Taxonomic differences shape the responses of freshwater aerobic anoxygenic phototrophic bacterial communities to light and predation

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Abstract

Aerobic anoxygenic phototrophic (AAP) bacteria are a phylogenetically diverse and ubiquitous group of prokaryotes that use organic matter but can harvest light using bacteriochlorophyll *a*. Although the factors regulating AAP ecology have long been investigated through field surveys, the few available experimental studies have considered AAP as a group, thus disregarding the potential differential responses between taxonomically distinct AAP assemblages. Here, we used sequencing of the *pufM* gene to describe the diversity of AAPs in ten environmentally distinct temperate lakes, and to investigate the taxonomic responses of AAP communities in these lakes when subjected to similar experimental manipulations of light and predator removal. The studied communities were clearly dominated by *Limnohabitans* AAP but presented a clear taxonomic segregation between lakes presumably driven by local conditions, which was maintained after experimental manipulations. Predation reduction (but not light exposure) caused significant compositional shifts across most assemblages, but the magnitude of these changes could not be clearly related to changes in bulk AAP abundances or taxonomic richness of AAP assemblages during experiments. Only a few OTUs, which differed taxonomically between lakes, were found to respond positively during experimental treatments. Our results highlight that different freshwater AAP communities respond differently to similar control mechanisms, highlighting that in-depth knowledge on AAP diversity is essential to understand the ecology and potential role of these photoheterotrophs.

INTRODUCTION

The aerobic anoxygenic phototrophic (AAP) bacteria are a group of photoheterotrophs capable of obtaining energy from the oxidation of organic matter and from light using bacteriochlorophyll *a* (Bchl*a*). Since their discovery last decade, multiple studies have tried to understand the role that these widely distributed prokaryotes play in natural aquatic ecosystems and what their main drivers are (Koblížek, 2015). Due to their photoheterotrophic potential, particularly high growth rates and large cell sizes, it has been hypothesized that AAP may have light-driven competitive advantages over other heterotrophic bacteria, but that they may be particularly vulnerable to grazing (e.g., Ferrera *et al.* 2011, Kirchman *et al.*, 2014, García-Chaves *et al.*, 2015, Koblížek, 2015). Light availability and predation pressure have thus been invoked as major drivers of AAP ecology (Koblížek, 2015), but still basic questions such as the role of light on AAP ecology or their contribution to carbon flow in aquatic trophic webs remain unsolved (García-Chaves *et al.*, 2016; Yurkov & Hughes, 2017). This is probably because, although it is well established that AAP communities comprise phylogenetically and metabolically diverse taxa (Ferrera *et al.*, 2014; Koblížek, 2015; Ferrera *et al.*, 2017b; Lehours *et al.*, 2018; Auladell *et al.*, 2019), studies exploring AAP drivers have often characterized the AAP guild in terms of its bulk properties, such as the total Bchl*a* concentration or cell numbers (Waidner & Kirchman, 2007; Hojerová *et al.*, 2011; Lamy *et al.*, 2011; Ruiz-González *et al.* 2013; Fauteux *et al.*, 2015; García-Chaves *et al.*, 2015; 2016, Kolářová *et al.*, 2019). Given that the role of AAP in aquatic ecosystems and their responses to controlling factors may be modulated by their taxonomic structure, it is key to understand what factors shape the composition of natural AAP communities, and in turn, how changes in community structure influence their ecological roles.

Research on AAP diversity in natural communities has been based on the sequencing of the taxonomic marker *pufM* gene, which codifies for the M subunit of the bacterial photosynthetic reaction center. Such investigations have established that both marine and freshwater AAP assemblages display pronounced spatial (Salka *et al.*, 2008; Waidner & Kirchman, 2008; Cottrell & Kirchman, 2009; Cottrell *et al.*, 2010; Ferrera *et al.*, 2017b; Lehours *et al.*, 2018) and seasonal (Lamy *et al.*, 2011; Ferrera *et al.*, 2014; Bibiloni-Isaksson *et al.*, 2016; Auladell *et al.*, 2019) patterns, likely driven by strong environmental selection. Most of these studies, however, have used clone libraries or fingerprinting approaches (Jiao *et al.*, 2007; Waidner & Kirchman, 2008; Cottrell *et al.*, 2010; Ritchie & Johnson, 2012; Ferrera *et al.*, 2017b; Lehours *et al.*, 2018) that target only the most abundant members of these guilds, limiting the information about the ubiquity or the dynamics of a large fraction of the taxa. Although current sequencing technologies allow a much deeper characterization of bacterial assemblages, so far only a few studies have described AAP communities using 454-pyrosequencing (Ferrera *et al.*, 2014; Zheng *et al.*, 2015) or Illumina sequencing (Bibiloni-Isaksson *et al.*, 2016; Auladell *et al.*, 2019) of the *pufM* gene in marine waters. To our knowledge, however, no study has conducted such a deep characterization of AAP assemblages in freshwater ecosystems. In addition, most available studies on AAP diversity are descriptive and based on field surveys, and there has been no attempt to experimentally assess how different AAP assemblages change their taxonomic structure in response to environmental forcing.

Focusing on total AAP abundance or activity, experimental manipulations have provided key insight into the environmental and biological controls of AAP in natural ecosystems. For example, AAP bacteria as a whole can be strongly grazed by flagellates in marine

waters (Ferrera *et al.*, 2011; Sato-Takabe *et al.*, 2018) and by protists and larger zooplankton in lakes (García-Chaves *et al.*, 2015). Similarly, experimental light exposure has resulted in a range of bulk responses in terms of incorporation of radioactive leucine by AAP cells from lake, estuarine or marine waters (Kirchman *et al.*, 2014; Stegman *et al.*, 2014; Garcia-Chaves *et al.*, 2016), and growth of marine AAP bacteria (Ferrera *et al.*, 2017a). However, the compositional basis of these responses remain unknown; we ignore whether grazing selectively removes or favors certain AAP taxa, or whether different AAP groups display differential vulnerability to top-down control or variable capacities to benefit from light exposure. Determining the existence of specific taxonomic groups within AAP communities that respond coherently to a given forcing factor across environmentally different sites may help better understand or predict the ecological preferences and roles of these photoheterotrophic microorganisms.

Here, we designed a cross-lake comparative study to experimentally assess the taxonomic responses of freshwater AAP bacteria to grazing and light. Combining for the first time experimental manipulations with high-throughput sequencing of the *pufM* gene, we monitored changes in the taxonomic composition of AAP communities in experiments where bacteria from 10 environmentally different temperate lakes were released from predator pressure through dilution and/or exposed to light or dark conditions. In a subset of lakes, the taxonomic responses were compared to changes in total AAP bacterial abundance during experiments to explore whether the observed compositional changes in AAP assemblages can lead to detectable changes in total AAP numbers. Finally, in order to investigate whether predation or light exposure favors specific AAP groups (e.g., whether the same taxa are preferentially grazed across taxonomically different assemblages), we identified the taxa most strongly responding to both treatments in each lake and compared

them across the studied lakes.

MATERIALS AND METHODS

Study sites and sample collection

Ten temperate lakes from Québec were sampled once between July and August 2013, during summer stratification (Table 1). Lake Croche is located in the Laurentian region north of Montréal (46.01°N, 74.15°W), Canada, and the rest of the lakes belong to the Eastern Townships region of southeastern Québec (45.24°N, 72.12°W). Lakes were selected to cover a gradient in dissolved organic carbon (DOC), chlorophyll *a* (Chl*a*) and nutrient concentrations, so that they differed greatly in trophic status and light availability (Table 1). Vertical profiles of temperature and dissolved oxygen were measured *in situ* with a YSI (Yellow Springs, OH, USA) Pro Plus multiparameter probe to avoid sampling hypoxic zones that may harbor anaerobic Bchl*a*-containing phototrophic cells. Underwater profiles of photosynthetically active radiation (PAR) were measured with a LI-COR LI-190 Quantum Sensor (Lincoln, NE, USA).

At each lake, 40L of water were collected by pumping water into acid-rinsed 20L polycarbonate containers from a depth corresponding to an irradiance of 200–300 $\mu\text{mol photons m}^{-2} \cdot \text{s}^{-1}$ and transported refrigerated in the dark to the laboratory within 3-4 hours after collection. Additional samples were filtered *in situ* through 0.45 μm PES cartridge filters and stored in acid-washed glass vials for DOC analyses. *In situ* concentrations of DOC, Chl*a*, total phosphorus (TP), and total nitrogen (TN) were determined following standard procedures as in García-Chaves *et al.* (2015).

Experimental setup: release of predator pressure and resource competition and light exposure

To assess the effect of predation on structuring lake AAP communities, two experimental treatments were performed at each lake: A control treatment (CT), consisting of unfiltered lake water, and a predator-reduced treatment (PR), consisting of a 1:4 dilution of unfiltered lake water with 0.2 μm -filtered lake water to reduce predator encounter rates, which was prepared in an acid-rinsed 20L polycarbonate container. These two types of water (unfiltered lake water and 1:4 diluted lake water) were sampled immediately for initial AAP communities (see below) and were then distributed into duplicate or triplicate 2.4L-Polyethylene terephthalate transparent bottles. Approximately 15L of each type of water was used in experiments done with triplicate bottles (lakes Montjoie and Bowker), and 10L in experiments done with duplicate bottles (the rest of the lakes). We acknowledge that besides reducing the encounter rates with predators, the PR-treatment might also release competition for resources due to a decrease in the total number of bacteria, and thus our PR-treatment likely integrates the effect of both predator reduction and release of resource competition. However, due to the large size of freshwater AAP bacteria (Cuperová *et al.*, 2013, Fauteaux *et al.* 2015, García-Chaves *et al.* 2016) and their potential particle-attached preference (Masín *et al.*, 2012), the removal of predators by filtration through 0.8 μm or 1 μm pore-size filters as performed in previous studies (Ferrera *et al.*, 2011; Sato-Takabe *et al.*, 2018) could have resulted in a fundamental change in the original composition of our AAP assemblages. Moreover, the only AAP study that compared the effect of dilution (as performed in our study) with that of removing grazers through filtration showed very similar responses of AAP in both treatments, and concluded that in the dilution treatment most of the effect was due to the release of predation pressure

(Ferrera *et al.* 2011).

To assess the effect of light on structuring lake AAP communities or on the predation-driven AAP responses, duplicate bottles from the treatments CT and PR were kept either in the dark (by wrapping bottles with black adhesive tape) or in the light (cycles of 8h darkness and 16h of artificial PAR, at an irradiance of $150 \mu\text{mol photons m}^{-2} \cdot \text{s}^{-1}$ provided by UV-free fluorescent lamps (45-W Sun Blaster)).

Samples were taken before distributing the water into the experimental bottles (time = 0) for initial conditions, and from each experimental bottle when the experiment was stopped, usually between 48 and 60 hours. During experiments, the experimental bottles were incubated horizontally at close to *in situ* temperature ($20 \pm 2^\circ\text{C}$, Table 1) in a circulating water tank within a temperature-controlled room. The light intensity in the incubation tank was measured with a HR 4000 spectrometer PAR sensor (Ocean Optics).

Abundance of total prokaryotes, total viruses, and AAP bacteria

At each lake, a 25-ml sample was taken for microscopic enumeration of AAP and total bacterial cells present in the ambient surface waters (before the experiments). For logistic reasons the preservation of the samples failed in a set of samples, so the AAP abundances after the experimental treatments could only be quantified in 5 of the 10 lakes. 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- μm pore-size black polycarbonate filters (Merck Millipore GTBP, 25 mm) and stored at -80°C . Total bacteria and AAP cell abundance were determined from microscope images of 4',6-diamidino-2-phenylindole (DAPI), BChl *a* (IR autofluorescence), Chl *a* and phycoerythrin-

positive cells using an Olympus Provis AX70 microscope. The microscope was fitted with a charge-coupled-device camera capable of infrared detection (Intensified Retiga Extended Blue; Qimaging), and the quantification of AAP and total prokaryotes was conducted using an automated image analysis software (ImagePro Plus, Media Cybernetics) as described previously (Cottrell *et al.*, 2006; García-Chaves *et al.*, 2015).

The viral abundance at each lake was estimated on fixed samples by standard epifluorescence microscopy as described previously (Suttle and Furhman 2010). In brief, water samples were preserved in glutaraldehyde (0.5% final concentration), flash-frozen in liquid N₂ and stored at -80°C until 1-4 µL of the preserved sample was filtered on 0.02-µm Anodisc filter. The filter was stained by placing it on top of 80 µL SYBR solution (400-fold diluted) for 15 min in the dark. The dye solution was removed using vacuum and the Anodisc filter was dried by placing it on a Kimwipe paper in the dark. The filter was laid on a glass slide using AF1 mounting solution and covered with a slide. Bright pinpricks of light were counted as particle viruses by epifluorescence microscopy, when excited with blue light and observed under at 1000× magnification objective.

Prokaryote heterotrophic production

In situ prokaryotic biomass production rates were estimated from initial samples by the H³-leucine assimilation method described by Kirchman *et al.* (1985) with the modifications of Smith and Azam (1992). Three replicates of 1.5 ml plus one 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 processing by the centrifugation method (Smith & Azam, 1992).

pufM gene diversity

Prokaryotic biomass for DNA extraction was collected from each lake *in situ*, and from experimental bottles at both initial and final times by filtering 300–500 ml of water onto 0.22 µm pore-sized polycarbonate filters. A total of 107 samples were collected. Genomic DNA was extracted using the MoBio PowerWater DNA extraction kit (Qiagen, Carlsbad, CA) following the manufacturer's protocol. Primers *pufMF* (5'-TACGGSAACCTGTWCTAC-3') and *pufM_WAWR* (5'-AYNGCRAACCACCANGCCCA-3') were used for amplifying a ~245 base-pair (bp) fragment of the *pufM* gene (Béjà *et al.*, 2002; Yutin *et al.*, 2005). The primers were chosen based on a previous analysis where the performance of the available *pufM* primers was tested using a clone library containing 193 freshwater AAP clones, some of which were retrieved from one of the studied lakes (Croche) and another lake in the area (Cromwell, Mesa-Ortiz 2014). In addition, these primers have been used in recent marine studies in which high-throughput sequencing of this marker gene was applied (Ferrera *et al.* 2014; Auladell *et al.* 2019).

PCR amplifications were performed using the SsoFast EvaGreen Supermix that contains the Sso7d fusion polymerase (BioRad), with the following conditions: An initial denaturalization step at 98°C for 2 min followed by 40 cycles of 5 s at 98 °C (5s), 58°C (5s), 72 °C (5s), and a final elongation step at 65°C for 10 min. The amplicons were sequenced on an Illumina MiSeq2000 in Génome Québec (<http://www.genomequebec.com>) following a paired-end approach (Caporaso *et al.*, 2012). Paired-end reads were assembled with PEAR (Zhang *et al.*, 2014) and 200–230bp sequences were used for downstream analyses in QIIME to remove primers and low-quality (Phred scores <30) reads (Caporaso

240 *et al.*, 2010). Sequences were clustered into Operational Taxonomic Units (OTUs) using
241 the SWARM 2.0 algorithm (Mahé *et al.*, 2014). We applied a local clustering

242 threshold of $d=1$ and the fastidious option, which tends to optimize the precision of OTU
243 delineation and reduces the number of singletons and doubletons, by grafting them onto
244 more abundant clusters based on their similarity with the seed sequences (Mahé *et al.*,
245 2015). Chimeric sequences were detected and removed using UCHIME with the *de novo*
246 option (Edgar *et al.*, 2011). OTUs with <10 sequences were discarded in order to minimize
247 sequencing errors. To enable comparisons between samples, the OTU table was randomly
248 subsampled to ensure an equal number of sequences per sample based on the sample with
249 the fewest reads (10,000 sequences), with the exception of six samples that had fewer reads
250 (range 3,376-9,197 reads) that were however also kept for analyses. Raw sequences are
251 available at the European Nucleotide Archive (acc. no. PRJEB36763).

252 The representative sequence of each OTU was used for taxonomic identification. A
253 custom ARB database (Ludwig, 2004) based on the sequences reported by Salka *et al.*
254 (2011), Cuperová *et al.* (2013), Cáliz and Casamayor (2014), Ferrera *et al.* (2017b), and
255 reference sequences extracted from NCBI's non-redundant database
256 (<http://www.ncbi.nlm.nih.gov>) was used for taxonomic identification, which was performed
257 by using local BLAST (e value=0.01, percent identity=80). Sequences were mapped to
258 specific groups based on the phylogenetic analyses of the corresponding reference
259 sequences.

Statistical analyses

The Shannon index was calculated as an estimate of AAP taxonomic diversity. Communities were clustered using non-metric multidimensional scaling (NMDS) analysis (R Vegan metaNMDS, Oksanen *et al.*, 2015) based on Bray-Curtis distances. The Vegan *envfit* function was used to fit environmental vectors onto the ordination space. Differences in taxonomic composition among treatments were tested using ANOSIM. The effect of geographic position or local lake conditions on AAP community composition was assessed through the correlation between the compositional dissimilarity among lake communities and either the environmental or geographic distance using Mantel tests. Pearson's correlation coefficient was calculated to establish linear relationships between individual variables. All analyses were done with R 3.0.0 software (R Core Team, 2013, Vegan package).

RESULTS

The studied lakes differed largely in terms of water color, transparency, DOC, chlorophyll *a* and nutrient concentrations (Table 1). For example, the PAR attenuation coefficient (K_{PAR}) varied between 0.3 m^{-1} in the oligotrophic lake Bowker and 1.57 m^{-1} in Coulombe. DOC concentrations varied fivefold ($2.7\text{--}14\text{ mg L}^{-1}$), and Chl*a* concentrations ranged from $0.7\text{ }\mu\text{g L}^{-1}$ in Nicolet to $11\text{ }\mu\text{g L}^{-1}$ in the most eutrophic lake Waterloo (Table 1). Epilimnetic water temperature averaged $22\pm 2\text{ }^{\circ}\text{C}$, and except for lake Waterloo, all lakes were stratified during the sampling period. Most lakes had a fully aerobic water column, but in those presenting an anoxic hypolimnion at the time of sampling (Table 1), this was physically separated from the sampled oxic epilimnetic waters by a clear thermocline. The

choice of sampling sites was intended to minimize the possibility of detecting another group of *Bchl*a-containing bacteria, the purple sulfur bacteria, which are present in the anoxic hypolimnia of lakes (see below).

In situ AAP abundances, measured by infrared microscopy, varied from 0.2 to 4.7×10^5 cells mL⁻¹, representing 1%-22% of total prokaryote abundance (Table 1). K and DOC concentration were the only variables explaining AAP abundance across lakes ($R=0.81$, $p=0.008$ and $R=0.72$, $p=0.03$, respectively, $n=9$, Figure S1a,b).

Taxonomic composition of in situ AAP communities across lakes

Sequencing of the *pufM* gene resulted in 4,182,541 quality sequences, which clustered into 9,685 OTUs after removing 235 OTUs classified as purple sulfur bacteria. Sulfur bacteria comprised less than 2.5% of the total sequences per lake in all cases except in lake Aylmer, where they accounted for 13% of the sequences (Table 2), even though this lake showed a fully aerobic water column (Table 1). After rarefaction to 10,000 reads per sample, 1,052,968 total sequences and 7,665 OTUs were retained.

The number of *pufM* OTUs per lake varied between 119 and 449 (Table 2), and the Shannon diversity index ranged from 2.2 to 4.3. This variability in richness and diversity was not statistically related to any of the measured environmental variables, but both indexes were positively related to total viral abundances (Fig S1c,e) and to prokaryotic heterotrophic production measured as ³H-leucine incorporation rates (Figure.S1d,f). Individual OTU accumulation curves tended to reach an asymptote in most cases, suggesting that we had a good coverage of the *pufM* diversity within each lake (Fig. S2a) and also when considering all lakes together (Fig. S2b).

The studied lake communities showed a similar structure where betaproteobacterial and alphaproteobacterial AAP made up ca. 50% and 30% of the detected OTUs, respectively (Fig. 1a). Even the different subgroups within *Alphaproteobacteria* showed remarkably similar proportions across lakes. In terms of relative sequence abundance, the structure of AAP communities varied more across lakes, but in all cases betaproteobacterial OTUs comprised most *pufM* sequences (range 54%-86%) at *in situ* conditions, followed by variable proportions of the different alphaproteobacterial subgroups (Fig. 1b). Interestingly, betaproteobacterial sequences decreased towards lakes with higher pH (Fig. 1c,d), while alphaproteobacterial AAPs showed the opposite trend.

At finer taxonomic levels, we also observed a strikingly similar distribution of OTUs among the different AAP genera, with *Limnohabitans* (Betaproteobacteria) being the most abundant one, comprising roughly 50% of the detected OTUs across all lakes (Fig. 2a). *Limnohabitans* also dominated community sequences in most cases (range 33%-77% of *pufM* sequences per community), yet the relative abundances of the other genera showed more variation across the studied assemblages (Fig. 2b).

Considering exclusively *in situ* lake communities, 2153 OTUs were detected, of which only 3 OTUs were present across all lakes, and in general the most abundant OTUs were also the most widely distributed (Fig. S3). When all the *in situ* and experimental communities were considered (n=107), 87 out of the 7,665 OTUs showed presence across all 10 lakes, but these common taxa comprised between 45% and 85% (avg. 67%) of the sequences in each lake (details not shown). Between 8% to 38% of OTUs in a given lake were not detected in other lakes, suggesting that all lakes also harbor a set of unique AAP OTUs (Table 2).

Conductivity (and to a less extent Chl_a) were the only variables correlating significantly to the two axes of the NMDS of ambient lake AAP communities (Fig. 3a). Differences in conductivity (Euclidean distances) across lakes were also significantly correlated to taxonomic changes (Bray-Curtis dissimilarity) between *in situ* AAP communities (Mantel R=0.52, p=0.004, Table S1). Interestingly, moreover, differences in biotic properties such as total prokaryotic or viral abundance, or prokaryote heterotrophic production, were also significantly correlated to taxonomic differences in AAP assemblages between lakes (Table S1). Geographic location (latitude and longitude), instead, was not significantly related to either of the NMDS axes, and there was no significant relationship between the geographic distance and the taxonomic dissimilarity between AAP communities (Mantel R=-0.27, p=0.97).

Taxonomic shifts in AAP communities upon light exposure or release of predator pressure

The lake-specific clustering of AAP assemblages shown in Fig. 3a persisted even after the taxonomic shifts caused by experimental manipulations (Fig. 3b,c, ANOSIM_{BYLAKE} R=0.93, p<0.0001). In general, the dominance of betaproteobacterial AAP was maintained in most cases after the enclosure of lake communities in the bottles for 2-3 days (Table S2, Fig. S3). Only in the case of lake Bowker, the AAP community composition shifted largely during incubation, mostly due to the growth of a single alphaproteobacterial OTU belonging to the alpha2 phylogroup (affiliated to *Methylobacterium populi*, Fig. 3b-c, Fig. S4). However, this happened in the Control (CT) and Predator-reduced (PR) treatments, as well as under dark and light conditions, suggesting that the observed abundance increase of this OTU was due to some kind of bottle effect rather than to specific responses to the

experimental treatments.

Light exposure did not result in significant changes in the overall taxonomic structure of AAP communities relative to dark treatments or ambient communities (Fig. 3b, Table 3), but as we discuss below, certain OTUs did show significant responses. In contrast, the PR-treatment caused substantial shifts in overall AAP community structure in 8 out of the 10 lakes (Fig. 3b, Table 3). These compositional responses to the experimental treatments were not always associated to changes in taxonomic richness of communities (i.e., we did not find any consistent increase or decrease in OTU number during incubations associated to a given treatment across lakes, Fig. 4a). A significant decrease in taxonomic richness was only observed for lake Bowker after the PR incubation, probably caused by the growth of the single alpha2 OTU shown in Fig. S4. In other four cases, communities showed significantly higher number of OTUs after incubations (Fig. 4a), pointing to the recruitment of taxa that were rare in the original assemblages.

The response of communities to our experimental treatments in terms of total AAP abundances also varied across lakes, ranging from no change during incubations to 3-fold increases in AAP relative abundances in lake Waterloo after both CT and PR treatments (Fig. 4b). Such abundance changes, however, were not clearly associated to variations in taxonomic richness or in community structure during incubations in the five lakes where AAP counts were available. In other words, greater community shifts during incubations (Table 3) were not necessarily accompanied by more pronounced increases or decreases in AAP relative abundances (Fig. 4b), nor by larger changes in taxonomic richness (Fig. 4a).

AAP OTUs selectively responding to experimental treatments: PR-shifters and Light-

374 *shifters*

375 Regardless of the presence or absence of major compositional changes at the community
376 level, we detected a subset of OTUs that did respond positively to exposure to light
377 conditions (hereafter ‘Light-shifters’, which may represent OTUs that are favored upon
378 light exposure) or to the reduction of grazers (hereafter ‘PR-shifters’, which may represent
379 OTUs that were preferentially grazed *in situ*). We operationally identified ‘Light-shifters’
380 as those OTUs that met the following three criteria: i) they contributed at least 1% of the
381 community sequences by the end of the light control (CT) incubations in at least one
382 replicate; ii) they increased in relative abundances during the light CT incubations; and iii)
383 at the end of incubations, they were on average at least two fold more abundant in the light
384 than in the dark treatment. As our analysis relies on relative abundances, we acknowledge
385 that we may be missing other responsive taxa that changed in number but not in their
386 contribution to total AAP communities. However, we were interested in structural changes
387 in AAP assemblages, so our ‘shifter’ categorization enabled the identification of specific
388 taxa that became enriched in AAP assemblages after the experimental manipulations.

389 Overall we recovered 33 Light-shifter OTUs (Fig. 5a). In lakes Bowker, Lovering and
390 Coulombe no OTU met the selection criteria, and in the rest of the lakes, the number of
391 Light-shifter OTUs recovered ranged between 1 and 8 (Fig. 5a), and their cumulative
392 relative abundances accounted for 4%-30% of the total sequences after light CT
393 incubations. In terms of taxonomic composition, the phylogenetic identity of the Light-
394 shifters differed across lakes (Fig. 5a), although OTUs associated to the genus
395 *Limnohabitans* were detected among Light-shifters in all cases but lake Nicolet.

To identify ‘PR-shifters’, we selected OTUs that met the following three criteria: i) they contributed at least 1% of the community sequences by the end of the PR incubations in at least one replicate; ii) they increased in relative abundances during PR incubations; and iii) at the end of incubations, they were on average at least two fold more abundant in PR than in the CT treatment. This was done separately for dark and light conditions, resulting in the identification of both PR_dark-shifters and PR_light-shifters. Based on these criteria, and by pooling the results from the 10 lakes together, we recovered 52 PR_dark-shifter and 32 PR_light-shifter OTUs (Fig. 5b,c), 20 of which showed both behaviors (i.e., were identified as both PR_dark and PR_light shifters). No OTU met the selection criteria in the case of lake Bowker, where a single alpha2 OTU grew in all treatments (Fig. S4), and no PR_light-shifter was detected in lake Lovering (Fig. 5c). In the rest of the lakes, the number of PR_dark-shifters ranged from 2 in lake Coulombe to 12 in Nicolet and Montjoie (Fig. 5b), and the number of PR_light-shifters ranged from 2 in Nicolet to 10 in Magog. Their cumulative relative abundances accounted for up to 47% and 40% in the case of PR_dark- and PR_light-shifters, respectively, after dark or light PR incubations (Fig. 5b,c).

In terms of taxonomic composition, alpha- and betaproteobacterial PR-shifters were detected in every lake, but their identity clearly differed between lakes at finer taxonomic levels (Fig. 5b,c). In addition, although again OTUs associated to the genus *Limnohabitans* were the most cosmopolitan PR-shifters, since they were identified as such in the majority of cases (Fig. 5b,c), the identity of the taxa responding to the PR-treatment generally differed between light and dark incubations within a given lake (Fig. 5b,c).

In general, the increase in relative abundance of Light-shifters or PR-shifters within a given lake was consistent between replicates (Fig. 5). The magnitude of the PR-treatment

effect, i.e. the difference in final relative contribution of PR-shifters between CT and PR treatments (Fig. 5b,c), varied widely among lakes, but we could not relate this metric to any of the measured environmental or biological variables. Similarly, the light stimulation of the Light-shifter OTUs (Fig. 5a) was not related to any of the measured environmental or biological variables. The fact that not the same OTUs were categorized as PR_{dark} or PR_{light}-shifters within a given lake, and that the observed responses of PR-shifters differed between dark and light conditions, indicate a certain interaction (yet of unknown nature) between both treatments.

Abundance and presence of PR-shifter and Light-shifter OTUs across other lakes

Most OTUs identified as shifters in a given lake were rare *in situ*, accounting for less than 1% of community sequences or being even undetected in the original lake communities (Fig. 6a-c). In order to explore whether the PR- or the Light-shifters identified in a given lake were present across other lakes, as opposed to comprising a pool of OTUs unique to each lake, we searched for the shifters identified at a given lake across all the other lakes pooling together all *in situ* and experimental samples. We observed that whereas in some cases the PR-shifters found in a lake were almost exclusively present in that lake (e.g. lakes Lovering and Magog, Fig. 6e or lakes Magog and Montjoie, Fig. 6f), in most occasions PR-shifters from one lake were present across several other lakes reaching abundances of up to 40% of community sequences (Fig. 6e,f). In the case of the Light-shifters, they were always detected in more than one lake (Fig. 6d) and their abundances across other lakes ranged from rare to highly abundant (75%).

DISCUSSION

Since the discovery of AAP bacteria (Kolber *et al.*, 2000), multiple studies have attempted to gain insight into the factors that determine their distribution and activity, as well as the ecological and biogeochemical role they may play in natural aquatic ecosystems (Koblížek, 2015). Still, however, evidence is fragmented and often contradictory, and even basic questions such as the role of light in conferring a competitive advantage to AAP remain unsolved (Yurkov & Hughes, 2017). This is could be due to the fact that AAP are often approached as a coherent functional group, in particular in the few experimental studies performed so far with natural aquatic communities (Ferrera *et al.*, 2011; García-Chaves *et al.*, 2015; 2016; Ferrera *et al.*, 2017a; Sato-Takabe *et al.*, 2018). By combining for the first time experimental manipulations with high throughput sequencing of the *pufM* gene, here we show that comparable experimental treatments lead to different community responses across compositionally distinct lake AAP assemblages.

Environmentally different but closely located lakes harbor different AAP taxonomic communities

The sampled lakes differed largely in terms of light attenuation, DOC, Chla and nutrient concentration, all factors that have been shown to be important drivers of AAP abundances and taxonomic composition (Masín *et al.*, 2012; Cuperová *et al.*, 2013; Cáliz & Casamayor, 2014; Fauteux *et al.*, 2015; Ferrera *et al.*, 2017b). Accordingly, the relative abundance of AAP varied largely across our lakes (1%-22%) following DOC and water transparency gradients, and AAP communities showed a clear lake-specific taxonomic segregation that appeared related to changes in conductivity and chlorophyll *a* concentration. Geographic

position did not explain the spatial structuring of the ten AAP assemblages, suggesting that at least at the spatial scale of the study ($<500 \text{ km}^2$) dispersal limitation of taxa due to geographic location was not a major driver of communities. This agrees with the results from a previous survey, conducted at a much larger spatial scale, where lake AAP communities from different continents (assessed by clone libraries) clustered based on local environmental conditions and regional climatic variables, but not on geographic distance (Ferrera *et al.*, 2017b). In addition, differences in biotic parameters such as the *in situ* abundance of prokaryotes or viruses were related to the taxonomic changes between the studied AAP assemblages, and the number of different AAP OTUs per community increased towards lakes with higher viral abundances. This indicates that besides the local environment, the observed lake-specific segregation may be driven by local biotic processes such viral lysis or predation, both which were shown to have a simultaneous impact on AAP growth in marine waters (Ferrera *et al.* 2011), or by interactions with other prokaryotic or eukaryotic microorganisms. For example, AAP are often observed in association to phytoplankton, although the nature of this relationship is unclear and may be species-specific (see refs. in Koblížek). It is thus possible that differences in the phytoplankton, bacterioplankton, viral or grazer communities across our lakes may determine to a certain extent the taxonomic structure of AAP assemblages, an issue that deserves further attention.

Despite these inter-lake differences, all lakes showed a similar structure in terms of broad phylogenetic groups characterized by a dominance of betaproteobacterial OTUs, followed by different alphaproteobacterial subgroups. The taxonomic distribution of *pufM* OTUs was also strikingly similar even at finer taxonomic levels, showing a clear

dominance of *Limnohabitans*-like OTUs across all lakes as well as very similar proportions of the other detected genera. This dominance of Alpha- and Betaproteobacteria is consistent with previous surveys of lake AAP composition at both small and large spatial scales (Salka *et al.*, 2011; Cuperová *et al.*, 2013; Cáliz & Casamayor, 2014; Ferrera *et al.*, 2017b), and the absence of gammaproteobacterial OTUs agrees with the notion that these AAPs are restricted to marine waters (Cáliz & Casamayor, 2014).

Within our lakes, the relative abundance of these two major taxonomic groups varied along a pH gradient, with Betaproteobacteria being proportionally more abundant in neutral than in more alkaline waters. This points to a certain niche partition between alpha- and betaproteobacterial AAPs in these temperate Canadian lakes, but contrasts with a previous study on ten German lakes reporting the exact opposite pattern between pH and alpha- and betaproteobacterial AAPs (Salka *et al.*, 2011). However, whereas in our lakes betaproteobacterial *pufM* sequences were clearly dominated by the genus *Limnohabitans*, the abovementioned study found a prevalence of *Rhodoferrax*-like betaproteobacterial AAP, which could explain the contrasting patterns. This example illustrates the need to gain knowledge of AAP taxonomy at fine levels in order to understand AAP ecology and their potential responses to environmental queues.

Several studies in marine waters have described AAP communities using high throughput sequencing approaches (Ferrera *et al.*, 2014; Zheng *et al.*, 2015; Bibiloni-Isaksson *et al.*, 2016; Auladell *et al.*, 2019), yet to our knowledge all previous research on freshwater AAP diversity has been based on clone libraries or fingerprinting approaches that target only the most abundant community members (Salka *et al.*, 2011; Cuperová *et al.*, 2013; Ferrera *et al.*, 2017b). For instance, using clone libraries Ferrera *et al.* (2017b)

identified between 3 and 23 OTUs per community across a set of 23 globally-distributed lakes, whereas the number of OTUs identified in our ten lakes (at a sequencing depth of 10,000 reads/sample) ranged from 174 to 449 OTUs, increasing up to 600 OTUs during some incubations. Our results show that lake AAP communities can be highly diverse and that their taxonomic richness can change largely between lakes. Interestingly, the most taxonomically diverse AAP communities were also associated to the highest prokaryote heterotrophic activity measured at each lake. By measuring the uptake of ^3H -leucine by individual AAP cells in a subset of these lakes (García-Chaves *et al.* 2016), we estimated that AAP may contribute up to 20% of the total bacterial biomass production, so it is possible that changes in AAP communities can be reflected into processes at the whole bacterial community level. However, this relationship was not maintained after experimental incubations (details not shown), suggesting that different AAP taxa may contribute differently to the bulk AAP activity. In addition, the fact that some assemblages gained up to 200 new OTUs in 2-3 days indicates that rare AAP taxa may be key players in aquatic ecosystems, and highlights the relevance of high throughput sequencing methods for gaining insight into how AAP communities respond to environmental changes.

Responses of lake AAP communities to experimental manipulations: light exposure and predator reduction by dilution

We chose light exposure and predator reduction as our experimental treatments because, although these two factors have been repeatedly invoked as key drivers of AAP communities (Koblížek, 2015), so far no study had addressed whether or how variations in the responses of AAP assemblages to these factors may be driven by compositional differences between communities. Our results show that the lake-specific taxonomic

signature of AAP communities was maintained after exposure to the different treatments. This indicates that the pool of taxa responding to the experimental manipulations differs between lakes, because if the same taxa had responded during experiments, we would have expected a homogenization or convergence in the structure of AAP communities after manipulations. Light exposure did not result in detectable changes in the overall taxonomic composition of AAP communities compared to dark conditions, which agrees with the absence of clear light-driven effects on AAP heterotrophic activity previously reported for a subset of these same lakes (Garcia-Chaves *et al.*, 2016). The PR-treatment, however, led to significant overall compositional shifts most lakes. This agrees with a recent study performing similar experiments with whole marine bacterial communities, where predator removal also caused larger changes in composition than light exposure, by promoting the growth of certain taxa that are likely strongly grazed *in situ* (Sánchez *et al.*, 2017; Teira *et al.*, 2019).

Lack of taxonomic signal in responsive AAP taxa across lakes

Of the 7,665 OTUs, only a small sub-set (33 OTUs) appeared to be favored under light exposure with increasing contribution in relative abundance. These Light-shifters belonged to different taxonomic groups in each lake, such that responsiveness to light does not appear to be associated to any particular AAP phylogroup, at least at the scale of our study. Since the discovery of AAP and their photoheterotrophic potential, many efforts have been devoted to understand under which circumstances the capacity to use light may confer them an ecological advantage, but we still lack conclusive evidence of obvious positive effects of light on AAP communities in ambient waters. For example, light did not enhance AAP single-cell uptake of leucine in experimental incubations in estuarine, lake or coastal waters

(Kirchman *et al.*, 2014; Stegman *et al.*, 2014; Garcia-Chaves *et al.*, 2016), and only recently, Ferrera *et al.*, (2017a) reported for the first time a light-driven increase in growth rates of natural AAP populations from a coastal marine site. Differences in taxonomic composition may thus underlie the differences in bulk AAP level responses to light between ecosystems or over time reported in the abovementioned experimental studies (Kirchman *et al.*, 2014; Stegman *et al.*, 2014; Garcia-Chaves *et al.*, 2016; Ferrera *et al.*, 2017a). Our results highlight the unlikelihood of finding coherent light-driven patterns in bulk properties such as total AAP numbers or activity when taxonomically different communities are compared.

On the other hand, the structural shifts due to the PR treatment observed in most lakes may suggest a widespread top-down control of certain AAP members, or alternatively, the presence of opportunistic of AAP groups that are limited by the available resources *in situ* and that grow fast when the number of bacterial competitors is reduced. Even though we cannot discern whether the PR-shifters were responding to experimental changes in top-down or bottom-up controls, the only other AAP study performing a manipulation similar to our PR-treatment (DL-treatment in Ferrera *et al.* 2011) concluded that the dilution effect on the growth of marine AAP was mostly caused by a decrease in the grazing pressure, rather than by the release of resource competition, which was shown to have a rather small effect.

The hypothesis that AAP bacteria may be subjected to strong selective losses has been based on the fact that AAP bacteria are on average larger and more active than the other heterotrophic cells in bacterioplankton communities (Koblížek *et al.*, 2007; Liu *et al.*, 2010; Kirchman *et al.*, 2014; Stegman *et al.*, 2014; Fauteux *et al.*, 2015; Koblížek, 2015). To our

knowledge, only three studies (two in marine sites (Ferrera *et al.*, 2011; Sato-Takabe *et al.*, 2018) and one in lakes (García-Chaves *et al.*, 2015)) have shown that AAP bacteria as a whole grow faster and are preferentially grazed compared to other prokaryotes. The finding that the identity of the PR shifters varies between lakes further suggests that not all AAP taxa are equally susceptible to predation, such that the strength of top-down control may vary widely over time or space depending on AAP composition or on the nature of the grazer communities, that may also differ largely between such environmentally different lakes. In addition, the observation that different taxa were identified as PR-shifters under dark or light conditions points to a certain interaction between these two potential drivers of AAP. Understanding the nature of such interaction would require much more controlled experimental setups, as the effects of light on aquatic microorganisms are extremely complex and can be either direct or indirect by simultaneously affecting multiple trophic levels (e.g., Ruiz-González *et al.*, 2013b).

The lack of a phylogenetic signal in the pool of AAP OTUs responding positively to the experimental manipulations (i.e., the light- or PR-shifters) was not necessarily expected, as for example similar manipulations with marine bacterial communities often lead to predictable responses where a few opportunistic groups within Gammaproteobacteria, Rhodobacteraceae or Flavobacteriales often grow and dominate experimental treatments (e.g. Ferrera *et al.* 2011, Teira *et al.* 2019). In soils, bacterial responses to rewetting were phylogenetically conserved, suggesting that certain strategies are specific to particular taxa groups (Placella *et al.*, 2012). Our results suggest instead that AAP community responses are not easily predictable, and call for the need of a deeper understanding of the ecology of different AAP community members in natural ecosystems. Finally, the fact most of the

responsive OTUs were rare and even undetected in the original waters means that field surveys conducted so far may have disregarded a key component of these AAP communities that is prone to respond upon changes in environmental conditions, and which may be driving the observed responses at the whole community level.

Limnohabitans as an ubiquitous responsive AAP community member

Only OTUs associated to the betaproteobacterial genus *Limnohabitans* seemed to consistently respond to predator reduction and light exposure in most lakes. *Limnohabitans* is a ubiquitous component of freshwater bacterioplankton communities, known to include taxa with opportunistic strategies (Kasalický *et al.*, 2013; Salcher, 2014; Jezberová *et al.*, 2017). The reported higher growth and substrate uptake rates, metabolic versatility, large cell sizes and higher mortality rates from bacterivory of this genus would explain its recurrent presence among our ‘shifter’ OTUs. Although initially this genus was believed to contain only heterotrophic species (Hahn *et al.*, 2010), recent studies have detected the *pufM* gene in multiple *Limnohabitans* strains (Zeng *et al.*, 2012; Kasalický *et al.*, 2018). OTUs identified as *Limnohabitans* comprised 33%-77% of the AAP sequences (and *ca.* 50% of the OTUs) detected initially in our lakes, supporting their ubiquity and potential role in these aquatic ecosystems. Our results thus support that members of this genus may be a key component of AAP communities across environmentally different lakes, which readily responds to changing conditions.

Methodological considerations

Unlike in field surveys, where drivers of AAP communities are deduced from links between spatial or temporal changes in taxonomic composition and the environment,

experimental manipulations allow a more direct evaluation of the responses of a given AAP community to particular factors. However, we acknowledge that our manipulations have limitations, and that the observed experimental outcomes may be the consequence of several unconsidered factors. For example, besides reducing the encounter rates with predators, the PR-treatment might also have released competition for resources due to a decrease in the total number of bacteria. Nonetheless, due to the large size of freshwater AAP bacteria reported in these (Fauteux *et al.*, 2015; Garcia-Chaves *et al.*, 2016) and other lakes (Cuperová *et al.*, 2013; García-Chaves *et al.*, 2015), and their potential preference to be attached to particles (e.g. Masín *et al.*, 2012), removing predators by 0.8µm or 1µm-filtration as performed in previous studies (Ferrera *et al.*, 2011; Sato-Takabe *et al.*, 2018) could have resulted in fundamental changes in the original composition of AAP assemblages.

On the other hand, interpreting light responses of bacterial communities has also proven difficult, as light effects can be direct or indirect (e.g., stimulation of activity due to light-enhanced photosynthate release by algae) and may be influenced by the light exposure history of assemblages (Ruiz-González *et al.*, 2013b). In any case, all current knowledge about light and predation on natural AAP communities derives from experimental manipulations similar to those conducted in our study (Ferrera *et al.*, 2011; García-Chaves *et al.*, 2015; Sato-Takabe *et al.*, 2018). Our aim was thus to keep the experimental setup as comparable as possible to those previous studies to address whether similar experimental manipulations trigger different responses when taxonomically different communities are targeted.

In addition, we acknowledge that diversity studies based on PCR amplification of

marker genes may suffer from primer biases, i.e. low phylogenetic coverage or misrepresentation of the relative abundances of certain populations. In fact, a recent evaluation of the primer set used in this study unveiled that some phylogenetic groups present in metagenomes were missed by these primers, but they recovered the major metagenome-identified AAP groups and more importantly, the seasonal diversity patterns unveiled with both methodologies were remarkably similar (Auladell et al. 2019). In any case, even if our data do not fully capture the AAP diversity present in these lakes, our study represents not only the deepest characterization of freshwater AAP communities conducted so far, but also the first attempt to assess compositional responses of aquatic AAP communities by combining high-throughput sequencing with experimental manipulations. Such a deep description is actually needed to follow the dynamics of rare community members that may become abundant upon changes in conditions like those imposed by our experimental manipulations, but also to understand the environmental tolerances and preferences of the different components within these complex AAP assemblages.

Conclusions

This study represents the first in-depth description of lake AAP targeting both abundant and rare community members. We show that different lakes harbor taxonomically distinct AAP communities, and that these differences are mostly explained by local environmental or biotic conditions rather than by geographic position. Predator removal resulted in larger compositional changes than light exposure, suggesting that grazers may further shape these communities by controlling the growth of different phylogroups. Regardless of the ubiquitous presence of certain AAP OTUs, the pool of taxa responding to the experimental

manipulation changed largely between lakes, showing no clear phylogenetic affiliation. Only OTUs belonging to *Limnohabitans* were consistently found among the most responsive OTUs, highlighting a relevant role of this genus across heterogeneous AAP assemblages. Our results suggests a scenario where environmental conditions strongly influence the local composition of the AAP assemblage, which in turn modulates how the assemblage as a whole will respond to grazing, light, and perhaps other environmental and biological drivers. The freshwater AAP are clearly much more complex and diverse from an eco-physiological standpoint than what has been assumed in previous experimental studies, and thus understanding AAP ecology and role in ecosystems necessarily requires incorporating the taxonomic dimension.

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Author contributions. MCGC and PdG designed the study and the sampling scheme. MCGC performed the experiments, collected and processed the samples, and extracted the DNA. JPNG and CRG processed the sequence data. IF performed the phylogenetic analyses and taxonomic assignation of the sequences. CRG conceived the idea of the manuscript, compiled the needed data, performed all analyses and wrote this article. All authors made relevant comments and editions to the text.

Data accessibility. DNA sequences and associated metadata: European Nucleotide Database (ENA) under accession number PRJEB36763.

REFERENCES

- Auladell, A., Sánchez, P., Sánchez, O., Gasol, J.M. & Ferrera, I. (2019) Long-term seasonality of marine photoheterotrophic bacteria reveals low cohesiveness within the different phylogroups. *The ISME Journal*, 13:1975–1987
- Béjà, O., Suzuki, M.T., Heidelberg, J.F., Nelson, W.C., Preston, C.M., Hamada, T., Eisen, J.A., Fraser, C.M. & DeLong, E.F. (2002) Unsuspected diversity among marine aerobic anoxygenic phototrophs. *Nature*, **415**, 630–633.
- Bibiloni-Isaksson, J., Seymour, J.R., Ingleton, T., Van de Kamp, J., Bodrossy, L. & Brown, M.V. (2016) Spatial and temporal variability of aerobic anoxygenic photoheterotrophic bacteria along the east coast of Australia. *Environmental Microbiology*, **18**, 4485–4500.
- Cáliz, J. & Casamayor, E.O. (2014) Environmental controls and composition of anoxygenic photoheterotrophs in ultraoligotrophic high-altitude lakes (Central Pyrenees). *Environmental Microbiology Reports*, **6**, 145–151.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, J., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G. & Knight, R. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, **6**, 1621–1624.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kellye, S.T., Knight, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunencko, T., Zaneveld, J. & Knight, R. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, **7**, 335–336.
- Cottrell, M.T. & Kirchman, D.L. (2009) Photoheterotrophic Microbes in the Arctic Ocean in Summer and Winter. *Applied and Environmental Microbiology*, **75**, 4958–4966.

- Cottrell, M.T., Mannino, A. & Kirchman, D.L. (2006) Aerobic anoxygenic phototrophic bacteria in the Mid-Atlantic Bight and the North Pacific Gyre. *Applied and Environmental Microbiology*, **72**, 557-564.
- Cottrell, M.T., Ras, J. & Kirchman, D.L. (2010) Bacteriochlorophyll and community structure of aerobic anoxygenic phototrophic bacteria in a particle-rich estuary. *The ISME Journal*, **4**, 945-954.
- Cuperová, Z., Holzer, E., Salka, I., Sommaruga, R. & Koblízek, M. (2013) Temporal changes and altitudinal distribution of aerobic anoxygenic phototrophs in mountain lakes. *Applied and Environmental Microbiology*, **79**, 6439.
- Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C. & Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, **27**, 2194–2200.
- Fauteux, L., Cottrell, M.T., Kirchman, D.L., Borrego, C.M., Garcia-Chaves, M.C. & 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**, e0124035.
- Ferrera, I., Borrego, C.M., Salazar, G. & Gasol, J.A. (2014) 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., Sánchez, O., Kolářová, E. & Gasol, J.M. (2017a) Light enhances the growth rates of natural populations of aerobic anoxygenic phototrophic bacteria. *The ISME Journal*, **10**, 2391-2393.
- Ferrera, I., Gasol, J.M., Sebastian, M., Hojerova, E. & Koblizek, M. (2011) Comparison of Growth Rates of Aerobic Anoxygenic Phototrophic Bacteria and Other Bacterioplankton Groups in Coastal Mediterranean Waters. *Applied and Environmental Microbiology*, **77**, 7451-7458.
- Ferrera, I., Sarmiento, H., Priscu, J.C., Chiuchiolo, A., González, J.M. & Grossart, H.P. (2017b) Diversity and distribution of freshwater aerobic anoxygenic phototrophic bacteria across a wide latitudinal gradient. *Frontiers in Microbiology*, **8**, 175.
- Garcia-Chaves, M.C., Cottrell, M.T., Kirchman, D.L., Ruiz-González, C. & del Giorgio, P.A. (2016) Single-cell activity of freshwater aerobic anoxygenic phototrophic bacteria and their contribution to biomass production. *The ISME Journal*, **10**, 1579-1588.
- García-Chaves, M.C., Cottrell, M.T., Kirchman, D.L., Derry, A.M., Bogard, M.J. & 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**, 71-83.
- Hahn, M.W., Kasalický, V., Jezbera, J., Brandt, U., Jezberová, J. & Šimek, K. (2010) *Limnohabitans curvus* gen. nov., sp. nov., a planktonic bacterium isolated from a freshwater lake. *International Journal of Systematic and Evolutionary Microbiology*, **60**, 1358–1365.
- Hojerová, E., Masin, M., Brunet, C., Ferrera, I., Gasol, J.M. & Koblizek, M. (2011) Distribution and growth of aerobic anoxygenic phototrophs in the Mediterranean Sea. *Environmental Microbiology*, **13**, 2717-2725.
- Jezberová, J., Jezbera, J., Znachor, P., Nedoma, J., Kasalický, V. & Šimek, K. (2017) The *Limnohabitans* genus harbors generalistic and opportunistic subtypes: evidence

774 from spatiotemporal succession in a canyon-shaped reservoir. *Applied and*
775 *Environmental Microbiology*, **83**, e01530.

776 Jiao, N., Zhang, Y., Zeng, Y., Hong, N., Liu, R., Chen, F. & Wang, P. (2007) Distinct
777 distribution pattern of abundance and diversity of aerobic anoxygenic phototrophic
778 bacteria in the global ocean. *Environmental Microbiology*, **9**, 3091-3099.

779 Kasalický, V., Jezbera, J., Hahn, M.W. & Simek, K. (2013) The Diversity of the
780 Limnohabitans Genus, an Important Group of Freshwater Bacterioplankton, by
781 Characterization of 35 Isolated Strains. *Plos One*, **8**, e58209.

782 Kasalický, V., Zeng, Y., Piwosz, K., Simek, K., Kratochvilova, H. & Koblizek, M. (2018)
783 Aerobic anoxygenic photosynthesis is commonly present within the genus
784 Limnohabitans. *Applied and Environmental Microbiology*, **84** e02116-17.

785 Kirchman, D., Knees, E. & Hodson, R. (1985) Leucine incorporation and its potential as a
786 measure of protein-synthesis by bacteria in natural aquatic systems. *Applied and*
787 *Environmental Microbiology*, **49**, 599-607.

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

791 Koblížek, M. (2015) Ecology of aerobic anoxygenic phototrophs in aquatic environments.
792 *Fems Microbiology Reviews*, **39**, 854-870.

793 Koblížek, M., Masin, M., Ras, J., Poulton, A.J. & Prasil, O. (2007) Rapid growth rates of
794 aerobic anoxygenic phototrophs in the ocean. *Environmental Microbiology*, **9**,
795 2401-2406.

796 Kolářová, E., Medová, H., Piwosz, K. & Koblizek, M. (2019) Seasonal dynamics of
797 aerobic anoxygenic phototrophs in freshwater lake Vlkov. *Folia Microbiologica*, **64**,
798 705-710.

799 Kolber, Z.S., Van Dover, C.L., Niederman, R.A. & Falkowski, P.G. (2000) Bacterial
800 photosynthesis in surface waters of the open ocean. *Nature*, **407**, 177-179.

801 Lamy, D., Jeanthon, C., Cottrell, M.T., Kirchman, D.L., Van Wambeke, F., Ras, J., Dahan,
802 O., Pujo-Pay, M., Oriol, L., Bariat, L., Catala, P., Cornet-Barhau, V. & Lebaron, P.
803 (2011) Ecology of aerobic anoxygenic phototrophic bacteria along an oligotrophic
804 gradient in the Mediterranean Sea. *Biogeosciences*, **8**, 973-985.

805 Lehours, A.C., Enault, F., Boeuf, D. & Jeanthon, C. (2018) Biogeographic patterns of
806 aerobic anoxygenic phototrophic bacteria reveal an ecological consistency of
807 phylogenetic clades in different oceanic biomes. *Scientific Reports*, **8**, 4105.

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

811 Ludwig, W. (2004) ARB: a software environment for sequence data. *Nucleic Acids*
812 *Research*, **32**, 1363-1371.

813 Mahé, F., Rognes, T., Quince, C., de Vargas, C. & Dunthorn, M. (2014) Swarm: robust and
814 fast clustering method for amplicon-based studies. *Peer J*, **2**, e593.

815 Mahé, F., Rognes, T., Quince, C., de Vargas, C. & Dunthorn, M. (2015) Swarm v2: highly-
816 scalable and high-resolution amplicon clustering. *Peer J*, **3**, e1420.

817 Masín, M., Cuperova, Z., Hojerova, E., Salka, I., Grossart, H.-P. & Koblížek, M. (2012)
818 Distribution of aerobic anoxygenic phototrophic bacteria in glacial lakes of northern
819 Europe. *Aquatic Microbial Ecology*, **66**, 77-86.

820 Mesa-Ortiz, M.C. (2014) Diseño de una métrica para la evaluación de la capacidad

- fotoheterótrofa *in situ* de bacterias fotótrofas anoxigénicas aerobias (AAP). Msc Thesis, Universidad Nacional de Colombia (<http://bdigital.unal.edu.co/46177/>)
- Oksanen, J., F., Blanchet, G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P.M., Stevens, H.H. & Wagner, H. (2015) Vegan: Community Ecology Package. R package version 2.3-0.
- Placella, S.A., Brodie, E.L. & Firestone, M.K. (2012) Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. *Proc Natl Acad Sci USA*, **109**, 10931-10936.
- Ritchie, A.E. & Johnson, Z.I. (2012) Abundance and genetic diversity of aerobic anoxygenic phototrophic bacteria of coastal regions of the Pacific Ocean. *Applied and Environmental Microbiology*, **78**, 2858.
- Ruiz-González C, Proia, L., Ferrera, I., Gasol, J.M. & Sabater, S. (2013a). Effects of large river dam-regulation on bacterioplankton community structure. *FEMS Microbiology Ecology* **84**, 316–331
- Ruiz-González, C., Simó, R., Sommaruga, R. & Gasol, J.M. (2013b) Away from darkness: a review on the effects of solar radiation on heterotrophic bacterioplankton activity. *Frontiers in Microbiology*, **4**, 131.
- Ruiz-González, C. (2020) *pufM* genes from ten different temperate Canadian lakes and experimental manipulations of light and predator removal; European Nucleotide Database (ENA) (<https://www.ebi.ac.uk/ena/data/view/PRJEB36763>)
- Salcher, M.M. (2014) Same same but different: ecological niche partitioning of planktonic freshwater prokaryotes. *Journal of Limnology*, **73**, 74-87.
- Salka, I., Cuperova, Z., Masin, M., Koblížek, M. & Grossart, H.-P. (2011) Rhodoferrax-related *pufM* gene cluster dominates the aerobic anoxygenic phototrophic communities in German freshwater lakes. *Environmental Microbiology*, **13**, 2865-2875.
- Salka, I., Moulisova, V., Koblížek, M., Jost, G., Jurgens, K. & Labrenz, M. (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**, 4398-4404.
- Sánchez, O., Koblížek, M., Gasol, J.M. & Ferrera, I. (2017) Effects of grazing, phosphorus and light on the growth rates of major bacterioplankton taxa in the coastal NW Mediterranean. *Environmental Microbiology Reports*, **9**, 300-309.
- Sato-Takabe, Y., Hamasaki, K. & Suzuki, S. (2018) High temperature accelerates growth of aerobic anoxygenic phototrophic bacteria in seawater. *Microbiology Open*, e00710.
- Smith, D. & Azam, F. (1992) A simple, economical method for measuring bacteria protein synthesis rates in seawater using ³H-leucine. *Marine Microbial Food Webs*, **6**, 107-114.
- Suttle, C. A., and J. A. Fuhrman. 2010. Enumeration of virus particles in aquatic or sediment samples by epifluorescence microscopy, p. 145–153. In S. W. Wilhelm, M. G. Weinbauer, and C. A. Suttle [eds.], *Manual of Aquatic Viral Ecology*. ASLO.
- Stegman, M.R., Cottrell, M.T. & Kirchman, D.L. (2014) Leucine incorporation by aerobic anoxygenic phototrophic bacteria in the Delaware estuary. *The ISME Journal*, **8**, 2339–2348.
- Teira, E., Logares, R., Gutierrez-Barral, A., Ferrera, I., Varela, M.M., Morán, X.A.G. & Gasol, J.M. (2019) Impact of grazing, resource availability and light on prokaryotic

- growth and diversity in the oligotrophic surface global ocean. *Environmental Microbiology*, **21**, 1482–1496.
- Waidner, L.A. & 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**, 3936–3944.
- Waidner, L.A. & Kirchman, D.L. (2008) Diversity and distribution of ecotypes of the aerobic anoxygenic phototrophy gene *pufM* in the Delaware estuary. *Applied and Environmental Microbiology*, **74**, 4012–4021.
- Yurkov, V. & Hughes, E. (2017) Aerobic Anoxygenic Phototrophs: Four Decades of Mystery. *Modern Topics in the Phototrophic Prokaryotes* (ed. by P. Hallenbeck), pp. 193–214. Springer Nature, Gewerbestrasse, Switzerland.
- Yutin, N., Suzuki, M.T. & Béjà, O. (2005) Novel primers reveal wider diversity among marine aerobic anoxygenic phototrophs. *Applied and Environmental Microbiology*, **71**, 8958–8962.
- Zeng, Y., Kasalický, V., Šimek, K. & Koblížek, M. (2012) Genome sequences of two freshwater betaproteobacterial isolates, *Limnohabitans* species strains Rim28 and Rim47, indicate their capabilities as both photoautotrophs and ammonia oxidizers. *Journal of Bacteriology*, **194**, 6302–6303.
- Zhang, J., Kobert, K., Flouri, T. & Stamatakis, A. (2014) PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*, **30**, 614–620.
- Zheng, Q., Liu, Y., Steindler, L. & Jiao, N. (2015) Pyrosequencing analysis of aerobic anoxygenic phototrophic bacterial community structure in the oligotrophic western Pacific Ocean. *Fems Microbiology Letters*, **362**, fnv034.

Table 1. *In situ* environmental or biotic properties in the epilimnetic waters of the studied lakes. Lakes are ordered following a gradient of increasing DOC concentrations.

Lake	Bowker	Nicolet	Croche	Magog	Stukely	Lovering	Montjoie	Waterloo	Aylmer	Coulombe
Latitude	45.4067	45.8378	45.9922	45.3244	45.3639	45.1661	45.4156	45.3350	45.8253	45.8400
Longitude	-72.2269	-71.5569	-74.0053	-72.0275	-72.2531	-72.1494	-72.1036	-72.5175	-71.3783	-71.4658
Temperature (°C)	21.4	20.1	20.9	23.8	24.3	24.0	23.7	25.2	20.4	19.1
Sampling depth (m)	6.0	3.5	1.5	4.0	2.0	3.0	3.0	2.0	1.0	0.5
Fully aerobic water column	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes	No
Conductivity (µS cm ⁻¹)	58	49	18	148	58	104	70	94	84	42
pH	8.20	7.47	6.75	8.51	7.99	8.18	7.43	8.51	7.02	7.48
DOC (mg L ⁻¹)	2.70	3.51	4.80	4.90	5.66	6.83	6.96	7.80	10.13	13.84
K _{PAR} (m ⁻¹)	0.31	0.52	0.67	0.50	0.53	0.64	0.67	1.34	1.05	1.57
Dissolved oxygen (%)	109.70	94.10	95.90	107.80	96.69	101.70	92.00	103.30	95.20	89.40
Total Nitrogen (mg L ⁻¹)	0.15	0.29	0.16	0.25	0.19	0.28	0.26	0.49	0.45	0.44
Total Phosphorus (µg L ⁻¹)	3.30	2.71	2.67	12.73	4.03	8.51	6.10	6.20	11.72	16.02
Chl <i>a</i> (µg L ⁻¹)	0.84	0.73	1.05	2.90	2.60	1.80	2.40	11.00	3.30	3.69
Total prokaryotes (10 ⁶ cells ml ⁻¹)	0.94	0.70	0.92	-	1.60	2.39	3.30	3.76	2.54	2.13
Total viruses (10 ⁸ ml ⁻¹)	2.42	3.72	2.25	6.47	3.46	4.43	-	5.70	4.09	3.47
Prok. heterotrophic prod. (pmol ³ H-Leu L ⁻¹ h ⁻¹)	2.3	31.8	-	187.6	6.5	0.1	3.7	406.6	138.9	35.3
AAP bacteria (10 ⁵ cells ml ⁻¹)	0.32	0.71	0.40	-	0.16	1.94	0.05	2.76	0.66	4.74
% AAP	3.5	10.1	4.3	-	12.2	1.1	1.5	7.3	2.6	22.2

Table 2. Average number of operational taxonomic units (OTU), Shannon diversity index and percentage of unique OTUs (i.e., OTUs detected only in one lake) in each of the studied lakes, considering all *in situ* and experimental samples together. OTU richness and diversity were calculated from the rarefied OTU table, whereas the % of unique OTUs was estimated from the non-rarefied database.

Lake	Number of OTUs	Shannon Index	% of unique OTUs	% purple sulfur bacterial sequences
Bowker	248	3.2	13.8	0.11
Nicolet	235	2.8	26.9	0.15
Croche	331	3.2	32.4	0.99
Magog	449	4.3	23.5	2.41
Stukely	119	2.2	7.8	0.33
Lovering	240	3.5	12.8	1.45
Montjoie	174	2.5	18.2	0.55
Waterloo	437	4.3	20.7	0.45
Aylmer	425	3.8	38.1	13.46
Coulombe	359	3.3	23.6	0.20

Table 3. ANOSIM *R* values (and *p* values) indicating differences in AAP taxonomic composition between control and predator-reduced treatments, and between dark and light treatments. Only composition at the end of the experiment was considered for these analyses. All *R* for Bray-Curtis distance matrices were calculated using 9999 permutations. n.s. = not significant

	ANOSIM <i>R</i> values	
	Control vs predator-reduced	Dark vs light
Bowker	0.55 (0.003)	n.s.
Nicolet	n.s.	n.s.
Croche	0.60 (0.03)	n.s.
Magog	0.65 (0.03)	n.s.
Stukely	n.s.	n.s.
Lovering	0.49 (0.03)	n.s.
Montjoie	0.92 (0.002)	n.s.
Waterloo	0.85 (0.03)	n.s.
Aylmer	0.67 (0.03)	n.s.
Coulombe	0.56 (0.03)	n.s.

FIGURE LEGENDS

Figure 1. Relative contribution of each phylogenetic group to the total diversity of the *in situ* AAP communities across lakes. Values calculated as the percentage of total OTUs (a) or total sequences (b) associated to each taxonomic rank within each lake before starting the experiments. Lakes are ordered along a gradient of increasing DOC concentration. Relative contribution of alphaproteobacterial (c) and betaproteobacterial (d) AAPs to total *pufM* sequences along the pH gradient. The black lines are Pearson's linear correlations. Shaded areas represent the 95% confidence intervals.

Figure 2. Taxonomic composition at the genus level of the studied lake AAP communities, considering only *in situ* AAP assemblages. Values are calculated as the percentage of total *pufM* OTUs (a) or total sequences (b) associated to each taxonomic rank within each lake. Lakes are ordered along a gradient of increasing DOC concentration.

921 **Figure 3.** NMDS ordination of AAP communities based on the Bray-Curtis
 922 dissimilarity, color-coded by lake. a) *In situ* AAP communities; the size of the dot is
 923 proportional to the number of OTUs identified in each lake. The arrows indicate the
 924 direction at which the environmental vectors fit the best (using the envfit function) onto the
 925 NMDS ordination space, and the size of the arrow is proportional to the strength of the
 926 correlation of each variable. b-c) All communities before (Initial) and after experiments; the
 927 shape indicates the different treatments shown (control –CT- versus predator-reduced –PR-
 928 in b, and light versus dark in c). Chla, Chlorophyll *a* ($\mu\text{g L}^{-1}$); Cond, conductivity ($\mu\text{S cm}^{-1}$).
 929 Normalized stress values are shown for each plot.

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Figure 4. a) Changes in the number of *pufM* OTUs per community during experimental incubations in each lake. b) Changes in the relative abundance of AAP bacteria (% of total prokaryotic counts) during experimental incubations in the five lakes where AAP were quantified by infrared microscopy. Shown are the mean and standard deviation of the experimental replicates in each case. Different letters indicate significant differences between AAP communities before and after the experiments (Tukey's test, $p < 0.001$).

Figure 5. (a) Relative abundance of the AAP OTUs that were categorized as Light-shifters before and after dark and light incubations. The pie charts indicate the taxonomic composition of the Light-shifters at the end of the CT light incubations, expressed as percent contribution of each taxonomic group to the total Light-shifter sequences after light incubations. (b-c) Relative abundance of the AAP OTUs that were categorized as PR-shifters before and after control (CT) and Predator-reduced (PR) treatments, incubated either in the dark (b) or in the light (c). In the case of the PR-shifters (b,c), the initial communities of both the PR and CT communities are plotted together as 'Initial' for simplification. The pie charts indicate the taxonomic composition of the PR-shifters at the end of the PR-dark (b) or PR-light (c) incubations, respectively, expressed as percent contribution of each taxonomic group to the total PR-shifter sequences after PR incubations. In all cases, the numbers on top indicate the number of OTUs identified as Light-shifters or PR-shifters in each lake.

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Figure 6. Relative abundance of Light-shifter (a) or PR-shifter (b,c) OTUs in the original lake AAP communities. Note that in most cases, OTUs identified as shifters were rare or even not detected *in situ*. (d-f) Contribution to community sequences of Light-shifters (d) PR_dark-shifters (e) or PR_light-shifters (f) identified in a given lake (indicated by the asterisk) across all the other lakes, pooling all *in situ* and experimental samples of each lake together.