Horizontal heterogeneity of seston, organic carbon and picoplankton in the photic zone of Lago Maggiore, Northern Italy

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ABSTRACT

The horizontal heterogeneity of seston, dissolved (DOC) and particulate (POC) organic matter, Chlorophyll-a (Chl) and of autotrophic (APP) and heterotrophic (HPP) picoplankton was evaluated seasonally during a two-year period in Lago Maggiore, a large deep oligotrophic lake in Northern Italy. The dissolved fraction resulted homogeneously distributed in the epilimnion during the whole sampling period. The particulate fraction showed the higher heterogeneity in winter 2000 and 2001. For those years, POC and Chl heterogeneity was, respectively, 3-5 and 5-7 times higher than DOC. A similar trend was evident for Chl and for APP. The spatial heterogeneity of HPP resulted always smaller than the analytical variability, thus preventing any consideration about the spatial distribution of HPP. POC and Chl concentration exhibited a recurrent pattern of their spatial heterogeneity, possibly in relation with the location of the part of the drainage basin conveying higher amounts of nutrients to the lake.

Key words: TOC, picoplankton, horizontal distribution

1. INTRODUCTION

Spatial heterogeneity, or patchiness, has long been recognized in biological communities of lakes (Downing 1991) such as phytoplankton (Platt *et al*. 1970; George & Heaney 1978) and zooplankton (George & Jones 1987; Laybourn Parry *et al*. 1990; Malone & McQueen 1983). Its occurrence has been attributed to combinations of organism's behaviour (e.g. feedback between water chemistry and organisms, predator - prey interactions) and physical processes (e.g. wind-induced turbulence, internal seiches, currents). On the other hand, organisms and parameters of the microbial food web are often studied under the assumption that epilimnetic waters are horizontally homogeneous, at least in the pelagic zone. According to this hypothesis, also in relatively large lakes (surface in the order of 10^2 km^2) samples for dissolved and particulate organic matter, seston, chlorophyll and picoplankton are often taken only for a single site and the measures from this station are considered to be representative of the entire lake.

With this research is offered an analysis of spatial variance of seston, dissolved (DOC) and particulate (POC) organic matter, Chlorophyll-*a* (Chl) and of autotrophic (APP) and heterotrophic (HPP) pico-plankton in Lago Maggiore, a large deep oligotrophic lake in Northern Italy. With a two-year data set we evaluated whether and to what extent the horizontal homogeneity assumption is acceptable in a water body of that size and shape.

2. METHODS

2.1. Study site

Lago Maggiore is a deep oligotrophic subalpine lake (surface area 212 km^2, z_{max} 372 m) in Northern Italy (de Bernardi *et al*. 1996). Many papers documented its trophic evolution in the last 50 years (e.g. Mosello & Ruggiu 1985; Manca *et al*. 1992) and its present oligotrophic conditions after the restoration process (Calderoni *et al*. 1998). The lake is narrow, elongated in a north-south direction, and irregular in shape due to the presence of bays and side arms (Shoreline Development Index, DL = 3.29) (Fig. 1). It is feed by 13 main tributaries, two of them (Ticino River, Northern edge, and Toce River, Western harm edge) convey 134 m^3 s^{-1} which accounts for 71% (yearly average of period 1978-2001) of total water inflow. The very shape of its basin is thus a potentially important determinant of the heterogeneous distribution of particles and organisms (de Bernardi *et al*. 1984).

2.2. Sampling

Samples for horizontal heterogeneity study were collected in the pelagic zone along seven transects, with a total of 18 sampling stations, crossing the main axes of Lago Maggiore (Fig. 1). Samples were seasonally taken during years 2000 and 2001 from 0-20 m layer (approximately corresponding to the photic zone), with an integrating sampler which collects a 5 litre sample in a single operation (Bertoni, pat. 96/A 000121). In addition, monthly (in autumn and winter) and fortnightly (in

spring and summer) samplings were performed at the station of maximum depth (station 5 in Fig. 1).
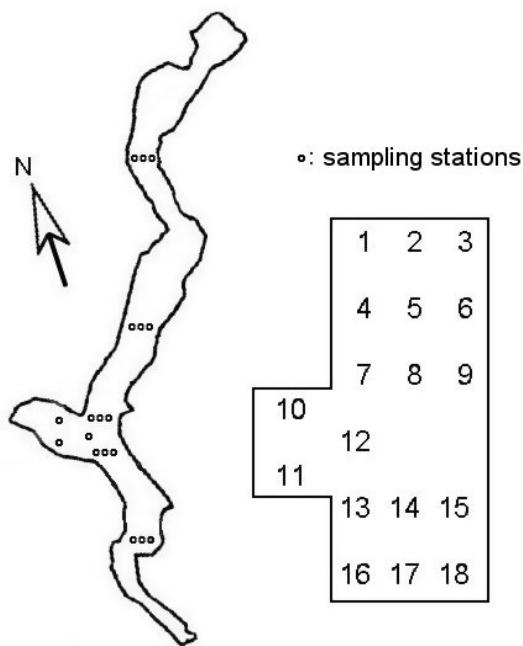

Fig. 1. Location of sampling stations in Lago Maggiore and position of corresponding data in tables 1 and 2.

2.3. Analytical methods

Duplicate subsamples where filtered through GF/C filters (Whatman) previously combusted at 450 °C for 3 hours and weighted. The filters where used to measure the total seston gravimetrically (Sartorius, 3311 microscale) and to perform Particulate Organic Carbon (POC) analyses by CHN Elemental Analyser (Carlo Erba, ANA 1500) (Bertoni 1978). Dissolved Organic Carbon (DOC) was calculated subtracting POC from Total Organic Carbon (TOC), measured with a Shimadzu (5000A) TOC analyser.

Samples of HPP and APP were immediately fixed using 0.2 μm filtered cacodylate-buffered formaldehyde (final concentration 2% vol/vol). To evaluate APP and HPP abundance, 1 to 5 ml sample was filtered through 0.2 μm Anopore filter. HPP were counted after DAPI (α', 6-diamino-2-phenylindole) staining (final concentration 0.2% wt/vol) following Porter & Feig (1980). At least 400 bacterial cells were counted on at least 10 fields in individual filters. APP were counted by autofluorescence of phycobiliproteins under blue and green light (Zeiss Axioplan microscope equipped with an HBO 100 W lamp, a Neofluar 100× objective 1.25× additional magnification and filter sets for blue (BP450-490, FT510, LP520) and green (BP510-560, FT 580, LP590) light excitation). Chlorophyll-*a* (Chl) was fluorometrically determined by Perkin-Elmer LS-2 Filter Fluorometer in duplicate subsamples, filtered through GF/C (Whatman), after pigment extraction with

methanol (Talling & Driver 1961; Holm-Hansen & Riemann 1978).

2.4. Heterogeneity index

We defined the heterogeneity index (HI) according to Knauer *et al.* (2000) as a measure of the spatial heterogeneity of a variable at a given time as:

$$HI = \frac{\sqrt{S_s^2 - S_m^2}}{\text{mean concentration}} \times 100\% \tag{1}$$

where S_s is the "spatial" standard deviation among concentrations measured at the 18 sampling stations. In Eq. 1 it is assumed that analytical variability and spatial variability add in quadrature. The error of the measure is

$$S_m = S_r / \sqrt{2}$$

where S_r is the standard deviation of replicated analyses of the same sample. S_m is the correction for the component of S_s attributable to the analytical variability. For each sampling date, S_r is calculated from the differences between duplicate samples at each station,

$$S_r^2 = \sum_{i=1}^{18} \sum_{j=1}^{2} (x_{i,j} - \bar{x}_i)^2 / 18 \tag{2}$$

where $x_{j,1}$ and $x_{j,2}$ are the measurements at station i, and where

$$\bar{x}_i = (x_{i,1} + x_{i,2})/2 .$$

The heterogeneity index (HI) thus computed is meaningful when the spatial heterogeneity is much larger than the analytical variability. However, when these values are comparable, the estimated spatial heterogeneity may largely be an expression of the analytical variability. Then the true spatial heterogeneity is likely smaller and the analytical variability can be viewed as the upper limit of HI.

3. RESULTS AND DISCUSSION

During years 2000 and 2001 the seasonal evolution of seston and TOC (Fig. 2, upper panel), chlorophyll-*a* and bacterial number (Fig. 2, lower panel) followed the path observed in previous years (Bertoni & Callieri 1993), with all parameters peaking in spring and summer. The most striking feature of the study period is the seston peak of November 2000, related to an exceptional flood event which lasted from October 14th till October 24th rising the lake level of about 4 m above the mean level (Ambrosetti *et al.* 2001). Seston was composed by 93% of inorganic particles immediately after the flood while the average of the previous months of 2000 was 71%.

The flood event did not influence to the same extent the parameters under study: despite seston increased, organic carbon, chlorophyll-*a*, APP and HPP did not show any maxima mirroring that of seston.
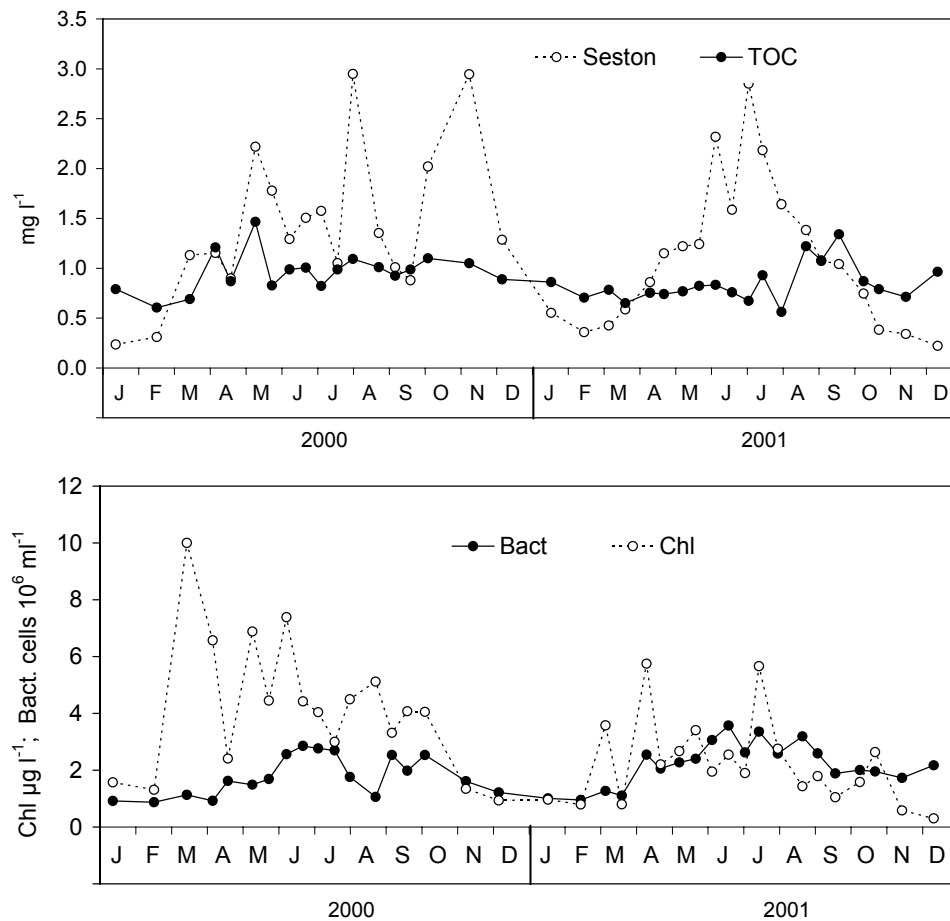
Fig. 2. Seasonal variation of Seston (mg l^{-1}) and Total Organic Carbon (TOC: mg l^{-1}) (upper panel), Chlorophyll-*a* (Chl: µg l^{-1}) and bacterial number (Bact.: cells 10^6 ml^{-1}) (lower panel), in the euphotic zone of Lago Maggiore, during years 2000 and 2001.

In November and December there were not evident flood-induced alterations of the variables considered here. Thus, the data collected for horizontal heterogeneity study appear well in context with the time series data set, with the exception of November 2000.

The heterogeneity index (HI, Fig. 3) provides an estimate of the spatial heterogeneity corrected by the component which can be ascribed to analytical variability, i.e. the variability between two subsamples.

The HI values show differences among the parameters and with the season. In general the lake appears more homogeneous in autumn (Fig. 3). The data of November 2000, taken immediately after the flood, show that this phenomenon, although increasing the in-lake concentration at least of seston, occurred with an intensity such to stir the upper layers and to homogeneously distribute the particles conveyed to the lake.

Among the particles related variables, POC and Chl resulted as the most heterogeneously distributed. This hold true also for seston, which is, however, mainly of biogenic origin in Lago Maggiore (Bertoni & Callieri 1989) except during important floods. The heterogene-

ity of Chl and POC decreased from winter to autumn. During the spring and summer of both years POC and Chl concentrations were 5 - 8 times and 5 - 6 times, respectively, higher than in winter. According to HI seasonal distribution of POC and Chl, their heterogeneity resulted higher when their concentration was lower. A possible explanation of this result is that in winter, when there is no production of new organic particles, any rare and small patch of detrital particles residual of previous season production, may result in high heterogeneity. Nevertheless, comparing non-productive and productive seasons it achieved a comparison between populations of particles with different densities. It is impossible to exclude completely that the HI we used, as the several indices proposed to compare the spatial aggregation of populations of unequal densities, do not contain mathematical artifacts bringing to misleading interpretations (Downing 1991).

As for the horizontal distribution of the picoplanktonic organisms, the spatial heterogeneity of the heterotrophic fraction (HPP) abundance resulted lower than the analytical variability in all samplings. This result suggests that the bacterial abundance from DAPI esti-
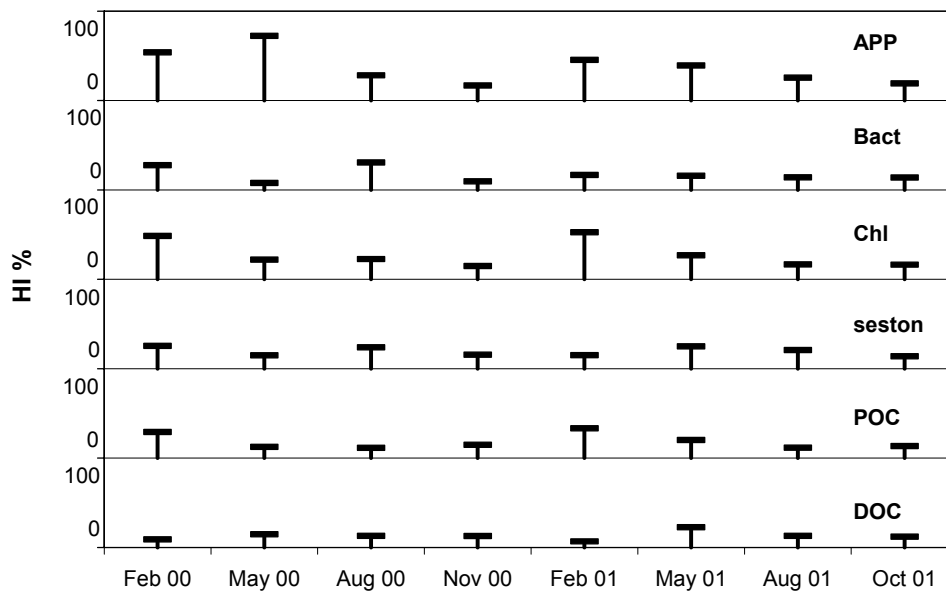
Fig. 3. Histograms of HI % for the different variables.

mates does not allow to highlight a spatial heterogeneity of bacterial community, also because of the low specificity of this method. It has, in fact, a delayed response to changes in bacterial abundance not allowing the discrimination of non-living "ghosts" or not-growing cells (Søndergaard 2000). The spatial heterogeneity of bacterial community could be better evaluated from changes in population diversity at genetic level (Yannarell *et al.* 2003).

The APP horizontal heterogeneity was high in winter and spring in both years and lower than the analytical variability only in autumn 2001.

To provide a visual assessment of the presence and of the spatial distribution of heterogeneity were prepared tables, schematically miming the shape of the lake. Figure 1 shows the correspondence between the sampling station location in the lake map and the schematic tables. In such tables marked in bold are the values where the concentration falls above the upper 95% confidence limit for variance associated to the analytical error (i.e., above the interval [l.m. ± t (a.v.)], where l.m. is the lake mean concentration, t = 2.11 (for P=0.05 and df = 17) and a.v. is the analytical variability). The stations in bold are those where the variability due to spatial heterogeneity significantly exceeded the analytical variability, i.e. where the average concentration was significantly higher respect to the homogeneous portion of the lake.

While the spatial heterogeneity of DOC, seston, autotrophic and heterotrophic picoplankton did not show any space-time pattern, the POC and Chlorophyll-*a* concentration exhibited a recurrent trend (Tab. 1, 2). It appears evident that both variables had significantly higher values in spring and summer samplings at the eastern side of the lake.

The eastward gradient of increasing POC and Chlorophyll-*a* can result from patches formation due to differences in phytoplanktonic production. This indicate a decline, although small, of the trophic state along the east coast of the lake. This decline can be due to the nutrient inputs to the lake mainly coming from the east coast (Tab. 3). The possibility that such distribution is an effect of a wind promoted accumulation is unlikely due to the shape of Lago Maggiore, lying in a deep valley north - south oriented as the dominant winds (Ambrosetti *et al.* 2001).

4. CONCLUSIONS

The horizontal heterogeneity of some variables related to microbial food chain is a measurable attribute of Lago Maggiore. Its occurrence affects particulate more than dissolved fraction and is unevenly distributed during the seasons. The spatial heterogeneity is higher in winter particularly for Chl and APP, when the concentration of both variables is lower. This is probably linked more to the residuals from the spoilage of patches of the previous season than to episodic growth, unlikely in winter. No indication can be inferred on bacterial horizontal heterogeneity in this lake since the analytical variance of bacteria counts always exceeded the spatial variance. To gather such information a qualitative approach should be considered. Only Chl and POC (in this lake mainly autochthonous) showed an heterogeneity pattern recurrent in time (spring and summer) and location (east coast) of occurrence. The localization of the part of drainage basin contributing the major nutrient input is consistent with the hypothesis that such input is the driving event of the observed heterogeneity.

Tab. 1. Particulate Organic Carbon concentration (POC, µg C l^{-1}) in 18 stations of Lago Maggiore during years 2000 and 2001.

02-2000

76	70	54
107	69	70
146	67	54
121		
	107	
134		
	105	68	**98**
	88	**110**	92

05-2000

336	392	**491**
410	442	**507**
406	442	**490**
335		
	426	
368		
	368	419	**376**
	427	**495**	455

08-2000

361	424	**460**
410	417	**500**
370	371	**465**
299		
	380	
355		
	404	368	**440**
	406	388	**406**

11-2000

93	101	80
129	**137**	94
104	105	105
116		
	124	
131		
	106	**120**	**133**
	143	127	125

02-2001

214	118	73
60	98	98
118	128	87
123		
	136	
202		
	124	106	101
	139	**193**	135

05-2001

221	199	246
267	266	396
293	299	**351**
274		
	273	
282		
	300	**453**	342
	303	**359**	345

08-2001

301	332	**407**
365	**434**	432
383	**425**	422
333		
	402	
295		
	348	377	**444**
	380	372	**421**

10-2001

242	**256**	237
202	202	168
202	173	165
183		
	207	
177		
	193	186	164
	196	184	174

Tab. 2. Chlorophyll-*a* concentration (Chl-*a*, µg l^{-1}) in 18 stations of Lago Maggiore during years 2000 and 2001.

02-2000

0.9	0.7	0.3
1.6	1.3	0.2
1.5	0.5	0.3
1.4		
	1.2	
2.1		
	1.5	0.8	**1.2**
	1.3	**1.7**	**1.4**

05-2000

3.7	4.4	7.4
5.8	6.2	**9.8**
7.7	**7.5**	**7.6**
5.5		
	7.3	
4.1		
	6.1	6.7	**7.6**
	6.6	**7.8**	7.7

08-2000

4.5	4.2	3.8
4.2	4.7	**5.5**
3.9	3.3	**5.7**
1.5		
	4.0	
3.1		
	4.1	**5.5**	5.1
	4.2	4.4	**4.8**

11-2000

0.6	0.6	0.5
0.8	0.7	0.6
0.7	0.7	0.6
0.9		
	0.9	
0.9		
	0.7	0.8	0.8
	0.7	0.7	**0.8**

02-2001

2.1	2.0	0.9
0.6	1.3	1.3
2.8	1.3	0.9
2.2		
	2.5	
1.7		
	2.3	1.6	1.1
	2.7	**2.8**	2.1

05-2001

2.9	2.6	3.5
4.4	4.7	**5.7**
4.4	**5.5**	7.3
5.2		
	5.1	
5.1		
	4.0	**5.8**	5.9
	5.5	**7.4**	7.2

08-2001

2.6	2.8	4.2
4.1	4.3	4.3
4.3	3.9	**5.0**
2.8		
	3.8	
2.8		
	3.5	3.3	**5.6**
	2.5	2.8	3.3

10-2001

1.5	**1.6**	1.5
1.3	1.2	1.1
1.3	1.0	1.0
1.0		
	1.3	
0.9		
	1.1	1.0	1.0
	1.2	1.0	1.1

Tab. 3. Areal contribution of total N and total P from drainage basin of Lago Maggiore (average of years 2000-2001 data) (Calderoni *et al*. 2001).

	Total N (g m^{-2} y^{-1})
Northern basin (Switzerland)	1.504
Western basin (Piedmont, Italy)	1.612
Eastern basin (Lombardy, Italy)	2.300
	Total P (g m^{-2} y^{-1})
Northern basin (Switzerland)	0.015
Western basin (Piedmont, Italy)	0.038
Eastern basin (Lombardy, Italy)	0.075

The large flood event occurred during this research did not produce a larger spatial variance of dissolved and of particulate matter of microscopic size. It rather resulted in higher homogeneity of the epilimnetic zone of the lake.

The observed magnitude and persistence of horizontal heterogeneity indicates that a single sampling point cannot be used for lake-scale characterization, at least for some variable which are of paramount importance in assessing the trophic status of the lake. Fairly, a sufficient number of distinct samples must be collected to quantify the spatial variance of seston, POC, Chl and APP. Furthermore, since we did not base sampling times on the occurrence of major meteorological phenomenon and sampling site on particular hydrological feature, it seems likely that the degree of heterogeneity that we observed may be a common occurrences in lakes such as Lago Maggiore. Further studies, conducted with higher spatial and temporal resolution, are needed to quantify specific mechanisms controlling the spatial distribution of microscopic size components of the food chain.

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