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TOWARDS AN UNDERSTANDING OF THE REGULATION AND THE ECOLOGY OF AEROBIC ANOXYGENIC PHOTOTROPHIC BACTERIA IN LAKES

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A few months ago, a friend asked me a question that got stuck in my mind trying to be answered. He wanted to know why doctoral students should invest so much time and effort in getting a doctoral degree. Despite the high costs payable could be clear for him, he wondered about the kind of benefits that future PhDs should get in order to counterbalance the sum of the costs spent. It has taken me a long while to find an answer to that question. Indeed, it is likely that there is more than one answer, but I will take a few lines of this section of my thesis to express the kind of things motivated me during these five years, and my analysis of how all the invested experience resulted in a positive net balance.

Opportunity is the word that best describes, in my opinion, the answer to the question on the benefit for which my friend was timely asking. Doing a doctorate provides an extensive range of opportunities. In my case, the doctorate study gave me the opportunity to be a student once again, better yet, to be a foreign student and experience all the challenges involved in being away from home, family, in being immersed in a different educational and cultural system, and deal with another language and another type of society. Thus, this opportunity resulted in perceiving new ways to act, and to meet great individuals.

From a particular perspective, the process of formulating, developing and writing this thesis gave me the opportunity to address a range of challenges such as meeting deadlines, learning new skills, balancing workload, getting access to equipment in other labs, and starting a new process almost every day with the feeling of having unfinished the previous one. Such a daily exploration demands honest perseverance, organization, self-confidence, and what I found even

more valuable is that by experiencing every difficulty, I've gained the ability to connect with my colleagues and to find in this interaction the support needed for overcoming all sort of challenges. This connection with the others deeply facilitated me the process of growing as a scientist and as a person. The doctorate pathway provides these and a long list of other opportunities; and the key to get a benefit from that is to recognize the opportunity and take pride in it, and be able to honor and make something out of it. My friend's question was a good chance to reflect.

Looking back now, I am aware of the many opportunities I have been given, and also of the critical role of the people who have helped me to seize these opportunities. I would like to express my deepest gratitude to my supervisor, Paul del Giorgio, for his patience, his excellent guidance, and for the excitement and energy with which he leads CARBBAS group. I would like to thank Matthew Cottrell, for his willingness to help, for the relevance that his practical experience had for the development of this project, and for his promptness in reading over drafts. I would also like to thank David Kirchman, for giving me the opportunity to make use of all the facilities in his lab, as well as, for pointing useful suggestions for my research. Likewise, I would like to express my gratitude to Pep Gasol, for his joy and for the key hints that I've received from him when I was writing my proposal. I would like to express my profound gratitude to Clara, who patiently corrected all my writings, for being always willing to help before any difficulty, and moreover, for showing me the way to enjoy the research. I would like to thank Juan, for pushing me to do the doctorate, and for being always ready to help me.

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GENERAL ABSTRACT

The main purpose of this thesis is to gain insight into the ecology of aerobic anoxygenic photototrophic (AAP) bacteria in freshwater ecosystems by exploring how different ecophysiological aspects of these bacteria are affected by potential regulatory factors such as light availability, predation pressure, nutrients and dissolved organic carbon. Specifically, the three chapters of this thesis explore the regulation of AAP bacteria on different ecophysiological aspects. On one hand we studied the regulation of net growth, cell size, and relative abundance of AAP bacteria. On the other, we looked at the regulation of the gross growth rate and the single-cell activity of AAP bacteria. With respect to regulatory factors, we centered our attention on exploring the effect of grazing on regulating the abundance, net growth, and cell size of AAP bacteria, although we also investigated the role of nutrients. As to the regulation of the activity and growth rate of AAP bacteria, we focused on the effect of light, dissolved organic carbon and nutrients. By combining empirical and experimental approaches we explored the patterns in single-cell activity, gross growth, pigment production, phototrophic potential, mortality, cell size, abundance and biomass of AAP in the natural environment, as well as the underlying regulatory processes in experimental conditions. This mixed approach involved the combination of experiments at mesocosm and microcosm scales, the collection of water samples in several lakes that cover a wide range of environmental gradients, and the use of a variety of microbiological tools that include infrared epifluorescence microscopy, kinetic fluorometry, microautoradiography, and quantitative real time PCR (qPCR).

Our main results demonstrate that AAP bacteria are a highly dynamic component of freshwater bacterial communities. Compared to the bulk bacterial community, AAP are on average larger, more active and faster growing, although this varies largely among systems and even within a given community. These features seem to render them very attractive to predators, including metazoan zooplankton. Consequently, AAP bacteria appear to be subjected to a strong grazing pressure in freshwater systems, which may explain their very low ambient net growth rates and low relative abundances. Nevertheless, their strong predation likely results in an effective carbon transfer from the microbial food web to higher trophic levels.

Our exploration of the effect of light on key physiological processes of freshwater AAP communities demonstrates how complex the interactions between the environment and the physiology of AAP can be. Our results suggest that, contrary to expectations, the higher apparent growth rates and potential competitiveness of AAP bacteria may not be directly linked to the ability of AAP bacteria to use light energy. The potential lack of a direct effect of light on the growth and activity of AAP does not imply the absence of a benefit from the phototrophic function, but the

actual nature of this advantage is still not understood. We confirmed that light has a direct effect on the synthesis of BChl*a* in natural AAP communities. Furthermore, we suggest that pigment production may be linked to other cellular functions that are not directly linked to growth but which may nevertheless represent an advantage for AAP cells. Such cellular functions could include the activation of cells, the regulation of the expression of their large set of enzymes, or the production of carotenoids that are hypothetically associated with protection from phototoxicity.

We conclude that few inferences can be made concerning the ecological role and performance of AAP bacteria based solely on their abundance patterns. While the question concerning the actual advantage of the phototrophic function of AAP bacteria remains unsolved, this thesis makes a significant contribution to the understanding of the role of light by providing an integrative scenario that examines the potential links between light, AAP growth, activity, regulation and photosynthetic potential. We also suggest a number of questions and hypotheses that may encourage future studies that will help resolving some of the uncertainties associated to this functional group of prokaryotes.

Key words: aerobic anoxygenic phototrophic (AAP) bacteria, freshwater, light, grazing, growth, activity, ecophysiology.

RÉSUMÉ GÉNÉRAL

Le principal objectif de cette thèse consiste à mieux comprendre l'écologie des bactéries aérobiques anoxygéniques phototrophes (AAP) dans les écosystèmes d'eaux douces et ce, en explorant comment leurs différentes composantes écophysiologiques sont affectées par des facteurs régulateurs tels que la disponibilité de la lumière, la prédation, les nutriments et le carbone organique dissous. Les trois chapitres de cette thèse explorent plus particulièrement la régulation des bactéries AAP selon différents aspects écophysiologiques. D'une part, nous avons étudié les facteurs qui régulent la croissance nette, la taille des cellules et l'abondance relative des bactéries AAP dans les communautés bactériennes. D'autre part, nous avons observé la régulation du taux de croissance brut et de l'activité de cellules individuelles des bactéries AAP. Par rapport aux facteurs ayant le potentiel de régir les communautés APP, nous avons centré notre attention sur l'effet du broutage et des nutriments sur la régulation de l'abondance, de la croissance nette et de la taille cellulaire. Par rapport aux facteurs ayant le potentiel de régir l'activité et la croissance des bactéries AAP, nous avons centré notre attention sur les effets de la lumière, des nutriments et du carbone organique dissous. En combinant des approches empirique et expérimentale, nous avons exploré à la fois les patrons des activités des cellules individuelles, des taux de croissances bruts, de la production de pigments, du potentiel phototrophique, de la mortalité, de la taille cellulaire, de l'abondance et de la biomasse des AAP dans leurs écosystèmes naturels ainsi que les processus réglementaires sous-jacents en conditions expérimentales. Cette approche diverse inclue la combinaison d'expérimentation à l'échelle des mésocosmes et des microscomes, la collecte d'eau dans plusieurs lacs couvrant des grands gradients environnementaux et l'utilisation d'une variété d'outils microbiologiques incluant la microscopie à fluorescence infrarouge, la fluorimétrie cinétique, la microautoradiographie et la PCR quantitative en temps réel (qPCR).

Nos résultats principaux démontrent que les bactéries AAP sont une composante hautement dynamique des communautés bactériennes d'eaux douces. Par rapport à la totalité de la communauté bactérienne, les bactéries AAP sont en moyenne plus grosses, plus actives et ont un taux de croissance plus rapide, qui varie cependant entre les systèmes et au sein même d'une même communauté. Ces caractéristiques semblent les rendre plus attrayantes aux prédateurs, en incluant le zooplancton métazoaire. Par conséquent, les bactéries AAP semblent être soumises à une forte pression de broutage dans les écosystèmes d'eaux douces, ce qui pourrait expliquer leur très faible taux de croissance ambient et leurs abondances relatives très basses. En vertu de ceci, le broutage préférentiel des AAP résulte probablement dans un transfert efficace du carbone de la toile microbienne vers des niveaux trophiques plus élevés.

Notre exploration de l'effet de la lumière sur les processus physiologiques clés

des communautés de bactéries AAP démontre la complexité des interactions entre l'environnement et la physiologie des AAP. Nos résultats suggèrent que, contrairement à nos prévisions, les taux de croissances accrues et le potentiel compétitif des bactéries AAP ne peuvent pas être directement liés à la capacité de ces bactéries à utiliser l'énergie lumineuse. Le manque potentiel d'un effet direct de la lumière sur la croissance et activité des AAP n'implique pas l'absence d'un bénéfice de la fonction phototrophe, mais suggère plutôt que cet avantage n'est pas encore compris. Nous avons confirmé que la lumière a un effet direct sur la synthèse de la BChl*a* dans les communautés naturelles de bactéries AAP. De plus, nous suggérons que la production de pigments peut être corrélée avec d'autres fonctions cellulaires qui ne sont pas directement reliées à la croissance mais qui peuvent néanmoins représenter un avantage significatif pour les cellules AAP. Ces fonctions cellulaires pourraient inclure l'activation des cellules, la régulation de l'expression de leurs grands ensembles d'enzymes ou la production de caroténoïdes qui sont hypothétiquement associées avec la protection de la phototoxicité.

Pour conclure, peu d'inférences peuvent être apportées concernant le rôle écologique et la performance des bactéries AAP basé seulement sur leurs patrons d'abondance. Tandis que la question concernant l'avantage de la fonction phototrophe des bactéries AAP demeure inexpliquée, cette thèse apporte une contribution significative à la compréhension du rôle de la lumière en apportant un scénario intégratif qui examine les liens potentiels entre la lumière, la croissance des bactéries AAP, l'activité, la régulation et le potentiel photosynthétique. Nous suggérons aussi quelques questions et hypothèses qui pourront encourager des études futures à résoudre certaines incertitudes associées à ce groupe de procaryotes.

Mots clés : bactéries aérobiques anoxygéniques phototrophes (AAP), eau douce, lumière, broutage, croissance, activité, écophysiologie.

INTRODUCTION

In the late 1970's Harashima and Shiba (Harashima et al., 1978 ; Shiba et al., 1979) described for the first time an interesting and colorful group of cultivable heterotrophic bacteria, the aerobic anoxygenic phototrophic (AAP) bacteria. These prokaryotes produce the light-harvesting pigment Bacteriochlorophyll a (BChl*a*), which is incorporated into a functional photosynthetic system similar to that of anaerobic phototrophic purple non-sulfur bacteria (Koblížek, 2015 ; Yurkov and Csotonyi, 2009). Contrary to their anaerobic counterparts, the photosynthetic system of AAP bacteria is functional only under aerobic conditions (Garcia et al., 1994 ; Okamura et al., 1985 ; Yurkov and Beatty, 1998). Thus, whereas the purple bacteria can either grow photoautotrophically under anaerobic conditions or chemoheterotrophically under aerobic conditions, AAP bacteria are able to grow aerobically using simultaneously light and organic matter as energy sources (Kolber et al., 2001); this defines AAP as a functional group of strict aerobic bacteria that carry out a photoheterotrophic metabolism (Eiler, 2006 ; Koblížek, 2015).

The potential ecological relevance of AAP bacteria remained unexplored until Kolber and collaborators revived the issue in 2000 by reporting not only their widespread presence in a variety of coastal and oceanic environments, but also by demonstrating their photosynthetic competence in situ (Kolber et al., 2000 ; Kolber et al., 2001). This "rediscovery" of AAP marked the beginning of investigations aiming to explore their biogeochemical role, since it suggested the existence of a new path of light-energy input to the aquatic system that had not been captured by any primary production method, since it does not involve CO_2 fixation, does not produce oxygen, and uses a pigment different from Chlorophyll *a*. Moreover, the discovery of AAP bacteria has challenged the models of aquatic food webs by suggesting that light may have a direct influence on the processing and incorporation of carbon

into the bacterial biomass (Karl, 2002 ; Koblížek, 2015 ; Moran and Miller, 2007).

The following sections present an overview of the current knowledge of the ecology of AAP bacteria that place our work in context, and describe the objectives and the conceptual and methodological approach that we propose to achieve these objectives.

0.1 State of knowledge

0.1.1 AAP bacteria and their ability to use light energy

0.1.1.1 Basic information on the physiology of AAP bacteria

Most of the knowledge regarding the physiology of AAP bacteria has been gained from studies with isolates. These studies have revealed distinctive characteristics of these bacteria, which are relevant to understand the potential role of this functional group in aquatic environments. For example, in contrast to other photosynthetic microorganisms, AAP cells produce photosynthetic complexes with low levels of BChl*a*, i.e the main light-harvesting pigment. A reaction center of an AAP cell generally contains about 34 molecules of BChl*a*, more than 10-fold lower than those produced by anaerobic anoxygenic phototrophic bacteria or by cyanobacteria (Koblízek et al., 2005 ; Kolber et al., 2001). This feature constrains the rate of absorption of photons and, consequently, the amount of energy potentially gained from phototrophy in AAP bacteria is theoretically smaller than that of other phototrophs (Kirchman and Hanson, 2013).

A common feature of AAP cultured strains is their diverse range of intense colors, conferred by abundant carotenoids. Only a small fraction of these carotenoids,

however, are linked to the photosynthetic function; the vast majority appears to be associated with protection from phototoxicity, a phenomenon that results from the simultaneous presence of light, BChl*a* biosynthesis intermediates, and oxygen (Beatty, 2002 ; Koblížek, 2015 ; Yurkov and Csotonyi, 2009).

Another interesting feature of AAP cells is that the synthesis of BChl*a* is generally inhibited by light. The clustering of photosynthesis-related genes in a superoperon (*pufLM* operon) favors a tight regulation of their expression, which involves a elevated energetic cost; during the early stages of light exposure, energy is invested in suppressing the synthesis of BChl*a* and activating the cell detoxification system against reactive oxygen species. Similarly, during dark growth, the cells invest energy for the novo synthesis of the photosystems units (Tomasch et al., 2011). A wider range of regulatory factors involving not only light intensity, but also oxygen concentration and carbon availability have also been reported (Nishimura et al., 1996 ; Ouchane et al., 2004 ; Sato et al., 1985 ; Spring and Riedel, 2013 ; Yurkov and Beatty, 1998). Therefore, according with theoretical calculations of the bioenergetics of AAP bacteria, it appears that the net energetic benefit of phototrophy for AAP bacteria may be just enough to compensate the high metabolic costs associated to the production and turnover of the photosynthetic apparatus (Kirchman and Hanson, 2013).

0.1.1.2 The debate on the advantages of phototrophy in AAP

There is an ongoing debate concerning the metabolic benefit that AAP bacteria derive from phototrophy, which originates from the contradictory results that have been reported regarding the influence of light on AAP bacteria. While experimental studies with AAP bacterial isolates have repeatedly demonstrated a positive influence of light

on different aspects of the physiology and metabolism of AAP bacteria, (Biebl and Wagner-Döbler, 2006 ; Hauruseu and Koblizek, 2012 ; Soora and Cypionka, 2013; Spring et al., 2009, Yurkov and Vangemerden, 1993), less clear results have been obtained when assessing the effect of light on natural AAP communities. Field studies have shown both positive and negative correlations between light availability and the patterns in AAP abundance; for example, studies conducted in Mediterranean coastal waters reported a positive correlation of AAP abundance and day length (Ferrera et al., 2013). On the contrary, two large surveys of AAP abundance in freshwater systems showed negative correlations of AAP abundance and water transparency (Cuperová et al., 2013 ; Fauteux et al., 2015). Moreover, the few recent studies that have experimentally exposed marine and estuarine bacterial communities to light and dark conditions have failed to demonstrate any light stimulation of the incorporation of radioactive leucine in natural AAP assemblages (Kirchman et al., 2014 ; Stegman et al., 2014). At the same time, however, one of these studies showed that the percentage of active AAP cells was negatively correlated to water column light attenuation, suggesting that the proportion of active AAP cells may be enhanced by light (Stegman et al., 2014).

Another uncertainty about the benefit from light derived energy on AAP communities emerges from the surveys of BChl*a* content per cell in aquatic systems. While experimental studies have shown that cultivated AAP bacteria modulate the cellular pigment content in response to light availability (Koblízek et al., 2003 ; Li et al., 2006), similarly to photoadaptation by phytoplankton that results in high cell pigment concentration in low-light environments (Cullen, 1982), field studies have indicated that the large variation in the BChl*a* content per cell in natural communities is not explained by photoadaptation (Cottrell et al., 2010). In contrast, these studies have reported a decline in the pigment content per AAP cell with lake depth (Fauteux et al., 2015), or a lack of correlation between the cell BChl*a* content and turbidity (Cottrell et al., 2010). A more intriguing issue is the larger concentrations of BChl*a* content found in

cells associated with particles relative to free-living AAP bacterial communities, which suggest that AAP bacteria may modulate the cellular pigment content in response to higher concentrations of dissolved and particulate substrates and not light attenuation (Cottrell et al., 2010). The controversy surrounding these reports is not solved yet and we still have little information about the factors that regulate the production of BChl*a*.

0.1.2 Ecology and distribution of AAP bacteria

0.1.2.1 Patterns of abundance of AAP bacteria

Surveys of AAP abundance over the last 15 years have confirmed the widespread distribution and the ubiquitous character of this group of bacteria. AAP bacteria have been found in a wide variety of aquatic systems ranging from the open-ocean (Ferrera et al., 2013 ; Lami et al., 2007 ; Lamy et al., 2011 ; Lehours et al., 2010 ; Nikrad et al., 2012 ; Palovaara et al., 2014 ; Salka et al., 2008 ; Waidner and Kirchman, 2007) to freshwater ecosystems (Cuperová et al., 2013 ; Eiler et al., 2009 ; Mašín et al., 2012 ; Mašín et al., 2008 ; Ruiz-González et al., 2012). In spite of the fact that they are found across highly variable environmental conditions, it is remarkable that the contribution of AAP bacteria to total bacterial abundance is often very low (average 5%), and varies only in the range of 1 to 20% among aquatic systems (Koblížek, 2015).

Interestingly, the abundance of AAP bacteria tends to increase together with total prokaryotic abundance along environmental gradients (Fauteux et al., 2015 ; Jiao et al., 2007 ; Liu et al., 2010 ; Salka et al., 2008), a covariation that implies that AAP bacteria respond to the same environmental drivers than the bulk of the bacterial community. Therefore, it is not surprising that temperature, phosphorus, chlorophyll *a*,

and dissolved organic carbon have been identified as some of the environmental drivers explaining the variation in AAP abundance (Cuperová et al., 2013 ; Hojerová et al., 2011 ; Mašín et al., 2006, 2012 ; Medová et al., 2011 ; Salka et al., 2008). The observed patterns of variation in AAP abundance are, however, in conflict with the presumed advantage of possessing the ability to use both light and organic carbon as a source of energy. First, the positive trend observed between AAP abundance and trophic status (Hojerová et al., 2011 ; Mašín et al., 2012) does not support the hypothesis, initially held by some studies (Kolber et al., 2001 ; Mašín et al., 2008), that the ability of these bacteria to use light should be especially advantageous in nutrient-poor environments. Another unexpected pattern, which is contrary to the hypothesized positive effect of light on this photoheterotrophic group, is the negative relationship of AAP abundance and water transparency observed in marine and freshwater sites (Cuperová et al., 2013 ; Fauteux et al., 2015). In addition, it has been also surprising to find BChl*a*-containing AAP cells in conditions where the ability to harvest light energy would seem to be unnecessary, such as under the ice in northern lakes and in the Arctic Ocean in winter when light intensities are near zero (Cottrell and Kirchman, 2009; Fauteux et al., 2015). Therefore, these findings question the actual advantage of AAP of having a photoheterotrophic metabolism, and have generated a lot of uncertainty surrounding the environmental conditions or the specific situations where the ability to harvest light would provide a competitive advantage to AAP over the rest of heterotrophic bacteria.

0.1.2.2 Activity patterns of AAP bacteria and their ecological role in aquatic systems

In contrast to the accumulating information regarding the abundance and distribution of AAP bacteria, little is known about their role in the natural environment. Over the last 15 years, most of the studies have been focused on demonstrating their relevance by exploring

their abundance dynamics, concluding that their widespread distribution must mean that these photoheterotrophs are important players in aquatic ecosystems. While these surveys have yielded valuable insight into the ecology of AAP in natural ecosystems, little information can be extracted about the ecological relevance of AAP in aquatic systems. This has motivated a line of research focusing on the exploration of the heterotrophic activity and the growth of marine AAP communities (Ferrera et al., 2011 ; Koblízek et al., 2005 ; Liu et al., 2010 ; Stegman et al., 2014), with the aim to better characterize and understand the ecological role of these prokaryotes in microbial food webs.

The results from these marine studies have suggested that AAP bacteria may be a highly active component of microbial communities. Experiments carried out in coastal waters of the Mediterranean Sea revealed that AAP bacteria grew about 2-fold faster than the total bacterial community (Ferrera et al., 2011). Other studies using different approaches to calculate growth rates of AAP have reported even higher differences in their growth rate relative to total bacteria. For example, Liu et al. (2010) reported a 4-fold higher average AAP growth rate, estimated on the basis of the frequency of dividing cells. Koblížek et al. (2007), on the other hand, reported AAP growth rates in the Atlantic ocean, based on the diurnal decay in BChl*a*, that were on average 10-fold higher than the average bulk bacterial growth rates that have been measured in those sites (Ducklow, 2000). Recent studies have developed a new technique involving microautoradiography (MAR) that has just opened the way to exploring the in situ patterns of activity of AAP bacteria (Stegman et al., 2014), since it allows the direct quantification of the incorporation of radiolabelled organic substrates by individual AAP bacteria. The only two studies applying this technique (Kirchman et al., 2014 ; Stegman et al., 2014) have been conducted in marine and estuarine waters and have shown that AAP cells seem to be on average more active in substrate uptake than the bulk bacterial cells.

An intriguing issue about AAP ecology is that despite their high activity and potential

fast growth, the contribution of AAP bacteria to total bacterial abundance is low; AAP relative abundances are seldom higher than 5% across freshwater and marine systems. Experimental evidence from the only study that simultaneously explored bottom and top-down factors that may regulate AAP densities more intensely than those of the rest of bacteria (Ferrera et al., 2011), showed that protist grazing was the main regulator of the abundance of marine AAP bacteria. This experimental proof of the relevance top-down regulation in AAP bacteria, together with the fact that AAP cells tend to be larger and more active than the rest of heterotrophic bacteria (Fauteux et al., 2015 ; Hojerová et al., 2011 ; Sieracki et al., 2006), have led to the hypothesis that the generally observed low AAP abundances are due to a strong, size-selective grazing pressure (Fauteux et al., 2015 ; Ferrera et al., 2011 ; Koblížek, 2015 ; Stegman et al., 2014). Whether this is the only process explaining the low abundance of AAP across different aquatic ecosystems has yet to be determined.

The fact that AAP bacteria display some systematic differences in terms of cell size, activity and growth rates compared to the average heterotrophic bacteria in the aquatic systems has led to the hypothesis that the higher cell sizes, fast-grow capacities and higher activity of AAP bacteria may be directly attributed to their capacity to obtain energy from light. However, although as mentioned before the positive influence of light on the growth of AAP isolates has been demonstrated in laboratory experiments (Biebl and Wagner-Döbler, 2006 ; Spring et al., 2009 ; Yurkov and Vangemerden, 1993), under natural conditions the effects of light on the functioning of AAP seem to be less obvious or at least more difficult to elucidate; the only two studies so far assessing the effect of light on the heterotrophic activity of natural AAP communities found that light did not enhance the individual incorporation of leucine by AAP cells in experimental incubations relative to dark controls (Kirchman et al., 2014 ; Stegman et al., 2014). However, the heterotrophic activity of AAP was found to be positively correlated to light availability in the water column, suggesting that light

may influence the ecology of these prokaryotes in some way (Stegman et al., 2014). Thus, whether the capacity to use light is directly linked with the fast growth and the apparent competitiveness observed in AAP communities is still an open question.

0.1.3 AAP bacteria in freshwater systems

In contrast to the increasing amount of information regarding the distribution, activity, regulation and phylogenetic composition of marine AAP bacteria, very little is known about freshwater AAP bacteria. The first data about freshwater AAP species go back to 1990's with the isolation of several AAP strains from alkaline mat surfaces (Yurkov and Gorlenko, 1992). A few more cultivation efforts led to the description of new AAP bacteria strains from various freshwater lakes (Gich and Overmann, 2006 ; Page et al., 2004). Our knowledge on the ecology and distribution of freshwater AAP in natural conditions, however, is based on a very limited number of culture-independent studies of a few sampled rivers (Ruiz-González et al., 2012 ; Waidner and Kirchman, 2005) and lakes (Eiler et al., 2009; Caliz and Casamayor, 2014) or from few large surveys of European and Canadian lakes (Cuperová et al., 2013 ; Fauteux et al., 2015 ; Mašín et al., 2012 ; Mašín et al., 2008).

These large surveys, which include Austrian, Czech, German, Polish, Finish and Canadian lakes, have demonstrated the widespread presence of AAP bacteria in a broad range of biological, chemical and physical freshwater conditions (Cuperová et al., 2013 ; Fauteux et al., 2015 ; Mašín et al., 2012 ; Mašín et al., 2008). Some of these studies have confirmed the previous marine observation that AAP bacteria are larger than the average bacterial cells in aquatic communities. Interestingly, the greater environmental heterogeneity of inland aquatic systems relative to marine

environments have been shown to led to a larger spatial and seasonal variability in the relative and absolute abundance of freshwater AAP relative to the marine AAP communities, with relative abundances oscillating from almost zero in the late winter period to up to 30% during the summer (Fauteux et al., 2015 ; Koblížek, 2015). Regarding the drivers of the spatial variation, it has been found that AAP bacterial abundance seem to increase with overall system productivity (Cuperová et al., 2013 ; Fauteux et al., 2015). Moreover, DOC availability, or DOC quality, assessed as the DOC:Chl*a* ratio, has been reported as one of the main factors influencing AAP distribution across alpine and boreal lakes (Cuperová et al., 2013 ; Fauteux et al., 2015 ; Koblížek, 2015).

In contrast to the predominantly aerobic marine environments, hypolimnetic anoxia is relatively common across lakes (Carignan et al., 2000 ; Wetzel and Likens, 1979). The presence of anoxic layers in the water column has complicated the lake AAP surveys since BChl*a*-containing anaerobic phototrophic bacteria inhabiting these anoxic zones cannot be discriminated from AAP bacteria by IR epifluorescence microscopy or any of the techniques based on the presence of BChla (Koblížek, 2015 ; Yurkov and Csotonyi, 2009). Nevertheless, a judicious choice of lakes and sampling scheme, as well as a proper analysis of the resulting data have been incorporated into freshwater AAP survey studies in order to minimize the uncertainties about the truly identity of AAP (Fauteux et al., 2015 ; Mašín et al., 2008).

Genomic approaches applied in the analysis of the *puf*M gene sequences, which as stated before codifies for the M subunit of the bacterial reaction center (Rathgeber et al., 2004), have been used to identify potentially different AAP ecotypes. These studies have reported a large diversity in the *puf*M gene, which may explain the wide range of temporal and spatial variation found in the freshwater AAP abundance surveys (Caliz and Casamayor, 2014 ; Cuperová et al., 2013 ; Salka et al., 2011 ; Waidner and Kirchman, 2005). A common observation of all these studies is the widespread

occurrence of Betaproteobacterial AAP phylotypes with a smaller contribution of various subgroups of Alphaproteobacteria. Interestingly, the analysis of the diversity of AAP communities in German lakes showed that the occurrence of particular groups of AAP bacteria belonging to the genera *Methylobacterium* and *Sphingomonas* was related to the acidic conditions and/or the recalcitrant nature of the DOC in humic lakes (Salka et al., 2011). This observation has led to the hypothesis that changes in the composition of the AAP community, with a dominance of AAP taxa that can participate in the degradation of humic compounds, may explain the increase of AAP abundance along the DOC:Chla gradient reported in northern Québec lakes (Fauteux et al., 2015).

Although as previously mentioned there have been some attempts to assess the activity and growth patterns of marine AAP bacteria (Kirchman et al., 2014 ; Stegman et al., 2014), all of the research on freshwater AAP bacteria has focused on exploring their abundance or diversity, and therefore very little is known about relevant aspects of the ecology of freshwater AAP communities. In particular, there have been no experimental studies with freshwater AAP, and as a result, issues like the effects of light on the magnitude and regulation of freshwater AAP bacterial activity, the variability of AAP growth rates, or the losses of these bacteria due to predation remain completely unknown. The fact that the composition of AAP communities seems different between marine and freshwater systems, with AAP representatives from the Alpha and Gamma proteobacteria dominating the marine AAP communities in contrast to a freshwater AAP community dominated mainly by Betaproteobacterial phylotypes (Koblížek, 2015 ; Salka et al., 2011), further suggests that the results derived from the study of marine AAP bacterial communities cannot be extrapolated to freshwater AAPs. Therefore, there is a clear need to develop experimental studies that will allow a more precise understanding of some of the facets of AAP that may be more directly linked to their performance in aquatic ecosystems.

0.1.4 Current uncertainties in the role of AAP bacteria in the aquatic environments

In general, the research that has been done on AAP bacteria over the last 15 years is limited to either a cellular or an ecosystem level, with both approaches attempting to unravel the ecological role of AAP in the aquatic systems. Most of the physiological studies of AAP bacteria has been based on cultivable members of AAP bacteria, whereas most studies on their ecology have been focused on describing the patterns of AAP bacterial abundance and the environmental drivers that explain such patterns. The uncertainties associated to our understanding of the ecology of AAP bacteria derive from the difficulty to link the information about AAP physiology obtained from culture studies to the observations derived from field surveys and vice versa. A good example of this are the contrasting results mentioned above about the effect of light on AAP bacteria; while in experimental studies with AAP bacterial isolates light has been shown to positively affect the growth rate and other aspects of AAP bacterial activity, its effect on natural AAP communities remains unclear. This lack of coherence between both approaches has also resulted in overlooking some of the available information. For example, it has been experimentally demonstrated that AAP cells are able to downregulate the synthesis of BChl*a* and the photosynthetic apparatus in response to nutrient and carbon limitation as well as oxygen and light availability (Nishimura et al., 1996 ; Ouchane et al., 2004 ; Sato et al., 1985 ; Spring and Riedel, 2013 ; Yurkov and Beatty, 1998). This suggests that the expression of the pigment, and therefore of the phototrophic potential, may be highly dynamic under natural conditions, yet the quantification of AAP abundance in natural aquatic systems is still mostly based on the presence of BChl*a*. As a consequence, most ecosystem-level approaches do not incorporate on their surveys those AAP bacteria that may not express the pigment (i.e., those cells containing the genes encoding the photosynthetic apparatus but not expressing it, Du et al., 2006 ; Schwalbach and Fuhrman, 2005), and only a few studies

have explored the variations in the pigment content of AAP cells that do express this function. There is clearly a need to better link the information on the physiology and metabolism generated by the cell-level approaches to the study of the natural AAP bacterial communities in order to improve our understanding of the regulation and the ecological role of this group in natural aquatic systems. However, this will not be possible unless future studies move from a mostly descriptive strategy towards more experimental approaches that will allow linking particular ecological or physiological responses of AAP to their in situ patterns in abundance, activity and diversity.

0.2 Objectives of the thesis

The overall purpose of this thesis is to gain insight into the ecology of aerobic anoxygenic phototrophic (AAP) bacteria in freshwater ecosystems by exploring how different ecophysiological aspects of these bacteria are affected by two potential regulatory factors, the light availability and predation pressure. This main objective can be break down into the following three specific objectives:

1. Assess the role of freshwater grazers and nutrients in regulating the abundance, net growth, and cell size of AAP bacteria.

2. Explore the role of light, nutrients and dissolved organic carbon in regulating the single-cell heterotrophic activity of AAP and their contribution to total biomass production across a range of environmentally contrasting lakes.

3. Explore whether the responses of AAP bacteria to light and predation are linked to the pigment dynamics and the phototrophic potential.

0.3 General Approach

The conceptual framework of this thesis is built on exploring the regulation of two main aspects of AAP bacteria: on one side we studied the regulation of the net growth, the cell size, and the relative abundance of AAP bacteria. On the other side we explored the regulation of the gross growth rates and the single-cell activity of AAP bacteria (Figure 0.1). In each case, we centered the attention on exploring the effect of one main regulatory factor; in the first case we chose to assess the effect of grazing on regulating the abundance, the net growth, and the cell size of AAP bacteria. In the second case, we focused on the effect of light on regulating the activity and

growth of AAP bacteria. We used an approached that combined the simultaneous exploration of the patterns in the natural environment and the underlying processes in experimental conditions. We assessed both the patterns and the processes along a range of environmental gradients, which allowed us to further assess the effect of other environmental factors such as DOC and nutrient concentrations on the growth, the activity, the abundance and the cell size of freshwater AAP bacteria.

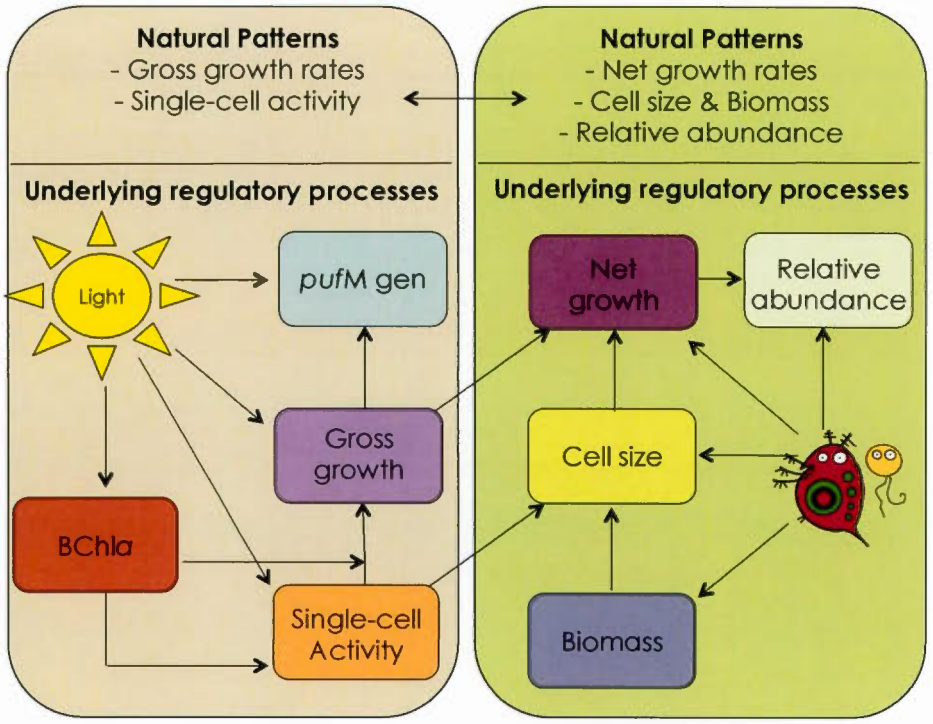

Figure 0.1. The aspects of the physiology, metabolism and ecology of AAP bacteria considered in this study and their potential link with light and predators

0.3.1 Data collection

The sampling was carried out in a few contrasting northern temperate lakes, that were located either in the Eastern Townships region of southeastern Québec or in the Laurentian region north of Montréal, Canada. The lakes wherein we collected the data of chapter II and III were chosen to cover a wide range in dissolved organic carbon (DOC) concentrations and a gradient in lake productivity, both in terms of chlorophyll*a* and nutrient concentrations, so that they differed greatly in trophic status, and light availability. In each case, the sampling involved the collection of water samples from the aerobic epilimnetic layer, the in situ measurement of oxygen, light and other key environmental variables and, the transportation of water samples to the laboratory in order to conduct the experiments.

All along our study we were aware of the fact that one of the main problems of the ecological studies of AAP on lakes is that available IR epifluorescence or pigment analysis techniques are not able to distinguish between AAP bacteria and other groups of BChl *a*-containing bacteria, such as purple sulfur and purple non- sulfur bacteria. Therefore, we explicitly incorporated this issue into the choice of lakes and sampling scheme, as well as in the analysis of the resulting data. During each sampling we carried out detailed O_2 and temperature profiles to clearly establish the boundaries of the oxygenated epilimnion of the sampled lakes, as well as to determine the potential distribution of anoxia in hypolimnetic waters and the potential for mixing between the two. We calculated the Schmidt stability index of those lakes with a well-developed anaerobic hypolimnion. A high thermal stability during stratification will prevent any significant mixing of aerobic and anaerobic layers, which minimizes the probability of upwelling events of the bacterioplankton from the deep layers to the metalimnion and eventually to the oxic surface waters. At this point, we discarded samples from lakes that at the time of sampling had an anoxic hypolimnion and a low Schmidt stability index. In

addition, there are some features that helped us to verify that the microscopic enumeration of bacteria and the extracted BChla originate from AAP bacteria and not from anaerobic phototrophic bacteria. It is well known, for example, that the cell-specific BChl*a* content in AAP cells is orders of magnitude lower than that of anaerobic phototrophic bacteria (Koblízek et al., 2005 ; Kolber et al., 2001). Therefore, the cell-specific BChl*a* values were a good estimator of a significant presence of anaerobic phototrophs. Finally, the analysis of the bacterioplankton composition and diversity of pufLM in the samples constitutes a good way to discriminate between a sample dominated by AAP and one dominated by anaerobic phototrophics. In this regard, we have collected DNA samples from all lakes and experiments. We have sequenced the 16S and *puf*M genes from almost 200 samples, and we are now in the process of analyzing these data, which are not shown here but rather will be part of a future paper that we will develop on the diversity and composition of AAP communities from these freshwater habitats.

0.3.2 Experimental approach

We conducted two main types of experiments that allowed us to obtain the set of variables related to the purposes of each chapter. We carried out a lake mesocosm experiment specifically aimed at quantifying the grazing of AAP bacteria by zooplankton and protists. This experiment was done in collaboration with Alison Derry, from the Université du Québec à Montréal (UQAM). We also conducted lake water dilution experiments to determine the gross growth and grazing mortality rates of AAP bacteria.

0.3.3 Experimental treatments

In order to explore the mechanisms behind the regulatory effects of light on the growth, the

activity, the pigment dynamics and the mortality of AAP, we designed a set of experiments that included two light conditions: 1) The light exposition treatment that involved cycles of 8h darkness and 16h of artificial PAR, at an irradiance between 150 and 200 µmol photons m^{-2} s^{-1} provided by UV-free 45-W fluorescent lamps 2) The dark treatment, attained by covering the incubation bottles with black adhesive contact paper.

In order to explore the mechanisms behind the regulation of AAP abundance by grazing, we set-up the following experimental treatments: 1) A control treatment, consisting of whole unfiltered lake water, 2) A reduced predation treatment, consisting of a 1:4 dilution of unfiltered lake water with 0.2 µm-filtered lake water to reduce predator encounter rates. In the case of chapter I, where we explored the regulation of AAP specifically by zooplankton or by protist, we included also treatments in mesocosm enclosures wherein we manipulated the presence of zooplankton.

0.3.4 Response parameters

0.3.4.1 Bacterial abundance and biovolume measurements

Total bacteria and AAP cell abundance and cell size were determined from images of 4',6-diamidino-2-phenylindole (DAPI), using an Olympus Provis AX70 microscope, fitted with a charge-coupled-device camera capable of infrared detection (Intensified Retiga Extended Blue; Qimaging), and image analysis software (ImagePro Plus, Media Cybernetics). This equipment, as well as the technical support, was provided by the collaboration with the group of D. Kirchman at the University of Delaware.

0.3.4.2 BChl*a* measurements

BChl*a* concentrations were measured by kinetic fluorometry using an FL3500-FT fluorometer (Photon Systems Instruments). The samples were always treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, 50 µM final concentration), an inhibitor of photosystem II in oxygenic phototrophs, so that BChl*a* fluorescence could be distinguished from Chl*a* fluorescence (Koblízek et al., 2005)

0.3.4.3 Quantification of *puf*M gene copies

Real time PCR (qPCR) was used to follow the relative abundance of *puf*M gene copies using a CFX96 thermocycler. The genomic DNA was extracted by filtrating the water samples onto 0.22 µm pore-size filters using the MoBio PowerWater DNA extraction kit (Carlsbad, CA, USA). The 245 base-pair (bp) partial *puf*M gene target was amplified using the forward (*puf*MF 5'-TACGGSAACCTGTWCTAC-3') (Tm, 50.9°C) and reverse (*puf*M_WAWr 5'-AYNGCRAACCACCANGCCCA-3') (Tm, 62.5°C) primers described in Béjà et al. (2002) and Yutin et al (2005).

0.3.4.4 Microautoradiography of AAP and total bacteria

The single-cell activity of AAP bacteria and bulk bacteria was determined using the AAP-MAR method, developed by Stegman et al. (2014). The use of this technique, as well as, the technical support was provided by the

collaboration with the group of D. Kirchman at the University of Delaware.

0.3.5 Estimating the impact of AAP bacteria on lake ecosystem processes: zooplankton production and CO_2 fluxes.

As an exercise of transposing the experimental results to a natural context, we estimated on a summer daily basis the average contribution of AAP bacteria to total zooplankton production, and CO_2 fluxes. For this purpose, we used the information available for Lac Croche, a reference lake located in the temperate region of Québec at the Station de Biologie des Laurentides, a field research facility of the Université de Montréal (www.sbl.umontreal.ca/index.html). We used the published data on zooplankton production (Sastri et al., 2014) and CO_2 fluxes (Vachon & del Giorgio, 2014) that have been measured on lake Croche. In addition we used the summertime data on total dissolved organic carbon (DOC) concentration (mg C l^{-1}), AAP abundances (%AAP), and bacterial biomass production (BP μg C l^{-1} d^{-1}) from a seasonal monthly survey that we carried out in this lake. It is worth mentioning that the data of this seasonal survey of AAP bacteria are not shown here but rather will be part of the above-mentioned future paper that we will develop on the diversity and composition of AAP communities on freshwater habitats.

The percentage of contribution of AAP to total bacterial biomass production (% contribution to BP) was derived from the relationship between the contribution of AAP to leucine incorporation and their contribution to abundance (Data chapter II). The leucine incorporation rate of AAP bacteria (BP_AAP μg C l^{-1} d^{-1}) was calculated from % contribution to BP and summer average of the total biomass production (BP μg C l^{-1} d^{-1}). The rate of DOC consumption attributed to AAP bacteria (C-consumption_AAP μg C l^{-1} d^{-1}) was calculated from the BP_AAP and assuming a bacterial growth efficiency (BGE_AAP) for AAP of 30% (Hauruseu et Koblizek,

2012). The respiration rate of AAP (μg C l^{-1} d^{-1}) was calculated from BP_AAP and C-consumption_AAP. For calculation purposes the lake was considered as composed of two well-mixed layers, and we calculated the values only for the epilimnion. The carbon transfer of AAP (C loss to ZP, μg C l^{-1} d^{-1}) to zooplankton biomass production (ZP) was calculated from BP_AAP, assuming the AAP mortality rates by protists and zooplankton measured in Chapter I and a zooplankton efficiency of 10%.

0.4 Thesis Structure

The main objective of this thesis is to explore the regulation of aerobic anoxygenic phototrophic (AAP) bacteria in order to gain insight into the ecology of this group of bacteria in freshwater ecosystems. The results of this thesis are presented in three chapters; two of them are already published and the third one is submitted.

Chapter I. Garcia-Chaves, M.C., Cottrell, M.T; Kirchman, D.L; Derry, A.M; Boggard, M.J and del Giorgio, P.A. 2015. Major contribution of both zooplankton and protists to the top-down regulation of freshwater aerobic anoxygenic phototrophic bacteria. Aquat Microb Ecol 76:71–83

Chapter II. Garcia-Chaves, M. C., Cottrell, M. T., Kirchman, D.L., Ruiz-González, C. and del Giorgio, P.A. 2015. Single-cell activity of freshwater aerobic anoxygenic phototrophic bacteria and their contribution to biomass production. ISME, 1-10. doi.org/10.1038/ismej.2015.242

Chapter III. Garcia-Chaves, M. C., Cottrell, M. T., Kirchman, D. L., Mesa, A.C. and del Giorgio, P. A. High intrinsic growth potential and selective mortality of freshwater aerobic anoxygenic phototrophic bacteria, and links to pigment production and phototrophic potential. Submitted to Environmental Microbiology.

CHAPTER I

MAJOR CONTRIBUTION OF BOTH ZOOPLANKTON AND PROTISTS TO THE TOP-DOWN REGULATION OF FRESHWATER AEROBIC ANOXYGENIC PHOTOTROPHIC BACTERIA

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N.B References cited in this chapter are presented at the end of the thesis.

1.1 ABSTRACT

Aerobic anoxygenic phototrophic (AAP) bacteria are photoheterotrophic prokaryotes that use light as a secondary energy source to complement the consumption of organic matter. Despite this metabolic flexibility and their widespread distribution, their low relative abundances suggest that they may be subjected to strong regulatory processes. However, there is still little information on the regulation of AAP abundance, particularly in freshwaters. Here, we used a lake mesocosm experiment to address the top-down regulation of freshwater AAP by protists and zooplankton under 2 contrasting nutrient regimes. Our results support the hypothesis that freshwater AAP are subject to intense top-down regulation, and are selectively removed by grazers. The average gross growth rate of AAP was ca. 1.5 times higher, and grazing loss rates 1.6 times higher than those of the bulk bacterial community. Our results further indicate that whereas protists are the main predators of AAP, zooplankton may account for over a third of AAP losses, and both exhibit a greater selectivity for AAP relative to total bacteria. The mechanistic underpinning of this selectivity is still unclear, but it may be related to the average larger cell size of AAP, and to their higher potential growth rates relative to the bulk bacterial community. Our results further suggest that AAP may play a disproportionate role in the nutrition of lake zooplankton, and in the trophic transfer of organic carbon in lake food webs.

1.2 INTRODUCTION

Aerobic anoxygenic phototrophic bacteria differ from their heterotrophic counterparts in their potential capacity to use light to supplement their heterotrophic diet of dissolved organic matter. This photoheterotrophic group of bacteria relies on heterotrophy for over 80% of their cellular energetic demands but can produce a photosynthetic apparatus that captures available energy from light-induced proton translocation (Kolber et al. 2001, Koblízek et al. 2003, Yurkov and Csotonyi 2009, Koblížek, 2015). This metabolic flexibility, together with the cosmopolitan nature of AAP, suggests that the light harvesting capacity may provide these bacteria with a significant ecological advantage, and this group has been hypothesized to play a significant role in the flux of carbon and energy in aquatic systems (Karl 2002, Fenchel 2008, Gasol et al. 2008).

Interestingly, AAP bacteria seldom represent more than 10% of the prokaryotic community across different types of aquatic ecosystems (Hojerová et al. 2011, Lamy et al. 2011, Mašín et al. 2012, Fauteux et al. 2015). Two possible hypotheses arise from their low in situ abundances: either light-derived energy has little effect on the growth and competitiveness of AAP, or, if there is an effect, there are other factors, unrelated to phototrophy, that may limit the ecological success of AAP bacteria more than that of other bacterial groups. Experimental evidence from marine environments suggests that AAP bacteria may have higher growth rates than the average bacteria (Koblízek et al. 2007, Ferrera et al. 2011, Kirchman et al. 2014, Stegman et al. 2014), and therefore their generally low abundance should be due to high losses, either via grazing or viral infection. Indeed, the only study so far to have explored the different controls of the abundance of this group showed that protist grazing was the main regulator of the abundance of marine AAP bacteria (Ferrera et al. 2011). Beyond this marine study, however, there is still little information on the regulation of AAP abundance and activity, especially for inland waters, despite the fact that these photoheterotrophic microbes

have also been shown to be widespread in freshwater planktonic food webs (Mašín et al. 2008, Medová et al. 2011, Mašín et al. 2012, Cuperová et al. 2013, Fauteux et al. 2015).

One of the major differences between freshwater and marine planktonic trophic webs is that, whereas in the latter the major grazers of bacteria are protozoans (i.e. ciliates and flagellates), in inland waters there are certain crustacean groups that may also act as potential bacterivores (Güde 1988, Jürgens et al. 1994, Bertilsson et al. 2003). In particular, some cladoceran groups have a filtering apparatus capable of effectively retaining a wide particle size spectrum, including bacteria, cyanobacteria, planktonic protists, and algae, and several field studies have shown a strong predatory control by this group on heterotrophic bacterioplankton in inland waters (Pace et al. 1990, Brendelberger 1991, Jürgens et al. 1994, Kim et al. 2000, Langenheder and Jürgens 2001, Degans and Zöllner 2002, Berga et al. 2015). The ability of cladocera to feed on bacteria differs among species and size classes within species, as a function of the size of their filtering apparatus, but larger bacterial cells in general have a higher probability of being retained (Brendelberger, 1991, Jürgens, 1994). Since AAP bacteria have been shown to be systematically larger than the average bacterioplankton cells (Sieracki et al. 2006, Kirchman et al. 2014, Stegman et al. 2014, Fauteux et al. 2015), this group may be more vulnerable to zooplankton grazing. As a consequence, freshwater AAP bacteria may be grazed by a wider variety of predators than in marine communities, resulting in an even stronger top-down regulation than in marine environments. In turn, these different consumption pathways have implications for the potential trophic role of AAP bacteria at the ecosystem level. If AAP bacteria are grazed mainly by protists, then relatively little of their production will reach higher trophic levels, whereas predation by metazooplankton will greatly increase the potential transfer of AAP production to higher trophic levels.

Here, we explore for the first time the relative contribution of freshwater zooplankton and protists in regulating the abundance of AAP bacteria. We carried out a lake mesocosm experiment specifically aimed at quantifying the grazing of AAP by zooplankton and protists. We further assessed whether AAP bacteria are selectively grazed relative to the bulk bacterial community, and how this biological regulation varies under different nutrient regimes, since prey selectivity may in turn be affected by nutrient availability (Simek et al. 2003, Jezbera et al. 2006). Our results show that freshwater AAP bacteria are indeed more intensely grazed than the bulk bacterial community, and suggest a significant role of cladocerans in selectively controlling the abundance of these ubiquitous photoheterotrophs.

1.3 MATERIALS AND METHODS

1.3.1 Study site and experimental design

The experiment was carried out in the oligo-mesotrophic Lake Cromwell, located in the temperate region of Québec, Canada (45°59' N 73°59' W) at the Station de Biologie des Laurentides, a field research facility of the Université de Montréal (www.sbl.umontreal.ca/index.html). The experiment consisted of 2 parts: (1) a field mesocosm experiment in which zooplankton was manipulated, and (2) a complementary predator-free laboratory re-growth incubation experiment that was carried out on samples taken from the experimental mesocosms. From this approach, we generated 4 basic treatments: (1) a protist only treatment, corresponding to Phase I of the mesocosm experiment; (2) a protist + zooplankton treatment, corresponding to Phase II of the mesocosm experiment; (3) absence of protists and zooplankton, corresponding to in vitro re-growth experiments using mesocosm samples; and (4) an unmanipulated protist + zooplankton control, corresponding to the ambient lake waters.

In order to assess the effect of zooplankton, and to compare the effect of top-down control on AAP and the bulk bacterial community, the mesocosm experiment involved enclosures where zooplankton were initially removed and subsequently added. We used 6 polyethylene mesocosms (1 m diameter, 6 m deep, 4700 l, closed bottom), anchored at the bottom, and attached to floating wooden frames in the middle of the lake (~8 m depth). Enclosures were filled by pumping surface water sequentially across 2 mesh screens of 110 and 54 μm to remove zooplankton. In order to investigate the variation of the top-down regulation under different nutrient regimes, 3 of the mesocosms each received nitrogen (N) and phosphorous (P) additions (nutrient-amended mesocosms); the other 3 mesocosms retained the original lake conditions (un-amended mesocosms). In nutrient-amended mesocosms, KH_2PO_4 and $NaNO_3$ were added to increase 5× and 2×, respectively, the ambient lake nutrient concentrations, to values of 50 μg P and 700 μg N l^{-1}.

The initial zooplankton-free phase was run for 6 d in order to quantify the response of AAP and bulk bacteria to exposure to protist grazing only. At Day 7 of the experiment, the 6 mesocosms were restocked with zooplankton at ambient lake concentrations, collected in the surrounding lake waters using a 54 μm Nitex net of 30 cm diameter and 1 m in length. This second experimental phase with zooplankton was run for 2 additional weeks. The development of the AAP bacteria and of the bulk community in the mesocosms was followed weekly over the course of 20 d, from 5 to 25 June 2012. Additionally, we measured zooplankton biomass and protists (heterotrophic nanoflagellates) abundances at those same time points.

1.3.2 Response parameters - sampling and analysis

Total phosphorus (TP), total nitrogen (TN), dissolved organic carbon (DOC), dissolved oxygen (DO), pH, conductivity (Cond) and temperature were measured weekly at 0.5 m depth in all bags. Entire water column profiles were taken from 1 enclosure per treatment, 2 times during the experiment (Days 1 and 12) using a YSI combination probe to assess the level of water column oxygenation. Photosyntetically active radiation (PAR) profiles were measured using a LI-COR LI-190 Quantum Sensor. In the lab, TP was quantified spectrophotometrically by the molybdenum-blue method following persulfate digestion. TN analysis was conducted as NO_3^- following alkaline persulfate digestion (Wetzel & Likens 1979). DOC was measured in 0.45 µm-filtered samples by wet oxidation using an O.I. Analytical Total Carbon Analyzer.

Chlorophyll *a* (Chl*a*) samples were filtered through GF/F filters, frozen and subsequently extracted with hot ethanol. Pigments were measured spectrophotometrically at 750 and 665 nm using a UV/Vis UltroSpec 2100 spectrophotometer. Bacteriochlorophyll *a* (BChl*a*) concentrations were measured on an FL3500-FT fluorometer (Photon Systems Instruments), using the standard calibration curve provided by the manufacturer. Samples were first treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, 50 mM final concentration), an inhibitor of photosystem II in oxygenic phototrophs, so that BChl*a* fluorescence could be distinguished from Chl*a* fluorescence (Koblízek et al, 2005).

Samples for the microscopic enumeration of total bacterial and AAP cells were fixed immediately after collection with 1% glutaraldehyde (final concentration) and kept refrigerated in the dark until filtration. In the lab, samples were filtered onto 0.2 µm pore-size black polycarbonate filters and immediately stored at –80°C. Total bacteria and

AAP abundance and cell size were determined by infrared epifluorescence microscopy, as described by Cottrell et al. (2006). In brief, a section of the black polycarbonate filter was stained with 1 mg ml^{-1} 4,6-diamidino-2-phenylindole (DAPI) for 5 min and then mounted on a microscope slide. AAP and total bacteria were counted using an Olympus Provis AX70 microscope, fitted with a charge-coupled-device camera capable of infrared detection (Intensified Retiga Extended Blue; Qimaging), and image analysis software (ImagePro Plus, Media Cybernetics). To enumerate total bacteria (DAPI-stained) and to discriminate and count AAP bacteria, 20 fields of view were assessed per slide, and 4 images were recorded for each field of view, each at a different excitation and emission wavelength. AAP bacteria, which fluoresce in the infrared (IR), were distinguished from cells containing Chl*a* and phycoerythrin-containing cells (PE), which fluoresce in the orange and red, respectively. In addition to abundance, cell size was also determined by image analysis from the DAPI-stained cells using the integration method (Sieracki et al. 1989).

To evaluate the changes in the rate of bacterial biomass production along the course of the experiment, 3 replicates of 1.5 ml of each mesocosm treatment plus 1 trichloroacetic acid-killed control were inoculated with 3H-leucine at a final concentration of 20 nM and incubated for 2 h in the dark and at 20 ± 2°C. Incubations were terminated by the addition of trichloroacetic acid and the samples were stored at 4°C until centrifugation Smith & Azam (1992).

Zooplankton samples were collected by taking one vertical haul from 5 m depth to the surface with a 15 cm diameter 54 µm mesh net (4.5% of enclosure volume). Zooplankton were anaesthetized via carbon dioxide exposure, and preserved in a buffered 4% sugar-formalin solution for later enumeration. Crustacean zooplankton were identified and population abundances were counted using a high-resolution dissecting microscope

(SZ2-IL-ST, Olympus SZ). Taxonomic keys used included Thorp and Covich (2010) for general identification, and Smith and Fernando (1978) for copepod identification. The taxonomic key of De Melo and Hebert (1994) was used for the Bosminidae identification, and Haney et al. (2013) as a general visual key. Crustacean zooplankton were enumerated with a protocol design to target mature individuals that would be unambiguously identified to species, as well as to detect rare species (Girard and Reid 1990). Subsamples (10 ml) were taken from a standardized 50 ml sample volume, and at least 250 individuals were counted so that no more than 50 copepodids per Order and no more than 30 nauplii per Order were included in the sum to 250 individuals, even though more were enumerated (Derry and Arnott 2007).

The abundance of heterotrophic nanoflagellates (HNF) was determined from fixed samples (1% glutaraldehyde, final concentration) by epifluorescence microscopy. Subsamples of 10 ml were stained for 20 min with 200 ml of DAPI (2 mg ml^{-1} final concentration) and filtered onto 0.8 mm black polycarbonate filters. At least 50 flagellates were enumerated on each filter by counting on randomly selected fields.

1.3.3 Estimation of total and AAP bacterial gross growth rates

In this paper, we refer to bacterial 'net growth rates' as the changes in abundance in the presence of grazers (protists, zooplankton or both), and to 'gross growth rates' as those observed in the absence of grazers. In order to estimate the gross growth rates (μ_G) of both total and AAP bacteria, we carried out a re-growth incubation experiment where we released bacteria from grazing. For that purpose, during Day 19 of the experiment, we collected water from the mesocosms and removed both types of grazers (i.e. protists and zooplankton) by filtration through GF/C filters (1.2 μm nominal size). The

filtered mesocosm water was then distributed into 2.4 l Nalgene transparent bottles and incubated in triplicate in a tank with circulating water held at in situ temperature. The incubation was performed under artificial lighting provided by 24 fluorescent Sun Blaster™ tubes under 16 h light and 8 h dark cycles; incident PAR averaged 200 µmol photons m^{-2} s^{-1}. The re-growth experiment was run for 3 d and samples for AAP and total bacterial abundance were taken every 12 h. Total and AAP bacterial gross growth rates were determined from the changes in the number of cells over the incubation time in the re-growth experiment, as the slope of the regression of the natural logarithm (ln) of N vs time in the filtered sample, where N is the cell abundance.

1.3.4 Calculation of grazing loss rates of bulk bacteria and AAP bacteria

In addition to measuring bacterial gross growth rates in the absence of grazing, we measured total and AAP bacterial net growth rates under the 2 experimental conditions: absence or presence of zooplankton in the mesocosms. These net growth rates were estimated as the slope of the regression of ln (N) vs time, where N is the cell abundance at the different phases of the mesocosm experiment. Grazing loss rates due to protists (G_p) were estimated by the difference between the gross growth rates (μ_G) obtained in the re-growth experiment described above, and the net growth rates observed during the initial experimental phase without zooplankton (μ_{N1}, Days 0 to 6): $G_p = \mu_G - \mu_{N1}$ (1)
Similarly, the grazing loss rates due to protists and zooplankton (G_T) were estimated as the difference between the gross growth rates and the net growth rates observed during the second experimental phase (μ_{N2}, Days 6 to 12), when both protists and zooplankton were present in the enclosures: $G_T = \mu_G - \mu_{N2}$ (2)
Grazing loss rates by zooplankton (G_Z) were then calculated as the difference between total grazing loss rates and grazing loss rates due to protists: $G_Z = G_T - G_p$ (3)

1.4 RESULTS

1.4.1 Mesocosm dynamics

The experimental mesocosms were deployed in Lake Cromwell, which is a typical temperate, oligo-mesotrophic shield lake (7.9 mg P l^{-1}), with relatively low algal biomass (average 2 mg Chl*a* l^{-1}), moderate water transparency (light extinction coefficient of 1 m^{-1}), and an average DOC concentration of 6.4 mg l^{-1} (Table 1.1). At

Table 1.1. Summary of the parameters measured in the mesocosms and in Lake Cromwell, Québec, Canada, during the experiment in June and July 2012. Nut-amended: nutrient-amended mesocosm; un-amended: un-amended mesocosm; K: light attenuation coefficient; DO: dissolved oxygen; DOC: dissolved organic matter; Chla: chlorophyll a; TP: total phosphorous; TN: total nitrogen.

Day	Treatment	K m^{-1}	Temp. °C	DO $mg*L^{-1}$	pH	DOC $mg*L^{-1}$	Chl*a* $\mu g* L^{-1}$	TP $\mu g* L^{-1}$	TN $mg* L^{-1}$
1	Nut-amended		18.7		7.1			72.2	
	Un-amended		18.7		7.4		1.63	12.37	
	Lake	1.05	18.7		6.8	6.4	1.55	10.68	0.23
6	Nut-amended	1.92	21.3	9.6	8.2	6.1	15.57	72.77	0.67
	Un-amended	0.88	21.1	8.4	6.8	5.9	6.34	17.25	0.29
	Lake		21.4	8.26		5.7	2.06	9.57	
12	Nut-amended	1.13	23.1	10.6	9.6	5.9	10.51	35.58	0.44
	Un-amended	1.05	23.1	8.2	7.1	5.6	2.24	9.69	0.24
	Lake						2.06	9.99	0.36
20	Nut-amended		23.4	8.2	9.6	8.1	6.79	22.34	0.34
	Un-amended		23.5	7	7.4	7.7	1.23	7.83	0.24
	Lake		23.1	6.59		7.4	1.29	8.38	0.27

the start of the experiment in early June 2012, the lake was thermally stratified (3.7 m thermocline depth), with an epilimnetic oxygen concentration of 8.3 mg l^{-1} and an oxic hypolymnion (6.4 mg l^{-1}) (Table 1.1). Total and AAP bacterial abundance in the epilimnion was 2.2×10^6 and 1.3×10^4 cell ml^{-1}, respectively (Supplementary Table S1.1a). The zooplankton community of lake Cromwell was dominated by 3 major crustacean zooplankton groups (calanoid and cyclopoid copepods, and cladocerans). Cladocerans were the most abundant group (1.43 ind. l^{-1}), composed of the species *Sinobosmina liederi, Daphnia ambigua, Daphnia longiremis, and Holopedium spp.* Cyclopod copepods were less abundant (0.93 ind. l^{-1}) and were dominated by *Cyclops scutifer* and *Acanthocyclops robustus*. Calanoid copepods were the least abundant (0.46 ind. l^{-1}), with *Leptodiaptomus minutus* as the only representative species. The abundance of heterotrophic nanoflagellates (HNF) ranged from $1–2 \times 10^3$ ml^{-1} across all treatments, and was on average higher in the nutrient-amended mesocosms (Supplementary Table S1.1a). The ambient lake density of HNF was relatively stable at around 1.9×10^3 ml^{-1}.

Nutrient enrichment triggered a rapid bacterial response: the initial sample was taken 36 h after the actual nutrient addition, and by that time total bacterial abundance and production were already 2-fold higher in the nutrient-amended than in the un-amended mesocosms (Supplementary Table S1.1a, Fig. 1.1a). AAP bacterial abundance and BChla concentration were also on average higher in the nutrient-amended relative to the un-amended mesocosms (Fig. 1.1a, b, c). Furthermore, cell size of total and AAP bacterial cells at the beginning of the experiment were both larger (1.5-fold on average) in the nutrient-amended compared with the un-amended mesocosm, and this difference in cell size persisted throughout the entire 3 wk of the experiment (Supplementary Table S1.1a).

The removal of zooplankton in the first phase of the experiment resulted in a 2 to 3-fold increase in total bacterial abundance (Fig. 1.1a), and a 27 to 34-fold increase in AAP abundance (Fig. 1.1a) across the un-amended and the nutrient-amended mesocosms, relative to the initial experimental conditions (Supplementary Table S1.1a). Since AAP abundance increased more than that of total bacteria, the relative proportion of AAP increased from less than 1% up to 9% during the first phase of the experiment in both the un-amended and the nutrient-amended mesocosm (Fig. 1.1b). The reintroduction of

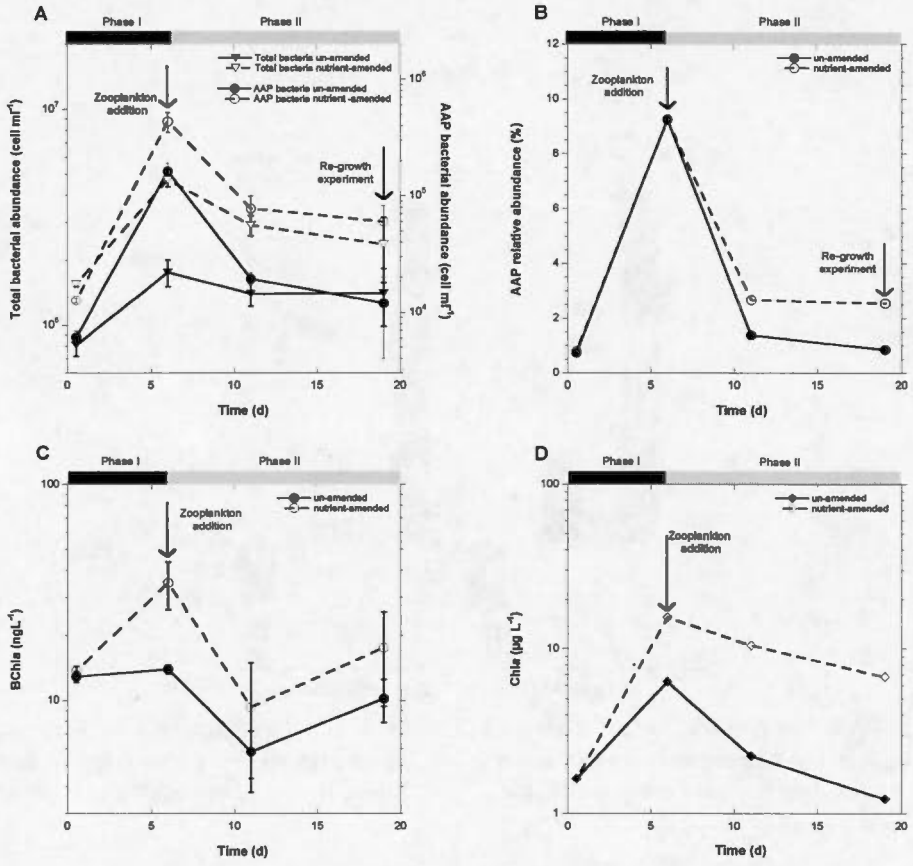

Figure 1.1. Dynamics of (A) AAP and total bacterial abundance, (B) Relative abundance of AAP bacteria (C) bacteriochlorophyll*a* (BChl*a*) concentration and (D) chlorophyll*a* concentration (Chl*a*) throughout the mesocosm experiment. Log_{10} transformed mean values (± SE) are presented for (A), (C) and (D). The arrows show the day zooplankton were added into the enclosures and the day of the re-growth experiment. Black and grey horizontal bars correspond to the time periods of the enclosure experiments without zooplankton (Phase I) and with zooplankton (Phase II), respectively. Means ± SE are shown.

zooplankton into the enclosures at the second phase of the experiment, at comparable densities to those in the ambient lake waters, resulted in a strong decline in both total and AAP bacteria, followed by a stabilization after week 2 (Figs. 1.1a,b). Chla followed the same pattern as bacteria in response to zooplankton, with an increase in the first phase, and a decline in the second (Fig. 1.1d), although the changes were much smaller than those observed for bacteria. The removal of zooplankton in Phase II resulted in a slight decrease in HNF abundance in the nutrient amended enclosures (Supplementary Table S1.1a).

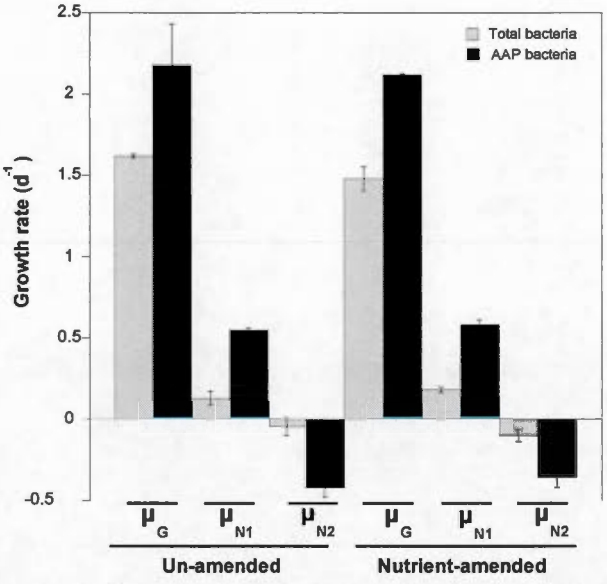

Figure 1.2. Growth rates (± SE) of total bacteria (grey bars) and AAP bacteria (black bars) derived from changes in abundance during the regrowth incubations (gross growth rate, μ_G) or during the Phase I without zooplankton (net growth rate, μ_{N1}) or Phase II with zooplankton (net growth rate, μ_{N2}) of the mesocosm experiment.

1.4.2 Bacterial gross and net growth rates

Gross bacterial growth rates, determined in the grazer-free re-growth experiments,

averaged 1.48 ± 0.07 SE and 1.62 ± 0.01 d^{-1} for the bulk bacteria in the nutrient-amended and un-amended treatments, respectively, whereas the gross growth rates of AAP bacteria averaged 2.12 ± 0.02 and 2.18 ± 0.25 d^{-1} in the nutrient-amended and un-amended treatments, respectively (Fig. 1.2). There were no significant differences (t-test, $p > 0.01$) in either total bacterial or AAP bacteria gross growth rates between nutrient-amended and un-amended samples, but gross growth rates of AAP bacteria were significantly higher (t-test, $p < 0.01$) than those of bulk bacteria in both treatments (Fig. 1.2).

Total bacterial net growth rates (based on changes in abundance in the mesocosms) in Phase I (absence of zooplankton), averaged 0.18 ± 0.02 and 0.13 ± 0.04 d^{-1} for

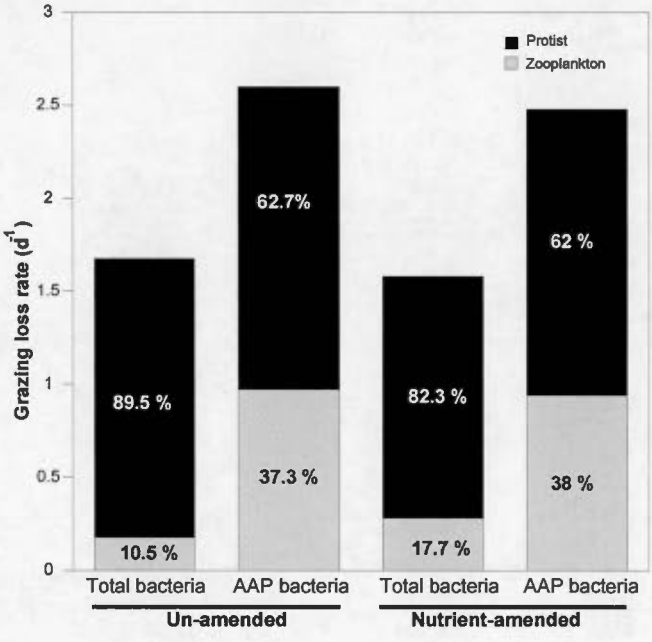

Figure 1.3. Mean absolute values of grazing loss rates of AAP and total bacteria due to protists (black bars) and multicellular zooplankton (grey bars). Mean relative values of protists or zooplankton contribution to grazing loss rates are presented in each bar for un-amended mesocosms and nutrient-amended mesocosms

nutrient-amended and un-amended mesocosms, respectively, and were not significantly different from each other (t-test, $p > 0.01$). Removal of zooplankton in Phase I resulted in even higher net growth rates for AAP bacteria, averaging 0.58 ± 0.03 and 0.55 ± 0.01 d^{-1} for nutrient-amended and un-amended treatments, respectively (Fig. 1.2). The AAP growth rates under zooplankton-free conditions did not differ between nutrient treatments (t-test, $p > 0.01$), and were significantly greater than total bacterial net growth rates (t-test, $p < 0.01$). The reintroduction of zooplankton to the mesocosms resulted in negative net growth in Phase II for total bacteria (–0.1 ± 0.04 and –0.05 ± 0.05 d^{-1}), and even more negative values for AAP bacteria (–0.36 ± 0.06 and –0.42 ± 0.06 d^{-1}) (Fig. 1.2). During the entire experimental period, bacterial and AAP abundances in the ambient lake waters were very stable (Supplementary Table S1.1a), with ambient net growth rates of 0.017 and 0.025 d^{-1} for total bacteria and AAP, respectively.

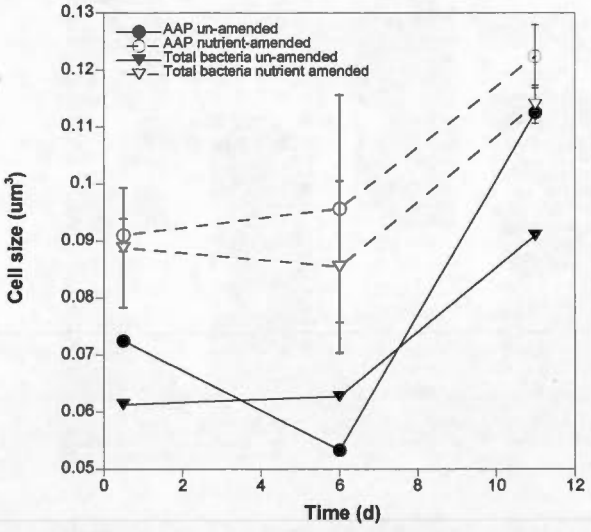

Figure 1.4. Changes in mean bacterial cell size through the first 6 d of the mesocosm experiment when zooplankton were absent (Phase I: day 1 to 6) and the next 6 d when zooplankton were present (Phase II: day 6 to 12).

1.4.3 Grazing loss rates of total and AAP bacteria

We estimated the grazing loss rates of total bacteria and of AAP from the difference between the gross and net growth rates in the 2 phases of the experiment, using Eqs. (1) & (2). Grazing loss rates due to protists were on average higher than grazing loss rates due to zooplankton (overall average of 1.48 and 0.60 d^{-1} by protists and by zooplankton, respectively). Grazing loss rates due to protists ranged from 1.3 to 1.50 d^{-1} for total bacteria, and from 1.54 to 1.63 d^{-1} for AAP bacteria (Fig. 1.3). Grazing loss rates due to zooplankton ranged from 0.17 to 0.28 d^{-1} for total bacteria, and from 0.94 to 0.97 d^{-1} for AAP bacteria and accounted for an average of 14% and 38% of the overall (protists + zooplankton) grazing loss rates of total bacteria and AAP, respectively (Fig. 1.3). Grazing loss rates by zooplankton were higher for AAP than for total bacteria (t-test, $p < 0.05$) (Fig. 1.3). There was no significant difference in grazing loss rates between nutrient-amended and un-amended mesocosms.

1.4.4 Cell size of AAP bacteria and total bacterial community

Mean cell size ranged from 0.06 to 0.12 μm^3 for total bacteria (average 0.090 μm^3), and 0.05 to 0.13 μm^3 (average 0.098 μm^3) for AAP bacteria. AAP cells were on average 10% larger than total bacterial cells, and this difference increased toward the end of the experiment; overall, there was a significant difference in size between the total and AAP cells (paired t-test, $p < 0.005$). Nutrient additions resulted in a systematic increase of approx. 25% in cell size, both for total bacteria and for AAP, and this difference persisted over the course of the experiment and under different grazing regimes (Fig. 1.4). There was a significant increase in mean cell size, both for the total and the AAP bacteria, upon the reintroduction of zooplankton in Phase II of the experiment (Fig. 1.4).

1.5 DISCUSSION

Much has been learned about the abundance and distribution of AAP bacteria in marine and freshwater systems (Koblízek, 2015) since Kolber and co-authors first demonstrated that these bacteria were widely distributed in surface waters throughout the oceans (Kolber et al. 2000). However, information on the regulation of AAP bacteria is still very sparse, and we are far from understanding the ecological role of this group in microbial food webs, and the contribution of AAP bacteria to carbon cycling in aquatic systems. In this paper we have specifically addressed the top-down regulation of AAP bacteria by protists and zooplankton in lakes. We explored whether AAP bacteria are selectively removed relative to the total bacterial community by the main classes of grazers in these systems, and further explored potential interactions between grazing and nutrient availability in shaping AAP dynamics in freshwater systems.

The approach we used was based on following the response of both total bacteria and AAP to the removal of zooplankton and of protists, and deriving from these observations the relative contribution of these 2 predator types to the loss rates of total bacteria and AAP bacteria. While it is possible to remove zooplankton from the mesocosms, it is very difficult to expose these bacterial communities to zooplankton only (without protists). To overcome this limitation, we initially removed zooplankton from our experimental mesocosms in order to subject bacteria only to protist grazing, and subsequently reintroduced zooplankton to the mesocosms and followed the ensuing total bacterial and AAP dynamics. In addition, we carried out re-growth experiments using the ambient mesocosm waters to estimate total bacterial and AAP growth rates in the absence of both protists and zooplankton grazing.

Our results revealed a systematic difference in growth rates of AAP and total bacteria in lake Cromwell; the average gross growth rate of AAP was about 1.5 times higher than that of total bacteria. We acknowledge that these gross growth rates may likely be underestimates, since both total bacteria and AAP were still subjected to some degree of viral infection, although the dilutions likely led to an initial decoupling between bacteria and viruses as well. Regardless of any potential viral or other effects on our estimates, our results are generally consistent with the handful of previous marine studies that have also reported high potential growth rates for AAP relative to the bulk community (Table 1.2). To our knowledge, similar estimates have not been reported for freshwaters. Ferrera et al. (2011) reported that AAP bacteria grew ca. 2-fold faster than the total bacterial community in the Mediterranean Sea, using a re-growth approach that was very similar to ours. Other studies using different approaches to calculate growth rates of AAP have reported even higher differences in their growth rate relative to total bacteria. Liu et al. (2010) reported a 4-fold higher average AAP growth rate, estimated on the basis of the frequency of dividing cells. Koblízek et al. (2007), on

Table 1.2. Summary of growth rates of total bacteria (Total) and AAP bacteria (AAP) from marine and freshwater systems and the corresponding mean relative abundance of AAP. NR: no data reported.

System	Treatment	Growth rates (day^{-1}) for group		Mean AAP abundance (%)	References
		Total	AAP		
Lake	Dilution	1.5–1.6	2.1–2.2	0.6–0.8	This study
Coastal	Dilution	1.3	2.4–2.6	5-7	(Ferrera et al. 2011)
Sea	Untreated	0.23–0.45	0.5–2.9	2–2.6	(Liu et al. 2010)
Bay	Untreated	NR	1.4	3.8	(Hojerová et al. 2011)
Sea	Untreated	NR	1.1	3.1	(Hojerová et al. 2011)
Ocean	Untreated	NR	0.7–0.9	2-4	(Koblízek et al. 2007)
Ocean	Untreated	NR	1.3–1.7	NR	(Koblízek et al. 2007)
Ocean	Untreated	NR	1.4–2.1	NR	(Koblízek et al. 2007)
Sea	Untreated	NR	1.4–2.2	3-10	(Koblízek et al. 2005)

the other hand, reported AAP growth rates in the Atlantic ocean, based on the diurnal decay in BChla, that were on average 10-fold higher than the average bulk bacterial growth rates that have been measured at those sites (Ducklow 2000). Clearly, AAP bacteria appear to grow faster than the bulk bacterial community across a diverse range in aquatic habitats, although methodological differences in rate estimates across studies currently prevent us from drawing strong quantitative conclusions about patterns in AAP growth rates.

Although it is unclear why the potential growth rate of AAP bacteria appears to be higher than that of the average bacteria, a basic hypothesis is that light-derived energy has a positive effect on the growth and competitiveness of AAP bacteria (Koblízek et al. 2010, Hauruseu and Koblížek 2012, Kirchman and Hanson 2012). Two recent studies have measured the activity of total bacteria and AAP cells by quantifying their incorporation of leucine using microautoradiography (Kirchman et al. 2014, Stegman et al. 2014), and concluded that AAP bacteria were indeed up to 40 to 60% more active than average cells in the community. Interestingly, light did not enhance AAP cell activity, neither in the experimental incubations of Delaware estuary samples nor in the West Antarctic Peninsula water, so the higher apparent intrinsic growth rates of AAP bacteria may not be directly related to phototrophy. It is clear that there is much still to learn on the regulation of the performance of this group.

The re-growth experiments from which we derived the gross growth rates were carried out during the third week of the experiment. Although such timing is unlikely to have influenced the difference in growth rates between AAP and total bacteria, it could have biased the absolute rates obtained, especially if the mesocosms had diverged greatly over time, rendering the resulting rates less applicable to the initial conditions. Our results suggest, however, that bacterial biomass and production were relatively stable

in the mesocosms through time (< 40% total variability). Moreover, our estimated gross growth rates for the whole bacterial community agree well with those reported for 20 lakes located in this same region of Québec, Canada, which ranged from 0.14 to 1.4 d^{-1} (Smith and Prairie 2004). We conclude that the pattern in gross growth rates between treatments and between total and AAP bacteria that we observed in the re-growth experiments is likely applicable to the dynamics that occurred over the first 2 wk of the experiment. There is the possibility, however, that the actual growth rates may have been somewhat higher in the initial weeks, which would result in a slight underestimation of the total grazing rates, but without necessarily biasing the relative contribution of protists and zooplankton that we report here.

The nutrient addition led to increases in Chl*a* relative to the un-amended mesocosms (Fig. 1.1d, Table 1.1). There was a rapid increase in bacterial abundances together with a 20 to 30% increase in the cell volume during the first day following the nutrient enrichment, and this difference remained for the length of the experiment (Figs. 1.1a and 1.4). In spite of this initial deviation in bacterial cell abundance and cell size, the average gross and net growth rates for AAP and total bacteria were similar between amended and un-amended treatments, suggesting that either bacterial growth was not nutrient-limited, or that nutrient-driven initial differences in growth were matched by proportional losses, either from grazing or viral infection.

The higher average potential growth rates of AAP bacteria should result in a significant contribution to total community biomass, and yet AAP bacteria are consistently a small proportion of total bacterial abundance across fresh, estuarine, and marine waters (Mašín et al. 2012, Cuperová et al. 2013, Kirchman et al. 2014, Fauteux et al. 2015, and see additional references in Table 1.2). This was also the case in Lake Cromwell (this study): we found that AAP bacteria also had high potential growth rates, yet

their relative abundance was less than 1%. One hypothesis to explain the low relative abundance of AAP bacteria, in spite of their higher potential growth, is that this group is selectively removed, either by predators or by viruses. In this study, we did not test the potential role of viral lysis on the regulation of AAP bacteria, but our results amply support the hypothesis that this group is selectively grazed. The integrated loss rates of the bulk bacterial community due to the combined protist and zooplankton grazing ranged between 1.6 and 1.7 d^{-1} (Fig. 1.3), comparable to those reported in other studies (Yokokawa and Nagata 2005), whereas the integrated loss rates of AAP bacteria were on average 1.6 times higher (2.5 to 2.6 d^{-1}). It is clear from these results that AAP bacteria are subjected to very strong top down control in lakes.

It is interesting to note that whereas AAP loss due to protist grazing was only slightly higher than that of total bacteria (1.5–1.6 vs 1.3–1.5 d^{-1} for AAP bacteria and total bacteria, respectively), AAP loss due to zooplankton was 3 to 6-fold higher than that of total bacteria. Experimental studies have demonstrated that protist grazing is generally size-selective, with protists preferentially grazing on medium to larger-sized planktonic bacterial cells (Gonzalez et al. 1990, Simek and Chrzanowski 1992, Hahn and Höfle 2001, Jürgens and Matz 2002). Grazing of planktonic bacteria by cladocerans has also been shown to be size-selective, depending on their body size and on the configuration of their filtering apparatus (Jürgens 1994, Bertilsson et al. 2003). In this regard, our results suggest that AAP bacteria are on average larger than the average bacterial cells, a pattern that has been reported before both for marine (Sieracki et al. 2006, Koblízek et al. 2010, Hojerová et al. 2011, Kirchman et al. 2014) and freshwater communities (Cuperová et al. 2013, Fauteux et al. 2015). The differential size distribution of AAP bacteria may thus explain their increased vulnerability to grazing in general, leading to selective removal and low relative abundances. Previous studies have also shown that selective predation may respond not only to cell size but also to cell activity (del Giorgio et al. 1996, Jezbera et al. 2005, del Giorgio and Gasol 2008, Montagnes et al. 2008).

In this regard, our results further suggest that AAP bacteria may have morphological and physiological traits that render the group particularly vulnerable to grazing by cladocerans, which establishes a strong trophic link that goes beyond the microbial food web, and which suggests that AAP bacteria may in fact be disproportionately important for the nutrition of this keystone planktonic group.

Strong size-selective grazing should influence the cell size distribution within bacterial communities, and more particularly, within AAP bacteria, when grazers are experimentally removed or added, but these shifts were not obvious in our experimental phases. There was a very modest decrease in mean cell volume of AAP bacteria exposed to only protist grazing, and the reintroduction of zooplankton actually resulted in an increase of the mean cell size of both AAP and total bacteria, in both amended and un-amended mesocosms (Fig. 1.4). This increase in bacterial cell size under exposure to both protist and zooplankton grazing is rather counterintuitive in the context of direct grazing, but may be explained as the effect of indirect trophic cascade interactions associated with the presence of zooplankton (likely cladocerans).

The size-selective grazing of zooplankton on protists could change the size structure of protists and consequently the cell size distribution of the AAP bacteria and total bacteria. The importance of indirect effects of zooplankton on bacterial communities via trophic cascades has already been emphasized in previous studies (Zöllner et al. 2003, Brucet et al. 2008, Compte et al. 2009). Interestingly, our results show that mean bacterial and AAP sizes were influenced in a similar way by the experimental shifts in predation, suggesting that this mechanism influenced bacteria as a whole and not specific groups within the community.

Regardless of the underlying mechanisms involved, our experiments provide evidence

that freshwater AAP bacteria are subject to intense top-down regulation, and are selectively removed by grazers relative to the bulk bacterial community. Our results further demonstrate that whereas protists are the main predators of AAP bacteria, zooplankton (likely cladocerans) may nevertheless account for over a third of AAP losses, exhibiting a much greater selectivity for AAP bacteria than protists. The mechanistic underpinning of this selectivity is still unclear, but it may be related to the average larger cell size of AAP bacteria, and to the higher potential growth rates of AAP relative to the average bacterial community. This strong preference for AAP bacteria by cladoceran zooplankton establishes a direct trophic link between lake metazoans and the microbial food web, which coupled to the high potential for growth that characterizes AAP bacteria, as demonstrated here, implies that AAP bacteria may play a disproportionate role in the nutrition of lake zooplankton, and in the trophic transfer of organic C in lake food webs.

1.6 ACKNOWLEDGMENTS

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Date	Treatment	Bacterial production	Bacterial abundance		Bacterial cell volume μm^3		Heterotrophic nanoflagellates	Zooplankton abundance ind*L^{-1}			
		µgC L^{-1}d^{-1}	Total 10^6 cell*ml^{-1}	AAP 10^4 cell*ml^{-1}	Total	AAP	10^3 cell*ml^{-1}	Total	Cladoceran	Cyclopods copepods	Calanoids copepods
6-Jun	Nutrient amended	12±13.5	1.56±0.11	1.27±0.11	0.088±0.01	0.091±0.004	2.22±0.23	0	0	0	0
	Non amended	4.9±1.9	0.81±0.09	0.61±0.08	0.0593	0.061	1.08±0.35	0	0	0	0
	Lake	9.5±	2.19	1.3			1.9	2.82	1.43	0.93	0.46
13-Jun	Nutrient amended	53±43.8	4.72±0.34	43.58±8.54	0.0854±0.02	0.096±0.03	1.95±0.19				
	Non amended	8.4±1.6	1.76±0.25	16.32±2.89	0.0627	0.0533	1.21±0.81				
	Lake										
18-Jun	Nutrient amended	29±7.5	2.91±0.31	7.79±2.17	0.1139±0.005	0.1224±0.009	1.51±0.28	2.6±0.92	0.9±0.31	1.3±0.44	0.3±0.166
	Non amended	2.8±1.5	1.4±0.17	1.95±0.24	0.0909	0.1125	1.31±0.79	1.1±0.12	0.5±0.04	0.2±0.03	0.4±0.05
	Lake	10	1.21	1.02	0.0535±0.057	0.06±0.03					

Supplementary Table S1.1. Abundance (mean ± SE) of the planktonic microoganisms in the mesocosm and Lake Cromwell, Québec, Canada during the experiment in 2012. BP, bacterial production; Total, total bacteria; AAP, AAP bacteria; HNF (heterotrophic nanoflagellates); Clad, Cladoceran, Cycl, Cyclopoid copepods; Cala, Calanoid copepods.

CHAPTER II

SINGLE-CELL ACTIVITY OF FRESHWATER AEROBIC ANOXYGENIC PHOTOTROPHIC BACTERIA AND THEIR CONTRIBUTION TO BIOMASS PRODUCTION

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N.B References cited in this chapter are presented at the end of the thesis.

2.1 ABSTRACT

Aerobic anoxygenic phototrophic (AAP) bacteria are photoheterotrophs that despite their low abundances have been hypothesized to play an ecologically and biogeochemically important role in aquatic systems. Characterizing this role requires a better understanding of the *in situ* dynamics and activity of AAP bacteria. Here we provide the first assessment of the single-cell activity of freshwater AAP bacteria and their contribution to total bacterial production across lakes spanning a wide trophic gradient, and explore the role of light in regulating AAP activity. The proportion of cells that were active in leucine incorporation and the level of activity per cell were consistently higher for AAP than for bulk bacteria across lakes. As a result, AAP bacteria contributed disproportionately more to total bacterial production than to total bacterial abundance. Interestingly, although environmentally-driven patterns in activity did not seem to differ largely between AAP and bulk bacteria, their response to light did, and exposure to light resulted in increases in the proportion of active AAP bacteria with no clear effect on their cell-specific activity. This suggests that light may play a role in the activation of AAP bacteria enabling these photoheterotrophs to contribute more to the carbon cycle than suggested by their abundance.

2.2 INTRODUCTION

Aerobic anoxygenic phototrophic (AAP) bacteria are a cosmopolitan photoheterotrophic group of prokaryotes that inhabits the water column of all aquatic ecosystems (Koblížek, 2015; Yurkov and Csotonyi, 2009). The capacity of AAP bacteria to obtain energy from both the oxidation of organic matter and light-induced proton translocation has lead to explorations of the potential competitive advantage of AAP bacteria and their trophic and biogeochemical roles in aquatic ecosystems (Béjà and Suzuki, 2008 ; Ferrera *et al.*, 2011 ; Moran et Miller, 2007). Experimental studies with AAP bacterial isolates demonstrate that light stimulates biomass production and increases the efficiency of utilization of organic carbon sources (Biebl and Wagner-Döbler, 2006 ; Hauruseu and Koblizek, 2012 ; Spring *et al.*, 2009), suggesting that AAP cells benefit from the capacity to use light-derived energy. However, most in situ studies on the ecology of AAP bacteria have focused on exploring their abundance or diversity (Koblížek, 2015), and very little is known about the physiological status of AAP cells in natural aquatic environments, and whether and how sunlight influences the in situ dynamics and activity of AAP bacteria. This largely limits our understanding of the ecological importance of this group, and the potential global significance of the photoheterotrophic processes carried out by AAP bacteria in natural aquatic ecosystems.

Available data indicate that despite their low relative abundances and the apparently low amount of energy potentially gained through phototrophy (Kirchman and Hanson, 2013), AAP bacteria are more active, larger, and grow faster than the bulk bacterial community (Koblízek *et al.*, 2007; Ferrera *et al.*, 2011; Hojerová *et al.*, 2011; Kirchman *et al.*, 2014). It is not clear, however, whether these higher apparent growth rates and potential competitiveness of AAP bacteria can be directly attributed to a light-driven effect. Recently, Stegman et al. (2014) developed an approach that combines infrared

epifluorescence microscopy with microautoradiography (AAP-MAR) in order to directly assess the activity of AAP bacteria from natural communities by measuring the incorporation of radioactive substrates by individual AAP cells. Recent studies using this approach in the Delaware estuary and the coastal waters of the West Antarctic Peninsula (Kirchman *et al.*, 2014 ; Stegman *et al.*, 2014) confirmed that AAP cells were on average more active in substrate uptake than bulk bacterial cells, although there were large spatial and seasonal variations in the percentage of active AAP bacteria. Moreover, contrary to expectations, light did not enhance AAP single-cell activity in leucine incorporation in experimental incubations relative to dark controls, although AAP activity was positively correlated with light availability in the water column. The evidence converges to suggest that in terms of activity, AAP bacteria must play a role that is disproportionately large relative to their biomass in aquatic systems, yet we are still far from understanding under what circumstances this role may be more or less important, under what scenarios this group has a competitive advantage relative to other prokaryotes, and how light influences the activity of this group of prokaryotes. In addition, all studies of AAP bacterial activity in natural environments have been carried out in estuarine or marine waters, and the magnitude and regulation of AAP bacterial activity in freshwater ecosystems remain completely unknown.

In this paper, we explore patterns in single-cell activity of freshwater AAP bacteria across a range of temperate lakes in Québec (Canada) that vary widely in trophic status and light availability. Using the AAP-MAR approach developed by Stegman et al. (2014) we investigated how the proportion of active AAP bacteria, and their single-cell activity in leucine incorporation vary among lakes, and how they compare to activity patterns of the bulk bacterial community. In addition, we explored the role of light in regulating the heterotrophic activity of bulk and AAP bacteria, by exposing lake samples to artificial photosynthetically active radiation (PAR). By quantifying the area of silver grains associated with active cells and comparing it with bulk bacterial production

rates, we were able to estimate, for the first time, the contribution of freshwater AAP bacteria to total bacterial biomass production in their natural setting.

2.3 MATERIALS AND METHODS

2.3.1 Study sites and sample collection

We sampled 7 northern temperate lakes, six of which were located in the Eastern Townships region of southeastern Québec (45.24°N, 72.12°W) and one in the Laurentian region north of Montréal (46.01°N, 74.15°W), Canada (Table 2.1). The lakes were chosen to cover a wide range in dissolved organic carbon (DOC) concentrations and a gradient in lake productivity, both in terms of chlorophyll a (Chl*a*) and nutrient concentrations, so that they differed greatly in trophic status, and light availability (Table 2.1). Each lake was sampled once between July and August 2013. During summer stratification, lakes may develop a hypoxic hypolimnion that makes the detection of AAP bacteria problematic, since it is not possible to distinguish bacteriochlorophyll a (BChl*a*) containing AAP bacteria from BChl*a*-containing anaerobic phototrophic cells. In order to avoid sampling in hypoxic zones, we measured vertical profiles of temperature and dissolved oxygen for all lakes and collected water samples from the aerobic epilimnetic layer. These profiles were recorded with a Yellow Springs Instruments (YSI) Pro Plus multiparameter probe and underwater profiles of photosynthetically active radiation (PAR) were measured with a LI-COR LI-190 Quantum Sensor.

Lake samples were collected by pumping water into 20 l polycarbonate containers from a depth corresponding to an irradiance in the range of 200-300 µmol photons m^{-2}

s^{-1} and transported refrigerated in the dark to the laboratory. Microautoradiographic incubations were carried out immediately upon returning to the laboratory (2 to 3 hours after collection). The relative abundance of AAP bacteria was estimated on fixed samples by standard IR epifluorescence microscopy (Cottrell *et al.*, 2006). AAP and total bacterial cell size was also determined by image analysis from the DAPI-stained cells using the integration method (Sieracki *et al.*, 1989).

Concentrations of total (TP) and dissolved phosphorus (TP, TDP) were measured by the molybdenum-blue method following persulfate digestion. Total and dissolved nitrogen (TN, TDN) were measured as nitrates after alkaline persulfate digestion. Dissolved organic carbon (DOC) was measured in 0.45 μm-filtered samples by wet oxidation using an O.I. Analytical Total Carbon Analyzer.

2.3.2 Chlorophyll a and Bacteriochlorophyll*a* concentrations

Water samples were filtered through GF/F filters, frozen and subsequently extracted with hot ethanol. Chl*a* in the extracts was measured spectrophotometrically at 665nm using an UV/Vis UltroSpec 2100 Pro spectrophotometer (Biochrom), correcting for turbidity at 750nm and for the presence of phaeophytin. BChl*a* concentrations were measured on a FL3500-FT fluorometer (Photon Systems Instruments), using the standard calibration curve provided by the manufacturer. Samples were first treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, 50 μM final concentration), an inhibitor of photosystem II in oxygenic phototrophs, so that BChl*a* fluorescence could be distinguished from Chl*a* fluorescence (Koblízek *et al.*, 2005).

2.3.3 Microautoradiography of AAP and total bacteria

The single-cell activity of AAP bacteria and bulk bacteria was determined using the AAP-MAR method, as described previously (Stegman *et al.*, 2014). In brief, 30 ml water samples were spiked with ^{3}H-leucine (20 nM final concentration) in 60 ml transparent polystyrene culture flasks, and incubated as described below. Incubations were ended with the addition of paraformaldehyde (2% final concentration), the samples were filtered onto 0.2-μm pore-size black polycarbonate filters, and then stored at -80°C for subsequent analysis. Sections (1/8) of filters were mounted on slides and first examined by IR epifluorescence microscopy to determine the exact location of microscope fields containing AAP cells. After identifying and counting the AAP cells, the filter sections were then taken off the microscope and subjected to the microautoradiography procedure as described by Cottrell and Kirchman (2003). A time series of autoradiographic exposures was used to select the shortest time required to identify the maximum number of active cells in each lake sample. The exposure time was 7 days for all samples except for the oligotrophic Lake Bowker, for which it was 35 days. After exposure, slides were developed and fixed, dried overnight, and the filter sections were carefully peeled off of the emulsion so that the cells remained stuck to the slide. The cells were then stained with DAPI, and the previously analyzed fields were re-located automatically under the epifluorescence microscope and analyzed again for the presence of silver grains around DAPI-stained cells. After analyzing all fields of view, ImagePro (Media Cybernetics, Rockville, MD, USA) was used to align DAPI images with the identified AAP cells from before and after the autoradiographic exposure. If the images matched, the two DAPI images were merged to create a composite image and the MicrobeCounter program (Cottrell and Kirchman, 2003) was used to count AAP and total bacteria, to quantify those with silver grains, and to estimate the size of the silver grain area around active cells. Since microscopic

counts of total bacteria include AAP bacteria, any comparison between AAP and total bacteria would be conservative even though AAP make up only a small fraction of the total community (see Results).

2.3.4 Effect of light on single-cell activity and production of AAP and total heterotrophic bacteria

In order to evaluate the influence of light on the single cell activity of AAP and total bacteria, AAP-MAR was applied to lake water samples that were incubated either under artificial PAR or in the dark. ^{3}H-leucine incubations were conducted in 60-ml transparent polystyrene culture flasks. Three dark, three light flasks plus one paraformaldehyde-killed control were attached to a surface-tethered array and incubated under artificial light at an irradiance of 150 μmol photons m^{-2} s^{-1} for 4 h, in a tank with circulating water to keep samples temperature at 20 ± 2°C. Illumination was provided by UV-free fluorescent lamps (45-W Sun Blaster™). The light intensity was measured with a HR 4000 spectrometer PAR sensor (Ocean Optics). The paraformaldehyde fixed samples were filtered onto 0.2-μm pore-size black polycarbonate filters and stored at -80° C for subsequent AAP-MAR analysis (see above).

To evaluate the influence of light on bulk bacterial production, three replicates of 1.5 ml of lake water plus one paraformaldehyde-killed control were inoculated with ^{3}H-leucine at a final concentration of 20 nM and incubated for 2 h either in the dark or under the same light and temperature conditions described above. Incubations were terminated by the addition of paraformaldehyde (2% final concentration) and the samples were stored at 4° C until processing by the microcentrifuge method (Smith and Azam, 1992).

Water color differed greatly among lakes, and thus we corrected for the possible disparity between the incident radiation and the actual incident light reaching the cells during the incubations. Measurements of incident radiation (I_0) and the extinction coefficient (k_d) of the lake water were used to calculate light intensity at the 1cm depth of the water sample inside the incubation flask (I_D), and the cell activity data obtained from the light incubations (percentage active cells, silver grain area, and rate of leucine incorporation) were corrected with the coefficient that relates I_0 and I_D.

2.3.5 Statistical analyses

Differences in activity between AAP bacteria and bulk bacteria and differences between light and dark experiments were assessed with the Kruskal-Wallis nonparametric test, or the paired t-test. Relative AAP bacterial abundance and percentages of active cells were arcsine transformed and silver grain areas were log transformed for parametric statistical analyses. Relationships between variables were investigated with linear correlation and regression techniques. All statistical analyses were performed using the JMP 9.0 statistical package (SAS Institute Inc., Cary, NC, USA).

2.4. RESULTS

We explored variations in abundance, cell size, and single-cell activity of AAP bacteria using the AAP-MAR approach, in seven different temperate lakes (Table 2.1). The attenuation coefficient of PAR (K_{PAR}) varied 4-fold (0.3 to 1.5 m^{-1}), DOC concentration

varied 5-fold (2.6 to 14 mg l^{-1}), and Chl*a* and TP concentrations varied 15-fold (0.7 to 11 μg l^{-1}) and 6-fold (2.7 to 16 μg l^{-1}), respectively, among the lakes (Table 2.1). All lakes except the shallow Lake Waterloo were stratified during the sampling period. Epilimnetic water temperatures averaged 21 ± 2°C. Lakes Bowker, Nicolet, Waterloo, and Aylmer had a fully aerobic water column, whereas lakes Magog, Coulombe, and Croche had an anoxic hypolimnion at the time of sampling. The high thermal stability in the latter three lakes during the time of sampling almost certainly prevented mixing events between the oxygenated surface layers and the deeper, anoxic layers. Considering that samples were always collected from fully aerobic subsurface waters, we can

Table 2.1. Environmental properties, prokaryotic abundances, activity and BChl *a* concentrations and content in the epilimnetic waters of the lakes studied

Environmental properties	Bowker	Waterloo	Magog	Coulombe	Aylmer	Nicolet	Croche
Max. depth (m)	63	6	11	9	14	29	11
Sampling depth (m)	6.0	2.0	4.0	0.5	1.0	3.5	1.5
K_{PAR} (m^{-1})	0.31	1.34	0.50	1.52	1.05	0.52	0.67
Temperature (°C)	21	25	23	19	20	20	21
Dissolved Oxygen (%)	110	103	108	89	95	94	96
pH	8.2	8.6	8.5	7.5	7.2	7.5	6.7
DOC (mg l^{-1})	2.7	7.8	4.9	14	10	3.5	4.8
TP (μg l^{-1})	3.3	7.3	12.7	16.0	11.7	2.7	2.7
DP (μg l^{-1})	2.5	6.2	5.5	8.4	7.3	1.6	1.3
TN (mg l^{-1})	0.15	0.49	0.25	0.44	0.45	0.29	0.16
DN (mg l^{-1})	0.13	0.48	0.21	0.37	0.41	0.28	0.15
Chl *a* (μg l^{-1})	0.8	11.0	2.9	3.7	3.3	0.7	1.1
Bacterial characteristics							
Total bacteria (10^6 cells ml^{-1})	2.65	5.27	3.43	2.42	2.98	1.38	0.92
AAP bacteria (10^4 cells ml^{-1})	3.05	13.5	7.78	6.71	9.57	4.16	4.00
AAP bacteria (% of DAPI counts)	1.15	2.57	2.27	2.78	3.22	3.01	4.34
BChl*a* (ng l^{-1})	5.94	24.29	10.52	9.80	5.16	8.53	12.57
Specific BChl*a* (10^{-1} fg $cell^{-1}$)	1.95	1.80	1.35	1.46	0.54	2.05	3.14

Abbreviations: AAP, aerobic anoxygenic phototrophic; K_{PAR}, attenuation coefficient of photosynthetically active radiation; DN, dissolved nitrogen; TN, total nitrogen; DP, dissolved phosphorus; TP, total phosphorous; Chl*a*, chlorophyll *a*; BChl*a*, bacteriochlorophyll *a*; DOC, dissolved organic carbon.

assume that the BChl*a*-containing bacteria and the BChl*a* concentrations observed originate from AAP bacteria, rather than from anaerobic phototrophic bacteria.

2.4.1 AAP and total bacterial abundance, cell size, and single-cell activity

In general, AAP bacterial abundances were low and varied 4-fold ($3x10^4$ – $1x10^5$ cell ml^{-1}) among the lakes (Table 2.1). AAP abundance co-varied with total prokaryotic abundance thus resulting in small variations in the proportion of AAP bacteria among lakes (1% to 4% of total DAPI counts, mean 2.4%, Table 2.1). Total bacterial cell size varied 1.5-fold among all samples, from 0.10 to 0.18 μm^3 (mean 0.13 ± 0.036 μm^3), whereas AAP bacterial cell size varied more than 2-fold (Fig. 2.1a), from 0.13 to 0.29 μm^3 (mean 0.17 ± 0.056 μm^3). The mean cell size of an AAP cell was 1.5-fold larger than that of an average cell in the total bacterial community (paired t-test, $p<0.0001$).

When all the lakes were considered together, the proportion of active cells was significantly higher for AAP bacteria than for total bacteria (paired t-test, combining light and dark incubations $p<0.0001$, Fig. 2.1b). The percentage of cells actively taking up ^{3}H-leucine varied remarkably among lakes, ranging from 2% to 44% of total bacteria and from 16% to 58% of AAP bacteria. Overall, the active fraction of AAP bacteria was almost 2-fold higher than the active fraction of the whole community.

Since the level of activity per cell can also vary greatly, we also quantified the size of the silver grain area (SGA) surrounding active cells. The SGA varied considerably among lake samples, with coefficients of variation of 49% and 72% for total bacteria and AAP cells, respectively. The mean SGA around active AAP cells was on average significantly larger than that for the bulk bacteria (paired t-test combining light and

dark incubations, $p<0.0001$, Fig. 2c). Overall, the mean SGA around AAP cells (2.6 μm^2) was on average almost 2-fold larger than the area around active cells from the bulk bacterial community (1.44 μm^2). There was no relationship between the mean cell size and the level of cell activity for AAP or total bacteria (data not shown), although it should be noted that the current version of the AAP-MAR method does not allow to discriminate between the cell sizes of active and inactive bacteria (Stegman *et al.*, 2014).

In order to account for the differences in size between AAP and total bacterial cells, the level of activity per cell (mean SGA) was normalized to the mean cell size (Fig. 2.1d). The mean SGA per unit cell volume of an active AAP bacterium was slightly higher than that of an active cell in the total bacterial community (11 vs 16 $\mu m^2 \mu m^{-3}$ for total bacteria and AAP, respectively), although this difference was not statistically significant ($p>0.05$; paired t-test combining light and dark incubations).

The variability in bacterial activity among replicate samples differed from lake to lake. The mean standard error (SE) of three replicate microautoradiographic assays ranged from 0.20% to 6.84% for total bacteria and from 1.45% to 18.1% for AAP bacteria (Supplementary Table S2.1). The SE associated with the measurements of SGA in three replicate samples ranged from 0.02 μm^2 to 0.53 μm^2 for active cells in the bulk bacterial community and from 0.1 μm^2 to 1.5 μm^2 for active AAP cells (Supplementary Table S2.1).

Table 2.2. Correlations between AAP and total bacterial activity or abundance and environmental parameters. Pearson correlation coefficient is shown for significant correlations (α = 0.05). Abundance was measured as cells ml^{-1}, normalized silver grain area (SGA) as $\mu m^2\ \mu m^{-3}$, BChl a as ng l^{-1}, and specific BChl a as fg $cell^{-1}$.

	Abundance		*% AAP bact.*	*% Active bacteria*		*Normalized SGA*		*BChla*	*Sp BChla*
	AAP	*TOT*	*AAP*	*AAP*	*TOT*	*AAP*	*TOT*	*AAP*	*AAP*
	0.33	0.26	0.31	-0.38				0.43	-0.41
	0.44	0.47	0.13	-0.35	-0.3	-0.27	-0.48	0.45	-0.58
	0.45	0.46	0.17	-0.31	-0.24	-0.27	-0.46	0.38	-0.67
	0.45	0.53		-0.14		-0.36	-0.48	0.24	-0.77
	0.16	0.26					-0.36	-0.3	-0.74
	0.47	0.57		-0.25	-0.23	-0.27	-0.33	0.91	
	0.24	0.16	0.31	-0.28	-0.11		-0.11		-0.58
ıla	-0.45	-0.66	0.18			0.38	0.45	-0.68	0.22
a	-0.33					0.43			

ons: AAP, aerobic anoxygenic phototrophic bacteria; TOT, total bacteria; SGA, silver grain area; K_{PAR}, attenuation coefficient of the photosynthetically activ ved nitrogen; TN, total nitrogen; DP, dissolved phosphorus; TP, total phosphorous; Chl*a*, chlorophyll *a*; BChl*a*, bacteriochlorophyll *a*; DOC, dissolved organic *cific* bacteriochlorophyll *a*.

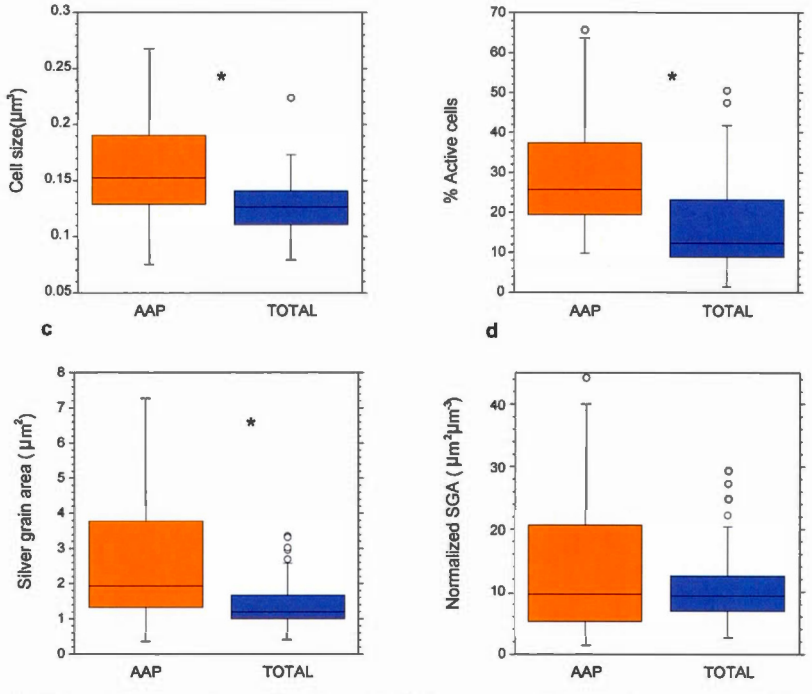

Figure 2.1. Cell size (a), percentage of active cells (b), average silver grain area around active cells (c), and average silver grain area (SGA) around active cells normalized for biovolume (d) of AAP and bulk bacterial cells. Central lines indicate median values and combine data from light and dark incubations, boxes indicate the lower and upper quartiles; whiskers depict the 10th and 90th percentiles, and the dots represent outliers. Stars indicate significant differences ($p<0.05$) between AAP and total cells.

2.4.2 Abundance and single-cell activity of total and AAP bacteria across environmental gradients.

We explored the patterns in abundance, proportion of active cells, and the level of cell-specific activity of AAP and total bacteria in relation to the environmental variables listed in Table 2.2 In general, total and AAP bacterial abundances responded similarly to changes in the measured environmental variables. The abundance of both groups increased with system productivity, being positively related with phosphorus, nitrogen, and Chl*a* concentration (Table 2.2). The concentration of BChl*a* was also positively correlated with phosphorous, nitrogen, and Chl*a* concentrations, as well as light attenuation (Table 2.2). In contrast, the percentage of active cells in both groups decreased with increasing nitrogen, Chl*a*, DOC and K_{PAR} (Table 2.2), and there was a strong positive relationship between the proportion of total and AAP active cells (Fig. 2.2). The volume-normalized SGA of AAP and total bacteria were also negatively correlated with Chl*a*, nitrogen, and phosphorus concentrations, and interestingly, the BChl*a* content per cell was positively correlated with the SGA of AAP cells (Table 2.2).

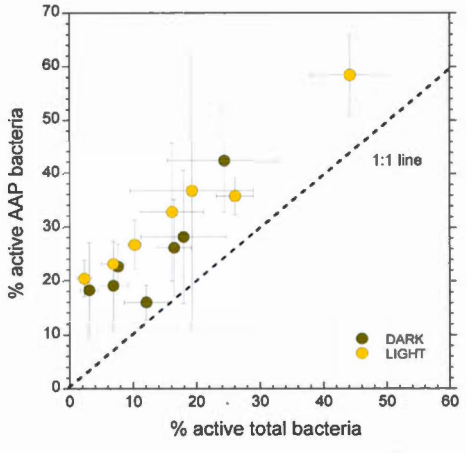

Figure 2.2. Comparison between the percentage of active AAP cells versus the percentage of active cells in the bulk bacterial community across all lakes. Data from the light and dark incubations for each lake are included, for a total of 14 observations. The dashed line indicates a 1:1 relationship between AAP bacteria and the total community. Error bars represent 1 standard deviation.

2.4.3 Light effects on AAP and total bacterial activity

Although AAP and total bacteria behaved similarly with respect to nutrient status, they differed in their responses to light. Overall, the exposure of bacterial communities to light significantly increased the percentage of active cells ($p<0.05$), but this increase was on average larger for AAP bacteria than for the bulk community; the mean enhancement of the relative number of active cells was 28% versus 37% for total and AAP bacteria, respectively. This enhancement varied greatly among lakes (Fig. 2.3), with the largest enhancement occurring in the lake with the highest light attenuation coefficient. The light-driven stimulation of the proportion of active cells in the bulk community was lower than that of AAP in four out of seven lakes (Fig. 2.3), and the highest stimulation was found in Lake Magog, which was the only case where an increase in the number

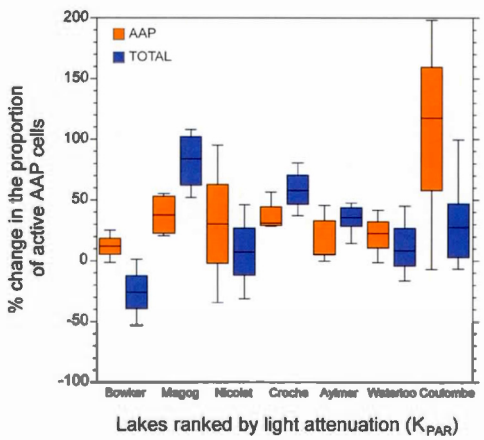

Figure 2.3. The response of AAP and total bacteria to exposure to photosynthetically active radiation (PAR) as determined by changes in the proportion of active cells, for each of the 7 lakes ranked by increasing light attenuation coefficients (K_{PAR}). The light-driven change in the percentage of active cells was calculated as (% $active_{Light}$ - % $active_{Dark}$)/% $active_{Dark}$. The central line indicates the median value of the three replicates; boxes indicate the lower and upper quartiles, and whiskers depict the 10th and 90th percentiles.

of total bacteria was detected after incubation in light conditions. AAP abundance never changed during light incubations when compared to dark-incubated samples or to killed-controls (data not shown).

In contrast to the effect on percent active, light had no significant effect on silver grain area for either active AAP bacteria or active cells in the bulk community, relative to the dark treatment (Fig. 2.4a). Accordingly there was no effect of light on bulk ^{3}H-leucine assimilation rates (Fig. 2.4b).

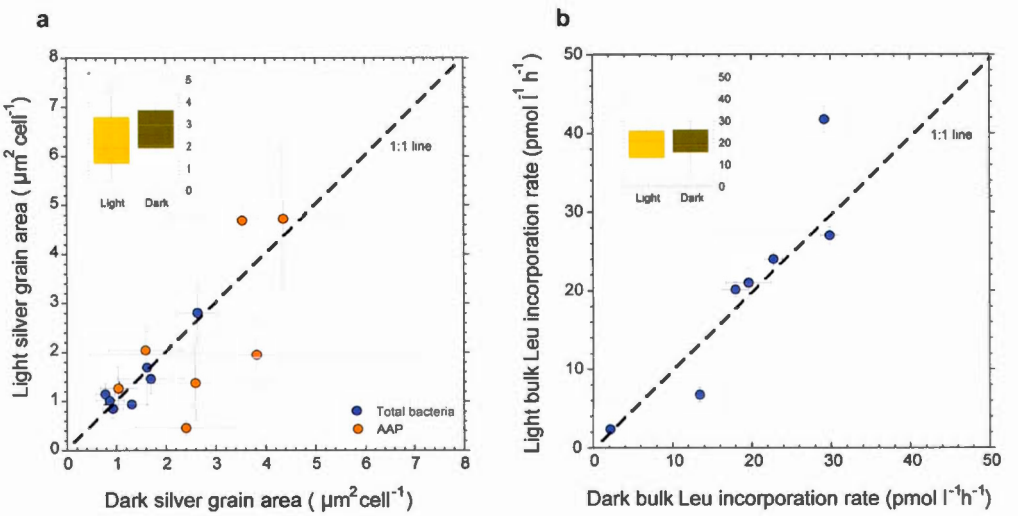

Figure 2.4. Comparison of the activity of AAP bacteria and of the total bacterial community in light versus dark incubations. Average silver grain area (SGA) associated with both active AAP cells and with active cells in the bulk community in light versus dark incubations (a), and bulk ^{3}H-leucine incorporation in light versus dark incubations (b). The dashed line indicates the 1:1 ratio. Error bars are 1 standard deviation. Insets in each panel show SGA values around active AAP (a) and bulk ^{3}H-leucine incorporation rates (b) between light and dark conditions pooling the 7 lakes together.

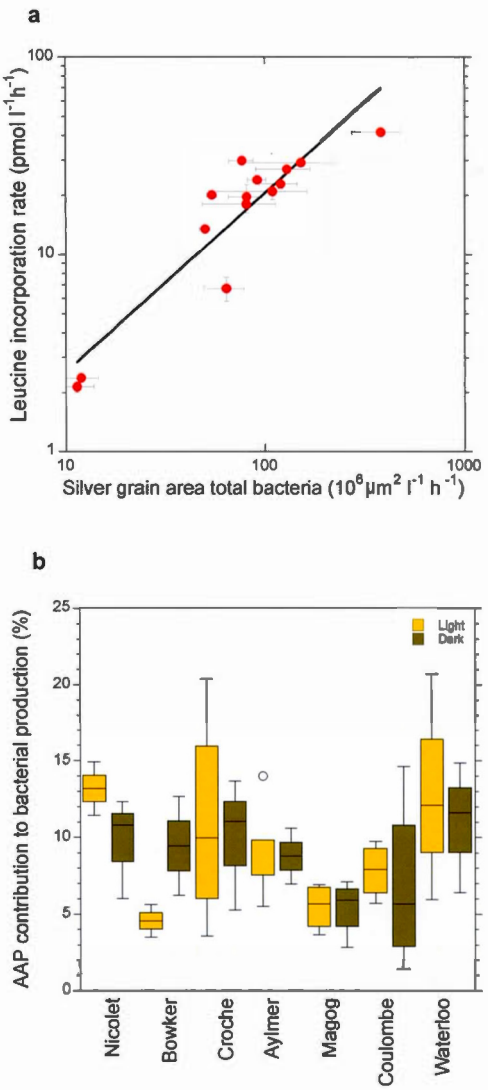

Figure 2.5. Relationship between bulk ^{3}H-leucine incorporation rate and the total average silver grain area associated with the total bacterial community (a). The numbers are means + SD calculated with data from both light and dark incubations for the total community. The solid line is a linear regression fit through all log-transformed data ($p<0.05$). Contribution of AAP bacteria to total bacterial production in the lakes, ranked by increasing system productivity (b). For each lake, yellow and green bars show the contribution estimated from light and dark incubations, respectively. Central lines indicate median values, boxes indicate the lower and upper quartiles; whiskers depict the 10th and 90th percentiles, and the dots represent outliers.

2.4.4 Contribution of AAP bacteria to total bacterial biomass production

The total SGA of all cells that took up leucine is strongly positively correlated with the corresponding bulk leucine incorporation rates as shown by Sintes and Herndl (2006) in the North Atlantic Ocean. We found a similar relationship for total bacteria in these

lakes (Fig. 2.5a). We used this relationship to estimate the potential contribution of AAP bacteria to total leucine incorporation (%BP) by calculating the ratio of SGA around active AAP cells to the SGA around all active bacterial cells. The contribution of AAP bacteria to total leucine incorporation varied among lakes, ranging from 5% to 13% (Fig. 2.5b). There was no significant difference between light and dark incubations when all the lakes were considered together, although the effect of light differed among lakes (Fig. 2.5b). We found no clear pattern in the contribution of AAP bacteria to total bacterial production along gradients of DOC, nutrients, or light attenuation.

2.5 DISCUSSION

The ecological and biogeochemical implications of photoheterotrophic pathways are receiving increasing attention, and during the last decade there have been remarkable advances in our understanding of abundance and distribution patterns of AAP bacteria in lakes and oceans (Hojerová *et al.*, 2011; Ferrera *et al.*, 2013; Fauteux *et al.*, 2015). One of the main challenges in furthering our understanding of the ecological role of this group, and in particular in determining the scenarios where they may have an ecological advantage over their heterotrophic bacterial counterparts, has been determining the actual activity of these bacteria in situ. It is well recognized that aquatic bacterial communities are composed of cells with a wide range of single-cell metabolic activities and physiological states, and that only a fraction of cells within these complex bacterial assemblages are metabolically active at a given time (del Giorgio and Gasol, 2008). This likely applies to AAP bacteria as well, but until recently it was technically impossible to determine the activity of AAP bacterial cells within bacterial assemblages. The recent development of the AAP-MAR method (Stegman *et al.*, 2014) opened the way to exploring the in situ patterns of activity of AAP bacteria. We applied this approach to 7 different lakes in Quebec, which represents the first

assessment of single-cell activity of freshwater AAP and their contribution to total biomass production in inland waters.

We chose leucine because it has been shown to be taken up by the widest spectrum of freshwater bacteria (see Salcher *et al.* 2013), and because it is the substrate most commonly used in aquatic studies for routine estimates of bulk bacterial production, therefore allowing for comparisons with previous results. The strong relationship we observed between the total SGA surrounding active cells and the bulk leucine uptake suggests that our approach effectively captures at least the fraction of the community that is active in leucine uptake. It is well recognized, however, that it is unlikely that a single method can be used to describe all the facets of bacterial activity (Smith and del Giorgio, 2003), and that the potential effect of light should be explored on other aspects of cell activity, such as respiration or in the incorporation of other substrates.

The fraction of total and AAP bacteria that took up leucine varied widely among the lakes we examined, as has been typically observed before for the total community (see Smith and del Giorgio, 2003). This variability suggests that there is a large heterogeneity in the proportion of cells that are active in substrate uptake among lakes, yet, in all cases, the active fraction of AAP bacteria was always higher than the active fraction of the whole community. In addition, the average specific rate of leucine uptake, based on the SGA around individual cells, was almost 2-fold higher for active AAP bacteria than for the average active bacterial cell in the community. This difference was slightly higher than that of the two previous MAR-AAP studies (1.6 and 1.4-fold, Stegman *et al.*, 2014, Kirchman *et al.* 2014), and is also in agreement with previous studies showing that AAP bacteria tend to grow faster than the bulk bacterial community across a diverse range in aquatic habitats (Koblízek *et al.*, 2007; Liu *et al.*, 2010; Ferrera *et al.*, 2011). We have further shown that AAP bacteria were on average larger than the average cell size in the community, a pattern that has been reported before (Sieracki

et al., 2006; Lamy *et al.*, 2011; Fauteux *et al.*, 2015). All this evidence indicates that at least a fraction of the AAP bacteria may be an intrinsically fast-growing component of aquatic bacterial communities. However, the large variability in these AAP activity patterns reported here and in the two previous studies (Kirchman *et al.*, 2014 ; Stegman *et al.*, 2014), suggests that AAP bacteria are far from being a homogeneous functional guild in terms of cell size, single-cell metabolic activity, and physiological status.

It is interesting to note that the variation among lakes in the proportion of metabolically active AAP cells was opposite to that in AAP cell abundance: Whereas total AAP bacterial abundance tended to increase with lake trophic status, a pattern that has been previously reported (Fauteux *et al.*, 2015 ; Hojerová *et al.*, 2011), the proportion of active AAP cells and their single-cell activity tended to decline along this same gradient. A similar trend was observed by Stegman *et al.* (2014) in the Delaware estuary, where the relative abundance of AAP bacteria was highest in the highly productive brackish waters and decreased towards less productive marine waters, but the opposite was true for the proportion of active AAP bacteria. This pattern suggests that the abundance of AAP cells in a system is not a simple function of their growth rate, but depends as well on the factors that regulate the loss and the persistence of the active and inactive pool of AAP cells. Regardless of the mechanism, our results indicate that the abundance and activity patterns of AAP bacteria are only loosely coupled, and that conclusions about the ecological role of these prokaryotes cannot be based on abundance patterns alone.

The covariation between AAP and total bacterial abundance observed in previous studies (Fauteux *et al.*, 2015 ; Hojerová *et al.*, 2011 ; Salka *et al.*, 2008) suggests that AAP bacteria respond to the same basic environmental drivers and are subject to a similar overall regulation as the bulk bacterial community. Our results support this hypothesis, since we show here that the abundance of active AAP bacteria tracked that of the total bacteria community across lakes. AAP cells appear, however, to be more

dynamic than the community as a whole, which was evidenced by a larger variability in the cell size, in the level of single-cell activity, and in the proportion of active cells, relative to the total community. This higher variability in size and activity, which has also been reported in previous studies (Stegman *et al.*, 2014; Kirchman *et al.*, 2014; Fauteux *et al.*, 2015), suggests that although the physiological structure of AAP bacteria and of the bulk community may be influenced by the same overall drivers, the response of AAP bacteria to variations in these drivers may be different and more dynamic than of other heterotrophic bacteria.

In particular, sunlight would be the most likely environmental factor differentially influencing the physiological structure of the AAP bacterial assemblage relative to the bulk bacterial community. In support of this idea, and in accordance to recent results (Stegman et al. 2014), we observed that the percentage of active AAP, but not that of the bulk bacteria, was negatively correlated to light attenuation, suggesting that the proportion of active AAP cells may be enhanced by light availability. We experimentally confirmed this pattern in our light/dark incubations, where we found a light-driven enhancement in the proportion of active AAP cells that was different to the light-driven responses in the bulk bacteria. This enhancement, however, was not accompanied by increases in the average SGA around active AAP cells. The observed light-driven increase in the proportion of active AAP cells could have been due simply to a light-enhanced division rate of active AAP cells, but the abundance of AAP cells did not increase after exposure to light compared with dark incubations or the killed control in any of the lakes (results not shown). These results suggest that light may play a role in the activation of AAP cells, regulating the passage of cells from dormant or slow growing states to an active growing state, but without necessarily enhancing the activity rates of individual cells. The hypothesis that light may play a role in the activation of AAP bacteria may in part explain why BChl*a*-containing AAP cells are found under the ice in northern lakes and in the Arctic Ocean in winter (Cottrell and

Kirchman, 2009; Fauteux *et al.*, 2015). Since the pigment cannot offer a metabolic advantage when light intensities are near zero, it may facilitate activation of AAP cells when light increases and other environmental conditions become more favorable in the spring.

We found a positive relationship between BChl*a* per AAP cell and activity per AAP cell as measured by SGA, suggesting that AAP cells may be more active because they have more pigment. This result supports the hypothesis that the small enhancement of AAP bacterial activity due to phototrophy can be accumulated and evidenced over the

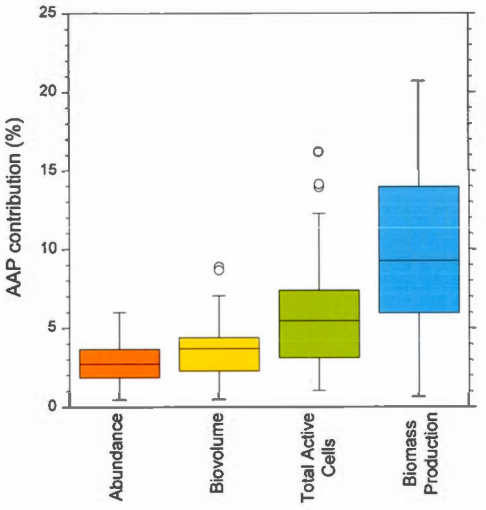

Figure 2.6. Contribution of AAP bacteria to total bacterial abundance, total bacterial biovolume, total active bacterial cells, and total bacterial production. The central line indicates the median value calculated with data from both light and dark incubations, the boxes indicate the lower and upper quartiles, the whiskers depict the 10th and 90th percentiles, and the circles represent outlier values.

lifetime of an AAP cell (see, Kirchman and Hanson, 2013; Kirchman *et al.*, 2014). Thus, it may be possible that light does increase AAP growth rates but that the enhancement is too small to be measurable in a short incubation experiment.

The larger cell sizes, higher activity and growth rates, and preferential grazing of AAP bacteria have repeatedly led to the hypothesis that this group plays a disproportionately large role in the cycling of carbon and nutrients in aquatic food webs (Kirchman *et al.*, 2014; Hojerová *et al.*, 2011; Koblížek, 2015). So far, however, no study had actually quantified their contribution to total bacterial activity in natural ecosystems. We estimated that the contribution of AAP cells to total bacterial community leucine incorporation averaged 10%, and varied from 5% to 17%. These estimated values of potential contribution of AAP bacteria are lower than those reported by Koblízek *et al.*, (2007) and Hojerová *et al.*, (2011) for marine waters, but those studies were based on growth rates estimated from the diurnal decay in BChl*a*. Regardless, all the evidence suggests that AAP bacteria contribute disproportionately to overall bacterial community activity in aquatic ecosystems.

Taken together, our results demonstrate that this ubiquitous group of bacteria represents a very active and dynamic component in freshwater microbial communities. On average, AAP bacteria accounted for 6% of all active cells, which was 2-fold higher than their average contribution to total bacterial abundance (Fig. 2.6). As a consequence of their high cell-specific activity and the high relative abundance of active AAP cells, the potential contribution of AAP bacteria to total biomass production was on average 2-fold higher than their contribution to total bacterial biovolume and 3-fold higher than their contribution to total bacterial abundance (Fig. 2.6). In addition to light and other environmental properties, grazing and viral lysis not considered here may differentially control the dynamics of the active and inactive pools of AAP cells relative to the rest of heterotrophic bacteria. Indeed, experimental evidence has shown that the larger

average cell size and higher cell activity of marine and freshwater AAP seem to render the group particularly vulnerable to grazing (Ferrera *et al.*, 2011, García-Chaves *et al.*, 2015). Since there is strong evidence that AAP cells are preferentially grazed even by freshwater crustacean zooplankton (M. Garcia-Chaves *et al.*, 2015), the contribution of AAP bacteria to the bacterial flow of carbon towards higher trophic levels may be even more important in freshwater ecosystems than in other aquatic habitats.

2.6 ACKNOWLEDGMENTS

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	% Active cells								Silver grain area (μm^2)							
	Total Bact.				AAP Bact.				Total Bact.				AAP Bact.			
Lake	*Light*	*SE*	*Dark*	*SE*	*Light*	*SE*	*Dark*	*SE*	*Light*	*SE*	*Dark*	*SE*	*Light*	*SE*	*Dark*	*SE*
Bowker	2.25	0.82	3.03	0.97	20.39	2.41	18.21	6.23	1.15	0.15	0.77	0.14	0.46	0.10	2.39	0.72
Waterloo	6.79	1.06	6.83	1.14	23.11	2.35	19.09	4.19	0.95	0.03	1.29	0.16	1.38	0.44	2.59	0.77
Magog	44.19	3.01	24.26	4.00	58.36	3.76	42.77	4.28	1.02	0.02	0.86	0.13	2.05	0.28	1.58	0.33
Coulombe	16.03	2.22	11.96	1.53	32.74	5.71	15.95	1.45	1.48	0.13	1.68	0.30	1.99	0.31	3.81	1.50
Aylmer	10.14	0.45	7.56	0.20	26.64	2.03	22.6	2.99	0.86	0.08	0.92	0.14	1.28	0.20	1.03	0.26
Nicolet	19.17	6.84	17.83	3.36	36.65	18.10	28.13	6.18	1.69	0.53	1.60	0.03	4.71	0.47	3.53	0.96
Croche	25.93	1.45	16.35	1.35	35.7	1.73	26.1	4.47	2.82	0.30	2.63	0.19	4.75	0.74	4.35	1.10

Supplementary Table S2.1. Activity of the total bacterial community ("Total Bact") and AAP bacteria ("AAP Bact") in light and dark leucine ^{3}H-incubations.

CHAPTER III

HIGH INTRINSIC GROWTH POTENTIAL AND SELECTIVE MORTALITY OF FRESHWATER AEROBIC ANOXYGENIC PHOTOTROPHIC BACTERIA, AND LINKS TO PIGMENT PRODUCTION AND PHOTOTROPHIC POTENTIAL

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N.B References cited in this article are presented at the end of the thesis.

3.1 ABSTRACT

Aerobic anoxygenic phototrophic (AAP) bacteria are ubiquitous photoheterotrophs, potentially playing significant roles in food webs and biogeochemical cycles in all aquatic ecosystems. However, basic aspects of their physiology, metabolism and ecology are still poorly understood. Here we assessed the links between four key facets of freshwater AAP bacteria eco-physiology: growth rates, grazing mortality, total and cell-specific pigment dynamics, and phototrophic potential. Our results indicate that grazing strongly influence net ambient growth rates of AAP bacteria, and that this strong top down regulation might reconcile the low relative abundance of AAP bacteria, commonly found in aquatic systems, and their high intrinsic growth rates measured here and in former experiments. Our results indicate that although light did not appear to directly influence AAP growth, pigment production may be partly regulated by growth, such that there may exist tradeoffs between growth and the expression and maintenance of the phototrophic apparatus. The relationship between pigment and cell dynamics was highly variable, and whereas in oligotrophic lakes, cell and pigment production were positively correlated, in mesotrophic lakes they were essentially uncoupled. These results suggest a range of strategies concerning pigment regulation across AAP bacterial communities, perhaps linked to phylogenetic composition.

3.2 INTRODUCTION

It is now recognized that photoheterotrophy, i.e., the capacity to use light and organic carbon as energy sources, is widespread among aquatic prokaryotes (Koblížek, 2015). This observation has prompted an increased interest in understanding the role of photoheterotrophic bacteria in the carbon and energy dynamics in freshwater and marine ecosystems (Eiler, 2006; Moran and Miller, 2007; Gasol et al., 2008). Aerobic anoxygenic phototrophic (AAP) bacteria constitute the second most abundant group of photoheterotrophic prokaryotes inhabiting the photic layer of aquatic ecosystems after the proteorhodopsin-based phototrophic (PRP) bacteria (Kolber et al., 2000; Béjà and Suzuki, 2008). AAP bacteria have been reported to be on average larger and more active than the average heterotrophic bacterial cells in the community, and this has led to the hypothesis that AAP bacteria are potentially a fast-growing component of bacterioplankton communities that may be subjected to strong selective losses (Koblízek et al., 2007; Liu et al., 2010; Ferrera et al., 2011; Fauteux et al., 2015; M. Garcia-Chaves et al., 2015). To our knowledge, however, only two studies have tested this hypothesis, one in a single marine site (Ferrera et al., 2011) the other in one freshwater site (Garcia-Chaves et al., 2015). Although both studies support the hypothesis that AAP bacteria grow rapidly and are preferentially grazed, whether this represents a general feature of the ecology and life strategy of AAP bacteria in other aquatic ecosystems has yet to be determined.

Another major outstanding issue is whether the high potential growth exhibited by AAP bacteria is linked to their capacity to derive energy from light. Culture studies have reported that the growth of various AAP bacterial isolates was positively influenced by light (Yurkov and Vangemerden, 1993; Biebl and Wagner-Döbler, 2006; Spring et al., 2009; Hauruseu and Koblizek, 2012), yet the few recent studies that have directly assessed the uptake of substrates by individual cells in natural AAP bacterial assemblages

have failed to observe any light enhancement of AAP cell-specific heterotrophic activity (Kirchman et al., 2014; Stegman et al., 2014; Garcia-Chaves et al., 2016). On the other hand, the observation that the abundances of active or total AAP bacteria seem to respond to light availability (Cuperová et al., 2013; Ferrera et al., 2013; Stegman et al., 2014) suggests that light may play a role in the ecology of these prokaryotes. Whether this role of light is associated with the growth rates of natural AAP bacterial consortia or to other facets of AAP bacteria metabolism remains largely unexplored.

Linked to the issue of the potential effects of light on natural AAP bacterial communities are the questions of how the expression of the light-harvesting pigment (bacteriochlorophyll a) varies under natural conditions, and how this variation may be linked to the activity and growth of AAP bacteria. To date, most of the information related to the variability in the expression of the photosynthesis-related genes comes from experimental studies with AAP bacterial isolates. The results converge to show a tight regulation of the photosynthetic gene expression in AAP bacterial isolates by a combination of nutrient and carbon limitation as well as oxygen and light availability (Yurkov and Vangemerden, 1993; Nishimura et al., 1996; Koblízek et al., 2010; Tomasch et al., 2011). This suggests that the expression of the phototrophic potential may be highly dynamic under natural conditions, both over time and along environmental gradients. However, the quantification of AAP bacterial abundance in natural aquatic systems is still mostly based on the presence of bacteriochlorophyll a (BChl a), an approach that assumes that AAP bacteria always produce this pigment, and few studies have explored the variations in the pigment content of AAP cells that do express this function (Cottrell et al., 2010; Lamy et al., 2011; Ferrera et al., 2013), so our knowledge of the regulation of this photoheterotrophic potential of AAP bacteria is limited. More importantly, no study to date has explored how or whether pigment content relates to the potential to grow of this group within natural communities.

There is clearly a need to better link these various aspects of the physiology, metabolism and ecology of AAP bacteria to improve our understanding of the regulation and the ecological role of this fascinating group in natural aquatic systems. Linking these aspects likely requires combining approaches that have typically been used in isolation. Here, we quantified four key facets of AAP bacterial ecophysiology, all of which are potentially linked to the ecological performance of the group in natural ecosystems: (i) gross growth rates, (ii) grazing mortality, (iii) total and cell-specific pigment dynamics, and (iv) phototrophic potential, the latter determined on the basis of the presence of the pufM gene, which is associated with the phototrophic function (Schwalbach and Fuhrman, 2005). We have measured these aspects in dilution culture experiments under dark and light conditions using water samples from five environmentally contrasting lakes in Québec. We explicitly tested the hypotheses that AAP bacteria have potentially high growth rates and that they are also subject to high selective grazing. We further tested whether light exposure leads to a higher growth of freshwater AAP bacterial consortia, and explored the links between AAP bacterial growth rates and BChl a dynamics. Finally, we explored the coherence in the dynamics of the functional genes associated with phototrophy, and the cells that actually express the function, as a way to gain further insight into the potential ecological role of phototrophy in this group.

3.3 EXPERIMENTAL PROCEDURES

3.3.1 Study sites and sample collection

Water samples for the experiments were collected in five temperate lakes, four of which are located in the Eastern Townships region of southeastern Québec and one in the Laurentian region north of Montréal, Canada (Table 3.1). Each lake was

sampled once during the summer of 2013. We measured the vertical profiles of temperature and dissolved oxygen for each lake, and collected water samples only from the aerobic epilimnetic layer. These profiles were recorded with a Yellow Springs Instruments (YSI) Pro Plus multiparameter probe and underwater profiles of photosynthetically active radiation (PAR) were measured with a LI-COR LI-190 Quantum Sensor. We collected 40 l of lake water in acid-washed, 20 l polycarbonate containers by pumping water from a depth corresponding to an irradiance of 200-300 µmol photons m^{-2} s^{-1}. Samples were kept cold and dark while transported to the laboratory where it was used to carry out regrowth experiments. Back in the lab, total phosphorus (TP), total nitrogen (TN), dissolved organic carbon (DOC) and chlorophyll *a* (Chl *a*) were determined as previously detailed (Garcia-Chaves et al., 2015).

Table 3.1. Physicochemical and biological characteristics of lakes sampled in the present study. Concentration data represent samples collected from the epilimnion.

Table 1

Environmental properties	Bowker	Coulombe	Nicolet	Stukely	Cromwell
Max. Depth (m)	63	9	29	28	12
Sampling depth (m)	6.0	0.5	3.5	2	1
K_{PAR} (m^{-1})	0.31	1.52	0.52	0.53	1.01
Temperature (°C)	21	19	20	24	23
Dissolved Oxygen (%)	110	89	94	97	99
pH	8.2	7.5	7.5	8	7.8
DOC (mg l^{-1})	2.7	14	3.5	5.4	5.7
TP (µg l^{-1})	3.3	16.0	2.7	4.4	4.2
TN (mg l^{-1})	0.15	0.44	0.29	0.20	0.22
Chl*a* (µg l^{-1})	0.8	3.7	0.7	2.6	1.8
BChl*a* (ng l^{-1})	5.94	9.80	8.53	11.56	1.33
Bacterial characteristics					
Total bacteria (10^6 cells ml^{-1})	2.65	2.42	1.38	1.39	1.08
AAP bacteria (10^4 cells ml^{-1})	3.05	6.71	4.16	3.75	0.35
AAP bacteria (% of DAPI counts)	1.15	2.78	3.01	2.67	0.30
Puf copy (10^3 puf gene ng^{-1} DNA)	4.90	7.29	7.80	3.30	n.d
Cell volume total bacteria (μm^3)	0.11	0.13	0.12	0.14	0.11
Cell volume AAP bacteria (μm^3)	0.16	0.17	0.13	0.20	0.13
Biovolume total bacteria (10^5 μm^3 ml^{-1})	2.17	3.15	1.61	1.90	1.21
Biovolume AAP bacteria (10^3 μm^3 ml^{-1})	3.17	10.9	5.39	7.64	0.46
Specific BChl*a* (10^{-1} fg $cell^{-1}$)	1.95	1.46	2.05	3.08	3.75

Abbreviations: K_{PAR}, attenuation coefficient of photosynthetically active radiation; DOC, dissolved organic carbon; TP, total phosphorus; TN, total nitrogen; Chl*a*, chlorophyll *a*; BChl*a*, bacteriochlorophyll *a*, AAP, aerobic anoxygenic photo determined.

3.3.2 Dilution culture setup

We conducted lake water dilution experiments to determine growth and grazing mortality rates of AAP bacteria and the total bacterial assemblage. Two experimental treatments were set up for each of the lakes: (i) a control treatment (CT), consisting of whole unfiltered lake water, and (ii), a reduced predation treatment (PR), consisting of a 1:4 dilution of unfiltered lake water with 0.2 μm-filtered lake water to reduce predator encounter rates.

For all lakes except Cromwell, dilution cultures also included experimental light treatments. In order to assess the influence of light on the growth rates of AAP bacteria and on that of the total bacterial assemblages, CT and PR water samples were incubated either in the light (cycles of 8h darkness and 16h of artificial PAR, at an irradiance of 150 μmol photons m^{-2} s^{-1} provided by UV-free fluorescent lamps (45-W Sun Blaster) or in the dark, attained by covering the incubation bottles with black adhesive contact paper. Dilution cultures from all lakes were performed in triplicate 2.4 L Polyethylene terephthalate transparent bottles and incubated for 3 days at 20± 2°C in a circulating water bath placed in a temperature-controlled room. The rationale to incubate at uniform temperature was to minimize any confounding effects of temperature variations on bacterial growth among experiments. The light intensity in the incubation bath was measured with a HR 4000 spectrometer PAR sensor (Ocean Optics). Cultures were sampled immediately upon setup (time = 0) for initial conditions, and then sampled at intervals of 12 h for 60 h.

At each time point, a 25-ml subsample was taken from the dilution cultures for the microscopic enumeration and biovolume determinations of AAP and total bacterial cells. These samples were fixed immediately after collection with 1% glutaraldehyde (final concentration) and kept refrigerated in the dark until filtration. Samples were

filtered onto 0.2-mm pore-size black polycarbonate filters and immediately stored at -80°C. Total bacteria and AAP cell abundance and cell size were determined from images of 4',6-diamidino-2-phenylindole (DAPI), BChl a (IR autofluorescence), Chl *a* and phycoerythrin-positive cells using an Olympus Provis AX70 microscope, fitted with a charge-coupled-device camera capable of infrared detection (Intensified Retiga Extended Blue; Qimaging), and image analysis software (ImagePro Plus, Media Cybernetics) as described previously (Cottrell et al., 2006; Garcia-Chaves et al., 2015). AAP and total bacterial cell size was determined by image analysis from the DAPI-stained cells using the integration method (Sieracki et al., 1989).

At times zero and 48h, a 40 ml subsample was taken from the dilution cultures for BChl *a* quantification. This analysis was not performed on the dilution culture from Lake Cromwell. The samples were treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, 50 μM final concentration), and BChl a concentrations were measured on an FL3500-FT fluorometer (Photon Systems Instruments) as described previously (Koblízek et al., 2005). The specific BChl *a* content per AAP cell (fg cell^{-1}) was calculated dividing the BChl *a* concentration (fg ml^{-1}) by the corresponding AAP bacterial abundance (cells ml^{-1}).

3.3.3 Quantification of pufM gene copies

Quantitative PCR (qPCR) was used to follow the relative abundance of *puf*M gene copies during the course of the dilution experiments. At time zero, 24h and 60h, a 200-500 ml subsample was taken for DNA extraction and the genomic DNA extracted was used for the subsequent quantification of *puf*M gene copies. This analysis was not performed on the dilution culture from lake Cromwell. Water samples were filtered onto 0.22 μm pore-size filters, and genomic DNA was extracted using the MoBio PowerWater DNA extraction kit (Carlsbad, CA, USA). The 245 base-pair (bp) partial *puf*M gene

target was amplified using the forward (*puf*MF 5'-TACGGSAACCTGTWCTAC-3') (Tm, 50.9°C) and reverse (*puf*M_WAWr 5'-AYNGCRAACCACCANGCCCA-3') (Tm, 62.5°C) primers described in Béjà et al. (2002) and Yutin et al (2005). To confirm the specificities of the primer sets, we examined the target region of forward primer *puf*MF and *puf*M_WAWr in 193 sequences, which included 96 *puf*LM sequences from two clone libraries that we had previously constructed from water samples from lakes Cromwell (sampled in this study) and the nearby lake Croche, from 97 *puf*LM fragments generated by PCR amplification from bacterioplankton in the Delaware River, and from sequences recovered from 43 temperate and boreal lakes in northern Québec (unpublished data). Of these sequences, fewer than 20% and 30% contained more than four mismatches at the 3' end of the primers *puf*MF and *puf*M WAWr, respectively.

Partial *puf*M gene copy abundance was determined in triplicate 15 µl qPCR reactions that contained the following: 1x SsoFast EvaGreen supermix (BioRad, 2x), 3 µl of DNA stock (DNA stocks: 3 ng μl^{-1}), 0.4 µM of each primer (10 µM stock) and sterile, nuclease-free water. Negative controls contained sterile, nuclease-free water and no template DNA. The qPCR assays were run on a CFX96 thermocycler in 96-well plates using the following thermal profile: 2 min at 98°C followed by 40 cycles of 5 s at 98°C, 5s at 58 °C and 5 sec at 72°C.

The number of *puf*M gene copies in each DNA sample was determined by comparing our samples to a serial dilution of standards containing a known number of *puf*M gene copies. Genomic DNA (gDNA) obtained from a cultured AAP strain (*Erythrobacter litoralis* ATCC 700002) was used as standard; the number of *puf*M gene copies in the gDNA serial dilution ranged from 370 to $5x10^6$ copies. Amplification efficiencies of qPCR reactions ranged from 91% to 103%, as determined by the slope of the regression of log copies with threshold cycle values Deriving an absolute number of gene copies in the original sample is in theory possible, but requires knowing the exact amount of

total DNA present in the original water sample and therefore, the accurate estimation of DNA extraction efficiency. Because there is much uncertainly in these two variables, we rather chose to work with the copy numbers of the *puf*M gene in each sample expressed relative to the mass of the initial template DNA added to the qPCR reaction (as copies $ngDNA^{-1}$). We used this as an index of the relative abundance of AAP cells having the potential for aerobic anoxygenic photosynthetic metabolism. Since there is only one copy of the *puf*M gene per AAP bacterial genome, increases in this relative copy number over the course of the experiments would suggest an enrichment in cells containing *puf*M (*puf*M+ cells) relative to the total (provided that the average DNA per cell remains relatively constant), and therefore that the growth rate of *puf*M+ cells is higher than that of the bulk bacterial community. Likewise, decreases in the relative copy number over the course of the incubations would suggest a depletion of *puf*M+ cells relative to the total, which could be associated with either lower AAP bacterial growth rates, or more likely, higher AAP bacterial loss rates. A constant relative copy number would suggest that the loss and growth rates of *puf*M+ cells and those of the total bacterial assemblage are similar, and therefore the proportion of AAP bacterial cells remains constant regardless of whether the absolute numbers are increasing or declining.

3.3.4 Calculation of gross, net growth rates, and grazing mortality rates of AAP and total bacterial cells

Gross growth rates of AAP and total bacteria were derived from the time course measurements of cell abundances in the lake water dilution treatment PR where mortality due to grazers was minimized. Additionally, net growth rates of both bacterial groups were calculated from the changes in abundance in the control treatment (CT, whole unfiltered lake water) under the presence of grazers and virus. Growth rates (d^{-1}) were estimated

under the assumption of exponential growth, as the slope of the regression of the natural logarithm of abundance (N, cells ml^{-1}) vs time (d) in each experimental treatment.

Loss rates due to grazers were estimated for AAP bacteria (L_{AAP}) and for total bacteria (L_{Tot}) as the difference between the net growth rates (μ_N), calculated from the control treatment, and the gross growth rates (μ_G) calculated from the PR treatment as described above: $L_P = \mu_N - \mu_G$ (Equation 3.1)

3.3.5 Rate of change in total BChl*a* concentration, BChl*a* per cell and *puf*M gene copies

The net change in BChl*a* concentration during the incubations was calculated as the slope of the regression of the natural logarithm of the concentration of BChl*a* (ng L^{-1}) vs time (d) in the light and dark PR treatment incubations. This is a net rate since it is the result of two different processes: the BChl*a* production and BChl*a* loss due to AAP bacterial mortality and pigment degradation. Assuming a high photostability of the AAP reaction center pigment and low probability of bleaching (Koblizek et al. 2005), the rates obtained in the PR treatment incubations (when AAP mortality is reduced) will be a function of the growth rate and the pigment content per cell. We calculated also the change in cell-specific BChl*a* content (fg $cell^{-1}$) as the difference in cell-specific BChl*a* content for AAP cells (fg $cell^{-1}$) at the end of the incubation (t = 48h) and their specific pigment content at time zero.

The rate of change in the relative abundance of *puf*M gene copies was derived from the changes in the relative abundance of *puf*M gene copies (copies ng DNA^{-1}) over time in the light and dark PR treatment incubations. The change in *puf*M

copies (d^{-1}) were estimated as the slope of the regression of the natural logarithm pufM gene copies (copies $ngDNA^{-1}$) vs time (d) in the experimental treatment.

3.4 RESULTS

3.4.1 Environmental characteristics and AAP and total bacterial abundance, cell size and biovolume of the sampled lakes.

Dilution culture experiments were carried out using freshwater waters collected at five different lakes that covered a range of DOC (2.7-14 mg l^{-1}), Chl*a* (0.8-3.7 µg l^{-1}) and light transparency (K_{PAR} 0.3-1.5 m^{-1}) (Table 3.1). All lakes were stratified during the sampling period, and epilimnetic water temperatures averaged 21 ± 2°C. At the time of sampling, lakes Bowker, Nicolet and Stukely had a fully aerobic water column, whereas lakes Coulombe and Cromwell had partial anoxia that occupied the bottom of the hypolimnia. However, the high thermal stability of the water column of these two lakes, inferred from the elevated Schmidt stability index values (95 and 150 J m^{-2} for Coulombe and Cromwell, respectively), likely prevented any significant mixing of surface aerobic and bottom anaerobic layers, minimizing the likelihood of upwelling of bacterioplankton from the deep anoxic layers to the metalimnion and eventually to the oxic surface waters where we sampled. We can thus safely assume that the BChl a-containing bacteria and the BChl*a* concentrations observed originate from AAP bacteria, rather than from anaerobic phototrophic bacteria from the hypolimnion. This is further supported by the fact that in a parallel study that assessed the composition of these bacterial communities based on 16S rDNA genes (Clara Ruiz-González pers. com.), we found very few sequences related to potential anaerobic phototrophic bacteria (0.006 to 0.05% of total sequences), whereas the relative number of sequences associated with potential AAP bacterial

genera matched well with our microscopic estimates of AAP bacterial abundance.

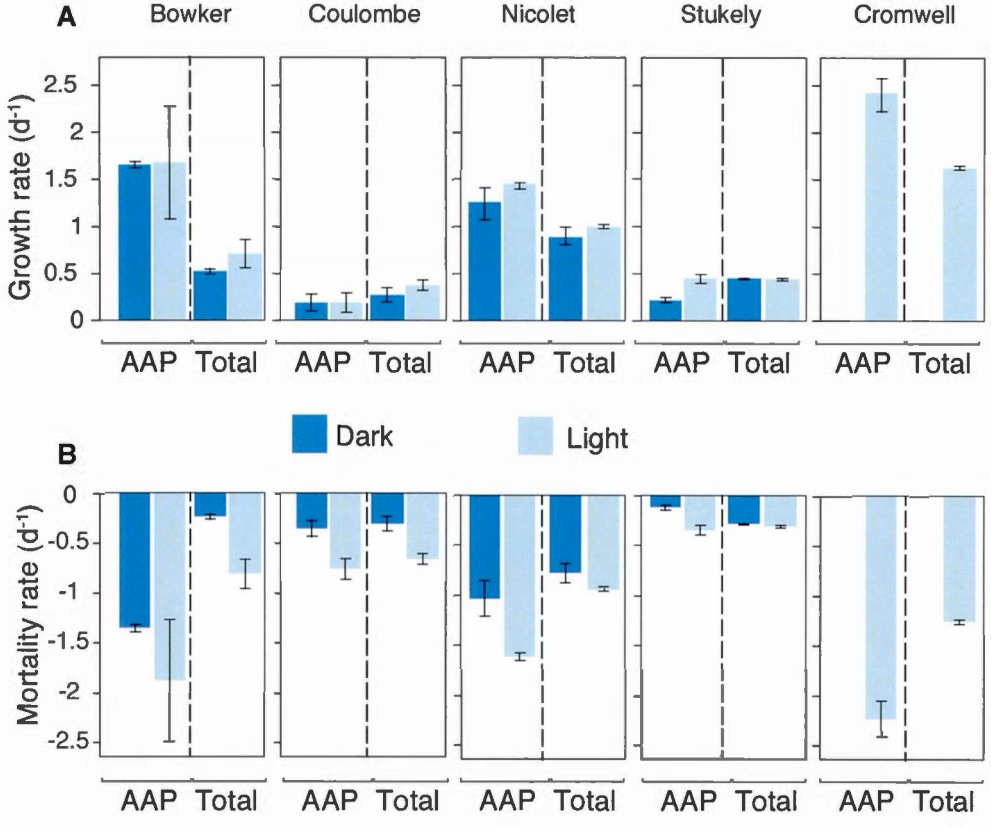

Figure 3.1. Gross growth rates of AAP bacteria (AAP) and total bacteria (Total) derived, for each lake, from the changes in cell abundance during light and dark incubations in the predator-reduced (PR) treatment (a). Grazing mortality rates of AAP and total bacteria, calculated as the difference in growth rates (day^{-1}) between control (CT) and PR treatments in light and dark incubations (b, see Materials and Methods for details). Error bars represent the standard error of the mean for three replicated incubations per lake.

The abundance of total bacterial cells ranged from 1.1 x 10^6 to 2.7 x 10^6 cells ml^{-1} among the lakes (Table 3.1). The relative abundance of AAP bacteria, enumerated by infrared epifluorescence microscopy, varied between 0.3 and 2.8% of total DAPI counts, while the relative abundance of *puf*M gene copies quantified by qPCR

ranged from 3.3 to 7.8 x 10^3 copies per nanogram of total genomic environmental DNA (Table 3.1). The cell size of AAP bacteria was on average 20% higher than that of the bulk bacterial community (Table 3.1). Total bacterial biovolume, calculated as the product of cell size and abundance, varied less than 3-fold (1.2- to 3.2 x 10^5 μm^3 ml^{-1}), whereas the total biovolume of AAP bacteria varied 10-fold (0.4 to 5.4 x 10^3 μm^3 ml^{-1}, Table 3.1). When all lakes were considered together, AAP cells contributed on average 2.7% to total bacterial biovolume.

Table 3.2. Summary of minimal and maximal growth rates, grazing mortality rates and relative abundance of AAP bacteria (AAP) obtained from marine and freshwater systems. NR, no data reported.

System	Approach	Growth rate AAP bacteria (d^{-1})	Grazing mortality rates AAP (d^{-1})	Relative Abundance (%)	Reference
Temperate lakes	Re-growth	0.33-2.41	0.07-3.3	0.3-3	This study
Freshwater Mesocosms	Re-growth	2.1-2.2	2.5-2.6	0.6-0.8	Garcia-Chaves et al., 2015
Mediterranean coast	Re-growth	2.38-3.71	1.4-1.8	5-7	Ferrera et al., 2011
Baltic sea	Diurnal decay in BChl*a*	0.7-2	NR	3-10	Koblízek et al., 2005
Sargasso sea	Diurnal decay in BChl*a*	0.91-1.03	NR	1.9-4.3	Koblízek et al., 2007
Atlantic equatorial ocean	Diurnal decay in BChl*a*	1.32-1.68	NR	0.8-18	Koblízek et al., 2007
North Atlantic ocean	Diurnal decay in BChl*a*	1.44-2.13	NR	NR	Koblízek et al., 2007
Catalan sea	Diurnal decay in BChl*a*	1.15-1.42	NR	2.6 – 4.2	Hojerová et al., 2011
Mediterranean bay	Diurnal decay in BChl*a*	2.35-3.01	NR	6.8-11	Koblízek et al., 2007
China sea	Frequency of dividing cells	0.5-2.9	NR	2-2.6	Liu et al., 2010

3.4.2 Potential growth and mortality of AAP bacteria and the influence of light.

The gross growth rates determined in dilution experiments of total and AAP bacteria varied among the lakes. Total bacterial growth rates varied from 0.3 to 1.6 d^{-1} (mean 0.68 d^{-1}, Table 3.2), whereas AAP bacterial growth rates were 2-fold more variable than that of the total bacterial community, ranging from 0.2 to 2.4 d^{-1} (mean 1.03 d^{-1}, Table 3.2). AAP bacteria did not always exhibit higher growth rates than the bulk bacterial community; whereas in the oligotrophic lakes Bowker and Nicolet, and in the mesotrophic lake Cromwell the growth rate of AAP bacteria was significantly higher than that of the total bacterial assemblage (paired t-test, combining light and dark incubations, $p<0.05$), in lakes Stukely and Coulombe AAP growth rates were similar to those of the whole bacterial assemblage (Fig. 3.1a). Overall, however, the mean growth rate of AAP bacteria was 1.5-fold higher than the mean growth rate of the whole community (paired t-test, combining light and dark incubations, $p<0.05$).

Total and AAP bacterial mortality rates also differed widely between lakes (Fig. 3.1b). The lowest mortality rates were observed in the mesotrophic lake Stukely with mean absolute values of 0.23 and 0.29 d^{-1} for AAP and total bacteria, respectively. Mortality rates were highest in the oligotrophic lakes Bowker and Nicolet. In the mesotrophic lake Cromwell mean mortality rates varied from 1.38 to 2.23 d^{-1} for AAP and from 0.58 to 1.24 d^{-1} for total bacteria. The mortality rates of AAP bacteria were significantly higher than those of the total bacterial assemblage in all lakes but Stukely, where the mortality rates of AAP bacteria did not differ from that of the total bacterial community (Fig. 3.1b). Overall, the grazing mortality rate of AAP bacteria was almost 2-fold higher than that of the total bacterial assemblage, and both were significantly different (paired t-test, combining light and dark incubations, $p<0.02$).

We further assessed the potential impact of light on the gross growth and grazing mortality rates of AAP and the total bacterial assemblage in 4 out of the 5 lakes. There was no significant difference in the gross growth rate of AAP bacteria or total bacteria between light and dark incubations (Fig. 3.1a). The mortality rates of AAP bacteria and that of the total bacterial community were significantly higher in light than in dark incubations in all lakes but Bowker, where the mortality rates of AAP in light did not differ from those at dark conditions (Fig. 3.1b).

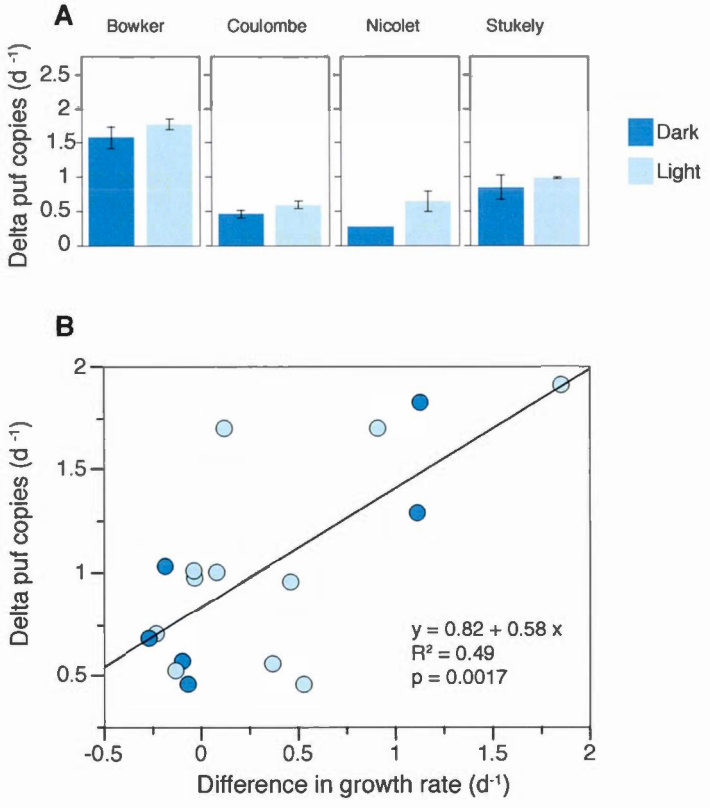

Figure 3.2. The rate of change in the relative abundance of the pufM gene copies during light and dark predator-reduced (PR) incubations. Error bars represent the standard error of the mean for three replicated incubations (a). Relationship between the rate of change in the number pufM gene copies and the difference in growth rates between AAP bacteria and the total bacterial community (b). This difference was calculated as the average gross growth rate of the pigmented AAP bacteria minus the average gross growth rate for the total bacterial community derived for each replicated light or dark incubation.

3.4.3 Dynamics in *puf*M gene copy numbers

It is possible under natural conditions that AAP cells do not express enough pigment to be detected by epifluorescence microscopy. To explore the potential covariation between cells that contain the gene and cells that produce the pigment, we also quantified the specific rate of change over time in the relative abundance of the *puf*M gene copies during the light and dark dilution incubations. This rate of change of *puf*M copies was always positive and varied among lakes from 0.5 to 1.7 d^{-1}. Again, we found no significant differences in these rates of change between light versus dark incubations (Fig. 3.2a). Interestingly, the variation in the rate of change in relative *puf*M copy number was positively correlated with the difference in the gross growth rate between AAP and total bacteria (Fig. 3.2b), suggesting that this apparent enrichment in *puf*M+ cells was the result of the higher average growth rates of AAP bacteria relative to the total bacterial assemblage. This result further suggests that there is a close coupling between the presence of the functional gene and the expression of this function in AAP cells.

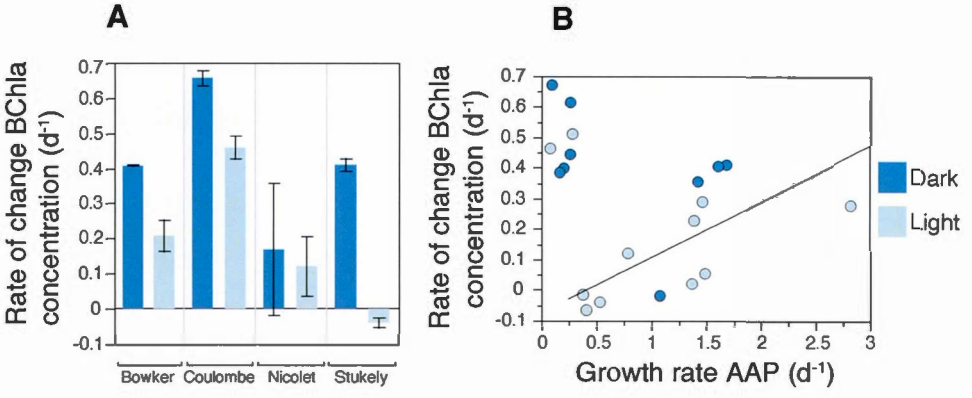

Figure 3.3. Rate of change in the BChla concentration derived for each lake from changes in pigment concentration during light and dark predator-reduced (PR) incubations (a) and its relationship with the growth rate of AAP bacteria (b). The solid line is a linear regression fit through data of lakes Nicolet and Bowker ($P<0.05$). Error bars represent the standard error of the mean for three replicated incubations.

3.4.4 Total BChl*a* and cell-specific BChl*a* dynamics and their relationship with growth rate.

In order to explore the links between growth, pigment production and light in AAP bacteria, we analyzed the changes in BChl*a* concentration (ng l^{-1}) along the time course of the light and dark dilution incubations. We observed positive values for the rates of change in BChl*a* in all lakes except in Stukely under light conditions (Fig. 3.3a), and in all cases, the rates of change were higher in the dark than in the light incubations. The rates were highest in the dark incubations of the high DOC and highly colored lake Coulombe (0.65 d^{-1}), and the lowest in the light incubations of the mesotrophic lake Stukely and in the oligotrophic lake Nicolet (-0.04 and 0.17 d^{-1}, respectively, Fig. 3.3a). The rate of change in BChl*a* concentration was positively related to the mean gross growth rates of AAP bacteria in lakes Bowker and Nicolet, but not in lakes Stukely and Coulombe ($r = 0.52$ $p = 0.0015$; Fig. 3.3b). Interestingly, we observed that the pigment content per cell declined greatly during the incubations with samples from the oligotrophic lakes Bowker and Nicolet (data not shown), and the change in cell-specific BChl*a* content was negatively correlated with AAP growth rates (Fig. 3.4a). The average cell specific pigment contents measured at the end of the experimental manipulations were not significantly correlated with the estimated AAP growth rates (Fig. 3.4b).

3.5 DISCUSSION

Our results indicate that freshwater AAP bacteria have highly variable growth rates that on average exceeded those of the total bacterial assemblage, but not in all cases. Consistent with our results, the few previous studies that have assessed AAP bacterial growth rates have shown that AAP bacteria have the potential to grow two to four times faster than the total bacterial community (Table 3.2). Interestingly, however,

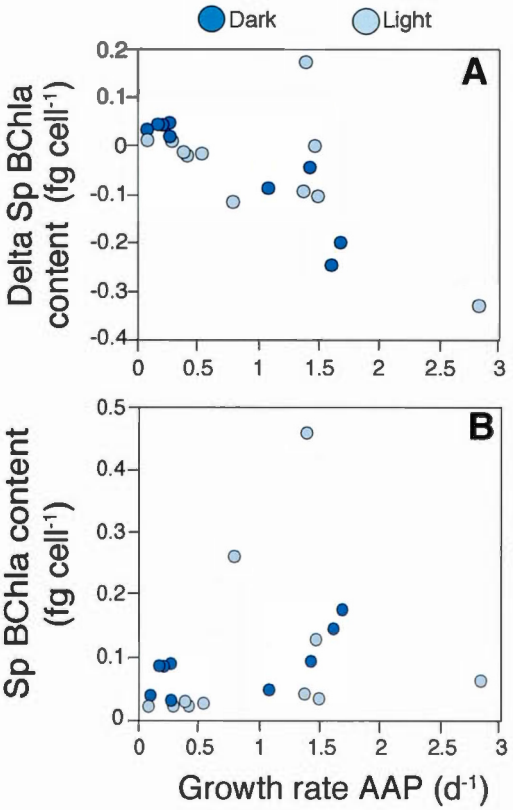

Figure 3.4 Change of cell-specific BChla content (a) and cell-specific BChla content (b) as a function of the growth rate of AAP bacteria. The plots comprise data estimated from lakes Bowker, Coulombe, Stukely and Nicolet in light and dark incubations. The delta of cell-specific BChla content was calculated as the average cell-specific BChla content at the end of the incubation (t = 48h) minus the average cell-specific pigment content at time zero for each replicated light or dark incubation. The cell-specific BChla content plotted in (b) corresponds to the average cell-specific BChla content at the end of the incubations (t = 48h).

these high growth rates do not translate into high cell densities, since the relative abundance of AAP bacteria in our study lakes never exceeded 3% (Table 3.1). Previous studies have also shown that AAP bacteria are consistently a small fraction of the total bacterial community across fresh, estuarine, and marine waters (Fauteux

et al., 2015; Koblížek, 2015). One plausible explanation is that these bacteria do not attain higher abundance because they are subjected to strong top-down regulation.

In support of this hypothesis, we observed that the mortality rates of AAP bacteria were on average two-fold higher than those measured for the bulk bacterial community, although again this varied greatly among lakes (Table 3.2). This is in agreement with the only two previous studies that have specifically quantified mortality rates of marine and freshwater AAP bacteria, which also showed that these bacteria are subjected to a stronger grazing pressure than the total bacterial community (Ferrera et al., 2011; Garcia-Chaves et al., 2015). It is well known that protistan grazing can be highly selective, and that the underlying basis of this selectivity is mostly prey cell size and activity (Jürgens and Massana, 2008; Sintes and Del Giorgio, 2014). In this regard, the larger AAP cell sizes (Fauteux et al., 2015; Koblížek, 2015; Sieracki et al., 2006) and the high single-cell activity of AAP bacteria (Kirchman et al., 2014; Stegman et al., 2014; Garcia-Chaves et al. 2016) may explain the preference of grazers for these photoheterotrophic bacteria. Our own results suggest that grazing might strongly influence net ambient growth rates of AAP bacteria, and also potentially their abundance and size structure, since there was an overall negative relationship between the average loss rates in our incubations and the relative AAP bacterial abundance and AAP cell size in the lakes (Fig. 3.5). This strong top down regulation constitutes a relevant link between AAP growth and the microbial food web, and implies a disproportionate role of AAP bacteria in the process and transfer of organic matter through lake food webs. If light harvesting were an important aspect of AAP bacterial metabolism, we would expect that most of the growing AAP cells would express their phototrophic potential and synthesize BChl*a*. We explored this hypothesis by comparing AAP bacterial dynamics revealed by microscopic counts, which relies on the presence of the pigment, and by *puf*M gene copies, which represent phototrophic potential (Schwalbach and Fuhrman, 2005; Du et al., 2006). Interestingly, we found that regardless of the

light condition, the change in the number of *puf*M gene copies was always positive, implying that total DNA became enriched in *puf*M copies with time. The observed increase in the proportion of pufM copies could have been due simply to a decrease or even to a constant amount of total DNA over time in the experiments. Considering that the total bacterial community did indeed grow, total DNA could remain constant or decrease only if the amount of DNA per cell decreased over time. However, the cell size of the total bacteria increased and so did the total DNA concentration during the experiments (Supplementary Fig. S3.1). Therefore, a more plausible explanation is that the enrichment in pufM copies was due to a higher growth of the AAP bacteria compared to the total bacterial assemblage. If the growing AAP cells were all expressing their phototrophic potential, we could expect the responses at the level of *puf*M gene copies to match those in AAP abundance based on the presence of the pigment, which is indeed what we observed; there was a positive correlation between changes in the rate of change in pufM copies and the difference in the growth rate for

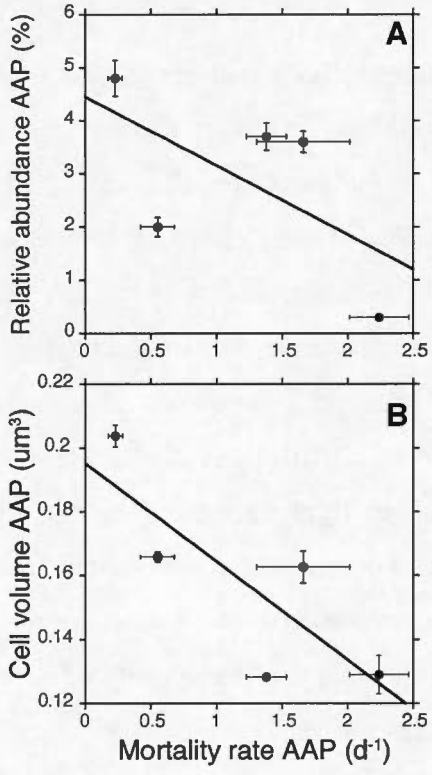

Figure 3.5 Relationship between the mean grazing mortality rates of AAP bacteria and their relative abundance (a), and their mean cell size (b) in the ambient lake water. The solid line is a linear regression fit through all data. Error bars represent the standard error of the mean.

AAP bacteria and the bulk community based on microscopic counts (Fig. 3.2b). This suggests that most *puf*M+ cells must have been expressing phototrophic functions, which in turn suggests some level of ecological advantage of this function regardless of light and other environmental conditions. Since the advantage of expressing *puf*M is likely linked to light, it becomes important to disentangle the potential influence of light on pigment dynamics, metabolism and growth of AAP bacteria. The simultaneous presence of light, BChl*a* biosynthesis intermediates and oxygen results in the generation of toxic reactive oxygen species. To minimize this negative effect, AAP cells are able to down-regulate the synthesis of pigments and the photosynthetic apparatus (Nishimura et al., 1996; Tomasch et al., 2011; Koblížek, 2015). In support of this idea, and in agreement with previous results (Koblízek et al., 2005, 2007), we observed that the rate of change of BChl*a* concentration not only varied greatly among lakes, but was overall significantly higher in dark than in light conditions (Fig. 3.3a), suggesting that the synthesis of BChl*a* is down-regulated in the presence of light.

Although there was a clear effect of light on pigment dynamics during the dilution incubations, we observed an intriguing lack of effect of light on the gross growth rates of AAP bacteria. A possible explanation is that the higher intrinsic growth rates of AAP bacteria may not be directly related to their capacity to derive energy from light. Indeed, previous studies that have experimentally exposed freshwater and marine bacterial communities to light and dark conditions have also failed to demonstrate any light stimulation of the uptake of leucine by natural AAP bacterial assemblages (Kirchman et al., 2014; Stegman et al., 2014; Garcia-Chaves et al. 2016). Field studies, on the other hand, have demonstrated an apparent link between light and several aspects of the ecology of AAP bacteria in ambient communities. For example, studies conducted in Mediterranean coastal waters reported a positive correlation of AAP abundance and day length (Ferrera et al., 2013). Moreover, recent assessments of the activity of AAP bacteria in the Delaware estuary and in temperate lakes showed that the percentage of

active AAP cells was negatively correlated to water column light attenuation, suggesting that the proportion of active AAP cells may be enhanced by light (Stegman et al., 2014; Garcia-Chaves et al., 2016). Indeed, in a previous study we found that the exposure to light seemed to trigger the passage of AAP cells from the inactive to the active pool, suggesting a role of light in the activation of AAP bacteria (Garcia-Chaves et al., 2016). We therefore hypothesize that whereas light-derived energy may have little direct effect on growth, at least in short term incubations, it may nevertheless positively influence other relevant aspects of AAP cellular metabolism and ecological performance.

Although light did not appear to directly influence AAP growth, we did observe a link between the pigment dynamics and cell growth in our dilution incubations. In two of the lakes (oligotrophic lakes Nicolet and Bowker) there was a significant positive relationship between AAP gross growth rates and change in BChl*a* concentration, whereas in mesotrophic lakes Stuckely and Coulombe, where AAP bacterial growth rates were the lowest, there was no relationship between growth and pigment production rate (Fig. 3.3b). This lack of relationship cannot be explained by a differential effect of light on BChl*a*, because there was no difference between light and dark treatments, and one plausible explanation is that different phylogenetic groups within the AAP bacterial guild may have different functional links between growth and pigment dynamics. Previous reports showed that composition of AAP bacterial communities is highly variable in freshwaters, with individual AAP bacterial groups having specific environmental preferences (Salka et al., 2011; Cuperová et al., 2013). Lehours and colleagues (2010) found that meso-eutrophic and oligotrophic sites in the Mediterranean were dominated by distinct AAP phylogenetic groups, and Ferrera and colleagues (2013) linked the seasonal dynamics in cell specific pigment content in a coastal Mediterranean site to the seasonal AAP succession. The fact that AAP bacteria appeared to intrinsically grow slowly in lakes Stukely and Coulombe, with high rates of BChl*a* production, and that the AAP community in lakes Bowker and Nicolet were

intrinsically fast growing with lower associated rates of BChl*a* production, suggest that oligotrophic and mesotrophic lakes may be dominated by different phylogenetic AAP groups which differ in life strategies and cell-specific pigment regulation.

Interestingly, we found a significant inverse relationship between the change in cell-specific BChl*a* content during incubations and the corresponding AAP growth rates, with cellular pigment content declining greatly in samples that had the highest growth rates. This observation suggests that BChl*a* losses exceeded BChl*a* production in these lakes. It was surprising to observe declines in cell specific BChl*a* content even in the dark incubation because degradation processes such as non-photochemical quenching or pigment bleaching are known to be light dependent (Koblízek et al., 2005). An alternative explanation is that the pigment synthesis can be modulated depending on the growth conditions, and that under environmental conditions that promote high growth rates, cells invest proportionally less in pigment production or turnover than in conditions of low growth. As a result, there was only a weak relationship between growth rates and the average cell specific pigment content in these manipulations, suggesting that high growth rates may be associated with high and low cell pigment contents, independent of the short term light exposure.

The production and turnover of the photosynthetic apparatus is associated with relatively high metabolic costs (Kirchman and Hanson, 2013), including the cost of biosynthesis and down regulation during diurnal light/dark cycles (Tomasch et al., 2011). Culture studies have demonstrated that pigment expression in AAP bacteria is facultative (Yurkov and Vangemerden, 1993; Nishimura et al., 1996; Biebl and Wagner-Döbler, 2006; Koblízek et al., 2010; Tomasch et al., 2011), and it would be expected that in circumstances where the photosynthetic system becomes a net energy sink, for example under extended periods of darkness, cells would stop its expression. Our results based on the *puf*M gene suggest, however, that most AAP

cells express the pigment to some degree, and this in turn would suggest that the benefit of maintaining the photosynthetic machinery must outweigh its energetic cost. There was indeed an overall positive relationship between the average cell specific pigment content and growth rates. This is particularly intriguing since our results suggest that there is no measurable pigment-mediated light effect on growth. One possible explanation is that the amount of energy gained by AAP cells from phototrophy is probably too small to quantitatively detect in a short-term experiment (Kirchman and Hanson, 2013). According to previous estimations, the expected light enhancement of AAP growth per hour would be around 2% (Kirchman et al., 2014); a difference which is too difficult to be measured in our dilution incubations.

These results allow us to begin to piece together the potential links that exist between AAP growth, regulation and photosynthetic potential, and collectively suggest a scenario that deviates somewhat from current models. We have found that there was a strong coherence in the dynamics of cells that carry the phototrophic potential (*puf*M+ cells) and the cells that express this potential by producing pigment, and this suggests that phototrophy is widely expressed and therefore that it may confer a consistent ecological advantage. The nature of this advantage is still unclear, however. Our results confirm that light has a direct effect on AAP pigment dynamics, with BChl*a* production apparently being down-regulated under light exposure, and there is further evidence of multiple strategies concerning the regulation of cellular pigment content among AAP bacterial communities. This scenario suggests that pigment production and turnover may support a cellular function linked to metabolism, protection or survival, which may be indirectly linked to growth. In fact, our results suggest that pigment dynamics during these dilution incubations may be at least partly regulated by the magnitude of growth, and not vice-versa. Under this scenario, there may be tradeoffs between growth and the expression and maintenance of the phototrophic apparatus, which complicates the use of BChl*a* dynamics as a proxy of AAP growth (Koblízek et al., 2005, 2007;

Hojerová et al., 2011). The nature of the ecological advantage conferred by the phototrophic function, and the bioenergetics and ecological tradeoffs associated with this function remain to be understood, but our results suggest that the higher intrinsic growth potential of this group may be somehow linked to this phototrophic function.

3.6 ACKNOWLEDGMENTS

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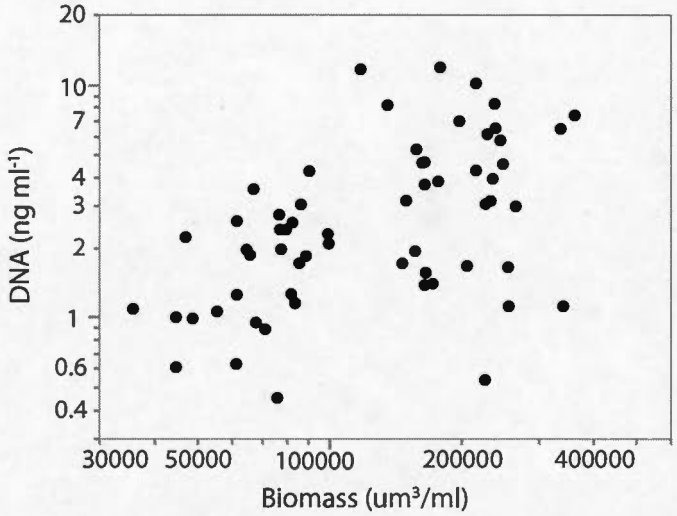
Supplementary Figure 1



Supplementary Figure S3.1 Relationship between bacterial biomass and the DNA concentration.

GENERAL CONCLUSIONS

4.1 Main contributions

The main contribution of this thesis is that it provides a comprehensive view of the ecology of freshwater aerobic anoxygenic phototrophic (AAP) bacteria, which, although believed to be key players of aquatic ecosystems, had been much less studied than their marine counterparts. We have used an innovative experimental approach that allowed us to assess and to piece together unknown aspects of the physiology, the metabolism and the ecology of freshwater AAP bacteria to improve our understanding of the regulation and the ecological role of this peculiar group of bacteria in freshwater ecosystems. Using a combination of tools that includes infrared epifluorescence microscopy, kinetic fluorometry, microautoradiography and qPCR, we investigated the regulatory processes underlying the patterns in the abundance, activity, growth, cell-size and mortality of AAP in lakes. By carrying out mesocosms and re-growth experiments we were able to address, for the first time, the role of freshwater zooplankton and protist in regulating the biomass, abundance, cell size and net growth of AAP bacteria. An important part of this work was devoted to better understanding the role of light in regulating the activity, the potential to grow, the pigment dynamics and the *puf*M gene dynamics of AAP bacteria, since although they are the second most abundant groups of prokaryotic photoheterotrophs, the influence of light on the ecology of AAP remains obscure. The simultaneous exploration of the presumed most important regulatory processes of AAP and their patterns in cell size, abundance, activity, growth, pigment and *puf*M gene dynamics allowed us to describe the links between different ecophysiological aspects of AAP with light and grazing pressure as depicted in figure 0.1. Most of these links had never been explored before within natural communities

and, therefore, this thesis provides an original assesment of the interactions between the environment, both physical and biological, and the physiology of freshwater AAP bacteria.

In particular, the following key elements emerge from each chapter:

1. Freshwater AAP bacteria, similar to their marine counterparts, exhibit higher growth rates than the bulk bacteria community. In spite of their fast growth, AAP are not a dominant component of the freshwater communities since their abundance is strongly regulated by grazers. Moreover, this top-down regulation on AAP seems to differ from that in marine systems since AAP are not only preferentially predated by protists but also by metazooplankton. These different grazing pathways have implications for the potential trophic role of AAP bacteria at the ecosystem level that need to be further explored.

2. In accordance with their higher growth rates, freshwater AAP cells are also on average more active than an active cell of the total bacterial community. Moreover, since the fraction of active AAP cells is also higher than that of the bulk bacterial community, their potential contribution to total biomass production is disproportionately higher than their contribution to total bacterial abundance. The high variation in the heterotrophic activity of freshwater AAP cells is only partly explained by light availability, since light does not have a clear effect on their cell-specific activity but it does appear to influence positively the proportion of active AAP bacteria.

3. The last chapter integrates basic elements of the former two chapters. We explored the regulation of light and mortality on key eco-physiological aspects of AAP bacteria. By these means, we propose a new scenario of the potential links between

light, activity, growth and pigment production, and corroborate empirically the role of grazers on regulating the abundance and the cell size of natural AAP communities. In this new scenario, we propose that although light did not appear to have a direct influence on AAP growth, the photosynthetic function appears to provide an ecological advantage to AAP cells that we still ignored. This phototrophic function appears to be somehow linked to growth in a way that the factors that regulate the growth may indirectly regulate the cell pigment content.

4.2 Novel insight and implications

This thesis provides the only experimental study so far conducted in freshwater ecosystems with the aim to elucidate diverse aspects of these prokaryotes, as well as the mechanisms that control the activity and the abundance of AAP bacteria in freshwater systems. In spite of the accumulating knowledge regarding the abundance and the diversity of freshwater AAP bacteria, nothing was known about their role in the freshwater environment. By using this approach, we moved beyond the abundance data and assessed the growth, the single-cell activity, the mortality and the pigment production of this functional group, and therefore this new approach allowed us to further expand the current knowledge of the role of AAP in freshwater ecosystems.

The results from chapter I and III support the only previous marine study (Ferrera *et al.* 2011) that indicates that AAP are subjected to a stronger top-down control by grazers than the average bacteria, although mortality rates varied largely among lakes, ranging from 0.2 to 2.5 d^{-1}. This preferential predation of AAP may be due to their higher cell sizes and higher gross growth rates, since previous studies have shown that grazers preferentially predate on active and large bacteria (del Giorgio *et al.*, 1996; Jürgens and

Massana, 2008). Based on the results from chapter III, we conclude that this mechanism may be responsible for the observed patterns of low AAP relative abundance, the cell size structure of AAP communities, and therefore the net growth of the AAP in the natural environment. In addition, the results from chapter I indicate that this regulation may be stronger in freshwaters than in marine environments, since AAP cells are not only preferentially grazed by protists but also by cladocerans, which are the dominant members of crustacean zooplankton in lakes but not in the sea (Sommer and Sommer, 2006). Although this preferential predation of AAP bacteria by grazers had been previously postulated, the only previous evidence was based on the results reported by one marine study (Ferrera *et al.* 2011). Therefore, our results provide the first empirical evidence that this phenomenon may also be common in freshwater ecosystems, and further highlight that, due to this unexplored regulation of AAP bacterial biomass by zooplankton predation, AAP bacteria may play unique and disproportionate roles in the trophic transfer of organic C in lake food webs. Whether this transference of carbon is more efficient in freshwater systems than in the ocean, is an interesting question that emerges from this study, which could be eventually answered with further estimates on the contribution of marine and freshwater AAP to total zooplankton production.

It has been hypothesized that these high growth rates of AAP could be explained by the light harvesting capacity of AAP (Stegman *et al.*, 2014). In order to test this hypothesis, we carried out the first assessment of the single cell heterotrophic activity of AAP in freshwater ecosystems, as well as several experimental estimations of the gross growth rates of these bacteria under dark and light conditions. This allowed us to directly estimate, for the first time, the light-driven activity, the growth responses to light of natural AAP communities as well as their contribution to bacterial biomass production in freshwater ecosystems. The results from chapters II and III are in accordance with previous studies showing that AAP bacteria tend to grow faster, and are more active and larger than the bulk bacterial community (Fauteux *et al.*, 2015; Ferrera *et al.*, 2011;

Hojerová *et al.*, 2011; Stegman *et al.*, 2014). However, the intriguing absence of light stimulation on the uptake of radioactive leucine by natural AAP assemblages, shown in chapter I and by previous marine studies (Kirchman *et al.*, 2014; Stegman *et al.*, 2014), as well as the lack of effect of light on the gross growth rates of AAP communities observed in chapter III, preclude concluding whether there is a direct contribution of the phototrophic pathway to the growth and activity of AAP bacteria. We observed in the experiments, however, that light seems to trigger the passage of AAP cells from the inactive to the active state, and we also found that the percent of active AAP bacteria did have a negative correlation with light attenuation. This, together with previous observations that the proportion of active AAP increase along a gradient of water transparency in estuarine waters (Stegman *et al.* 2014), suggest that light may indeed play a role in the ecology of AAP, although the nature of this role remains unclear.

If light harvesting is an important aspect of AAP bacterial metabolism, a deeper understanding of the regulation of BChl*a* production may provide insight into alternative mechanisms involved in AAP responses to light. The results from chapter III suggest that regardless of the variability in environmental conditions, the dynamics of the *puf*M containing bacteria and the AAP cells actually expressing the pigment are relatively similar. This indicates that both pools of AAP cells are not differentially regulated, which may suggest that most AAP cells are indeed expressing the phototrophic function and thus that AAP cells likely benefit from the phototrophic function. The highly dynamic BChl*a* synthesis observed in our experiments agrees with previous *in situ* observations (Cottrell *et al.*, 2010; Koblízek *et al.*, 2007; Koblízek *et al.*, 2005), and indicates that the benefit of maintaining the photosynthetic machinery should outweigh its energetic cost, which in turn suggests that light harvesting is an important aspect of AAP bacterial metabolism. One possible explanation could be that pigment production may not support in itself a function that is directly linked to growth, but rather contributes to some other cellular function linked to the metabolism that may be

indirectly linked to growth, such as the regulation of the expression of the large enzyme inventory typically found in AAP members (Fuchs *et al.*, 2007; Koblízek *et al.*, 2010; Yurkov and Csotonyi, 2009), or the synthesis of the abundant amounts of carotenoids hypothetically associated with protection from phototoxicity (Beatty, 2002; Yurkov and Csotonyi, 2009). Certainly, while the advantages of possessing the photosynthetic function need to be further investigated, this new working hypothesis may lead future studies to explore aspects of the metabolic versatility of AAP bacteria tat have not been addressed to date.

Paradoxically, the variability in cell-specific BChl*a* content and the factors that explain its natural variation are among the most underexplored aspects of the ecology of AAP. Therefore, a significant contribution of this thesis emerges from the exploration of BChl*a* dynamics and how this variation may be linked to the activity and growth of AAP cells. The results of chapter III support the scarce evidence that natural AAP communities show a large variability in the cell-specific BChl*a* content (Cottrell *et al.*, 2010; Fauteux *et al.*, 2015; Ferrera *et al.*, 2013; Lamy *et al.*, 2011), but, more importantly, suggest that pigment dynamics is at least partly regulated by the magnitude of growth, which implies that the factors that regulate the activity and growth of AAP bacteria will influence also the pigment content of the AAP cells in their natural environment. Perhaps what is even more interesting and, in some respect, surprising, is that the production of the pigment is apparently modulated in such a way that fast-growing cells tend to produce proportionally less pigment than slowly growing cells. From a physiological point of view, our results imply, in accordance to previous studies (Cottrell *et al.*, 2010 ; Fauteux *et al.*, 2015) that the pigment content per cell may not be controlled by the light availability, but may be influenced by other factors, likely nutrients or DOC, that are known to regulate the heterotrophic activity of the bacterial cells. From an ecological point of view, these results imply that the conditions that favor the phototrophy in AAP may be not the same that favor their growth and in fact

can even be completely the opposite. This issue raises the question of which aspects of AAP bacteria should be considered in order to inquire into the significance of their ecological role.

Despite 15 years of efforts aiming to demonstrate the trophic and biogeochemical relevance of AAP bacteria in the aquatic carbon cycle, quantitative estimates of the contribution of AAP bacteria to ecosystem processes were still missing. This may be in part due to the fact that most of the information on the spatial and temporal distribution of AAP bacteria in marine and freshwater environments, as well as the factors regulating their dynamics across environmental gradients, has been obtained from surveys of AAP abundances (Koblížek, 2015). Our experimental approach allowed us to explore not only the abundance but also other aspects of these prokaryotes that may be more directly linked to the role of AAP in natural communities, and thus this thesis provides the first actual attempt to estimate quantitatively the significance of AAP in aquatic ecosystems. By these means, in Chapter II we demonstrated a decoupling between the abundance and activity patterns of AAP bacteria, which indicates that the abundance and activity patterns of AAP bacteria are only loosely coupled. This suggests that the factors that control the abundance of AAP bacteria do not necessarily coincide with the ones involved in the regulation of their activity and growth, and implies that conclusions about the ecological role of AAP bacteria cannot be based on abundance or on activity patterns alone. Furthermore, by quantifying the incorporation of radioactive leucine by individual AAP cells from natural lake communities, we provide, in chapter II, the first estimate of the potential contribution of AAP to total bacterial biomass production. We show that despite their very low abundances, AAP bacteria may contribute from 5% to 17% of total bacterial biomass production, supporting the idea that AAP bacterial communities must be relevant players of aquatic microbial food webs.

In order to transpose these experimental findings to a natural context, we did the

exercise of estimating the contribution of AAP bacteria to two key lake ecosystem processes: zooplankton production and CO_2 fluxes (Fig. 4.1). For this purpose, we used the information available for Lac Croche, a reference lake that has been the object of intense investigation for decades, located in the temperate region of Québec at the Station de Biologie des Laurentides of the Université de Montréal (www.sbl. umontreal. ca/ index.html). We exploited the advantage of having access to zooplankton production and CO_2 flux data that have been measured on this lake by a number of researchers, together with our own data on DOC concentration, AAP abundances, and bacterial biomass production from a seasonal survey carried out in this lake (for details see the section Approach in the Introduction). We derived from the average summer AAP abundances in lake Croche an AAP contribution to biomass production using the relationship observed in Chapter II, and used the AAP mortality rates by protists and zooplankton measured in Chapter I from nearby lake Cromwell to determine the fate of the organic carbon consumed by AAP bacteria. This exercise shows that, in spite of the fact that AAP bacteria represent only 2% of total bacterial abundance in lake Croche, they may disproportionately contribute to the overall bacterial production in the lake (6.5%), may contribute significantly to the total zooplankton production (5.2%), and their respiration may be responsible for up to 2% of the CO_2 emissions per day in the lake (Fig. 4.1).

We acknowledge that these are point estimates, and considering the high variability in the patterns of single-cell activity, growth and mortality rates of AAP bacteria observed among the limited number of lakes surveyed in this study, we may expect that the magnitude of these fluxes and the relative contribution of AAP bacteria to lake ecosystem processes will vary largely both temporally within this ecosystem and also across different lakes. Although light would be one of the factors that one might expect to explain this potential variability, our results from Chapter II show that the variability in the contribution of AAP bacteria to total bacterial production

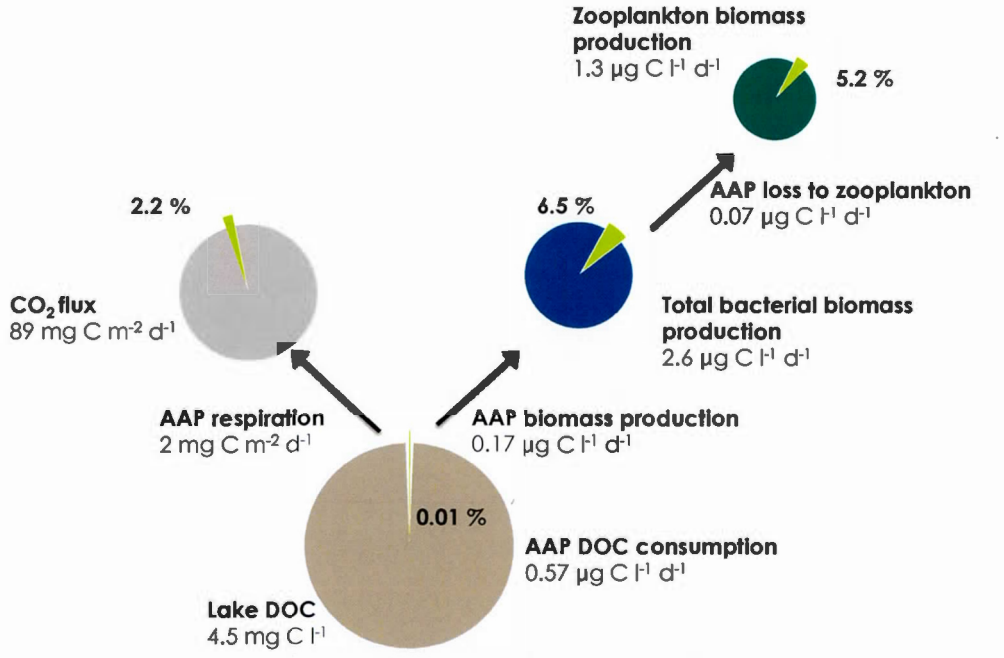


Figure 4.1 Estimation of the importance of AAP bacteria for lake Croche ecosystem processes: zooplankton production and CO_2 fluxes.

does not seem to depend on the light conditions. Accordingly, the results from the annual survey on lake Croche, where we measured the bulk ^{3}H-leucine assimilation rates in the dark and light conditions on a monthly basis, did not show any significant light-driven stimulation of the bacterial production (Fig. 4.2). Moreover, despite the large differences in physico-chemical conditions between our study lakes, none of the environmental factors considered in this thesis seemed to clearly explain the observed variability in AAP abundance, activity, mortality or contribution to biomass production. There may be other factors not assessed here that could explain the observed variability in AAP abundances and activity patterns, such as differences in taxonomic composition of AAP bacterial communities, changes in the composition of the communities of bacterivores, differential susceptibility of AAP taxa to viruses or changes in the quality of the dissolved organic carbon, among others. This highlights the importance of further exploring the factors that control the activity and growth

of AAP in aquatic systems, in order to identify in which situations, or under which conditions, these photoheterotrophic prokaryotes may be more successful or playing a more relevant role in the processing of carbon and energy in the ecosystem. In any case, our results provide a preliminary indication of the significance of the AAP role and a baseline for further comparative analyses of the ecological role of AAP bacteria in natural ecosystems.

In summary, this thesis demonstrates that AAP bacteria are a highly dynamic component of the freshwater bacterial communities. Compared to the bulk bacterial community, AAP seem to be on average larger, more active and growing faster,

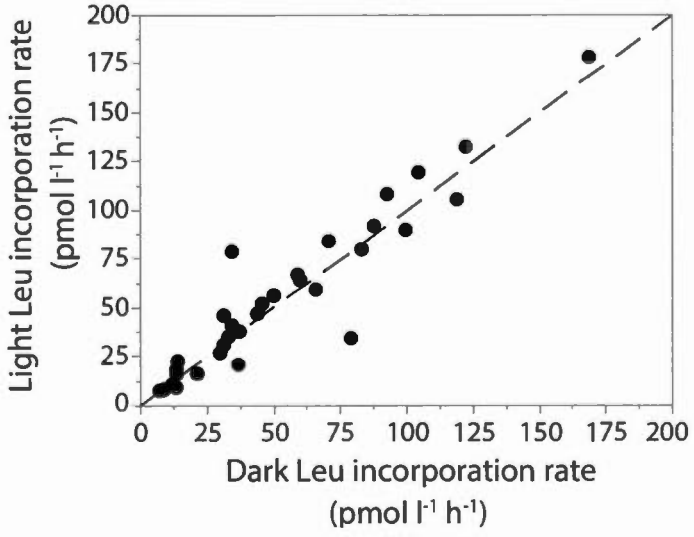


Figure 4.2 Comparison between bulk ^{3}H-leucine incorporation rates measured in light and dark incubations. The dashed line indicates the 1:1 ratio. The plot includes the data from a monthly survey carried out in lake Croche.

although these features vary greatly among systems. These features seem to render them very attractive to predators. Consequently, AAP bacteria appear subjected to a strong grazing pressure in freshwater systems, which may explain the very low net growth rates and low relative abundances commonly observed, but that likely results in an effective carbon transfer from the microbial food web to higher trophic levels. The abundance of AAP in aquatic systems is thus the result of the complex interaction of processes that govern the physiological state of AAP cells, their potential to grow and their loss in the natural environment, suggesting that few valid inferences about the ecological performance of AAP can be made based only on their abundance patterns. Our exploration of the effect of light on key physiological processes of freshwater AAP communities demonstrate how complex the interactions between the environment and the physiology of AAP can be. While the conundrum about the actual advantage of the phototrophic function for AAP remains, this thesis makes a significant contribution to the understanding of the role of light by providing an integrative scenario that examines the potential links between light, AAP growth, activity, regulation and photosynthetic potential. Our results suggest that, contrary to expectations, the higher apparent growth rates and potential competitiveness of AAP bacteria may not be directly linked to the ability of AAP bacteria to use light energy. The potential lack of a direct effect of light on the growth and activity of AAP does not imply the absence of a benefit from the phototrophic function, but certainly we still ignore the nature of this advantage. In addition, we confirmed that light has a direct impact on the synthesis of BChl*a* in natural AAP communities. Furthermore, we suggest that pigment production may be linked to other cellular functions such as the regulation of the expression of their large set of enzymes or the synthesis of large amounts of carotenoids that are hypothetically associated with protection from phototoxicity and that the exploration of this link may lead us to explain the nature of the advantage provided by the phototrophic function. Certainly, this and many other questions remain to be answered, such as the apparent regulation of the cellular pigment content by growth and the factors involved

in this regulation, the hypothetical tradeoffs between growth and the expression and maintenance of the phototrophic apparatus, the environmental drivers explaining the variability in single-cell activity and gross growth rates, the links between patterns in activity, growth, mortality, and pigment dynamic with the phylogenetic structure of AAP communities, and the role of viruses in regulating the abundance and the net growth as well as the physiological structure of AAP communities. However, this thesis provides a step forward towards a more complete understanding of the trophic role and the biogeochemical relevance of freshwater AAP bacteria in the carbon cycle, and will luckily encourage future studies that will help resolving some of the uncertainties associated to this functional group of prokaryotes.

REFERENCES

Beatty, J. T. (2002). On the natural selection and evolution of the aerobic phototrophic bacteria. Archives of Microbiology, 73(1-3), 109-114.

Béjà, O. and Suzuki, M. T. (2008). Photoheterotrophic marine prokaryotes. In David L. Kirchman (Ed), Microbial Ecology of the Oceans (2nd Edition, p. 131-157): John Wiley & Sons.

Bertilsson, S., Hansson, L., Graneli, W. and Philibert, A. (2003). Size-selective predation on pelagic microorganisms in Arctic freshwaters. Journal of Plankton Research, 25(6), 621-631.

Berga, M., Ostman, O., Lindström, E. S. and Langenheder, S. (2015). Combined effects of zooplankton grazing and dispersal on the diversity and assembly mechanisms of bacterial metacommunities. Environmental Microbiology.

Biebl, H. and Wagner-Döbler, I. (2006). Growth and bacteriochlorophyll *a* formation in taxonomically diverse aerobic anoxygenic phototrophic bacteria in chemostat culture: Influence of light regimen and starvation. Process Biochemistry, 41(10), 2153-2159.

Brendelberger, H. (1991). Filter mesh size of cladocerans predicts retention efficiency for bacteria. Limnology and Oceanography, 36(5), 884-894.

Brucet, S., Compte, J., Boix, D., López-Flores, R. and Quintana, X. D. (2008). Feeding of nauplii, copepodites and adults of *Calanipeda aquaedulcis* (Calanoida) in Mediterranean salt marshes. Marine Ecology Progress Series, 355, 183-191.

Caliz, J. and Casamayor, E. O. (2014). Environmental controls and composition of anoxygenic photoheterotrophs in ultraoligotrophic high-altitude lakes (Central Pyrenees). Environmental microbiology reports, 6(2), 145-51.

Carignan, R., Planas, D. and Vis, C. (2000). Planktonic production and respiration in oligotrophic Shield lakes. Limnology and Oceanography, 45(1), 189-199.

Compte, J., Brucet, S., Gascón, S., Boix, D., Sala, J., López-Flores, R. and Quintana, X. D. (2009). Impact of different developmental stages of *Daphnia magna* (Straus) on the plankton community under different trophic conditions. Hydrobiologia, 635(1), 45-56.

Cottrell, M. T. and Kirchman, D. L. (2003). Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the

Delaware estuary. Limnology and Oceanography, 48(1), 168-178.

Cottrell, M. T. and Kirchman, D. L. (2009). Photoheterotrophic microbes in the Arctic Ocean in summer and winter. Applied and Environmental Microbiology, 75(15), 4958-4966.

Cottrell, M.T, Mannino, A., and Kirchman, D.L. (2006). Aerobic anoxygenic phototrophic bacteria in the Mid-Atlantic Bight and the North Pacific Gyre. Applied and environmental, 72(1), 557-564.

Cottrell, M. T., Ras, J. and Kirchman, D. L. (2010). Bacteriochlorophyll and community structure of aerobic anoxygenic phototrophic bacteria in a particle-rich estuary. The ISME journal, 4(7), 945-54.

Cullen, J. J. (1982). The deep chlorophyll maximum: comparing vertical profiles of chlorophyll. Deep Sea Research Part B. Oceanographic Literature Review, 29(12), 787.

Cuperová, Z., Holzer, E., Salka, I., Sommaruga, R. and Koblízek, M. (2013). Temporal changes and altitudinal distribution of aerobic anoxygenic phototrophs in mountain lakes. Applied and environmental microbiology, 79(20), 6439-46.

Degans, H. and Zöllner, E. (2002). Rapid Daphnia-mediated changes in microbial community structure: an experimental study. FEMS microbiology ecology, 42, 137-149.

del Giorgio, P. A. and Gasol, J. M. (2008). Physiological structure and single-cell activity in marine bacterioplankton. In: D. L. Kirchman (Ed.), Microbial Ecology of the Oceans (Second Edition, p. 243-298). NJ, USA: John Wiley & Sons.

del Giorgio, P. A., Gasol, J. M., Vaque, D., Mura, P., Agustí, S. and Duarte, C. M. (1996). Bacterioplankton community structure : Protists control net production and the proportion of active bacteria in a coastal marine community. Limnology and Oceanography, 41(6), 1169-1179.

Derry, A. M. and Arnott, S. E. (2007). Zooplankton community response to experimental acidification in boreal shield lakes with different ecological histories. Canadian Journal of Fisheries and Aquatic Sciences, 64(6), 887-898.

Du, H., Jiao, N., Hu, Y. and Zeng, Y. (2006). Real-time PCR for quantification of aerobic anoxygenic phototrophic bacteria based on pufM gene in marine environment. Journal of Experimental Marine Biology and Ecology, 329(1), 113-121.

Ducklow, H. (2000). Bacterial production and biomass in the oceans. In: D.L. Kirchman (Ed.), Microbial ecology of the oceans (p. 85-120). [s.l.] : New York: Wiley-Liss.

Eiler, A. (2006). MINIREVIEW Evidence for the Ubiquity of Mixotrophic Bacteria in the Upper Ocean : Implications and Consequences. Applied and Environmental Microbiology, 72(12), 7431-7437.

Eiler, A., Beier, S., Säwström, C., Karlsson, J. and Bertilsson, S. (2009). High ratio of bacteriochlorophyll biosynthesis genes to chlorophyll biosynthesis genes in bacteria of humic lakes. Applied and Environmental Microbiology, 75(22), 7221-7228.

Fauteux, L., Cottrell, M. T., Kirchman, D. L., Borrego, C. M., Garcia-Chaves, M. C. and del Giorgio, P. A. (2015). Patterns in abundance, cell size and pigment content of aerobic anoxygenic phototrophic bacteria along environmental gradients in northern lakes. Plos One, 10(4), e0124035.

Fenchel, T. (2008). The microbial loop – 25 years later. Journal of Experimental Marine Biology and Ecology, 366(1-2), 99-103.

Ferrera, I., Borrego, C. M., Salazar, G. and Gasol, J. M. (2013). Marked seasonality of aerobic anoxygenic phototrophic bacteria in the coastal NW Mediterranean Sea as revealed by cell abundance, pigment concentration and pyrosequencing of pufM gene. Environmental microbiology, 16, 2953-2965.

Ferrera, I., Gasol, J. M., Sebastián, M., Hojerová, E. and Koblízek, M. (2011). Comparison of growth rates of aerobic anoxygenic phototrophic bacteria and other bacterioplankton groups in coastal Mediterranean waters. Applied and environmental microbiology, 77(21), 7451-8.

Fuchs, B. M., Spring, S., Teeling, H., Quast, C., Wulf, J., Schattenhofer, M., Yan, S., Ferriera, S., Johnson, J., Glöckner, F. O. and Amann, R. (2007). Characterization of a marine gammaproteobacterium capable of aerobic anoxygenic photosynthesis. Proceedings of the National Academy of Sciences of the United States of America, 104(8), 2891-6.

Garcia, D., Richaud, P., Breton, J. and Verméglio, A. (1994). Structure and function of the tetraheme cytochrome associated to the reaction center of Roseobacter denitrificans. Biochimie, 76(7), 666—673.

Garcia-Chaves, M., Cottrell, M., Kirchman, D., Derry, A., Bogard, M. and del Giorgio,

P.A (2015). Major contribution of both zooplankton and protists to the top-down regulation of freshwater aerobic anoxygenic phototrophic bacteria. Aquatic Microbial Ecology, 76(1), 71-83.

Garcia-Chaves, M. C., Cottrell, M. T., Kirchman, D. L., Ruiz-González, C. and del Giorgio, P. A. (2016). Single-cell activity of freshwater aerobic anoxygenic phototrophic bacteria and their contribution to biomass production. ISME, 1-10.

Girard, R. and Reid R.A. (1990). Dorset Research Centre study lakes: sampling methodology (1986-1989) and lake morphometry. Toronto, Ont: Data report, Ontario Ministry of the Environment.

Gasol, J., Pinhassi, J., Alonso-Sáez, L., Ducklow, H., Herndl, G., Koblízek, M., Labrenz, M., Luo, Y., Morán, X., Reinthaler, T. and Simon, M. (2008). Towards a better understanding of microbial carbon flux in the sea. Aquatic Microbial Ecology, 53(September), 21-38.

Gich, F. and Overmann, J. (2006). *Sandarakinorhabdus limnophila* gen. nov., sp. nov., a novel bacteriochlorophyll a-containing, obligately aerobic bacterium isolated from freshwater lakes. International journal of systematic and evolutionary microbiology, 56, 847-54.

Girard R, Reid, R.A. (1990). Dorset Research Centre study lakes: sampling methodology (1986-1989) and lake morphometry. Data report, Ontario Ministry of the Environment, Toronto.

Gonzalez, J. M., Sherr, E. B. and Sherr, B. F. (1990). Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Applied and environmental microbiology, 56(3), 583-9.

Güde, H. (1988). Direct and indirect influences of crustacean zooplankton on bacterioplankton of Lake Constance. Hydrobiologia, 73, 63-73.

Hahn, M. W. and Höfle, M. G. (2001). Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS microbiology ecology, 35(2), 113-121.

Haney J, Aliberti M, Allan E, Allard S, Bauer JD, and Beagen W (2013) An image-based key to the zooplankton of North America. Center for Freshwater Biology, University of New Hampshire

Harashima, K., Shiba, T. and Totsuka, T. (1978). Occurrence of bacteriochlorophyll *a* in a strain of an aerobic heterotrophic bacterium. Agricultural and Biological Chemistry, 42(8), 1627-1628.

Hauruseu, D. and Koblizek, M. (2012). Influence of light on carbon utilization in aerobic anoxygenic phototrophs. Applied and Environmental Microbiology, 78(20), 7414-7419.

Hojerová, E., Mašín, M., Brunet, C., Ferrera, I., Gasol, J. M. and Koblížek, M. (2011). Distribution and growth of aerobic anoxygenic phototrophs in the Mediterranean Sea. Environmental Microbiology, 13(10), 2717-2725.

Jezbera, J., Hornák, K. and Simek, K. (2006). Prey selectivity of bacterivorous protists in different size fractions of reservoir water amended with nutrients. Environmental microbiology, 8(8), 1330-9.

Jezbera, J., Hornák, K. and Simek, K. (2005). Food selection by bacterivorous protists: insight from the analysis of the food vacuole content by means of fluorescence in situ hybridization. FEMS Microbiology ecology, 52(3), 351-363.

Jiao, N., Zhang, Y., Zeng, Y., Hong, N., Liu, R., Chen, F. and Wang, P. (2007). Distinct distribution pattern of abundance and diversity of aerobic anoxygenic phototrophic bacteria in the global ocean. Environmental microbiology, 9(12), 3091-9.

Jürgens, K. (1994). Impact of Daphnia on planktonic microbial food webs-A review. Marine Microbial Food Webs, 8(1-2), 295-324.

Jürgens, K. and Matz, C. (2002). Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. Antonie van Leeuwenhoek, 81(1-4), 413-34.

Jürgens, K. and Massana, R. (2008). Protist grazing on marine bacterioplankton. In: Microbial Ecology of the Oceans (p. 383-441).

Karl, D. M. (2002). Hidden in a sea of microbes. Nature, 415(6872), 590.

Kim, H., Hwang, S. and Joo, G. (2000). Zooplankton grazing on bacteria and phytoplankton in a regulated large river (Nakdong River, Korea). Jounal of Plankton Research, 22(8), 1559-1577.

Kirchman, D. L. and Hanson, T. E. (2013). Bioenergetics of photoheterotrophic bacteria in the oceans. Environmental Microbiology Reports, 5(2), 188-199.

Kirchman, D., Stegman, M., Nikrad, M. and Cottrell, M. T. (2014). Abundance, size, and activity of aerobic anoxygenic phototrophic bacteria in coastal waters of the West Antarctic Peninsula. Aquatic Microbial Ecology, 73(1), 41-49.

Koblížek, M. (2015). Ecology of aerobic anoxygenic phototrophs in aquatic environments. FEMS Microbiology Reviews, (January), 1-17.

Koblízek, M., Béjà, O., Bidigare, R. R., Christensen, S., Benitez-Nelson, B., Vetriani, C., Kolber, M. K., Falkowski, P. G. and Kolber, Z. S. (2003). Isolation and characterization of Erythrobacter sp. strains from the upper ocean. Archives of microbiology, 180(5), 327-38.

Koblízek, M., Masín, M., Ras, J., Poulton, A. J. and Prásil, O. (2007). Rapid growth rates of aerobic anoxygenic phototrophs in the ocean. Environmental microbiology, 9(10), 2401-2406.

Koblízek, M., Mlcousková, J., Kolber, Z. and Kopecký, J. (2010). On the photosynthetic properties of marine bacterium COL2P belonging to Roseobacter clade. Archives of microbiology, 192(1), 41-9.

Koblízek, M., Shih, J. D., Breitbart, S. I., Ratcliffe, E. C., Kolber, Z. S., Hunter, C. N. and Niederman, R. A. (2005). Sequential assembly of photosynthetic units in Rhodobacter sphaeroides as revealed by fast repetition rate analysis of variable bacteriochlorophyll a fluorescence. Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1706(3), 220-231.

Koblízek, M., Stoń-Egiert, J., Sagan, S. and Kolber, Z. S. (2005). Diel changes in bacteriochlorophyll a concentration suggest rapid bacterioplankton cycling in the Baltic Sea. FEMS microbiology ecology, 51(3), 353-61.

Kolber, Z., Gerald, F. and Lang, A. (2001). Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. Science, 292, 2492-2495.

Kolber, Z. S., Van Dover, C. L., Niederman, R. a and Falkowski, P. G. (2000). Bacterial photosynthesis in surface waters of the open ocean. Nature, 407(6801), 177-179.

Lami, R., Cottrell, M., Ras, J. and Ulloa, O. (2007). High Abundances of Aerobic Anoxygenic Photosynthetic Bacteria in the South Pacific Ocean. Applied and environmental microbiology, 73(13), 4198-4205.

Lamy, D., Jeanthon, C., Cottrell, M. T., Kirchman, D. L., Van Wambeke, F., Ras, J., Dahan, O., Pujo-Pay, M., Oriol, L., Bariat, L., Catala, P., Cornet-Barthaux, V. and Lebaron, P. (2011). Ecology of aerobic anoxygenic phototrophic bacteria along an oligotrophic gradient in the Mediterranean Sea. Biogeosciences, 8(4), 973-985.

Langenheder, S. and Jürgens, K. (2001). Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnology and Oceanography,

46(1), 121-134.

Lehours, A.-C., Cottrell, M. T., Dahan, O., Kirchman, D. L. and Jeanthon, C. (2010). Summer distribution and diversity of aerobic anoxygenic phototrophic bacteria in the Mediterranean Sea in relation to environmental variables. FEMS microbiology ecology, 74(2), 397-409.

Li, Q., Jiao, N. and Peng, Z. (2006). Environmental control of growth and BChl a expression in an aerobic anoxy- genic phototrophic bacterium, *Erythrobacter longus* (DSMZ6997). Acta ocenologica sinica, 25(6), 138-144.

Liu, R., Zhang, Y. and Jiao, N. (2010). Diel variations in frequency of dividing cells and abundance of aerobic anoxygenic phototrophic bacteria in a coral reef system of the South China Sea. Aquatic Microbial Ecology, 58(2007), 303-310.

Mašín, M., Čuperová, Z., Hojerová, E., Salka, I., Grossart, H. and Koblížek, M. (2012). Distribution of aerobic anoxygenic phototrophic bacteria in glacial lakes of northern Europe. Aquatic Microbial Ecology, 66(1), 77-86.

Mašín, M., Nedoma, J., Pechar, L. and Koblížek, M. (2008). Distribution of aerobic anoxygenic phototrophs in temperate freshwater systems. Environmental microbiology, 10(8), 1988-96.

Mašín, M., Zdun, A., Stoń-Egiert, J., Nausch, M., Labrenz, M., Moulisová, V. and Koblížek, M. (2006). Seasonal changes and diversity of aerobic anoxygenic phototrophs in the Baltic Sea. Aquatic Microbial Ecology, 45(3), 247-254.

Medová, H., Boldareva, E. N., Hrouzek, P., Borzenko, S. V, Namsaraev, Z. B., Gorlenko, V. M., Namsaraev, B. B. and Koblížek, M. (2011). High abundances of aerobic anoxygenic phototrophs in saline steppe lakes. FEMS microbiology ecology, 76(2), 393-400.

Melo D.R. and Hebert P.D. (1994). A taxonomic reevaluation of North American Bosminidae. Can J Zool 72:1808–1825

Montagnes, D., Barbosa, A., Boenigk, J., Davidson, K., Jürgens, K., Macek, M., Parry, J., Roberts, E. and Imek, K. (2008). Selective feeding behaviour of key free-living protists: avenues for continued study. Aquatic Microbial Ecology, 53(September), 83-98.

Moran, M. A. and Miller, W. L. (2007). Resourceful heterotrophs make the most of light in the coastal ocean. Nature reviews. Microbiology, 5(10), 792-800.

Nikrad, M. P., Cottrell, M. T. and Kirchman, D. L. (2012). Abundance and single-cell activity of heterotrophic bacterial groups in the western Arctic Ocean in summer and winter. Applied and environmental microbiology, 78(7), 2402-9.

Nishimura, K., Shimada, H., Ohta, H., Masuda, T., Shioi, Y. and Takamiya, K. (1996). Expression of the puf operon in an aerobic photosynthetic bacterium, *Roseobacter denitrificans*. Plant & cell physiology, 37(2), 153-9.

Okamura, K., Takamiya, K. and Nishimura, M. (1985). Photosynthetic electron transfer system is inoperative in anaerobic cells of *Erythrobacter* species strain OCh 114. Archives of Microbiology, 142(1), 12-17.

Ouchane, S., Steunou, A., Picaud, M. and Astier, C. (2004). Aerobic and anaerobic Mg-protoporphyrin monomethyl ester cyclases in purple bacteria: a strategy adopted to bypass the repressive oxygen control system. The Journal of biological chemistry, 279(8), 6385-94.

Pace, M., McManus, G. and Findlay, S. (1990). Planktonic structure determines the fate of bacterial production in a temperate lake. Limnology and Oceanography, 35(4), 795-808.

Page, K. A., Connon, S. A. and Giovannoni, S. J. (2004). Representative freshwater bacterioplankton isolated from Crater Lake, Oregon. Applied and environmental microbiology, 70(11), 6542.

Palovaara, J., Akram, N., Baltar, F., Bunse, C., Forsberg, J., Pedrós-Alió, C., González, J. M. and Pinhassi, J. (2014). Stimulation of growth by proteorhodopsin phototrophy involves regulation of central metabolic pathways in marine planktonic bacteria. Proceedings of the National Academy of Sciences, 111(35), 3650-3658.

Pernthaler J. (2005). Predation on prokaryotes in the water column and its ecological implications. Nat Rev Microbiol 3:537–546 PubMed.

Rathgeber, C., Beatty, J. T. and Yurkov, V. (2004). Aerobic Phototrophic Bacteria: New Evidence for the Diversity, Ecological Importance and Applied Potential of this Previously Overlooked Group. Photosynthesis Research, 81(2), 113-128.

Ruiz-González, C., Proia, L., Ferrera, I., Gasol, J. M. and Sabater, S. (2012). Effects of large river dam regulation on bacterioplankton community structure. FEMS microbiology ecology, 1-16.

Salcher, M. M., Posch, T. and Pernthaler, J. (2013). In situ substrate preferences of abundant bacterioplankton populations in a prealpine freshwater lake. The ISME journal, 7(5), 896-907.

Salka, I., Cuperová, Z., Mašín, M., Koblížek, M. and Grossart, H.-P. (2011). *Rhodoferax*-related pufM gene cluster dominates the aerobic anoxygenic phototrophic communities in German freshwater lakes. Environmental microbiology, 13(11), 2865-75.

Salka, I., Moulisová, V., Koblízek, M., Jost, G., Jürgens, K., Labrenz, M. and Ju, K. (2008). Abundance, depth distribution, and composition of aerobic bacteriochlorophyll a-producing bacteria in four basins of the central Baltic Sea. Applied and environmental microbiology, 74(14), 4398-404.

Sastri, A. R., Gauthier, J., Juneau, P. and Beisner, B. E. (2014). Biomass and productivity responses of zooplankton communities to experimental thermocline deepening. Limnology and Oceanography, 59(1), 1-16.

Sato, K., Hagiwara, K. and Shimizu, S. (1985). Effect of cultural conditions on tetrapyrrole formation, especially bacteriochlorophyll formation in a facultative methylotroph, *Protaminobacter ruber*. Agricultural and Biological Chemistry, 49(1), 1-5.

Schwalbach, M. and Fuhrman, J. (2005). Wide-ranging abundances of aerobic anoxygenic phototrophic bacteria in the world ocean revealed by epifluorescence microscopy and quantitative PCR. Limnology and Oceanography, 50(2), 620-628.

Shiba, T., Simidu, U. and Taga, N. (1979). Distribution of aerobic bacteria which contain bacteriochlorophyll a. Applied and Environmental, 38(1), 43-45.

Sieracki, M., Gilg, I., Thier, E., Poulton, N. J. and Goericke, R. (2006). Distribution of planktonic aerobic anoxygenic photoheterotrophic bacteria in the northwest Atlantic. Limnology and Oceanography, 51(1), 38-46.

Sieracki, M., Viles, C. and Webb, K. (1989). Algorithm to estimate cell biovolume using image analyzed microscopy. Cytometry, 10, 551-557.

Simek, K. and Chrzanowski, T. H. (1992). Direct and indirect evidence of size-selective grazing on pelagic bacteria by freshwater nanoflagellates. Applied and environmental microbiology, 58(11), 3715-20.

Simek, K., Hornák, K., Masín, M., Christaki, U., Nedoma, J., Weinbauer, M. and

Dolan, J. (2003). Comparing the effects of resource enrichment and grazing on a bacterioplankton community of a meso-eutrophic reservoir. Aquatic Microbial Ecology, 31, 123-135.

Smith, E. M. and Prairie, Y. T. (2004). Bacterial metabolism and growth efficiency in lakes: The importance of phosphorus availability. Limnology and Oceanography, 49(1), 137-147.

Sintes, E. and Del Giorgio, P. A. (2014). Feedbacks between protistan single-cell activity and bacterial physiological structure reinforce the predator/prey link in microbial foodwebs. Frontiers in microbiology, 5(September), 453.

Sintes, E. and Herndl, G. J. (2006). Quantifying substrate uptake by individual cells of marine bacterioplankton by catalyzed reporter deposition fluorescence in situ hybridization combined with microautoradiography. Applied and environmental microbiology, 72(11), 7022-8.

Smith, D. C. and Azam, F. (1992). A simple , economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. Marine Biology Research, 6(2), 107-114.

Smith, E. and del Giorgio, P. A. (2003). Low fractions of active bacteria in natural aquatic communities? Aquatic Microbial Ecology, 31, 203-208.

Sommer, U. and Sommer, F. (2006). Cladocerans versus copepods: The cause of contrasting top-down controls on freshwater and marine phytoplankton. Oecologia, 147(2), 183-194.

Soora, M. and Cypionka, H. (2013). Light enhances survival of Dinoroseobacter shibae during long-term starvation. PLoS ONE, 8(12), 6-12.

Spring, S. and Riedel, T. (2013). Mixotrophic growth of bacteriochlorophyll a-containing members of the OM60/NOR5 clade of marine gammaproteobacteria is carbon-starvation independent and correlates with the type of carbon source and oxygen availability. BMC microbiology, 13(1), 117.

Spring, S., Lünsdorf, H. H., Fuchs, B. M. B. M. and Tindall, B. J. (2009). The photosynthetic apparatus and its regulation in the aerobic gammaproteobacterium *Congregibacter litoralis* gen. nov., sp. nov. PloS one, 4(3), e4866.

Stegman, M. R., Cottrell, M. T. and Kirchman, D. L. (2014). Leucine incorporation by aerobic anoxygenic phototrophic bacteria in the Delaware estuary. The ISME

Journal, 1-10.

Tomasch, J., Gohl, R., Bunk, B., Diez, M. S. and Wagner-Döbler, I. (2011). Transcriptional response of the photoheterotrophic marine bacterium *Dinoroseobacter shibae* to changing light regimes. The ISME journal, 5(12), 1957-68.

Thorp AP, Covich J (eds) (2010) Ecology and classification of North American freshwater invertebrates. Elsevier Academic Press, London

Vachon, D. and del Giorgio, P. A. (2014). Whole-Lake CO_2 dynamics in response to storm events in two morphologically different lakes. Ecosystems, 1338-1353.

Waidner, L. A. and Kirchman, D. L. (2005). Aerobic anoxygenic photosynthesis genes and operons in uncultured bacteria in the Delaware River. Environmental Microbiology, 7(12), 1896-1908.

Waidner, L. A. and Kirchman, D. L. (2007). Aerobic anoxygenic phototrophic bacteria attached to particles in turbid waters of the Delaware and Chesapeake estuaries. Applied and Environmental Microbiology, 73(12), 3936-3944.

Wetzel, R. G. and Likens, G. E. (1979). Limnological Analysis. [s.l.] : W.B. Saunders Company.

Yokokawa, T. and Nagata, T. (2005). Growth and grazing mortality rates of phylogenetic groups of bacterioplankton in coastal marine environments. Applied and environmental microbiology, 71(11), 6799-6807.

Yutin, N., Suzuki, M. T. and Béjà, O. (2005). Novel primers reveal wider diversity among marine aerobic anoxygenic phototrophs. Applied and Environmental Microbiology, 71(12), 8958-8962.

Yurkov, V. and Beatty, J. T. (1998). Aerobic anoxygenic phototrophic bacteria. Microbiology and molecular biology reviews: MMBR, 62(3), 695-724.

Yurkov, V. and Csotonyi, J. T. (2009). New light on aerobic anoxygenic phototrophs. Advances in Photosynthesis and Respiration, 28, 31-55.

Yurkov, V.V., and Gorlenko, V.M. (1990). *Erythrobacter sibiricus* sp. nov, a new freshwater aerobic species containing bacteriochlorophyll a. Mikrobiologia 59: 120–126.

Yurkov, V., and Gorlenko, V.M. (1992). New species of aerobic bacteria from the genus *Erythromicrobium* contaning bacteriochlorophyll a. Mikrobiologia 61:248–255.

Yurkov, V. and Vangemerden, H. (1993). Impact of light dark regimen on growth-rate, biomass formation and bacteriochlorophyll synthesis in *Erythromicrobium-hydrolyticum*. Archives of Microbiology, 159(1), 84-89.

Zöllner, E., Santer, B., Boersma, M., Hoppe, H. G. and Jürgens, K. (2003). Cascading predation effects of Daphnia and copepods on microbial food web components. Freshwater Biology, 48(12), 2174-2193.