

## *Bacteria, Archaea, and Crenarchaeota* in the Epilimnion and Hypolimnion of a Deep Holo-Oligomictic Lake<sup>∇</sup>

Cristiana Callieri,<sup>1\*</sup> Gianluca Corno,<sup>1</sup> Emanuele Caravati,<sup>1</sup> Serena Rasconi,<sup>1,2</sup>  
Mario Contesini,<sup>1</sup> and Roberto Bertoni<sup>1</sup>

CNR-ISE, Institute of Ecosystem Study, Verbania Pallanza, Italy,<sup>1</sup> and UMR CNRS 6023,  
Université B. Pascal, Clermont Ferrand, France<sup>2</sup>

Received 29 May 2009/Accepted 15 September 2009

**In a deep, subalpine holo-oligomictic lake, the relative abundance of *Archaea* and *Crenarchaeota*, but not that of *Bacteria*, increases significantly with depth and varies seasonally. Cell-specific prokaryotic productivity is homogeneous along the water column. The concept of active *Archaea* observed in the deep ocean can therefore be extended to a deep oxie lake.**

The abundance, activity, and community composition of epilimnetic and hypolimnetic prokaryotes have been less thoroughly investigated in deep lakes than in oceans. Strong evidence that the depth gradient plays a role in modulating the balance between the domains of *Bacteria* and *Archaea* has been found in various marine systems (8, 12, 13, 20). It has been shown that the percentage of *Bacteria* in the deep marine hypolimnion decreases (up to 5,000 m) while, conversely, the percentage of *Archaea* increases. The percentage of *Crenarchaeota* is also higher in the mesopelagic zone than in surface waters (10).

Although *Archaea* have been found in a variety of freshwater habitats (3), little has thus far been published on differentiating between *Bacteria*, *Archaea*, and *Crenarchaeota* in the hypolimnion of deep lakes. An exception is a study of the high-altitude ultraoligotrophic Crater Lake (21, 22), where group I marine *Crenarchaeota* were observed in deep-water populations (22). This study and another study of various lakes from three continents (9) are based on summer sampling, making it impossible to ascertain the effects of temporal variability on the vertical distribution of *Archaea* and *Crenarchaeota*, as has been done for marine systems and shallow lakes (for examples, see references 8 and 11).

Our primary objective was to follow variations in the relative abundance of *Bacteria*, *Archaea*, and *Crenarchaeota* found in the hypolimnetic waters of a deep holo-oligomictic lake with a permanent oxie hypolimnion and compare them with those in the epilimnetic assemblages. We used the catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) technique and compared the data thus obtained with prokaryotic productivity.

**Environmental characteristics and samplings.** Lake Maggiore is a large, deep, subalpine lake (surface, 212 km<sup>2</sup>; maximum depth, 372 m) in Northern Italy included in the LTER network (<http://www.ise.cnr.it/lter>). The lake has recovered from an eutrophic state (15) and is now oligomesotrophic (2,

14). A particular hydrodynamic feature of Lake Maggiore is that the full winter overturn occurs only at the end of a particularly cold and windy period (1).

Temperature profiles (IDRONAUT OS316 multiparameter probe) indicated that stratification began in June (Fig. 1). The oxygen profiles showed that the water column was in a uniform oxie condition. Samples were taken in February, June, August, and October 2007 at 3 m, 10 m, 200 m, and 350 m, immediately fixed, and stored at –4°C (1 month).

**Prokaryote community composition.** CARD-FISH analyses for *Archaea*, *Bacteria*, and *Crenarchaeota* were performed in two replicates according to Pernthaler et al. (17) and Teira et al. (19). We used the following oligonucleotide probes (Thermo-Hybrid, Germany): *Archaea*, Arch915; *Bacteria*, Eub338, Eub338-II, and Eub338-III (5); and *Crenarchaeota*, Cren537. The hybridization for *Bacteria* ranged from 49% to 71% of total DAPI (4',6-diamidino-2-phenylindole) counts and was comparable to published data on aquatic systems (7, 16). The difference between the *Bacteria/Archaea* ratio in the hypolimnion and that in the epilimnion highlights the increasing relative contribution of *Archaea* to total DAPI counts in deep water layers. During late summer stratification, *Archaea* account for 47% of total counts at 350 m (Fig. 2). When all data were considered, the relative abundance of *Archaea* was significantly higher in the hypolimnion ( $P = 0.028$  by the Mann-Whitney U test) than in the epilimnion. *Bacteria* generally accounted for more than 50% of total DAPI counts. There was no significant difference in their relative abundances between the epilimnion and hypolimnion ( $P = 0.505$  by the Mann-Whitney U test). *Crenarchaeota* accounted for a significantly higher percentage in the hypolimnion ( $P < 0.001$  by the Mann-Whitney U test), following the same pattern as *Archaea*. They contributed from 30% (mean in epilimnion) to 70% (mean in hypolimnion) of the total archaeal counts.

Our results agree with those obtained in oceans (4, 8, 20), where pelagic *Archaea* and *Crenarchaeota* were found to increase at greater depths. Our data from a deep subalpine lake add new insight into the vertical distribution of *Bacteria*, *Archaea*, and *Crenarchaeota* under various limnological conditions during the year. There is a close relationship between marine *Crenarchaeota*

\* Corresponding author. Mailing address: CNR-ISE, Institute of Ecosystem Study, Largo Tonolli 50, 28922 Verbania-Pallanza, Italy. Phone: 39 0323 518320. Fax: 39 0323 556513. E-mail: c.callieri@ise.cnr.it.

<sup>∇</sup> Published ahead of print on 18 September 2009.

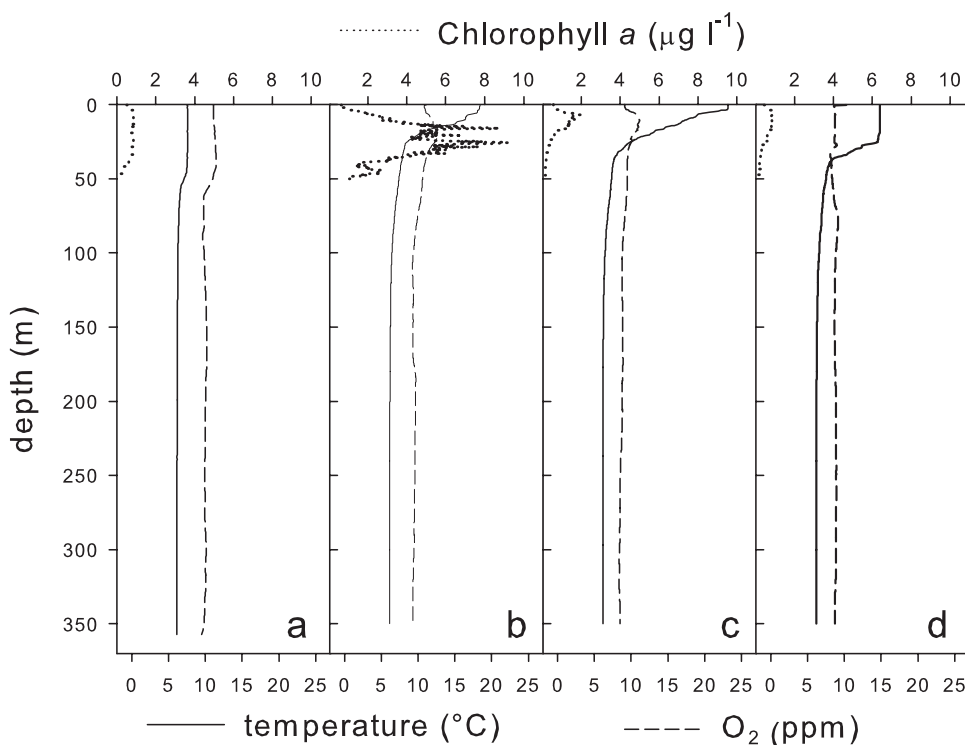


FIG. 1. Profiles of temperature, dissolved oxygen (O<sub>2</sub>), and in situ chlorophyll *a* in samples taken from Lake Maggiore on 14 February (a), 5 June (b), 7 August (c), and 23 October (d) 2007.

and their counterparts in large lakes, based on identical lipid structures (3). This evidence suggests that in the deep hypolimnion of lakes, the pelagic *Crenarchaeota* may act as chemoautotrophs, oxidizing ammonia to nitrate (6) and fixing inorganic carbon in the dark (23). The cold hypolimnion of Lake Maggiore is permanently oxygenated and may provide a niche for uncultivated mesophilic *Crenarchaeota*.

**Depth profiles of prokaryotic abundance and productivity.**

Total DAPI counts were used to calculate the abundance of *Bacteria*, *Archaea*, and *Crenarchaeota* from the percentage of hybridized cells. The average cell numbers of *Bacteria*, *Archaea*, and *Crenarchaeota* in the epilimnion and hypolimnion were  $25.6 \times 10^5$  to  $5.4 \times 10^5$  cells ml<sup>-1</sup>,  $7.4 \times 10^5$  to  $2.5 \times 10^5$  cells ml<sup>-1</sup>, and  $1.5 \times 10^5$  to  $1.6 \times 10^5$  cells ml<sup>-1</sup>, respectively

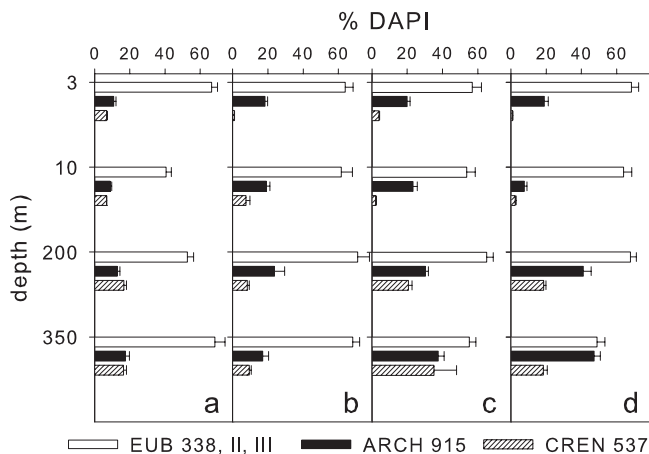


FIG. 2. Profiles of bacterioplankton composition from CARD-FISH with rRNA-targeted oligonucleotide probes for *Bacteria* (Eub338, EubII, and EubIII), *Archaea* (Arch915), and *Crenarchaeota* (Cren537). The histograms represent means  $\pm$  standard errors. Samples were taken from Lake Maggiore on 14 February (a), 5 June (b), 7 August (c), and 23 October (d) 2007.

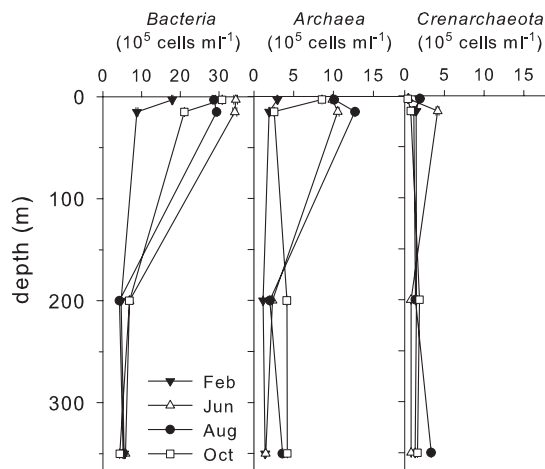


FIG. 3. Depth profiles of absolute abundance (cell ml<sup>-1</sup>) of *Bacteria*, *Archaea*, and *Crenarchaeota*. The bars represent standard errors. Samples were taken from Lake Maggiore on 14 February (filled inverted triangles), 5 June (open triangles), 7 August (filled circles), and 23 October (open squares) 2007.

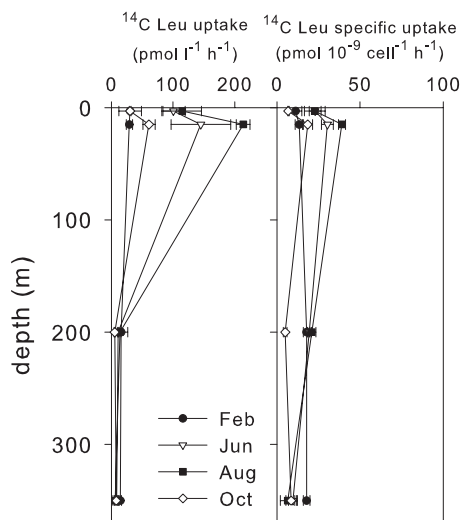


FIG. 4. Profiles of total productivity ( $^{14}\text{C}$ Leu uptake) and cell-specific productivity ( $^{14}\text{C}$ Leu-specific uptake) of prokaryotes. The bars represent standard errors. Samples were taken from Lake Maggiore on 14 February (filled circles), 5 June (open inverted triangles), 7 August (filled squares), and 23 October (open diamonds) 2007.

(Fig. 3). Abundances of *Bacteria* and *Archaea* were significantly different in the epilimnion and hypolimnion ( $P < 0.001$  [*Bacteria*] and  $P = 0.028$  [*Archaea*] by the Mann-Whitney U test), while no difference was observed in the abundances of *Crenarchaeota* ( $P = 0.645$  by the Mann-Whitney U test).

Prokaryotic productivity was measured by [ $^{14}\text{C}$ ]leucine ( $^{14}\text{C}$ Leu) according to Smith and Azam (18), with minor modifications. [ $^{14}\text{C}$ ]Leu uptake scaled to cell number showed a vertical homogeneous distribution in February, while in August there was a peak in the epilimnion ( $39 \text{ pmol } ^{14}\text{C} \text{Leu } 10^{-9} \text{ cell}^{-1} \text{ h}^{-1}$ ) (Fig. 4). No significant difference was observed between epilimnetic and hypolimnetic cell-specific [ $^{14}\text{C}$ ]Leu uptake ( $P = 0.113$  by analysis of variance). Conversely, total production levels were significantly different in the two zones ( $P < 0.001$  by analysis of variance) (Fig. 4).

The cell-specific productivity of prokaryotes did not change with depth. This evidence, together with the increasing relative abundance of *Archaea* and *Crenarchaeota* in the hypolimnion, supports the hypothesis that, in a deep lake, *Archaea* may be an active component of the prokaryotic community, as they have been found to be in oceans (20). Also, similar temporal fluctuations in archaeal abundances suggest that *Archaea* have an effective role in microbial communities, as has been observed in oceans (4).

**Conclusions.** Our results from in situ measurements demonstrated relatively unchanged cell-specific productivity along the water column, indicating that prokaryotes living in the hypolimnion are on average as active as those living in the epilimnion. The observed increment with depth in the relative abundance of *Archaea* and *Crenarchaeota* was always significant, although it varied unevenly through the seasons. The different abundances of *Bacteria* and *Archaea* in the epilimnion and hypolimnion suggest that they contribute differently to overall microbial activity in the two zones.

We thank Stefano Amalfitano (CNR-IRSA, Rome, Italy) for his support in executing the CARD-FISH protocol. We also thank our anonymous reviewers for their useful comments on the manuscript.

## REFERENCES

- Ambrosetti, W., L. Barbanti, and N. Sala. 2003. Residence time and physical processes in lakes. *J. Limnol.* **62**(Suppl.):1–15.
- Bertoni, R., R. Piscia, and C. Callieri. 2004. Horizontal heterogeneity of seston, organic carbon and picoplankton in the photic zone of Lago Maggiore, Northern Italy. *J. Limnol.* **63**:244–249.
- Casamayor, E. O., and C. Borrego. 2009. *Archaea*, p. 167–181. In G. E. Likens (ed.), *Encyclopedia of inland waters*, vol. 3. Elsevier, Oxford, United Kingdom.
- Church, M. J., E. F. DeLong, H. W. Ducklow, M. B. Karner, C. M. Preston, and D. M. Karl. 2003. Abundance and distribution of planktonic *Archaea* and *Bacteria* in the waters west of the Antarctic Peninsula. *Limnol. Oceanogr.* **48**:1893–1902.
- Daims, H., A. Bruhl, R. Amann, K. H. Schleifer, and M. Wagner. 1999. The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: development and evaluation of a more comprehensive probe set. *Syst. Appl. Microbiol.* **22**:434–444.
- Francis, C. A., J. M. Beman, and M. M. M. Kuypers. 2007. New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. *ISME J.* **1**:19–27.
- Glöckner, F. O., B. M. Fuchs, and R. Amann. 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. *Appl. Environ. Microbiol.* **65**:3721–3726.
- Karner, M. B., E. F. DeLong, and D. M. Karl. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* **409**:507–510.
- Keough, B. P., T. M. Schmidt, and R. E. Hicks. 2003. Archeal nucleic acids in picoplankton from great lakes on three continents. *Microb. Ecol.* **46**:238–248.
- Kirchman, D. L., H. Elifantz, A. I. Dittel, R. R. Malmstrom, and M. T. Cottrell. 2007. Standing stocks and activity of *Archaea* and *Bacteria* in the western Arctic Ocean. *Limnol. Oceanogr.* **52**:495–507.
- Lliros, M., E. O. Casamayor, and C. Borrego. 2008. High archaeal richness in the water column of a freshwater sulfurous karstic lake along an interannual study. *FEMS Microbiol. Ecol.* **66**:331–342.
- Massana, R., A. E. Murray, C. M. Preston, and E. F. DeLong. 1997. Vertical distribution and phylogenetic characterization of marine planktonic *Archaea* in the Santa Barbara Channel. *Appl. Environ. Microbiol.* **63**:50–56.
- Massana, R., L. J. Taylor, A. E. Murray, K. Y. Wu, W. H. Jeffrey, and E. F. DeLong. 1998. Vertical distribution and temporal variation of marine planktonic *Archaea* in the Gerlache Strait, Antarctica, during early spring. *Limnol. Oceanogr.* **43**:607–617.
- Morabito, G., D. Ruggiu, and P. Panzani. 2002. Recent dynamics (1995–1999) of the phytoplankton assemblages in Lago Maggiore as a basic tool for defining association patterns in the Italian deep lakes. *J. Limnol.* **61**:129–145.
- Mosello, R., and D. Ruggiu. 1985. Nutrient load, trophic condition and restoration prospects of Lake Maggiore. *Int. Rev. Gesamten Hydrobiol.* **70**:63–75.
- Pernthaler, A., J. Pernthaler, and R. Amann. 2002. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Appl. Environ. Microbiol.* **68**:3094–3101.
- Pernthaler, A., J. Pernthaler, and R. Amann. 2004. Sensitive multicolor fluorescence in situ hybridization for the identification of environmental microorganisms, p. 711–726. In A. D. L. Akkermans, F. J. de Bruijn, and J. D. van Elsas (ed.), *Molecular microbial ecology manual*, 2nd ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Smith, D. C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis in seawater using  $^3\text{H}$ -leucine. *Mar. Microb. Food Webs* **6**:107–114.
- Teira, E., T. Reinthaler, A. Pernthaler, J. Pernthaler, and G. J. Herndl. 2004. Combining catalyzed reporter deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by bacteria and archaea in the deep ocean. *Appl. Environ. Microbiol.* **70**:4411–4414.
- Teira, E., H. van Aken, C. Veth, and G. J. Herndl. 2006. Archaeal uptake of enantiomeric amino acids in the meso- and bathypelagic waters of the North Atlantic. *Limnol. Oceanogr.* **51**:60–69.
- Urbach, E., K. L. Vergin, G. L. Larson, and S. J. Giovannoni. 2007. Bacterioplankton communities of Crater Lake, OR: dynamic changes with euphotic zone food web structure and stable deep water populations. *Hydrobiologia* **574**:161–177.
- Urbach, E., K. L. Vergin, L. Young, A. Morse, G. L. Larson, and S. J. Giovannoni. 2001. Unusual bacterioplankton community structure in ultrahypolimnetic Crater Lake. *Limnol. Oceanogr.* **46**:557–572.
- Wuchter, C., S. Schouten, H. T. S. Boschker, and J. S. S. Damste. 2003. Bicarbonate uptake by marine *Crenarchaeota*. *FEMS Microbiol. Lett.* **219**:203–207.