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ORIGINAL ARTICLE

Micro-players for macro-roles: aquatic microbes in deep lakes

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ABSTRACT
Despite their relative low volume on a global scale, lakes represent a valuable reserve of accessible freshwaters. Any increase in our understanding of their functioning is a step towards their better exploitation and preservation. Microorganisms drive the metabolic processes that regulate the biogeochemical cycles in lakes; here, I will focus on Archaea and Cyanobacteria as micro-players playing macro-roles in lake ecosystems. In particular, I will present two examples to demonstrate the importance of prokaryotes in lakes: picocyanobacteria and mesophilic archaea. The role of prokaryotes in lakes will be discussed in relation to their taxonomy, dynamics and ecology using these two examples. The challenge, now, is to better understand the relationship between the diversity and the physiology of various ecotypes, how these interact with each other and with environmental factors allowing the proliferation of the most competitive genotypes.

Key words: Aquatic microbes; Synechococcus*; Thaumarchaeota; lakes.*

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PICOCYANOBACTERIA

The main components of autotrophic picoplankton in lakes are *Synechococcus*-type cyanobacteria, which generally exceeds the eukaryotic fraction. For this reason, autotrophic picoplankton is often considered as synonymous of picocyanobacteria. We know that the percentage of eukaryotic cells in the pico-size range increases in eutrophic or distrophic systems (Callieri, 2007) and in humic lakes (Jasser and Arvola, 2003). The ecological characteristics of picocyanobacteria and picoeukaryotes are different; however, their role in the trophic chain, as a carbon reserve for grazers, is similar due to their commensurate size-range.

Here I will report results from studies referred mainly to picocyanobacteria in deep lakes, where they play a significant role as primary producers (Callieri and Piscia, 2002). Two main genera, *Synechococcus* and *Cyanobium*, dominate in freshwaters. *Synechococcus* can be present in lakes both as single-cell and as microcolony (Passoni and Callieri, 2000; Callieri *at al.*, 2012) (Fig. 1). Microcolony formation can be facilitated by the presence of a regularly ordered globular protein (S-layer) on the cell surface (Ernst *et al.,* 1996), or even by rigid spinae, induced by grazers (Jezberová and Komárková, 2007). Another effective factor on microcolony formation is the ultraviolet radiation (UVR; 280-400 nm). It has been demonstrated that a selected *Synechococcus* ribotype, acclimated to low light, formed microcolonies as a quick-defence strategy (Callieri *et al.,* 2011). These monoclonal microcolonies, similar to those found in nature, should not be mistaken for aggregates like the ones that form after exposition to high blue light, as a result of the production of oxygen reactive radicals (Koblížek *et al.,* 2000). It has been suggested that microcolonies may be considered transition forms from single-cells to true colonial, changing the trophic role of *Synechococcus* in the food web (Callieri *et al.,* 2012). Microcolonies are generally present throughout the euphotic zone, with a peak of abundance in summer or autumn in a variety of lakes (Passoni and Callieri, 2000; Komárková, 2002; Crosbie *et al.,* 2003b; Mózes *et al.,* 2006; Ivanikova *et al.,* 2007). In general, single cells tend to prefer large, deep oligo-mesotrophic lakes, whereas microcolonies dominate in warm, shallow eutrophic lakes.

It is important to recall that *Synechococcus* are gram-negative bacteria with an outer membrane of lipopolysaccharide (LPS), and a peptidoglycan layer considerably thicker than that of most gram-negative bacteria (Hoiczyk and Hansel, 2000). They also have surface porins with a strict molecular size cutoff that potentially protect them from harmful agents (Hoiczyk and Hansel, 2000). As a consequence, due to their characteristics they are small sized autotrophic cells able to compete with larger phytoplankton in harsh conditions. An example of the different ecological niches occupied by picocyanobacteria and by the larger autotrophic fraction is clearly evident in North Patagonian ultraoligotrophic lakes (Fig. 2) (Modenutti *et al.,* 2004; Callieri *et al.,* 2007). In such extreme environments, picocyanobacteria are successful as they do not compete with phytoplankton, but rather take up the most unfavourable zone in the water column. This stratification takes place thanks to the small size of picocyanobacteria and to the phycobiliprotein content in the cell, which makes the increase of photosynthetic efficiency at low light possible (Callieri *et al.,* 2007).

Picocyanobacteria importance increases with their numbers relative to total phytoplankton in oligotrophic lakes, even if their abundances are higher in eutrophic lakes (Stockner *et al.*, 2000). The model proposed (Stockner *et al.*, 2000) was after enlarged by using data from 38 lakes, including high altitude clear lakes, deep large subalpine and ultraoligotrophic andine lakes, large shallow lakes, small reservoirs and fish ponds (Callieri *et al.,* 2007) (Fig. 3). In terms of activity, picophytoplankton contribution to total primary production increases in oligotrophic waters reaching up to 80% (Lake Baikal; Nagata *et al.,* 1994). During the process of Lake Maggiore oligotrophication a trend towards the increase of the percentage of picophytoplankton production on the total primary production was observed (Fig. 4); nevertheless, their numbers from 1992 to 2013 showed a pronounced decreasing trend (Fig. 5).

In eleven oligotrophic lakes of western Canada the relative contribution of picophytoplankton to total photosynthesis ranged from 29 to 53% (Stockner and Shortreed, 1991, 1994). A wider fluctuation has been found in Lake Constance, where the ^{14}C incorporation varied between 0.1 and 7.5 mg C m^{-3} h^{-1} with a relative contribution of 5-65% of total phytoplankton production. By examining the vertical profile of carbon uptake in Lake Maggiore, the percentage contributions of pico on total phytoplankton varied from year to year (ranging up to 40-80%) (Stockner *et al.,* 2000). In 2002, in Lake Maggiore the daily production was 13-266 mg C m^{-2} d^{-1} comprising up to 47% of total phytoplankton uptake (Fig. 6). Interestingly, the peaks of production were observed in the metalimnion at 25-10% of surface photosynthetic active radiation (PAR), even with only 1% of PAR on August, confirming the preference of picocyanobacteria for low light conditions (Fig. 7).

Light is known to be an important factor in niche differentiation for picocyanobacteria. *Synechococcus* ecotypes exhibit differences in their accessory pigments that affect their adaptation to spectral light quality (Haverkamp *et al.,* 2009). The pigment composition of picocyanobacteria has been associated with a particular underwater light quality (McMurter *et al.,* 1994). The influence of this factor on the selection of picocyanobacterial types with different pigment content has been studied in many lakes, covering a wide spectrum of trophic states and underwater light quality. In highly colored lakes, non-phycoerythrin cells (PC) dominated numerically, whereas in oligotrophic hard-water lakes phycoerythrin-rich cells (PE) were the most abundant (Pick, 1991; Vörös *et al.,* 1998). The growth response of PE and PC *Synechococcus* cells demonstrated the selective value of red light in stimulating the PC and in suppressing PE (Callieri, 1996). Conversely, blue and green lights are used more efficiently than red wavelengths of similar intensity by *Synechococcus* PE-cells (Glover *et al.*, 1985). In the field, PC are the majority when the red wavelength dominate, conversely PE exceed the PC when the green wavelength prevail (Fig. 8). The seasonal dynamics of *Synechococcus* population and subpopulations were demonstrated in Lake Constance (Becker *et al.,* 2002) and Lake Maggiore (Callieri, 2007). The rapid diversification of *Synechococcus* at different depths in lakes and in the Baltic Sea has been demonstrated in studies on the *cpc*BA operon sequences (Crosbie *et al.,* 2003a; Haverkamp *et al.,* 2009).

The phylogenetic studies on 16S rRNA gene of *Synechococcus* published to date have revealed the existence

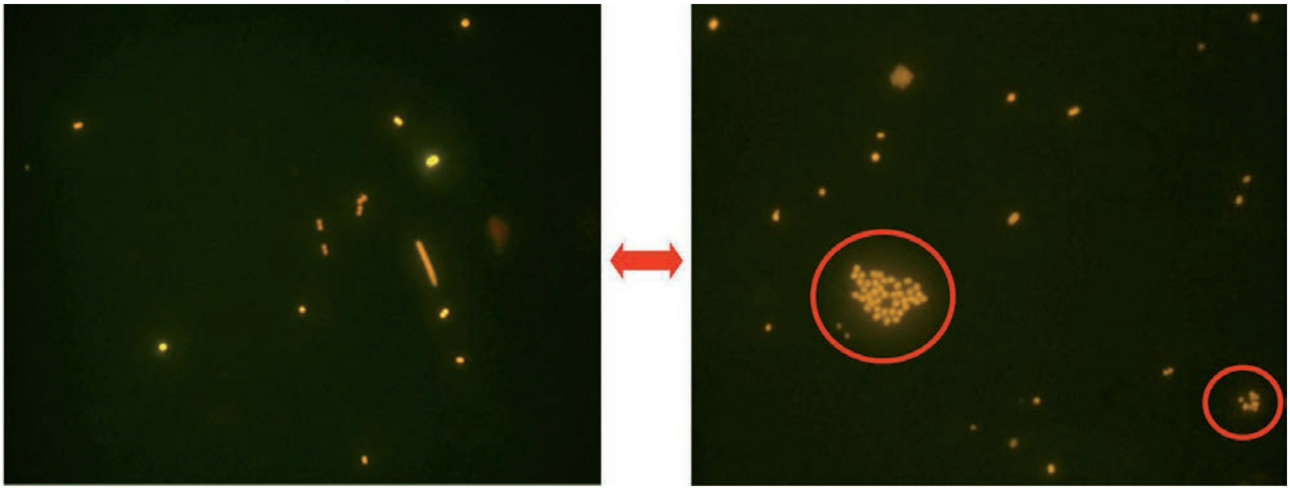

Fig. 1. Different morphotypes of picocyanobacteria (left panel) and microcolonies of different size (right panel) from Lake Maggiore.

of three marine sub-clusters: 5.1, 5.2 and 5.3 (Scanlan *et al.,* 2009), and around 13 clusters of non-marine picocyanobacteria (Callieri *et al*., 2013) (Fig. 9). Even if the majority of marine *Synechococcus* of open oceans belongs to sub-cluster 5.1, the less studied 5.2 and 5.3 appears to be very important to understand the phylogenetic evolution of *Synechococcus* and *Cyanobium* genera, as they have unexpected relations with the non-marine strains. The euryhaline PE-rich *Cyanobium*, isolated from the Arabian Sea, the *Synechococcus* 5.2 clade (Everroad and Wood, 2012) as well as the 5.3 clade, shared common ancestry with freshwater subalpine cluster II, with Lake Nahuel Huapi cluster and Halotolerant cluster (Athalassohaline crater-lakes) (Callieri *et al.,* 2013). The latter study, based on 16S rRNA, indicated new clades of halotolerants, shedding new light on the global distribution and evolution of picocyanobacteria.

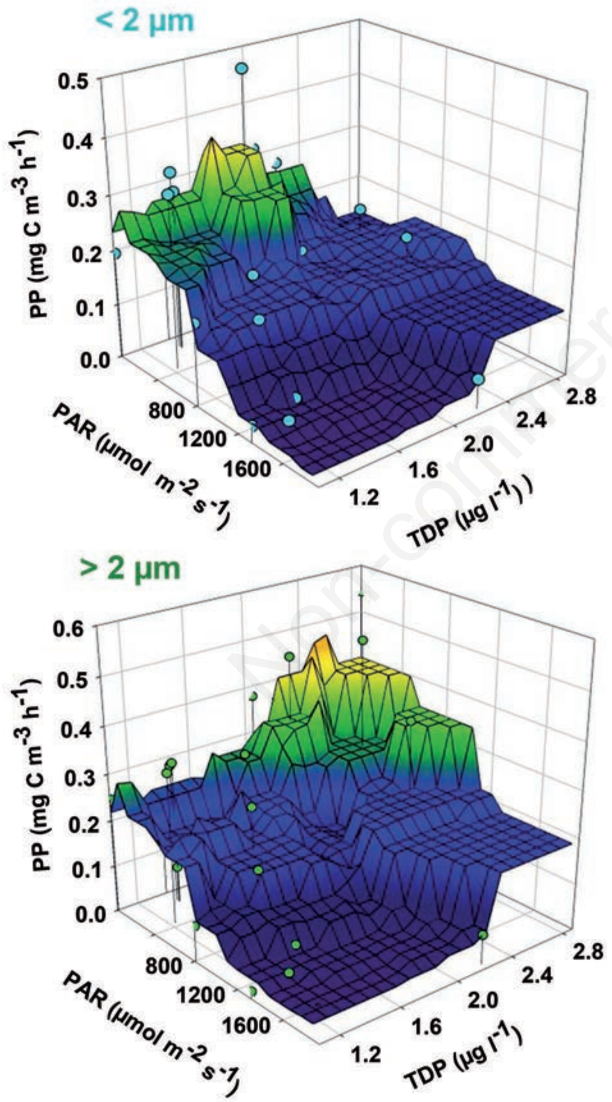

Fig. 2. Primary production of picophytoplankton (<2 µm, upper panel) and >2 µm fraction (lower panel) in relation with total dissolved phosphorus and irradiance. Dots represent the experimental points. From: Callieri *et al*., 2007.

THAUMARCHAEOTA

As a second example of microorganisms playing an important role in aquatic environments, I selected the mesophilic Archaea. In freshwaters, the presence of mesophilic Crenarchaeota (renamed Thaumarchaeota, Brochier-Armanet *et al.,* 2008) has been ascertained in the deep oxygenated hypolimnion of Crater Lake (589 m) (Urbach *et al.,* 2001), of Lake Maggiore (372 m) (Callieri *et al.,* 2009) and in the moderately deep (73 m) high altitude Lake Redon (Auguet *et al.,* 2012). Thaumarchaeota have also been found in the epilimnion and upper hypolimnion (~50 m) of other large lakes (Keough *et al.,* 2003).

Phylogenetically, Thaumarchaeota clearly separate from Euryarchaeota and comprise Marine Group I (MGI) commonly found in oceanic and freshwater plankton (formerly 1.1a), and in Soil Group (formerly 1.1b) (Pester *et al.,* 2011). The clade SAGMGC-1, sister to MG 1.1a, has been recently documented to be present at higher numbers in surface and lower at depth, in the high altitude Lake Redon (Auguet *et al.,* 2012).

The laboratory experiments by Könneke *et al.* (2005) showed Thaumarchaeota to oxidize ammonia to nitrite; in this process, they have the ability to chemolithotrophically fix inorganic carbon (Herndl *et al.,* 2005; Yakimov *et al.,* 2011).

Research efforts have focussed, on one hand, on quan-

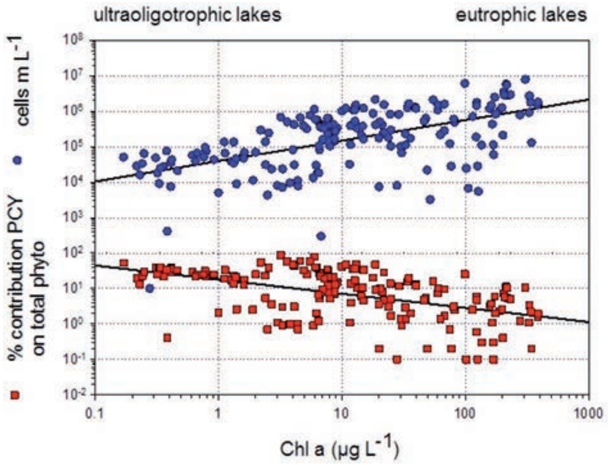

Fig. 3. Relationship between total autotrophic biomass (µg Chl *a* L^{-1}) and picophytoplankton abundance, and its percent contribution to total autotrophic biomass. Modified from Callieri *et al*., 2007.

tifying the genes that codify for archaeal ammonia monooxygenase (*amo*A), the first enzyme in the process of ammonia oxidation; and, on the other hand, on measuring the dark CO_2 fixation. Moreover, the discovery of the assimilation path used by autotrophic Thaumarchaeota, the 3-hydroxypropionate/4-hydroxybutyrate cycle (Berg *et al.,* 2007), has provided a molecular marker (*acc*C) to trace the Thaumarchaeota autotrophic activity (Auguet *et al.,* 2008). In the bathypelagic Mediterranean Sea the archaeal *amo*A gene abundance was lower than Thaumarchaeota abundance (De Corte *et al.,* 2009), indicating that these microorganism can perform other activities. Although a substantial fraction of Thaumarchaeota is chemoautotrophic, it has been found that they can contribute to heterotrophic activity. Using microautoradiography, it has been found that the MCGI group can contribute 32% to leucine incorporation, throughout the water column in the eastern Atlantic (Yokokawa *et al.,* 2012).

The *in situ* measurement of the dark CO_2 assimilation of Thaumarchaeota and Bacteria in the aphotic water column of Lake Maggiore (Northern Italy) was 65.8±5.2 mg C m^{-2} d^{-1}. This CO_2 sink in the hypolimnion, due to dark uptake, represented 87% of the total picoplanktonic primary CO_2 fixation taking place in the euphotic zone (Callieri *et al.,* 2014). Therefore the CO_2 uptake taking place in the hypolimnion is not negligible and constitutes a potentially significant CO_2 sink. These estimates were obtained using a new custom-made apparatus, which takes samples and adds tracers *in situ*. The new instrument was used at different depths and conditions, and compared with parallel conventional on board incubations. *In situ* dark CO_2 incorporations had lower standard deviation respect to the on board ones (Fig. 10).

In 2010, a study on the vertical distribution of Thaumarchaeota and Bacteria was performed on five subalpine lakes (Tab. 1) using CARD-FISH (see methods in Callieri *et al.,* 2009). The percentages of Thaumarchaeota (probe Cren537) of total DAPI counts increased with depth in all lakes, with values around zero in the upper 40 meters, whereas the percentage of Bacteria (probe EUB I-III) showed an opposite trend (Fig. 11). Highest relative abundances of Thaumarchaeota were recorded in Lake Como (19% of DAPI signals at 200 m depth), followed by Lakes

Tab. 1. Main morphometric characteristics of five subalpine lakes.

	Como	Zurich	Garda	Maggiore	Thun
Latitude N	46°00’	47°15’	45°42’	45°58’	46°41’
Longitude E	9°15’	8°41’	10°43’	8°39’	7°43’
Altitude (m asl)	198	406	65	193	558
Area (km^2)	146	88	368	213	48
Maximum depth (m)	410	136	350	370	217
Mean depth (m)	154	49	133	178	136

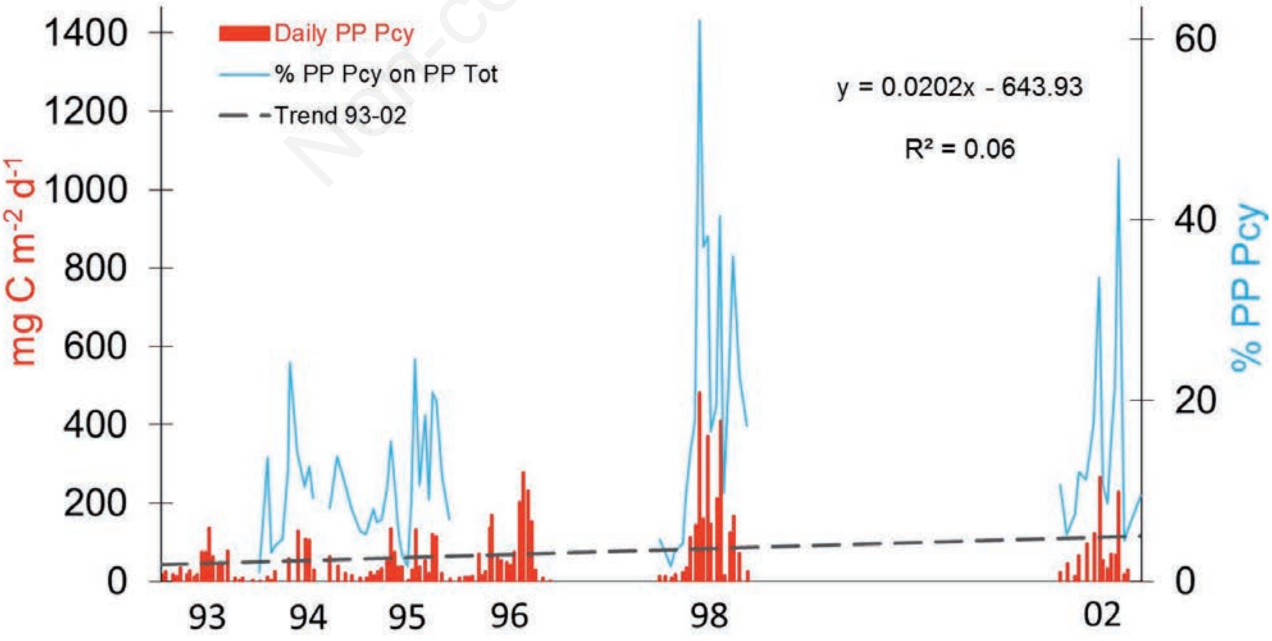

Fig. 4. Picophytoplankton primary production (PP pico: mg C m^{-2} d^{-1}) and percentage on total phytoplankton production in Lake Maggiore. The 1993 and 1996 data were estimated production. The equation of the regression and the r^2 value is reported.

Maggiore (14% of DAPI signals at 350 m depth, station Ghiffa), and Thun (14% of DAPI signals at 120 m depth).

Cell abundances of Thaumarchaeota and Bacteria calculated from CARD-FISH showed that despite the relative increase of Thaumarchaeota with depth, their abundances were always lower than those of Bacteria (Fig. 12). The highest Thaumarchaeota abundances were found in Lakes Como (188×10^3 cells mL^{-1}) and Thun (175×10^3 cells mL^{-1}). A positive significant correlation between Thaumarchaeota numbers and water depth (normalized data, Pearson product moment correlation, n=44, r=0.480, P=0.001), and an inverse significant correlation between abundances of Bacteria and water depth were found (normalized data, Pearson product moment correlation, n=44, r=-0.465, P=0.0015) (Fig. 13).

Recently, we extended the study to other subalpine lakes and to different seasons (Callieri *et al*., 2015). Although their percentages and abundances vary along the trophic gradient and with mixing conditions, a general pattern common to all the deep lakes was observed. In summer, during stratification, Thaumarchaeota were restricted to the oxygenated hypolimnion. In spring, even at partial water column mixing in oligomictic or meromictic lakes, Thaumarchaeota were also present in the epilimnion. Furthermore, temperature emerged as the main environmental driver of Thaumarchaeota abundance in the deep subalpine lakes. The presence and activity of Thaumarchaeota in the hypolimnion of deep lakes indicates that the mesophilic component of the domain of Archaea needs to be studied not only in the oceans but also in freshwater. Biogeochemical cycles in the deep hypolimnion can have comparable importance with those occurring in the photic zone.

CONCLUSIONS AND NEW PERSPECTIVES

Picocyanobacteria are an important component of the trophic structure of aquatic ecosystems. They are distrib-

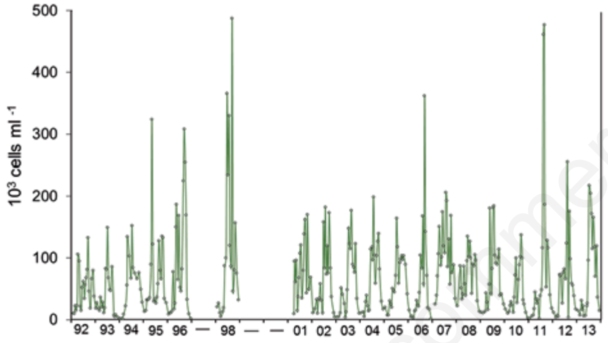

Fig. 5. Long-term picocyanobacteria abundance dynamics in Lake Maggiore, Northern Italy.

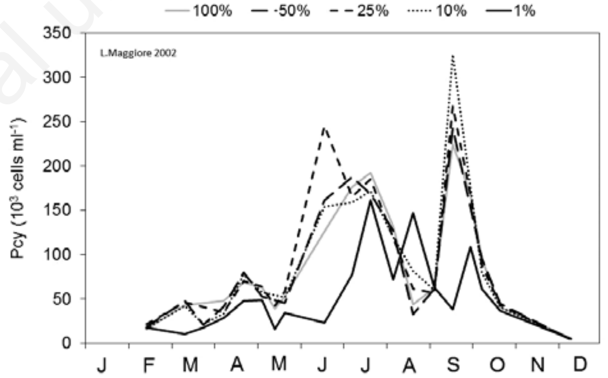

Fig. 7. Picocyanobacteria abundance at different water layer with different irradiance (100%, 50%, 25%, 10% and 1% of surface PAR), in Lake Maggiore, 2002.

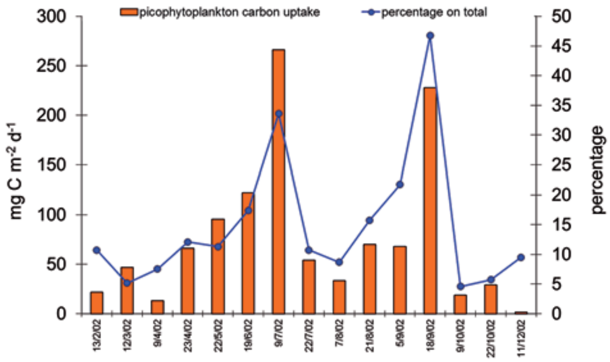

Fig. 6. Picophytoplankton primary production (mg C m^{-2} d^{-1}) and percentage on total phytoplankton production in Lake Maggiore, 2002.

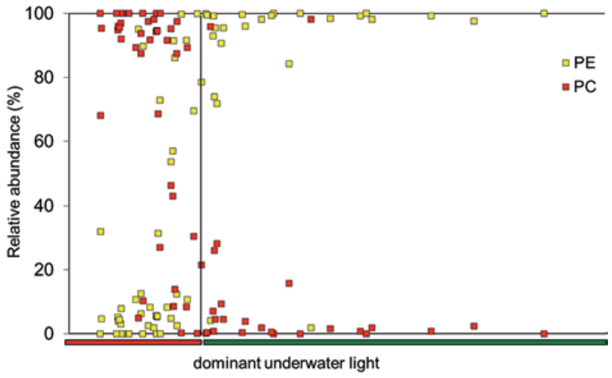

Fig. 8. Phycoerithryn (PE) and phycocyanin (PC) relative abundance in 32 lakes with different water quality. From: Vörös *et al*., 1998.

uted worldwide and are ubiquitous in systems of varying trophic state. Their significance has become clearer only in the last few decades, and this knowledge depended upon an improved understanding of the phylogenetic evolution of Cyanobacteria. It appears that the form-genus *Synechococcus* likely represents the ancestral morphology from which other types evolved, including colonial forms. We know that there are many clusters of non-marine picocyanobacteria that have been found within the picophytoplankton clade, and that phylogenies revealed a high diversity of *Synechococcus* strains. The local adaptation of different ecological niches can be related to the highly variable number of horizontally acquired genes, opening new perspectives to the understanding of the adaptation.

Thaumarchaeota numbers were higher in the hypolimnion of lakes than in the epilimnion. In the deep zone

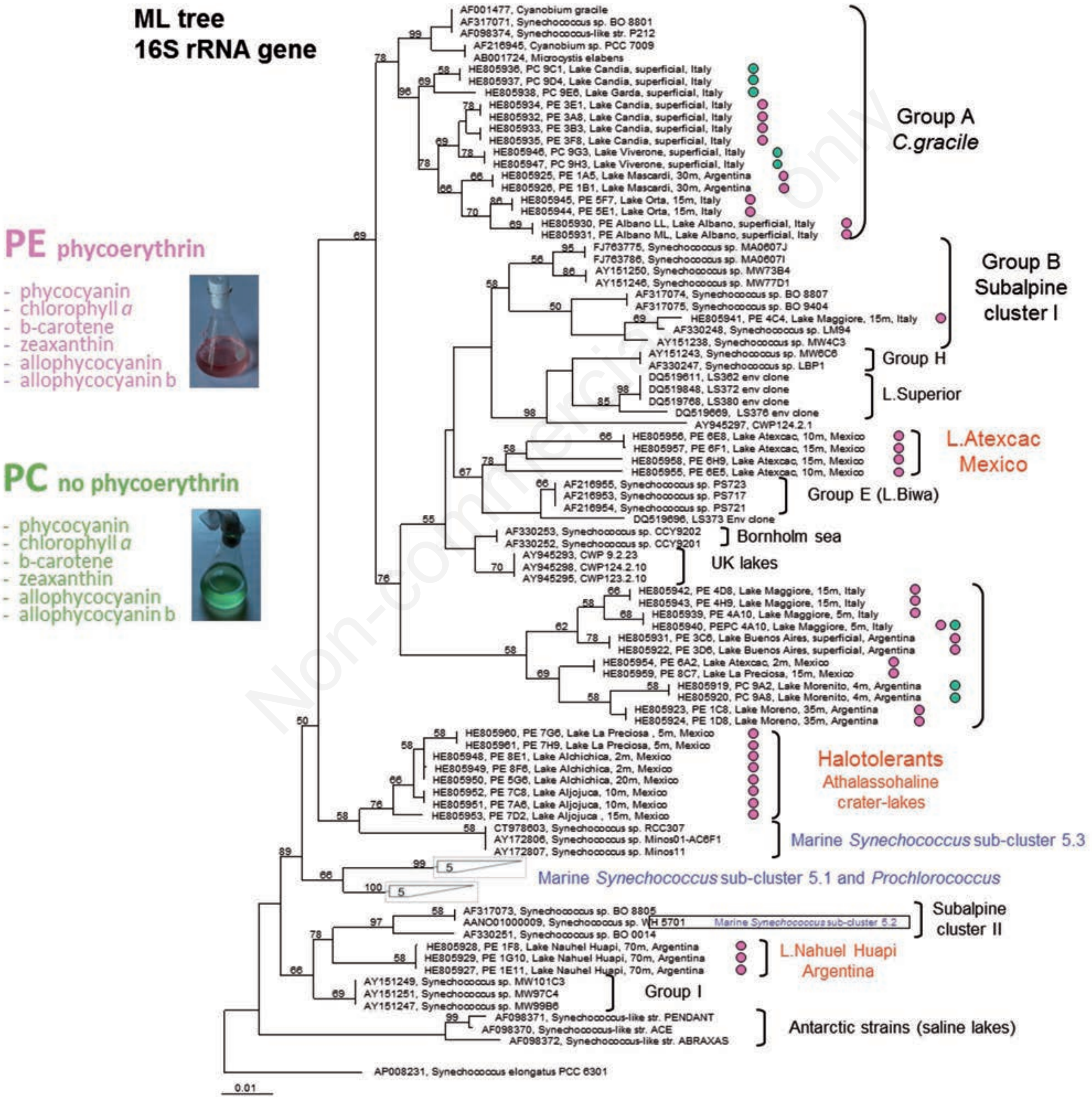

Fig. 9. Maximum-likelihood (ML) tree inferred from 16S rRNA gene sequences of 43 isolated strains from lakes in Italy, Mexico and Argentina. Terminal branches display GenBank accession numbers, name of the strains, of the lakes, depth and location. From: Callieri *et al*., 2013.

of the lakes they have the important role of ammonia-oxidizers. We measured *in situ* the Archaea CO_2 uptake in Lake Maggiore. In the hypolimnion of this lake, we recognized that Archaea are responsible for 28% (52.7±0.4 μg C m^{-3} d^{-1}) of the total dark CO_2 uptake. Their activity in the aphotic zone corresponds to 87% of picophytoplanktonic production taking place in the photic zone. Although deep hypolimnia of freshwater lakes comprised a lower percentage of Thaumarchaeota than bathypelagic marine waters, Thaumarchaeota abundances were higher (around 200×10^3 cells mL^{-1}). The studies on Thaumarchaeota indicated that the hypolimnia of deep lakes, often disregarded, are important in microbial metabolisms of carbon and nitrogen cycle, and dark CO_2 fixation rates can be comparable to the photosynthetic fixation occurring in photic zones.

Future research on aquatic microbial ecology should be directed towards picocyanobacteria, bacteria and Archaea at a single-cell level, to their association, competition and predation in the aquatic microbial trophic chains. Niche partitioning is the basis of community complexity as it avoids direct competition, enables adaptation to different micro-niches and fosters cooperative interaction among specialised microorganisms. Furthermore, the study at single-cell level, looking individually at relevant uncultured microorganisms, can highlight the large genetic repository of functions existing even within close phylogenetically-related organisms both in freshwater and marine systems.

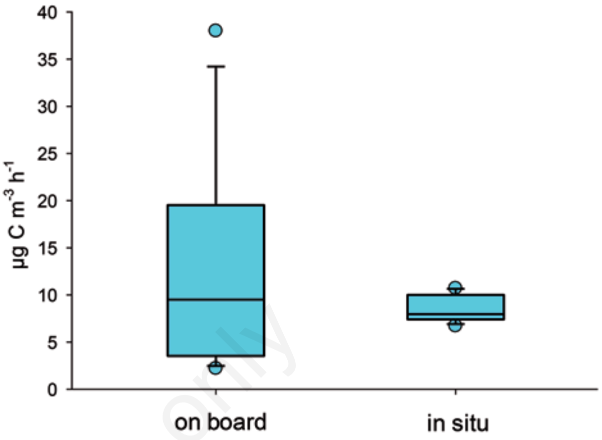

Fig. 10. Mean values and ranges of dark CO_2 uptake (µgC m^{-3} h^{-1}) obtained with *in situ* and on board incubations. Lines within the boxes, boundaries, error bars and dots mark are respectively median, 25th-75th percentiles, minimum and maximum values, and outliers. Modified from Callieri *et al*., 2014.

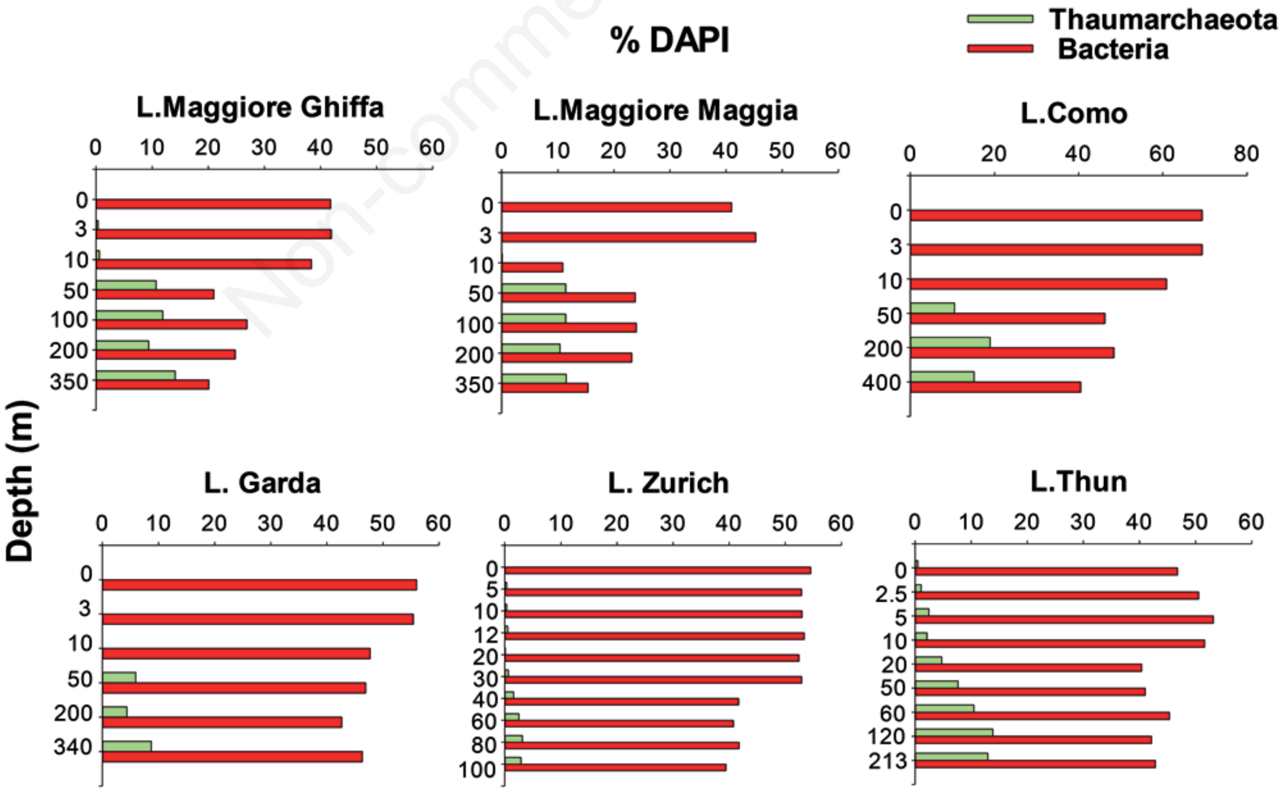

Fig. 11. Percentage of Thaumarchaeota and Bacteria of total DAPI counts in the depth profiles of five subalpine lakes during stratification. Lake Maggiore was sampled in two stations (Ghiffa and Maggia).

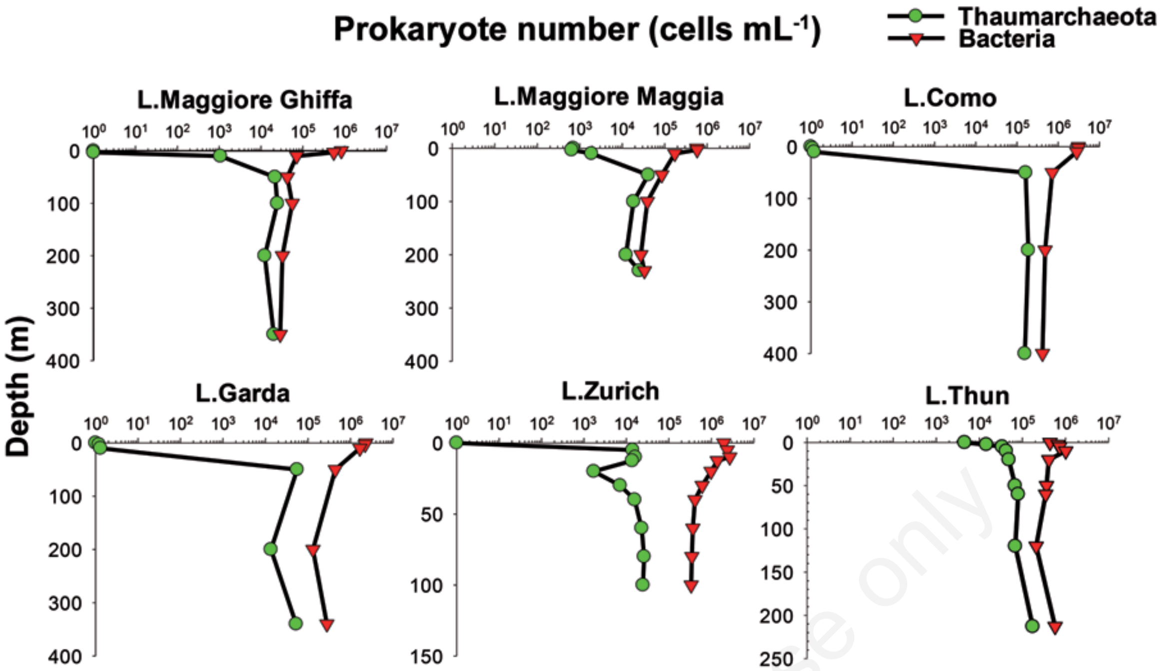

Fig. 12. Total cell number (cells mL^{-1}) of Thaumarchaeota (circles) and Bacteria (triangles) in the depth profiles of five subalpine lakes during stratification. Lake Maggiore was sampled in two stations (Ghiffa and Maggia).

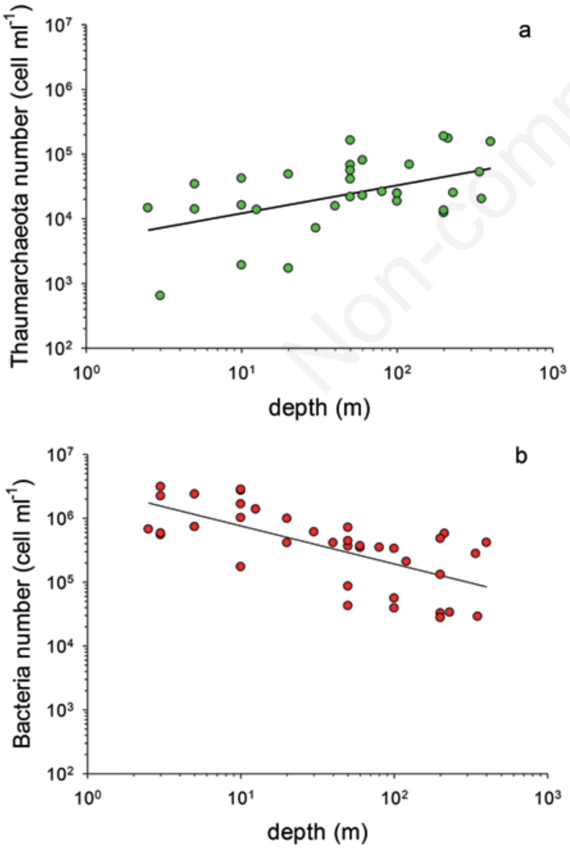

Fig. 13. Log-log relation between the depth and the number of Thaumarchaeota (a) and of Bacteria (b), in five subalpine deep lakes.

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