,8 ***	± *3,2*
C. snowii	0,62	**	*	69,5	± *4,4*	98,5 ***	± *5,1*
A. granulata	0,39	ns	*	159,8	± *15,4*	87,3 ***	± *6,4*
F. crotonensis	0,64	*	**	249,4	± *25,1*	50,3 ***	± *4,4*
P. muscicola	0,82	**	ns	60,7	± *4,5*	66,0	± *4,0*
M. flos-aquae	0,68	**	ns	44,7	± *2,7*	70,8 ***	± *1,8*
A. flos-aquae	0,42	**	ns	56,0	± *3,5*	78,8	± *2,9*
A. spiroïdes	0,45	**	ns	48,7	± *5,0*	61,1	± *2,6*

* $p<0.05$; ** $p<0.01$. *** significant difference between LLc and HLc. Refer to Fig. 5.4 for corresponding data.

Table 5.2

Species average Φ'_M-EC_{50} (nM atrazine) and SEM obtained in cells acclimated to low or high light condition (LL_c and HL_c) of 76 and 583 µmol photons m^{-2} s^{-1} and exposed to high actinic light intensity (HL_{AI} of 668 µmol photons m^{-2} s^{-1}) following 72 hr atrazine treatment.

Species	$LL_c + HL_{AI}$		$HL_c + HL_{AI}$	
	Φ'_M-EC_{50} (nM)	SEM	Φ'_M-EC_{50} (nM)	SEM
A. falcatus	75	± *14*	307 *	± *82*
P. morum	181	± *24*	317 *	± *22*
P. boryanum	55	± *8*	117 *	± *10*
C. snowii	135	± *30*	374 *	± *48*
A. granulata	424	± *67*	388	± *61*
F. crotonensis	2002	± *590*	94 *	± *21*
P. muscicola	16	± *2*	25	± *3*
M. flos-aquae	32	± *5*	108 *	± *39*
A. flos-aquae	52	± *15*	92	± *23*
A. spiroïdes	28	± *6*	18	± *6*

* *For each species individually, level significantly different by T-test ($p<0.05$)*

5.5 DISCUSSION

5.5.1 Effect of atrazine on photosynthesis

Despite differences in sensitivity to atrazine, the data presented in this study showed that this herbicide induced similar inhibitory effects on photosynthetic activity of cyanophytes and algae of different taxa. We showed that atrazine induced a decrease of the PSII operational quantum yield (Φ'_M) and the concentrations required to inhibit half of Φ'_M (Φ'_M-EC_{50}) among LL and HL acclimated phytoplankton varied between 18 nM (for *A. spiroïdes*) and 546 nM (for *F. crotonensis*). However, minimum, maximum and average Φ'_M-EC_{10} values were 10.1 nM, 98.9 nM and 34.4 ± 19.1 nM (data not shown). For all tested species, the consequence of Φ'_M inhibition was a decrease of the rETR and an inhibition of the maximal rate of photosynthesis (P_M). These data confirm that atrazine interfere directly with phytoplankton capacity to convert light energy into biologically available energy (ATP and NADPH), and significant effect was observed for algae and cyanobacteria. Although it is based on chlorophyll fluorescence technique, the measured effects are in agreement with previous observations of growth and oxygen production inhibition reported for similar organisms exposed to PSII inhibitors (Millie, 1992; Graymore et al., 2001). Furthermore, according to our data, the maximal rate of photosynthesis (P_M) was more inhibited by atrazine in HL adapted cyanophytes, *A. falcatus* and *P. morum*, while its sensitivity was lower or not modified by light acclimation for the other species. Since atrazine is known to block electron transfer between Q_A and Q_B (Jursinic and Stemler, 1983), it was not surprising to observe a strong decrease of Ψ_0 since this variable is a proxy of PSII electron transfer efficiency beyond Q_A (Force et al., 2003). As seen, this parameter was highly sensitive to atrazine with EC_{50} varying between 6 nM (*P. mucicola*) and 292 nM (*P. morum*) (Fig 1b) and EC_{10} between 0.9 nM and 68.5 nM (data not shown).

When comparing the relative sensitivity of the fluorescence parameters to atrazine, we found that Φ'_M was 2.7 times more resistant than Ψ_0. Taking into account the physiological information associated to these parameters, this discrepancy was informative. Indeed, Φ'_M represents PSII activity at steady state electron transport under continuous illumination and depends on the efficiency of the whole photosynthetic electron transport chain and processes linked to photosynthesis (Schreiber, 1986). In comparison, Ψ_0 measures the instantaneous electron transfer following 15 min of dark adaptation and reflects PSII activity independently of the following processes (Force et al., 2003). Hence, the lower sensitivity of Φ'_M observed here indicates that the blockage of Q_B binding sites by atrazine and the resulting diminution of electron transfer rate, was mitigated through processes activated under continuous illumination (for Φ'_M) but inactive in the dark (for Ψ_0). In algae and cyanobacteria, photoregulation and photoprotection processes are known to be activated in response to changing light environment or when facing light stress (Demmig-Adams and Adams 1996; Muller et al., 2001; Karapetyan, 2007). These processes include the redistribution of light harvesting antennae between the two photosystems (state transition), heat dissipation of excess energy and cyclic electron transport around PSI (Demmig-Adams and Adams 1996; Muller et al., 2001; Karapetyan, 2007). All these mechanisms modify the excitation pressure on the photosystems and our data suggested that they contributed to reduce the inhibiting effect of atrazine on phytoplankton photosynthesis.

Non-photochemical processes were shown to be very efficient mechanisms to dissipate excess energy that can be harmful to photosynthetic organism (Abeliovich and Shilo, 1972; Eloff et al., 1976; Barber and Anderson, 1992; Rutherford and Krieger-Liszkay, 2001). Indeed, these processes may prevent the production of reactive oxygen species (ROS) that can alter photosynthetic apparatus integrity. In this study, we showed that exposure to 250 nM atrazine significantly reduced the capacity to induce NPQ (lower NPQ_{HL}) for chlorophytes grown under LL and HL

and for baccilariophytes grown under LL. Our results are in accordance with previous studies demonstrating an inhibition of NPQ following atrazine treatment for other species of chlorophytes, baccilariophytes and cyanophytes (Chalifour and Juneau, 2011). Since NPQ represents an important photoregulatory process (Muller et al., 2001), our results suggest that atrazine decreases the capacity of phytoplankton to adjust photosynthetic electron transport to rapid fluctuating light environments. This inhibition may be explained by the indirect effect of atrazine on the transthylakoidal proton gradient since by inhibiting electron transport, atrazine also reduced this gradient required to activate NPQ (Horton et al., 2000; Lavaud and Krop, 2006). Thus, this indirect consequence of inhibition by atrazine enhanced its direct effect and lowered the photoprotection potential of NPQ needed for non-acclimated phytoplankton exposed to high light environments.

5.5.2 Photoacclimation, atrazine and light intensity

The data presented in this study showed that for all species tested, except for the baccilariophytes adapted to low light, the sensitivity to atrazine increased when phytoplankton are subjected to an increasing light intensity (Fig 3). For LL adapted cyanophytes and chlorophytes the relationship between Φ'_M-EC_{50} and light was semi-logarithmic. In HL adapted, this relationship remained semi-logarithmic for cyanophytes but it was linear for chlorophytes. Semi-logarithmic relationship indicates that the effect of light on atrazine sensitivity was greater at low light levels and became smaller above a certain point. Although inverted, such effect corresponds to the expected photosynthetic response observed between light limited and light saturated region in PE curves (MacIntyre et al., 2002). Thus the higher resistance following low light exposure relative to that observed following high light exposure observed for LL and HL adapted phytoplankton (Fig 3) may be related to photoregulatory changes affecting electron transport from limiting to saturating light

intensity. Under low light condition, phytoplankton are subjected to limited amount of photons and thus are not limited by the availability of quinones and other electron transporters, while under light saturating condition, availability of electron transport components becomes more limited (Anderson et al., 1995; MacIntyre et al., 2002). Thus, we may hypothesise that when cells acclimated to a specific light condition are exposed to light limiting conditions (low quinone requirement), a fixed number of atrazine molecules would block a small fraction of the quinones needed for photosynthesis and thus have small impact. The same phytoplankton suddenly exposed to light saturating conditions would require all available quinones to maximise light utilisation, thus atrazine would have a more pronounced effect by increasing the quinone limitation. Another indication in favour of this hypothesis was the lower sensitivity to atrazine in HL compared to LL adapted organisms (Fig. 4 and Table 1). While acclimating to high light, most algae and cyanobacteria tend to increase the PQ pool and associated electron transporters (Anderson et al., 1995) and hence, this increase dilutes the effect of atrazine by offering more binding sites. This may also explain the higher sensitivity of LL adapted cells exposed to HL compared to that obtained for HL adapted cells (Table 2) and the generally higher resistance to atrazine in the latter group (Table 1). In cyanophytes, the regression between Φ'_M-EC_{50} and light was semi-logarithmic for both LL and HL adapted cells suggesting that both light conditions correspond to light saturation region (small changes of PQ pool between LL and HL adapted cells) as opposed to chlorophytes for which LL adapted corresponded to light limiting region (low PQ pool) and HL adapted to light saturated region (higher PQ pool).

Because of these characteristics, comparison of sensitivity of LL adapted cells exposed to LL with the other extreme HL adapted cells exposed to HL, showed no significant difference except for cyanophytes and *F. crotonensis* (Fig 1). These results indicate that high light acclimation has a protective effect against atrazine as long as it induces an increase of the PQ pool and associated electron transporters.

Furthermore, we suggest that atrazine toxicity depends on simple dilution effects associated to the ratio of atrazine blocked quinones relative to global quinone availability and requirement.

We obtained contrasting results for baccilariophytes grown under LL since increasing light intensity tended to lower the effect of atrazine. This response may be explained by the high capacity and sensitivity of NPQ mechanisms for this algal group (Ruban et al., 2004). High NPQ capacity helps to decrease PSII excitation pressure observed under excess light condition, similar to that induced by atrazine, by decreasing the energy funnelled to PSII. This mechanism also down regulates photosynthetic electron transport and minimizes ROS formation and D1 photodamage (MacIntyre et al., 2002). Induction of NPQ increases with light intensity and may explain why both species were more protected against atrazine at higher light intensity. Finally, this mechanism was apparently not as efficient in HL adapted baccilariophytes and this may be attributed to changing photoacclimation strategy in this light condition, but further research is needed to identify the differences involved..

5.5.3 Conclusion

Difference of sensitivity was important between species, but also between light treatments and selected indicators (Φ'_M or Ψ_0) as seen by the variation of the obtained EC_{50}: 6.0 nM to 2002 nM (1.4 µg L^{-1} to 432 µg L^{-1}). This range corresponds to what was reported previously for phytoplankton, although some of our data stand in the lower portion of the published values (Huber, 1993; Solomon et al., 1996; Delorenzo et al., 2001). This can be explained by the higher sensitivity of chlorophyll fluorescence parameters compared to biomass or growth rate indicators, especially when assessing herbicide toxicity targeting PSII (Juneau et al., 2007). Our data also showed that sensitivity to atrazine was different between phylogenic groups where baccilariophytes and chlorophytes were generally more resistant to atrazine than cyanophytes. This is in accordance with a recent study comparing atrazine sensitivity at different water temperatures, where it was shown that a baccilariophyte (*Navicula pelliculosa*) was more resistant than a chlorophyte (*Scenedesmus obliquus*) and two cyanophytes (*Microcystis aeruginosa*) (Chalifour and Juneau, 2011). Higher sensitivity for cyanophytes was also reported in a mesocosm study where this group was eliminated by 100 µg L^{-1} of atrazine while the same treatment had small or no effect on baccilariophytes (Herman et al., 1986; Hamilton et al., 1988). In the environment, atrazine concentrations, in pulse events, often exceed 20 µg L^{-1} (93 nM) and can even reach 100 µg L^{-1} (464 nM), but the concentrations are generally lower than 5 µg L^{-1} (23 nM) (Solomon et al., 1996; Konstantinou et al., 2006; Giroux, 2010; Baxter 2011). Although the observed photosynthetic determined EC_{10} values were below the environmentally relevant concentrations, and assuming that growth is less sensitive to pollutants than photosynthesis (El Jay et al., 1997), our results tend to confirm that no significant effect should be expected on algae and cyanobacteria growth in the presence of atrazine (Baxter et al., 2011). On the other hand, in pulse events the most sensitive group should be cyanophytes and some chlorophytes, while baccilariophytes would only be affected in most extreme scenarios. However, in

natural aquatic environment, one could expect fluctuating light conditions, and our data clearly indicate that increased light intensity enhanced the deleterious effect of atrazine for all species (except LL grown baccilariophytes). This enhancing effect of light on atrazine toxicity was also reported in previous studies on some phytoplankton and macrophyte species (Brain et al 2012, Guash and Sabater 1998, Mayer et al 1998). In our study, the combination of HL exposure (> 668 µmol photons m^{-2} s^{-1}) and atrazine decreased the EC_{50} of cyanophytes below the aquatic protection criteria and suggests that in some cases, species from this group may be affected by atrazine at environmentally relevant conditions, because of surrounding light condition. Nevertheless, in a scenario where atrazine and water turbidity increase simultaneously following heavy rain or irrigation, our findings suggest that the decreased light intensity associated to turbidity would tend to protect all groups of phytoplankton by decreasing PSII excitation pressure. We also showed that pre-acclimation to high light tends to protect against atrazine toxicity thus, in a scenario where turbidity was already high in the environment, our data suggest that the resulting LL adapted phytoplankton would be more sensitive to atrazine compared to organism from an environment with low turbidity. Finally, we advance that protection in high light acclimated cells was due to increased photoregulation capacity and to a diluting effect caused by increased number of electron transporters which offers more atrazine binding sites relative to those available in low light acclimated cells.

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CONCLUSION GÉNÉRALE

Les relations entre photosynthèse, lumière et herbicides dans les écosystèmes aquatiques sont infiniment complexes et subtiles. Bien qu'aucun système *in vitro* ne puissent les reproduire exactement, il reste que des modèles intéressants peuvent être étudiés en laboratoire en isolant certaines variables et en permettant ainsi une plus grande compréhension des phénomènes impliqués au niveau des écosystèmes. C'est ce que les résultats compilés dans cette thèse contribuent à démontrer.

La photoacclimatation

Selon les résultats des chapitres I et II, la capacité d'acclimatation à la lumière des algues et des cyanobactéries est très grande. En effet, toutes les espèces étudiées dans cette thèse ont réussi à survivre et à croître dans une vaste gamme d'intensités lumineuses représentatives des milieux naturels allant de 14 à 1079 µmol de photons m^{-2} s^{-1}. Cette étonnante plasticité phénotypique souligne la grande capacité d'adaptation de ces microorganismes face aux contraintes de leur environnement et explique sans doute leur succès au niveau évolutif.

D'un point de vue cellulaire, la réponse de toutes les espèces exposées à un changement de l'intensité lumineuse était semblable et ce malgré des différences significatives au niveau de l'appareil photosynthétique des groupes phytoplanctoniques étudiés. Lorsqu'elles sont exposées à de fortes intensités lumineuses, la réponse des différentes espèces consiste en une diminution (plus ou moins importante) du contenu en pigments photosynthétiques, notamment en chlorophylle *a* et en phycobiliprotéine chez les cyanobactéries et cryptophytes. Parallèlement à ces modifications, toutes les espèces ont augmenté la proportion cellulaire de pigments de photoprotection relativement aux pigments photosynthétiques. Ces modifications, conformément à la théorie sur la photoacclimatation, ont permis de réduire l'efficacité d'absorption de la lumière au

niveau cellulaire en plus de diminuer la proportion d'énergie acheminée à l'appareil photosynthétique relativement à l'énergie totale interceptée par la cellule. Par ces ajustements, la pression énergétique sur les photosystèmes est minimisée ce qui diminue la production de radicaux libres et donc leurs effets néfastes pour la cellule. Ces travaux ont aussi permis d'obtenir un autre résultat significatif: montrer le lien étroit existant entre les réponses physiologiques associées à la photoacclimatation et la transition d'un environnement de lumière limitant pour la photosynthèse ($E : E_k < 1$) vers un environnement de lumière saturant pour la photosynthèse ($E : E_k > 1$). En fait, les caractéristiques principales de l'appareil photosynthétique en lumière saturante étaient :1) un rendement photochimique plus faible, 2) une grande capacité de dissipation thermique de l'énergie (NPQ) et, 3) une forte augmentation de l'état redox de la chaine de transport d'électrons (UQF_{REL}). De plus, la baisse d'efficacité du transport d'électrons à forte lumière était aussi reflétée dans la diminution (parfois forte) du rendement quantique de production d'oxygène. Paradoxalement, cette faible efficacité photosynthétique était largement compensée par la grande disponibilité de photons ce qui a permis à toutes les espèces d'atteindre des taux de croissance plus élevés que ceux mesurés à faible intensité lumineuse où l'efficacité photosynthétique était maximale.

D'un point de vue de la compétition entre les espèces, il semble que l'acclimatation à la lumière ne joue pas un rôle déterminant puisqu'aucune espèce n'était clairement avantagée par des intensités lumineuses faibles ou fortes. Néanmoins, les espèces de grandes tailles ou coloniales, comme certaines cyanobactéries et les baccilariophytes, ont atteint des taux de croissance plus faibles quelque soit l'intensité lumineuse, tandis que les organismes de petite taille et/ou flagellés ont atteint des taux de croissance plus élevés. Cependant, il est probable que ces caractéristiques ne soient plus aussi déterminantes dans les milieux naturels turbulents, dominés par les modifications rapides (journalières) de l'intensité lumineuse où la capacité de photorégulation peut jouer un rôle de sélection plus important en avantageant les espèces plus résistantes au stress lumineux (Lavaud et

al., 2007). En outre, les espèces de cette étude ont eu une période de temps illimitée pour s'acclimater à chaque condition de lumière et le fait qu'elles aient toutes réussi sous entend que la rapidité plutôt que la capacité d'acclimatation d'un organisme détermine son succès dans l'environnement.

Lumière et toxicité des cyanobactéries

La toxicité des fleurs d'eau de cyanobactéries est déterminée par 1) la présence de souches toxiques, 2) leur niveau de toxicité et 3) leur importance relative dans la population (Visser et al., 2005). Les résultats obtenus dans les chapitres II et III ont clairement montré que la lumière peut moduler chacun de ces aspects et peut donc influencer la toxicité des fleurs d'eau (Deblois et Juneau, 2010; Deblois et Juneau, *sous presse*). D'abord, la toxicité de *Microcystis aeruginosa* diminue en fonction de l'augmentation de l'intensité lumineuse conformément aux résultats du chapitre II et des études précédentes (Wiedner et al., 2003). Cependant, les résultats ont aussi démontré que la baisse de toxicité était étroitement corrélée aux changements photosynthétiques induits par la photoacclimatation notamment à la réduction de chlorophylle *a* et à l'augmentation du transport linéaire d'électrons (ETR) entre les PSII et PSI (Deblois et Juneau, 2010). Une étude précédente a montré que les microcystines sont majoritairement localisées sur les membranes thylakoïdiennes donc étroitement liées à l'appareil photosynthétique (Young et al., 2005). Cette information combinée aux résultats du chapitre II suggère que les microcystines, dont le rôle physiologique est encore inconnu, pourraient jouer un rôle au niveau de la régulation de la photosynthèse.

Les fleurs d'eau de cyanobactéries sont souvent visibles à la surface de l'eau. Dans ces conditions, les cellules à la surface sont exposées à une intensité lumineuse capable d'induire une forte photoinhibition voir même la mort par stress oxydatif (Abeliovich et Shilo, 1972; Eloff et al., 1976). Simultanément, quelques centimètres sous la surface, la disponibilité en lumière est extrêmement réduite et donc limitante.

Les résultats présentés dans le chapitre III ont montré que les souches toxiques de *M. aeruginosa* étaient plus résistantes lorsque exposées à de fortes intensités lumineuses (1200 µmol photons m^{-2} s^{-1}) comparativement aux souches non-toxiques. Les souches toxiques avaient aussi une capacité de récupération supérieure suite au stress lumineux et étaient avantagées (photosynthèse et croissance) par un cycle journalier de lumière (voir Fig. 2.1). Une des raisons évoquées pour expliquer ces différences est la plus faible pigmentation des souches toxiques ce qui aurait réduit l'absorption de lumière pour ces souches. De plus, des résultats récents sur ce sujet semblent démontrer que cette différence est essentiellement dûe à une plus grande capacité de photorégulation chez les souches toxiques (communication personnelle, Kui Xu). En résumé, les résultats de ce chapitre montrent que les fortes variations d'intensités lumineuses, auxquelles sont soumises les cyanobactéries pendant une floraison, peuvent sélectionner les souches toxiques au détriment des non-toxiques et augmenter le ratio de souche toxique : non-toxique et donc la toxicité de la floraison.

Lorsque l'on combine les résultats obtenus dans ces deux chapitres, il est possible d'établir deux scénarios extrêmes illustrant le rôle de la lumière comme facteur modulant la toxicité des floraisons en milieu naturel. Dans le premier scénario, le milieu est peu turbulent et les cellules sont en flottaison positive et forment donc des fleurs d'eau restant à la surface. Dans ces conditions, les souches toxiques sont dominantes puisque plus résistantes au stress lumineux associé aux conditions de lumière de la surface. Cependant, cet environnement est aussi plus saturé en lumière et les cellules présentent donc des caractéristiques associées à une acclimatation à de fortes intensités lumineuses et sont donc faiblement toxiques. Dans le second scénario, il y a de fortes turbulences et les cellules ne restent pas longtemps à la surface puisqu'elles sont fréquemment entrainées dans la colonne d'eau. Dans ces conditions, la disponibilité de lumière est limitante à cause de la forte densité cellulaire de la colonne d'eau et les souches non-toxiques ne sont donc plus désavantagées par de fortes intensités lumineuses. D'autre part, les souches toxiques ont maintenant des caractéristiques associées à de faibles intensités lumineuses et

elles sont significativement plus toxiques. Il y a donc proportionnellement moins de souches toxiques mais celle-ci contiennent plus de microcystines. Dans les milieux naturels, la situation est rarement aussi extrême et représente donc un mélange de ces deux scénarios. La toxicité réelle de la fleur d'eau est donc plus difficile à prédire mais, en montrant que la lumière est un facteur de modulation important, les résultats des chapitres II et III permettent de mieux comprendre la toxicité des fleurs d'eau mais aussi l'évolution de la toxicité au cours de la floraison.

Herbicides

Comme les études précédentes, les résultats obtenus dans les chapitres IV et V ont montré qu'il existe une grande variabilité dans la sensibilité du phytoplancton exposé à différents polluants utilisés en milieu agricole (DeLorenzo et al., 2001; Graymore et al., 2001). À l'exception de trois souches de *M. aeruginosa* exposées à l'oxadiazon, toutes les espèces étudiées dans cette thèse (n = 17) ont été affectées par la présence d'atrazine, de diuron ou d'oxadiazon. Parmi les variables mesurées et associées à l'appareil photosynthétique, les effets suivants ont été causé par ces herbicides: une diminution du contenu en chlorophylle *a* et une diminution du rendement photochimique des PSII (Φ'_M) résultant en une baisse du transport d'électrons (ETR) et de la capacité photosynthétique maximale (P_M). C'est ainsi que la production primaire mais aussi la croissance de plusieurs espèces de phytoplancton peuvent être inhibées en présence d'herbicides. En outre, en présence d'atrazine, certains mécanismes de photoprotection (NPQ) des différentes espèces étaient aussi affectés. En inhibant l'activité photosythétique et par le fait même la croissance en plus de réduire l'efficacité de certains mécanismes de photoprotection essentiels au maintien d'une espèce dans un environnement lumineux variable, l'ensemble des résultats obtenus jumelé à la sensibilité variable entre les espèces suggèrent que la présence d'herbicides en concentration suffisante peut modifier la structure de la communauté dans les milieux aquatiques où ils se retrouvent.

En se basant sur les résultats obtenus dans les études présentées ici, force est de constater que les effets des herbicides sur la communauté phytoplanctonique sont difficiles à prédire puisque aucune espèce, aucun genre ni groupe n'était systématiquement plus résistant ou plus sensible lorsque exposé à l'un ou l'autre des polluants étudiés. Par exemple, trois souches de *M. aeruginosa* étaient plus résistantes à l'oxadiazon et au diuron comparativement à deux autres cyanophytes et deux chlorophytes. Selon ces résultats, le genre *Microcystis* fréquemment rencontré au cours d'épisode de fleurs d'eau était donc favorisé voir même complètememt insensible dans le cas de l'oxadiazon. Les conclusions étaient bien différentes lorsque une espèce du même genre, M. *flos-aquae*, ainsi que trois autres espèces de cyanophytes retrouvés couramment dans les fleurs d'eau (des genres *Aphanizomenon*, *Anabaena* et *Phormidium*) ont été exposées à l'atrazine: elles étaient en effet significativement plus sensibles que les chlorophytes et les baccilariophytes testées. Dans ce dernier cas, les concentrations d'atrazine requises pour produire 50 % d'inhibition de l'activité photosynthétique étaient pour la grande majorité au-delà des concentrations habituellement mesurées dans l'environnement (< 5 µg L^{-1}) et il est probable qu'aucun effet sur la croissance et donc sur la communauté ne soit observable dans ces conditions.

Ce «non-effet» de l'atrazine dans l'environnement doit cependant être interprété avec une grande prudence puisqu'il a été obtenu dans des conditions qui ne représentent pas l'ensemble de celles qui se retrouvent typiquement dans les cours d'eau ou lacs affectés par les polluants agricoles. En fait, on retrouve fréquemment jusqu'à trente polluants différents dans l'eau des milieux naturels à proximité des terres agricoles et bien qu'individuellement peu d'entre eux ne dépasse les critères de protection de la santé aquatique, leur présence combinée peut représenter un problème important pour la communauté algale à cause des effets interactifs entre les différents polluants (Giroux 2010). Par ailleurs, les résultats présentés au chapitre V ont montré qu'il ne faut pas non plus négliger l'interaction entre la lumière et l'atrazine puisque la sensibilité à l'atrazine augmente significativement pour toutes

les algues exposées aux fortes intensités lumineuses représentatives des milieux aquatiques. Dans le cas des cyanophytes les plus sensibles, la combinaison lumière forte (> 668 µmol photons m^{-2} s^{-1}) et atrazine a donné une inhibition de la photosynthèse à des concentrations fréquemment mesurées dans l'environnement (<2 µg L^{-1}).

Finalement, en mettant en évidence l'importance de la lumière et des herbicides comme facteurs pouvant moduler la compétition entre les différentes espèces phytoplanctoniques, l'ensemble des travaux présentés dans cette thèse souligne, comme tant d'autre, l'importance de mieux encadrer les activités agricoles pour en contrôler les impacts sur les milieux aquatiques et les organismes qui y vivent.

ANNEXE 1: LA PHOTOSYNTHÈSE

On connait d'abord la photosynthèse comme étant la source de l'oxygène que nous respirons quotidiennement. Dans les faits, la production d'oxygène n'est pas la finalité de ce processus mais plutôt un résidu secondaire, son déchet. Le véritable rôle de la photosynthèse est biochimique et implique la conversion de l'énergie des photons provenant du soleil en une énergie chimique utilisable par les organismes vivants (Mauzerall et Greenbaum, 1989). Ce processus est rendu possible par l'action combinée de quatre complexes protéiques : les photosystèmes I & II (PSI & PSII), le cytochrome $b_6 f$ (Cyt $b_6 f$) et le complexe de synthèse d'ATP (ATPase). Ces complexes sont imbriqués dans une membrane plasmique qu'on appelle thylakoïde. Chez les cyanobactéries, les thylakoïdes se retrouvent à l'état libre dans le cytoplasme tandis que chez les organismes eucaryotes, ils sont enfermés dans une enveloppe membranaire appelée chloroplaste. Contrairement à certains constituants de l'appareil photosynthétique, la structure des PSI et II n'a pas beaucoup changé au cours de l'évolution. Chaque PS est composé de protéines spécifiques et d'un cœur constitué de deux molécules de chlorophylle *a* spécialisées qu'on appelle le centre réactionnel (RC). Le RC des PSII présente un pic d'absorption à la lumière rouge à 680 nm tandis que celui des PSI absorbe davantage dans le rouge lointain à 700 nm (Nelson et Yocum, 2006). Conséquemment, les abréviations P680 et P700 sont fréquemment utilisées pour désigner les RC du PSII et du PSI, respectivement. Les PS sont associés à des complexes apoprotéine-pigment appelés antennes collectrices de lumière (LHC). Ces antennes permettent d'élargir la gamme des longueur d'ondes pouvant être utilisées pour la photosynthèse ainsi que la surface d'absorption de lumière (Falkowski et La Roche, 1991; Huner *et al.*, 2003). La gamme des longueurs d'ondes de lumière utilisable pour la photosynthèse correspond à peu de chose près au spectre visible et se situe entre 350 et 700 nm (Wetzel, 2001). Les LHC renferment une grande diversité de pigments accessoires qui absorbent différentes longueurs d'ondes, comme la chlorophylle *a*, *b*, *c*, les phycobiliprotéines mais aussi les caroténoïdes qui

ont la capacité d'absorber la lumière sans nécessairement l'acheminer vers les PS. Comme nous le verrons plus loin, la quantité et le type de pigment formant les LHC peuvent changer en réponse aux variations lumineuses de l'environnement.

Maintenant que nous connaissons les différents constituants, voici comment ils fonctionnent. D'abord, le déroulement de la photosynthèse décrit ici suit les étapes du schéma en Z qui représente le transport linéaire d'électrons. À ne pas confondre avec les modes de transport non-linéaire qui ne sont pas traités ici. Le processus photosynthétique débute lorsqu'un photon est absorbé par un pigment des LHC. L'énergie de ce photon est transférée de pigments en pigments pour finalement atteindre le RC du PS associé. Ces événements se produisent en continu et chaque PS est donc constamment « alimenté » en énergie lumineuse. Au niveau du PSII, cette énergie fait passer le P680 de l'état stable vers l'état excité P680*. Pour retrouver l'état stable, les P680* perdent l'excédant d'énergie en donnant un électron à une molécule de transport étroitement associé au PSII appelé quinone (Qa). Après avoir absorbé un second électron par le même processus, les électrons se combinent à la plastoquinone (PQH_2) et se déplacent ensuite au travers de la membrane thylakoïdienne vers le Cyt b_6f. Le transfert des électrons de PQH_2 vers le Cyt b_6^f est couplé au transport de proton et à la formation d'ATP. La conversion de l'énergie lumineuse en énergie « biologique » commence donc ici. En outre, le passage des électrons établit un gradient de proton transthylakoïdale utilisable par les ATPases. Les ATPases utilisent l'énergie potentielle du gradient de protons pour convertir l'ADP en ATP. Les électrons provenant des PQ sont ensuite combinés à la plastocyanine (PC) qui les transporte à son tour vers un PSI où les électrons sont utilisés pour réduire un P700* en P700 stable. Une bonne partie de l'énergie potentielle provenant du photon a été perdue à chaque transfert ainsi qu'au profit du gradient transthylakoïdale de protons, mais chaque électron associé au PSI est tout de même plus énergétique qu'à son état initial. Chaque électron maintenant combiné a P700 est a nouveau excité (P700*) par l'absorption d'un photon des LHC dont

l'énergie est acheminé vers le P700. Par ce processus de double excitation, la première au PSII et la seconde au PSI, l'électron a maintenant suffisamment d'énergie pour permettre la conversion du NADP+ en NADPH grâce à l'action du complexe enzymatique Ferredoxine NADP réductase. C'est aussi par ce processus que le transport linéaire d'électrons se termine.

Rien ne se perd et rien ne se crée: chaque passage de l'état stable à l'état excité induit la perte d'électrons au niveau du P680 et du P700. Chaque électron doit donc être remplacé pour permettre au cycle photosynthétique de continuer. C'est ici qu'intervient le complexe de dégagement d'oxygène qui est associé au PSII. Ce complexe enzymatique est capable d'obtenir des électrons par hydrolyse d'une molécule d'H_2O. Durant ce processus, chaque molécule d'eau est scindée en un atome d'oxygène, deux protons et deux électrons. Les électrons permettent la réduction de P680* vers P680 et seront entraînés dans la chaîne de transport d'électrons décrite précédemment. Les protons libérés contribuent au gradient transmembranaire de protons utilisables par les ATPases (Mauzerall et Greenbaum, 1989; Falkowski et Raven, 1997). Le résidu de cette réaction d'hydrolyse est l'oxygène et c'est ce dernier qui est si vital pour la biosphère. Finalement, l'énergie lumineuse emmagasinée dans le NADPH et l'ATP grâce au transport linéaire d'électrons est ensuite utilisée pour le maintien de nombreux processus cellulaires énergivores, notamment pour la formation de matière organique via la fixation du carbone inorganique disponible sous forme de CO_2 (Raines, 2003).

ANNEXE 2 : AUTRES CONTRIBUTIONS SCIENTIFIQUES

Articles scientifiques

Juneau, P., Qiu, Baosheng., **Deblois, C.P.** 2007. Use of chlorophyll fluorescence as a tool for determination of herbicide toxic effect: Review. Toxicol. Environ. Chem. Vol 89 (4): 609-625.

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