

Ecological effects of multiple stressors on a deep lake (Lago Maggiore, Italy) integrating neo and palaeolimnological approaches

Piero GUILIZZONI,^{1*} Suzanne N. LEVINE,² Marina MANCA,¹ Aldo MARCHETTO,¹ Andrea LAMI,¹ Walter AMBROSETTI,¹ Achim BRAUER,³ Stefano GERLI,¹ Elisabetta A. CARRARA,^{1,5} Angelo ROLLA,¹ Licia GUZZELLA,⁴ and Davide A.L. VIGNATI⁴

¹National Research Council, Institute of Ecosystem Study (CNR-ISE), 28922 Verbania Pallanza (VB), Italy; ²Rubenstein School of Environment and Natural Resources, Aiken Center University of Vermont, Burlington, VT 05405, USA; ³GFZ German Research Centre for Geosciences, Section 5.2 Climate Dynamics and Landscape Evolution, 14473 Potsdam, Germany; ⁴National Research Council, The Water Research Institute (CNR-IRSA), UOS Brugherio, 20861 Brugherio (MI), Italy; ⁵Present address: Water Division, Bureau of Meteorology, Melbourne, Vic. 3008, Australia

*Corresponding author: p.guilizzoni@ise.cnr.it

ABSTRACT

To understand interactions of lake physical characteristics, trophic dynamics and climate in Lago Maggiore, we compare long-term limnological and meteorological monitoring data and results from sediment cores. We include analyses of nutrients, pigments, diatoms and cladoceran microfossils. Over the past decades, caloric content increased. Eutrophication from the 1960s to early 1980s was followed by oligotrophication. DDTs, PCBs and Hg showed high contamination in the '60s, compared to point source inputs in the '90s. Algal biomass was predicted by total pigments and some algal specific carotenoids. Following nutrient enrichment, Chydorus sphaericus, and total abundance of cladocerans changed inversely with trophic status. Fewer large Daphnia since the late '80s matched an increase in with subfossil Eubosmina micro lengths. Both were explained by the 10-fold increase in Bythotrephes longimanus from 1987 to 1993, when an increase of its mean annual population density occurred during warmer winter and springs. Bythotrephes remained abundant and further increased during the following 10 years as water temperature increased. We conclude that warmer water affects food chains indirectly by changing habitat use and predator-prey interactions. Relative abundances of Daphnia and its peak population density in the warm year of the oligotrophic period (2003) were close to the record from the mesotrophic period in 1982, supporting the hypothesis that warming can produce a eutrophication-like signal. The study illustrates the complexity of biological responses to synchronous changes in multiple drivers (e.g., eutrophication, fish introduction, ban of fish harvesting, chemical pollution, and climate) and, despite this complexity, how Lago Maggiore responded to multiple stressors.

Key words: human impact, climate change, palaeolimnology, time series.

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INTRODUCTION

Human impacts on Italian aquatic ecosystems have intensified considerably since ~1945 (Guilizzoni et al. 1982; Guilizzoni et al. 2011; Rose et al. 2011). Urbanization and industrial activities have increased, resulting in greater export of chemical pollutants, organic matter and nutrients to rivers and ultimately to lakes (Pagnotta and Passino 1992). Similarly, most lakes worldwide have experienced more-or-less intense eutrophication during the 20th Century (Codd et al. 2005; Levine et al. 2011). Despite major efforts to limit and reverse eutrophication through nutrient control, effective restoration has often required much more time than expected (Anderson et al. 2005; Battarbee et al. 2005; Chorus et al. 2010). Besides internal drivers such as nutrient efflux from enriched sediments, restoration measures may be countered by some of the nutrient-generating impacts of climate warming, for example increases in rates of mineralisation, decomposition, run-

off, and erosion (Schindler 2001; Saros 2009; Smol 2008, 2010).

Air temperatures in temperate latitudes of the Northern Hemisphere have increased over the last century, with an amplification of this warming trend over the past 20-25 years (Leavitt et al. 2009). The effects of recent temperature and solar radiation increases on trophic conditions of freshwater ecosystems may be blurred at temperate latitudes, as these regions are typically subjected to multiple stressors that can mask or override climatic and anthropogenic signals (Smol 2008; Thies et al. 2011).

Lago Maggiore is an extensively studied lake with a documented history of organic chemical pollution as well as eutrophication followed by successful oligotrophication (Mosello et al. 1991; CIP AIS 2007; Salmaso et al. 2007). An integrated network of meteorological stations around the lake and in the watershed has produced a long-

term data set of about 60 years that verifies a warming trend (Ambrosetti and Barbanti 1999). In this paper, we review the results of previously published palaeolimnological and neolimnological examinations of Lago Maggiore's ecosystem-scale responses to various anthropogenic stressors, including sewage discharge, whitefish introduction, a fishing ban, and industrial pollution (Persistent Organic Pollution, POPs and Hg), as well as the physical implications of recent climate warming, including an altered thermal stratification regime. To investigate possible additive (or ameliorating) effects, we integrate nutrient, pigment, diatom and Cladocera data from several high-resolution sediment records, with relatively long instrumental climate and lake monitoring data series, and historical records of flood events.

We hypothesize that changes in water temperature and thermal stratification regime (cf. Rühland et al. 2008) have influenced lake ecology directly through such impacts as a shortening of zooplankton development time and an increase in the number of generations produced per year (Hall and Burns 2002; Visconti et al. 2008a), and indirectly through such impacts as hypolimnetic oxygen depletion during thermal stratification. We pay special attention to altered phytoplankton abundance and composition (assessed through fossil pigments as well as monitoring data) and to zooplankton phenology and density. For the latter, we are especially concerned about climatic impacts on the invertebrate predator *Bythotrephes longimanus* (Cladocera: Onychopoda), which plays a key role in controlling populations of smaller herbivorous zooplankton and thus grazing impacts on algal biomass.

For the periods when biological monitoring data are lacking, we substitute data on remains of organisms when assessing the impacts of perturbations on food webs structure. By combining palaeolimnological and neolimnological data, we expect a better ecosystem-scale synthesis of the impacts on the pelagic food web than would obtain with either approach alone. The sediment data will then be compared to long-term chemical and bio-monitoring data to relate the changes in the sediment signals to past changes in the water column.

MATERIALS AND METHODS

Study site

Lago Maggiore (Fig. 1) lies just to the south of the Alps (45°56.70'N; 8°37.76'E, deepest area), along the border between Italy and Switzerland at an altitude of 194 m a.s.l. It is a large lake (212.5 km²; volume 37.5 km³), also very deep (mean and maximum depths, 177 and 370 m, respectively). Like most of southern alpine lakes, it lies in a cryptodepression, specifically a canyon deeply incised by the River Ticino during the late Miocene. The

depression was further reshaped and then dammed by frontal moraines at its southernmost edge during the Quaternary (Bini et al. 1978; Finckh 1978).

Lago Maggiore is one of the best-studied lakes in the world, with a long-term record of physico-chemical and phyto-zooplankton data dating back to the mid or even early 1900s for some variables (papers quoted in the special issue of the *Memorie dell'Istituto italiano di Idrobiologia* 46, 1989; Guilizzoni et al. 1992a). The drainage basin (including the lake area) covers 6,599 km², yielding a drainage basin/lake area ratio of 31:1. This high ratio along with unusually steep hillslopes substantially affects the lake's hydrology as well as many environmental variables. The geological features of the watershed have been influenced by alpine orogenesis as well as glaciation, and thus are complex. However, the major topographic feature is a narrow steep-sided valley through the Piedmont and Lombardy regions of Italy. The elevation of the watershed ranges from 193 m a.s.l. at the lake's outlet to approximately 4,600 m a.s.l. at the Monte Rosa crest (Montaldo et al. 2005).

Standard classification by thermal regime would designate Lago Maggiore *warm monomictic*. However, the lake's depth is such that winter turnover rarely homogenises the entire water column. The thickness of the mixed layer at the lake surface varies from year to year depending on winter meteorological conditions. In recent years, due to the warming of the lake, the complete winter turnover has become the exception rather than the rule (Ambrosetti and Barbanti 1999). It follows that Lago Maggiore should be more correctly classified as a hololimnetic lake.

Hypolimnetic temperatures in Lago Maggiore are highest in late autumn and winter, presumably as a result of the vigorous downward mixing of heat from the upper water column during these months. The diel temperature fluctuations recorded in the upper hypolimnion during the stratification period are weak and the occasional short-term temperature increases that occur in the hypolimnion can probably be attributed to the effects of plunging plumes from the River Toce (Kulbe et al. 2008).

An air-gun seismic reflection profile made by a 3.5 kHz echo sounder has revealed thick deposits of Pleistocene and Holocene sediment (Fig. 1). The stratigraphic sequence consists of three general elements: an upper interval (Unit A) 50-80 m thick and aged as late-glacial to Holocene, a middle interval (Unit B) with a thickness of 60-100 m and representing normal sedimentation of glaciolacustrine sediments, and a bottom element (Unit C) more than 300 m deep and consisting of sands, mud, tills and gravel and probably a product of glacier overriding and compaction (Finckh et al. 1984). Details concerning geology, mineralogy, chronology, physical limnology and hydroclimatic features are reported elsewhere (Bar-

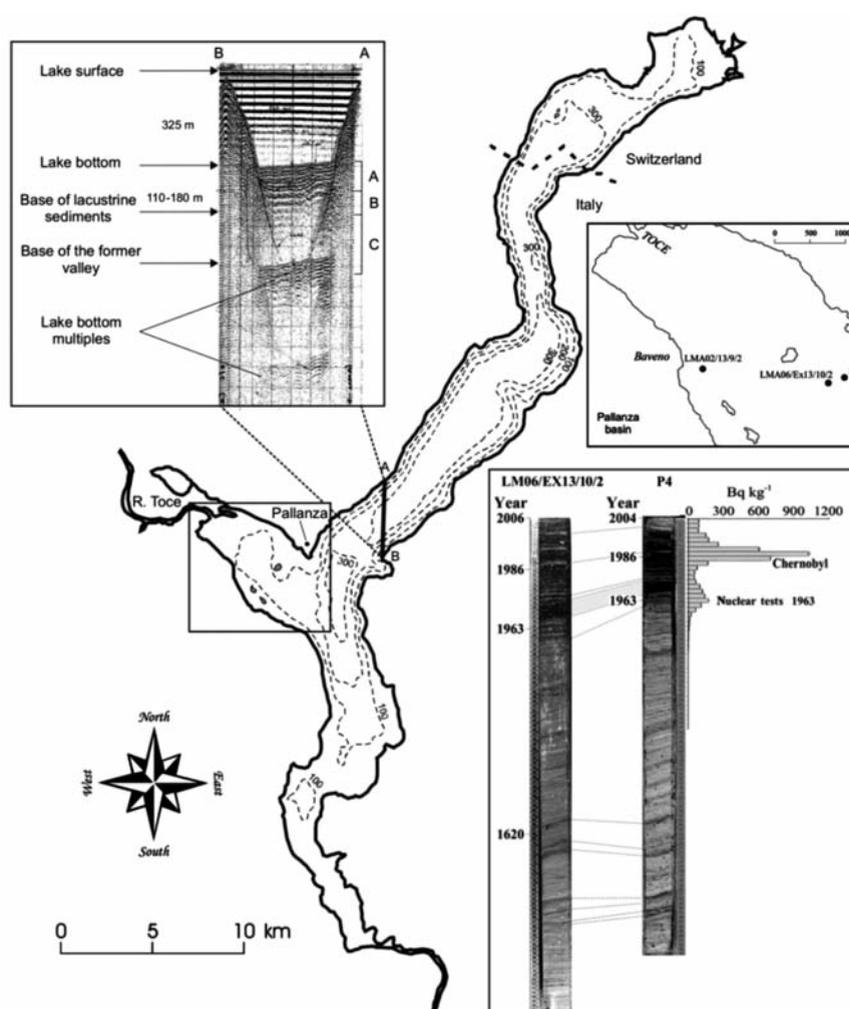


Fig. 1. Bathymetric map of Lago Maggiore. The North-South transect A-B (Laveno-S. Maurizio) for seismic reflection profile by a 3.5 kHz echo sounder (Finckh et al. 1984) shows the thicknesses of the Pleistocene and Holocene sediment infills (units A-C, and echo multiples are also indicated; see text). Core-to-core correlation from a dated core (P4; Putyrskaya and Klemt 2007) is also shown as well as the cores locations within the Pallanza Basin.

banti and Ambrosetti 1989; Carollo et al. 1989; Ambrosetti and Barbanti 1992). Nevertheless, some of these aspects will be reported in this paper when needed.

Meteorological and hydrological dataset

Daily monitoring data were gathered for nine meteorological variables (*e.g.*, temperature, precipitation, solar radiation, wind run) and two lake physical features, water level and water temperature. These data are available since the beginning of 1951 (Ambrosetti et al. 2006). Ongoing studies are directed towards determining climatic warming effects on 1) vertical mixing at the end of the thermal destratification phase; 2) deep hypolimnion oxygen content; 3) thermal energy distribution in the lake, and 4) lake water residence time. All four hydrological variables exert in turn major impacts on the functioning of the lake ecosystem as a whole.

Precipitation and temperature data collected since 1951 at the CNR meteorological station of Pallanza were analysed to compare with the timing of flood events and the winter North Atlantic Oscillation (NAO) index. Precipitation and temperature data (1951-2006) were organised as annual and seasonal means: autumn (September to November), winter (December to February), spring (March to May) and summer (June to August). Total annual inflow from both the entire watershed and the River Toce, was calculated for 1980 to 2006. The NAO winter index (December-March) is the normalized sea level-pressure difference between Lisbon, Portugal and Reykjavik, Iceland (Cook and D'Arrigo 2002) and was obtained from the Climate Analysis Section, NCAR, Boulder, USA (<http://www.cgd.ucar.edu/cas/jhurrell/indices.html>).

Coring sites

Two short cores collected in 2002 and 2006 by a gravity corer from the Pallanza Basin in the western part of Lago Maggiore (Fig. 1) were analysed for several geochemical variables, pigments, diatoms, and Cladocera subfossil remains. The sediment cores were sliced in layers of 1 cm thickness. The main inflow into the basin is the River Toce at the Basin's north western margin.

The core locations are labelled in fig. 1 as follows: LMA06 Ex13/10/2 and LM 02 13/9/2 (Tab. 1).

Several other cores collected in the previous years and at the same coring position, and mainly used for the chronological framework are also discussed here (*e.g.*, core P4, Fig. 1, Tab. 1).

These studies of sediment cores were integrated by high-resolution measurements of particle flux and water temperature in the Pallanza Basin designed to elucidate the mechanisms responsible for short-term anomalous events, as well as to determine the likely effects of longer-term anomalies such as those associated with climate change (Kulbe et al. 2008). Thus, between October 2004 and May 2006, sediment traps (either time-sequencing or single open traps), were deployed at four different depths in the water column (57, 72, 97, 117) and left to integrate sediment fluxes over periods of 7-21 days.

Chronology

Sediment chronology has been repeatedly established by several methods during the past 10 years: radiometric measurements (^{137}Cs and ^{210}Pb), (Marchetto et al. 2004; Putyrskaya and Klemm 2007; Putyrskaya et al. 2009) (Fig. 1), biological (historically documented changes in the diatoms abundance and composition, Marchetto et al. 2004) and chemical markers (*i.e.* the known increase of nutrients at the onset of eutrophication, AD 1960-1965). A ^{14}C date for a sediment layer from core LMA/ex13A/10/2 also has been obtained (terrestrial plant microscopic remains analysed by the Poznan Radiocarbon Laboratory, Poz. 25080). At 66-69 cm a date of 330 ± 70 calibrated years BP has been estimated using OxCal software (Reimer et al. 2004). The combination of all the methods provided a coherent and robust chronology.

Chemical and biological analyses

Dry mass and organic matter were measured by loss-on-ignition (LOI) at 70°C and 550°C , respectively (Dean 1974). Total carbon, sulphur and total nitrogen were obtained using a Carbon Hydrogen Sulphur analyzer (NA 1500 Fisons). Photosynthetic pigments were extracted from *ca* 1 g of wet sediment by soaking it in 5 mL 90% acetone overnight in the dark and under nitrogen atmosphere. The extract obtained was used to quantify the chlorophylls, their derivatives (Chlorophyll Derivatives

Tab. 1. Data on cores collected in the Pallanza Basin, including coring site location and water depth, and core length. (*) Putyrskaya and Klemm (2007).

Core name	Longitude (E)	Latitude (N)	Water depth (m)	Core length (cm)
LMA06/Ex13 /10 /2	8°32.89'	45°54.47'	134	72
LMA02/13 /9 /2	8°31.94'	45°55.48'	35	42
P4 (*)	8°33.02'	45°54.84'	150	93

Units, CD), and total carotenoids (TC) by spectrophotometry (for details see Lami et al. 2000). Individual carotenoids were detected by Reversed Phase High-Performance Liquid Chromatography using a Dionex SUMMIT with a DAD-UV detector (Lami et al. 2000). Carotenoid concentrations were expressed in nanomoles per gram of organic matter (nmol g^{-1} LOI, Züllig 1986) and chlorophyll derivatives in Units per gram of organic matter (U g^{-1} LOI, Guilizzoni et al. 1982). Guilizzoni et al. (1992b), Lami et al. (2000) and Guilizzoni and Lami (2002) provide a detailed account of the spectrophotometric and chromatographic analysis of the plant pigments, their identification, quantification and interpretation.

Chemical variables discussed here (total nitrogen, total and reactive phosphorus, oxygen, reactive silica) of water samples were analyzed using standard methods (spectrophotometry and ion chromatography, Tartari and Mosello 1997). Water samples were filtered through GFC glass filters to estimate chlorophyll- α concentrations. After 90% acetone extraction, chlorophyll concentrations were determined spectrophotometrically.

Total mercury concentrations in core LMA06 Ex13/10/2 were determined by thermal decomposition, amalgamation and atomic absorption spectrometry according to US EPA method 7473 (US EPA 1998) using an automated Hg Mercury Analyzer (AMA254, FKV, Bergamo, Italy). After freeze-drying and homogenizing, each section was analyzed in triplicate and precision was generally better than 5%. Accuracy was checked using certified reference materials BCR 280 from the Bureau Communautaire de Référence (0.670 ± 0.019 mg kg^{-1}) and GBW07305 from the National Standard Centre of China (0.1 mg kg^{-1}) along with internal reference sediment S7 from a ring test (AQUACON project) coordinated by the Joint Research Centre of ISPRA (1.72 ± 0.04 mg kg^{-1}). For all materials accuracy was within $\pm 10\%$ of certified values.

DDT and PCB analysis of sediment was performed, after lyophilisation, by a Soxhlet apparatus using a *n*-hexane/acetone mixture (3:1 v/v) with the addition of the recovery standards [$^{13}\text{C}_{12}$]CB 101 e 153 (Guzzella et al. 2011). The extract was concentrated under nitrogen flow and cleaned-up using a multilayer column (filled with acidified silica, potassium silicate and Florisil®). The column was eluted with *n*-hexane/dichloromethane 1:1 v/v,

then concentrated and analysed by GC-MS/MS using a Thermo Electron TraceGC 2000 coupled with a PolarisQ Ion Trap (ThermoElectron, Austin, Texas) mass spectrometer and equipped with a PTV injector and an AS 3000 autosampler. For PCB congeners and chlorinated pesticides, method performance was evaluated using 1939a SRM river sediment purchased from NIST (National Institute of Standard and Technology, Gaithersburg, Maryland).

Diatoms were prepared using standard H_2O_2 -HCl digestion (Renberg 1990) and mounted in Naphrax. On each slide, a minimum of 500 diatom valves were enumerated by light microscopy and taxonomical features confirmed by scanning electron microscopy procedures. Diatom abundance was expressed as relative percentages.

Cladoceran subfossil remains were analysed according to Frey (1986). About 4 g of wet sediment were deflocculated in 100 mL of 10% KOH at 100°C for 20 minutes and passed through a 40 μ m sieve. The concentrated remains were then transferred to 5% formalin. We identified and counted up to 200 cladoceran remains per sample (headshields, shells, post-abdomens, post-abdominal claws, mandibles and others) using the determination keys of Frey (1960) and a standard microscope at 100-200 magnification. Details about the diatoms and Cladocera subfossil analyses are reported elsewhere (Marchetto et al. 2004; Manca et al. 2007a).

RESULTS

Hydrodynamic variables

Meteorological monitoring in the Lago Maggiore watershed has revealed significant change over the past few decades in three variables that influence energy exchange between the lake surface and atmosphere (Figs 2 and 3): total annual and winter air temperature and solar radiation has increased (particularly evident since 1998), total winter wind run has dropped since the 1970s (Figs 2 and 3); mean winter air temperature has increased (Fig. 3a). Vertical mixing strength decreased due to convective motion that produced, at the end of limnological winter, a caloric content increase both in the mixing layer and the hypolimnetic layer: the result is a much higher thermal stability of the system. As a consequence, energy exchange and depth of mixing within the water body also has been altered, resulting in important modifications to the hydrodynamics, *e.g.* the water renewal time (Fig. 2c).

Over more than 50 years observation, annual solar radiation has shown a slight increasing trend, while a more evident increase in the duration of annual insolation (*ca* 100 hours) has occurred. The winter data on solar radiation show a more substantial increase (Fig. 2a, Ambrosetti et al. 2006).

One of the most evident consequences of the ongoing

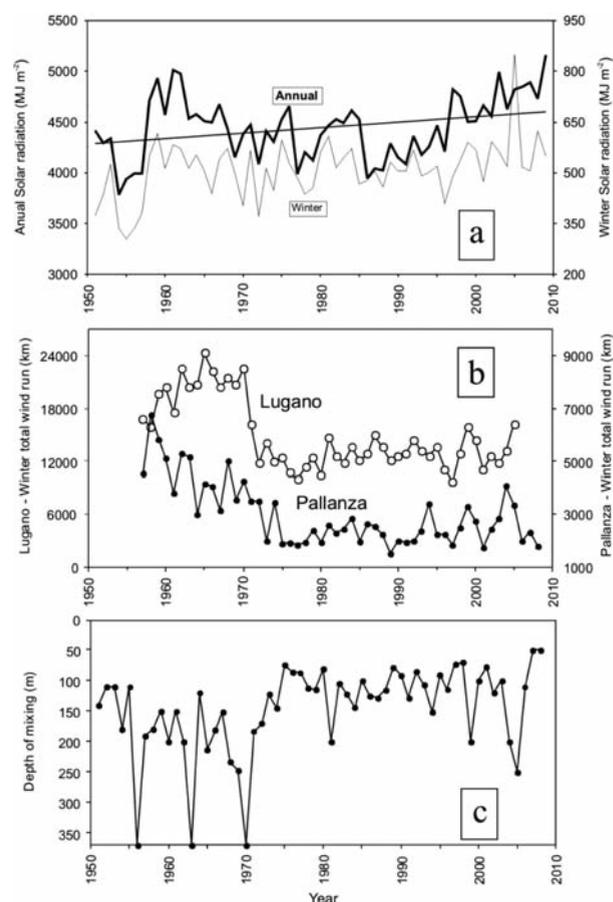


Fig. 2. (a) Solar radiation: winter (light line) and annual totals. The regression line (winter data) is also indicated. (b) Total winter wind run trend (km per month) recorded at Lugano and at Pallanza (on the right axis) from 1957 to 2009. (c) Depth of vertical convective mixing of Lago Maggiore water at the end of the limnological winter (March) in the period 1951-2008.

climate change for Lago Maggiore has been less deep convective mixing of the water body in winter. Fig. 2c shows what appears to be a 7-y cycle of complete mixing (1956, 1963, 1970) at the beginning of the record, followed by 38 yrs of winter convective mixing to depths of less than 250 m. The result has been isolation of deep hypolimnetic water, with consequences for its oxygen content, and return of nutrient stored in the hypolimnion back to the epilimnion. There also have been biological effects that we will discuss later.

Another notab. change has been an increase since 1963 in the total heat content of the lake (Figs 3b and 3c). Over 12-month intervals, heat content is strongly correlated with season, but the correlation coefficient returns to a value of 0.913 at the end of each cycle and the long-term upward trend is significant. By contrast, the deep lake layers show progressively less pronounced seasonal cycles over 40 years. By evaluating the heat content

within the water mass of the Lago Maggiore, and the mixing depth reached in winter, it has been possible to isolate a layer known as the deep hypolimnion. The upper limit of this layer, in the phase between the onset and the progressive development of thermal stratification, is the depth at which the forces of floating and vertical diffusion become equal (Michalski and Lemmin 1995). This deep layer can retain a sort of *climatic memory* sensu Ambrosetti and Barbanti (1999) and Ambrosetti and Sala (2006) in which the water mass hydrodynamics are strongly correlated with the dynamics of the atmosphere, thus making it possible to identify the lake's response to climate forcing (Fig. 3b). This increase of caloric content in the deep hypolimnion of the lake shows variations that fit into a longer time scale, comparable to that of changes in climate. This is how the deep water retains a *climatic memory*, from which it may be possible to gain information on past, present and future climate changes.

Discontinuities in the increasing trend in Fig. 3b are related to specific hydro-meteorological events, including the input of cold, high density water from tributaries and the so called conveyor belt mechanism, which transfers surface water into the deep water layers, radically changing the structure of the hypolimnion (see below, Ambrosetti and Barbanti 1992). The sediment trap study showed that diel temperature fluctuations of up to 0.8°C were recorded at 50 m depth from the end of April to November. On several occasions short-term temperature increases of up to 0.4°C occurred in the water column below 50 m depth. These can only be explained as plunging plumes of River Toce inflow.

Lake sediment characteristics: sedimentation rates, geochemistry

As expected for a lake with a large watershed area, several tributaries and high annual rainfall, Lago Maggiore collects sediment at an exceptionally rapid rate, albeit with considerable spatial heterogeneity (Marchetto et al. 2004). Along the main North-South axis, recent (last 50 years) sedimentation rate, as measured by radiometric and biological marker (diatoms), ranges from a high of 1.4 cm y^{-1} in the deepest area to a low of 0.31 cm y^{-1} near Arona in the lake's southern shallow area (Guilizzoni and Calderoni 2007). Rates are higher in the Pallanza Basin, from 0.6 cm y^{-1} far from shore to $2\text{-}15 \text{ cm y}^{-1}$ near the River Toce mouth. In the southern basin and central plain of the lake bottom, the sediments are fine-grained and show the characteristics of low-energy deposits. Autochthonous components prevail as indicated by abundant diatom frustules in the silt fraction.

The sediment trap study (Kulbe et al. 2008) showed an overall mean sediment accumulation rate at 57 m ($2.5 \text{ g m}^{-2} \text{ d}^{-1}$) is slightly lower than that measured at 117 m ($3.2 \text{ g m}^{-2} \text{ d}^{-1}$). The minimum accumulation rates were the

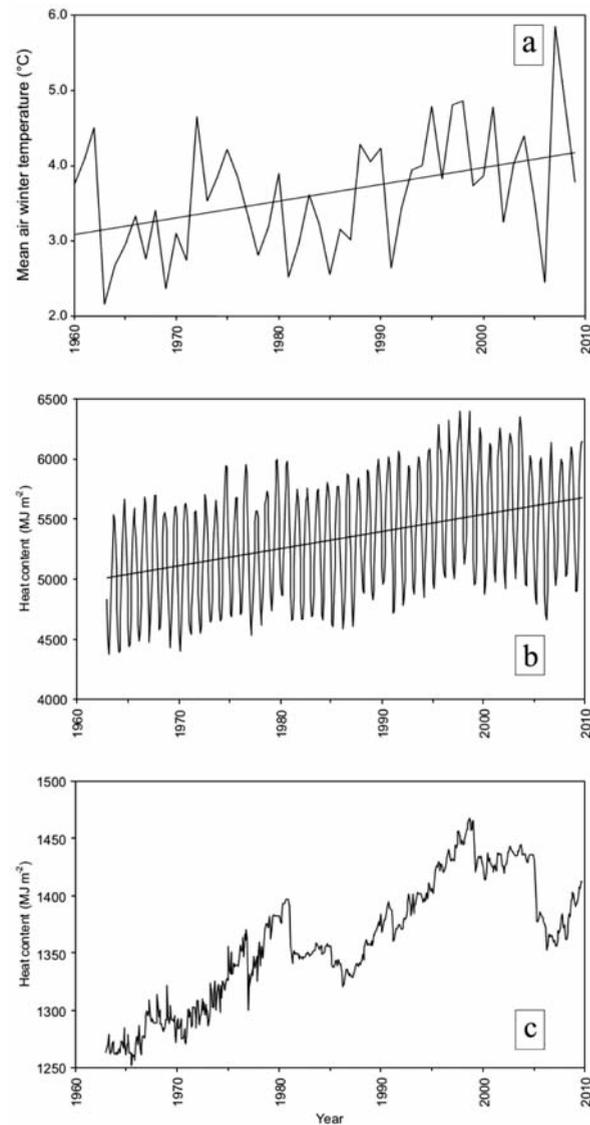


Fig. 3. (a) Average winter atmospheric temperature ($^{\circ}\text{C}$) recorded at Pallanza from 1960 to 2009. (b) Total water body heat content and climatic memory (c) in Lago Maggiore for 1963-2009 (see text).

same at both depths ($0.02 \text{ g m}^{-2} \text{ d}^{-1}$), whereas the maximum value at 57 m ($19.7 \text{ g m}^{-2} \text{ d}^{-1}$) was much lower than at 117 m ($30.8 \text{ g m}^{-2} \text{ d}^{-1}$). Both maxima occurred between October and November 2004. Allochthonous sediment appears to dominate in the Pallanza Basin, which is directly influenced by the River Toce. Here organic matter accounts for about 4-5% of dry weight and the TOC/TN ratio ranges from 11 to 31 (Damiani 1974). By contrast, autochthonous sediments are well represented on the bottom platform of the deeper main lake basin as well as in areas near urban centres. In these regions organic matter content can reach 25% of dry weight. The rivers Toce and Ticino appear to have major impacts on the distribution

of organic carbon (0.8-6% d.w.) and total nitrogen (0.1-0.6% d.w.), as well as sediment texture.

Macroscopic images of the sediments in cores (Fig. 1) reveal a succession of light marker layers in the upper 22-30 cm. These layers have thicknesses of millimetres to centimetres and consist of detrital matter. They are most discernable in sediment deposited during eutrophic conditions because the intervening sediment with high organic content is extremely dark. Microscopic analyses revealed that the detrital layers typically are graded, with coarse to medium-sized sand grains at the base and silt and clay further up in each layer (Kämpf et al. 2011).

Comparing these layers with historical flood statistics (Ambrosetti et al. 2006) permitted reconstruction of flood events in the Pallanza Basin during the past 5-6 decades.

High statistical correlations were found between floods, precipitation, River Toce discharge and total inflow to the lake ($p < 0.01$) (Tab. 2). General NAO index data were correlated with temperature ($p < 0.05$), but no relationships were found between the index and floods, total inflow from the watershed or River Toce inflow. A detailed analysis of the data on floods between 1868 and 2004 showed a rather complicated history, which we have divided into four periods, each with a flood frequency that was 18-33% of the total (Tab. 3). Floods were identified mostly in autumn. The period 1902-1970 was characterized by generally positive NAO indices and exceptionally low flood frequency (17.9%). In particular, there were no summer floods. The most recent period examined 1970-2004, also had a generally positive NAO index but, in this case, had an above average frequency of floods (33%) and, most significantly, a larger number of severe floods (Tab. 3).

Another feature of Lago Maggiore sediment that suggests climatic as well as anthropogenic influences is the

presence of vivianite concretions in sandy layers deposited in deep areas during the lake's eutrophication phase (Viel and Damiani 1985). Vivianite is a stable authigenic iron phosphate hydrate [$\text{Fe}_3(\text{PO}_4)_2 \cdot 8(\text{H}_2\text{O})$] generated by partial oxidation of Fe(II) into Fe(III). It is especially common in lakes poor in authigenic carbonates but rich in organic matter, with reducing conditions and a warm and humid climate (Kazuyoschi 2003). Its presence has been correlated with phases of higher primary productivity in the water column. In hundreds of sediment cores collected from Lago Maggiore over the past two decades, the vivianite found earlier has been absent, apparently having dissolved. This testifies to improved redox conditions at the sediment-water interface (unpublished data). Unfortunately it is unclear at present whether the dissolution observed is chemically (trophic state) or temperature controlled. Once this is resolved, vivianite may serve as a good proxy of reduced conditions or climate change.

Chemical contaminants: trace metals and organochlorine compounds

Sedimentary records of the pollution of Lago Maggiore with DDT, trace metals (especially mercury) and persistent organic pollutants (POPs) over the past *ca* 55 years have been obtained through the analysis of short cores collected during several sampling campaigns starting in 1963 (*e.g.*, Muntau 1981; Provini et al. 1995; Guzzella et al. 1998; Baudo et al. 2002). Detailed distribution maps for macro and trace elements have been drawn up based on the core data (Baudo et al. 1981; Viel and Damiani 1985; Rossi et al. 1993; Baudo et al. 2002; Bettinetti et al. 2005; Guzzella et al. 2007) and the total burdens of metals and POPs in sediments have been esti-

Tab. 2. Correlations (Pearson's correlation coefficients and sample size, n) between annual, autumn rainfall, River Toce discharge, NAO and flood events for the period 1951-2004. All correlations are significant ($p < 0.01$) except NAO vs floods.

	NAO		Total inflow		Rainfall		River Toce discharge
	Annual mean	Annual mean	Sep-Nov	Dec-Mar	Sep-Nov	Annual mean	Sep-Nov
n	24	23	16	10	18	24	9
Floods	0.118	0.635	0.792	0.814	0.696	0.604	0.971

Tab. 3. Number of historical floods (left) and severe floods (right) in Lago Maggiore since 1868. Percentages of events also are indicated. The water level of floods is >195.5 m a.s.l., while severe floods have levels >196.5 m a.s.l. The period between 1868 and 2004 was divided into four 34-year intervals.

Year	Total floods					Year	Severe floods				
	Spring	Autumn	Summer	Total/Period	Total/Period %		Spring	Autumn	Summer	Total/Period	Total/Period %
1868-1902	3	13	5	21	31.3	1868-1902	0	3	1	4	21.1
1902-1937	3	9	0	12	17.9	1902-1937	0	4	0	4	21.1
1936-1971	0	7	5	12	17.9	1936-1971	0	3	0	3	15.8
1970-2004	5	13	4	22	32.8	1970-2004	2	6	0	8	42.1
			TOTAL	67					TOTAL	19	

mated (Cenci et al. 1991; Provini et al. 1995; Guzzella, unpublished data). All work to date shows mercury (and other trace metals not shown) and DDT at highest concentrations in sediments deposited during northern Italy's industrial and economic boom of the 1960s (Fig. 4).

The concentrations of total DDT and total PCBs in the whole profile show and increase after the second world war reaching a maximum values of *ca* 13,000 ng g⁻¹ d.w. and 120 ng g⁻¹ d.w., respectively, whereas Hg starts to constantly increase at the beginning of the 20th Century. Background values of this metal are evaluated as 0.01-0.03 mg kg⁻¹ against a maximum concentration of *ca* 26 µg g⁻¹ d.w.

In 1996, however, a severe DDT pollution event occurred in Lago Maggiore, leading to a fishing ban for commercial fisheries from 1996 to 2005 (Guzzella et al. 1998). The source of pollution was liquid waste discharge into the River Toce by a chemical factory that produced DDT. DDT production was discontinued at this point and monitoring of the purging of DDT and its metabolites from the tissues of the two most important harvested fish species (*Coregonus* spp. and *Alosa agone* syn. *Alosa fallax*) as well as from sediments began. The whole ecosystem was adversely affected due to DDT transfer through pelagic and littoral food webs (Binelli et al. 2001; Bettinetti et al. 2010). In 2001 mean concentrations in sediments continued to exceed North American Sediment Quality Guidelines (MacDonald et al. 2000), suggesting potentially toxic conditions for benthos. A more recent assessment, however, showed improvements in surface sediment quality (Guzzella et al. in preparation).

All of the contaminants assessed were found at abnormally high concentrations in sediment deposited during seasons when tributary flooding carried large amounts of detrital material into the lake (Galassi et al. 2006; Guilizzoni and Calderoni 2007).

Sediment core analyses: the biological record

Pigments

Sediment profiles for total chlorophyll derivatives (CD), total carotenoids (TC) and specific algal pigments show substantial peaks in total algal biomass and primary production during certain periods within the past *ca.* 40 years (Fig. 4). Especially responsive appear to be the pigments associated with siliceous algae, Chlorophyta and cyanobacteria. Primary production in the Pallanza Basin was reconstructed based on fossil pigment accumulation rates (Guilizzoni et al. 1983) and compared both with sediment organic matter and nutrient profiles in the same sediments and with real time lake monitoring data. Pigments, organic matter and nutrients showed similar sediment profiles, and pointed to a marked productivity increase beginning in the 1960s. Only for some years in the 1990s did the inferred primary production curve disagree with

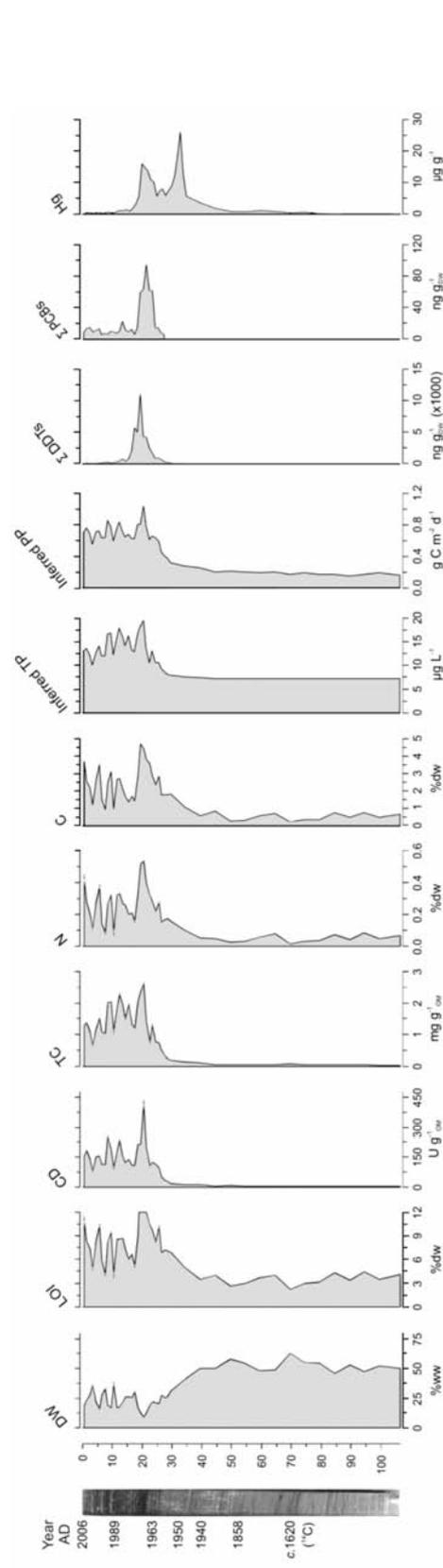


Fig. 4. Depth profile of several sediment variables from core LMA06 ex13A/10/2. Dry weight (at 60°C); LOI (Loss on Ignition at 550°C); CD (chlorophyll derivatives); TC (total carotenoids); C (total carbon), N (total nitrogen); inferred total phosphorus (TP) from a pigment model (Guilizzoni et al. 2011) and inferred primary productivity (PP) (Guilizzoni et al. 1982); sum of DDT, PCB, total mercury (Hg). Chronology is based on radiometric measurements (¹³⁷Cs) down to 25 cm and ²¹⁰Pb for older sediments. A ¹⁴C date on terrestrial plant remains also is reported. Measured TP and PP in 2006 was *ca* 10 µg L⁻¹ and 0.44 g C m⁻² d⁻¹, respectively.

that generated from in-lake measurements (Marchetto and Musazzi 2001; Marchetto et al. 2004). Differences were attributed to the higher sensitivity of the sedimentary pigment model and larger number of sampling dates. While the model appears to be quite accurate in estimating productivity and thus the assessment of spatial and temporal variability, global warming impacts on pigment sedimentation and preservation are yet to be determined.

Recently, a palaeolimnological method for inferring past total phosphorus (TP) concentrations in lake water from spectrophotometrically-measured sedimentary pigments, particularly total carotenoids, has been proposed (Guilizzoni et al. 2011). The results of its application agree well with TP measurements made by long-term water quality monitoring programs. The average pigment-inferred TP concentration was $7.0 \mu\text{g L}^{-1}$ with a standard deviation of $1.5 \mu\text{g L}^{-1}$ for the pre-eutrophication phase. In 2006, mean annual TP concentration measured at overturn ranged between 6 and $11 \mu\text{g L}^{-1}$ with a mean value of $8.5 \mu\text{g L}^{-1}$.

Diatoms

The sub-fossil diatom assemblages found can be divided into three zones (Fig. 5). At the bottom of the cores in sediments deposited prior to 1960 the assemblage is dominated by *Cyclotella* species, especially *C. comensis*, a species typical of oligotrophic lakes. Overlying this zone is one deposited between ca 1960-1980 and characterized by the dark black sediments typical of lake eutrophy. At the bottom of the middle zone, *Fragilaria crotonensis* is present, indicating that it was one of the earliest eutrophic species to respond to nutrient increase. The sequential appearance of a parade of meso- and eutrophic species followed, including *Tabellaria flocculosa*, *Aulacoseira islandica*, *A. ambigua*, and finally *Stephanodiscus minutulus* and *S. hantzschii*. The sediment layers deposited since 1980 make up a third zone indicating ongoing lake re-oligotrophication. In this zone, eutrophic species decline in abundance to be replaced again by *Cyclotella comensis*, which has not yet attained the relative abundance it had before 1950 (at the bottom of the cores) however (Fig. 5).

Cladocera

Cladocera abundance (Fig. 6) was relatively low in cores between 40-22 cm depth (ca 1943-1970) and particularly low between 31 and 28 cm (ca 1956-1961). Above this zone, in the 28-16 cm interval (deposited between ca 1961-1979), small increases in both chydorids and pelagic cladocerans are apparent. A marked increase follows at 16-13.5 cm. Between 13 and 4.7 cm (ca 1986-1996) however, there is a reversal of trends. First chydorids and then pelagic Cladocera decline in abundance,

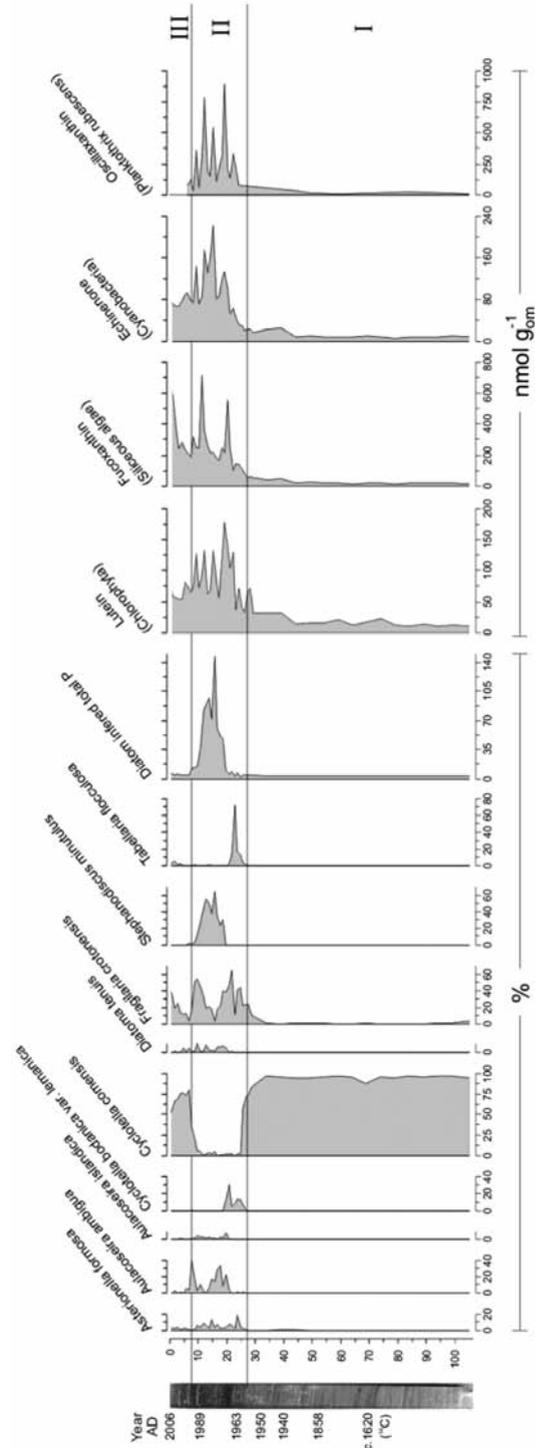


Fig. 5. Palaeoenvironmental changes in Lago Maggiore sediment core (LMA06 ex 13A/10/2) based on fossil diatoms and main specific pigments. Zones I-III mark pre-eutrophication, eutrophication and oligotrophication phases.

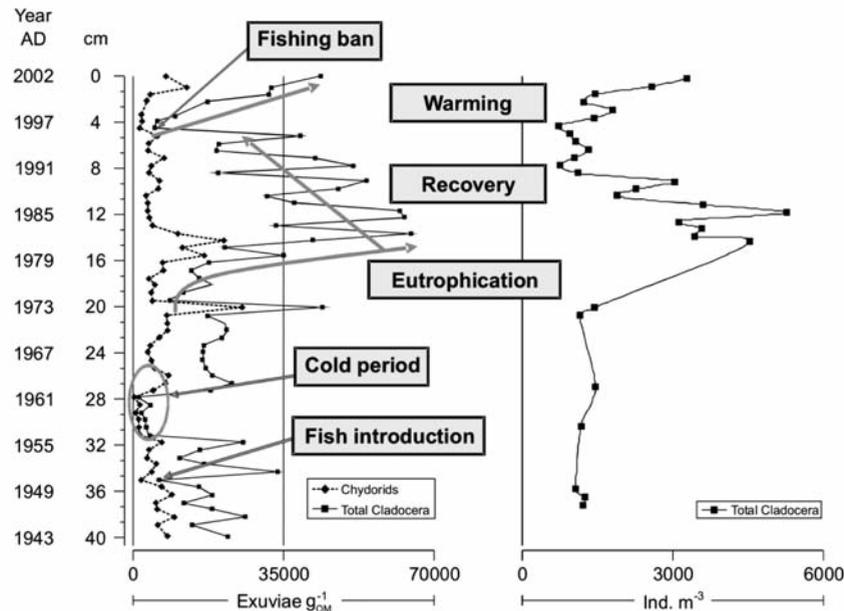


Fig. 6. Left panel: profile of changes in the abundance of cladocerans (total Cladocera and chydorids, as expressed on organic matter basis) sediment core from Lago Maggiore (LMA02/13//9/2) in relation with the main events during the last *ca* 60 years. Right panel: values from water column data. Main processes and forces also are indicated (redrawn from Manca et al. 2007a).

although the trend is obscured by large inter-annual variations. Finally, in the top few centimetres of the cores, a return to higher densities of pelagic cladoceran fossils in sediment is observed. This pattern of zooplankton species replacement in sediment cores agrees well with observational data from the long-term monitoring of lake communities (Fig. 6). The latter shows minimal cladoceran biomass in 1996 followed by a general increase from 1998-2002.

In addition to the recovery of substantial amounts of planktonic *Eubosmina longispina* from core sediments in the 20-8 cm interval, microfossils of *Bosmina longirostris* a littoral species common in deep lakes (de Bernardi et al. 1990) and also lakes with high fish predation (Kerfoot 1974), was found (Manca et al. 2007a). This finding concurs with the monitoring record, which shows *B. longirostris* present in the pelagic zone during the early 1980s (de Bernardi et al. 1990). The contribution of chydorids to the microfossil assemblage in sediment decreased during the lake's eutrophication phase with the exception of the continued presence of pelagic *Chydorus sphaericus* (Frey 1986; Korhola 1999). The abundance of *C. sphaericus* declined at the top of the core, but the species remained at higher densities than in sediments deposited prior to the lake eutrophication phase (28-20 cm). The proportion of pelagic to littoral cladocerans increased during eutrophication (not shown in the fig.) and remained high during oligotrophication to finally show a distinguishable decline at the end of the 1980s. A delayed re-

sponse of biota to nutrient input reduction also was recorded in the contemporary zooplankton data. The latter showed major changes only after 1988 (Manca et al. 1992). Although there was an overall trend towards decreasing density of planktonic subfossil Cladocera in sediment layers during oligotrophication, it was overlain by large short-term increases that appeared cyclic in nature. Cladoceran periodicity of 3-4 years also was observed for some taxa in the contemporary data series. The sediment data furthermore depict a recent increase in the abundance of Cladocera as a whole (Fig. 6), and particularly of *Daphnia* a trend that has been attributed to an earlier and faster population growth consequent to climate warming (Visconti et al. 2008a). Contrasted with the cladoceran peaks of warmer weather, there was a depression of total cladoceran abundance in the 35-28 cm layer representing deposition between *ca* 1950-1960. This was a generally cold period, particularly during the winter in 1956 (Manca et al. 2007a). Contemporary data show that the earlier start of population growth is related to an earlier thermal stratification. For prey organisms which are highly vulnerable to visual predation (*i.e.* *Bythotrephes*), changes in thermal stratification regime may lead to changes in the availability of a refuge from visual predation, which means that vulnerability to predation can change even at a stab. fish stock, as a consequence of climate change (Manca and DeMott 2009; Manca 2011).

Also from the Cladocera record we have shown good correspondence between the sediment and the contempo-

rary cladoceran record (Fig. 6), suggesting that a more complete understanding of changes in trophic dynamics in Lago Maggiore may be obtained using a combined palaeo-neolimnological approach than by relying on one of these alone.

The successful introduction of whitefish in 1950 was tracked in the sedimentary record through changes in the Cladocera community structure and the contribution of different size fractions to the total Cladocera bio-volume (Manca et al. 2007a). Fish predation indices (CPUE, Catch Per Unit Effort, and PI, Planktivory Index, not shown) calculated from sedimentary and observational data also closely matched the timing of the fishing ban (both professional and recreational) proclaimed because of DDT pollution of the lake (Guzzella et al. 1998). The ban lasted until 2005 after which it has been gradually released. Usually, a reduction in the abundance of planktivorous fish is expected during oligotrophication phase. As TP and Chl- α decline (Figs 7 and 8), zooplankton has less food and can support less tertiary productivity (Jeppesen et al. 2005).

The history of lake trophic evolution: comparison of neo- and palaeolimnological chemical data

From the sedimentary studies, the first evidence of eutrophication was detected from the late 1950s and particularly in the 1960-1970s, when an increase and subsequent peak of nutrients and fossil algal pigments was detected (Figs 4 and 8). Low values of primary production

and total phosphorus in the water were the normal situation for a long period prior to 1960. The long-term changes in the profiles for dry mass (DM), organic matter (LOI) and nitrogen reflect changes in the nutrient status of the lake (Fig. 4). LOI, carbon, nitrogen and pigments increased in the mid 1960s and peaked at the same time as the TP and chlorophyll a concentrations measured in water peaked (Fig. 7). Subsequently, they declined with the oligotrophication phase that bottomed out and stabilized around 1993.

Several short-term fluctuations of all variables in the 1990s reflect floods and complete vertical mixing events. Major floods occurred during the past 55 years and complete overturn through convective mixing was observed in the 1956, 1963 and 1970; these events being tracked by the LOI and DM profiles (Fig. 4). In contrast, the vertical mixing of lake water in 1981 (Ambrosetti et al. 1983) seems almost undetectable in the LOI and DM profiles, although it caused an overall decrease in the annual mean density of zooplankton because immature individuals trapped in deep water layers suffered high mortality (Manca et al. 2007a) (Fig. 6).

Nitrate data, first recorded in the late 1950s, indicated concentrations between 0.45 and 0.50 mg L⁻¹. From 1960 to 1980 the concentrations of N-NO₃ increased to values near 0.80 mg L⁻¹ and continued to increase after the phosphorus decline.

A comparison between total chlorophylls and total carotenoids in sediments with total nitrogen and chloro-

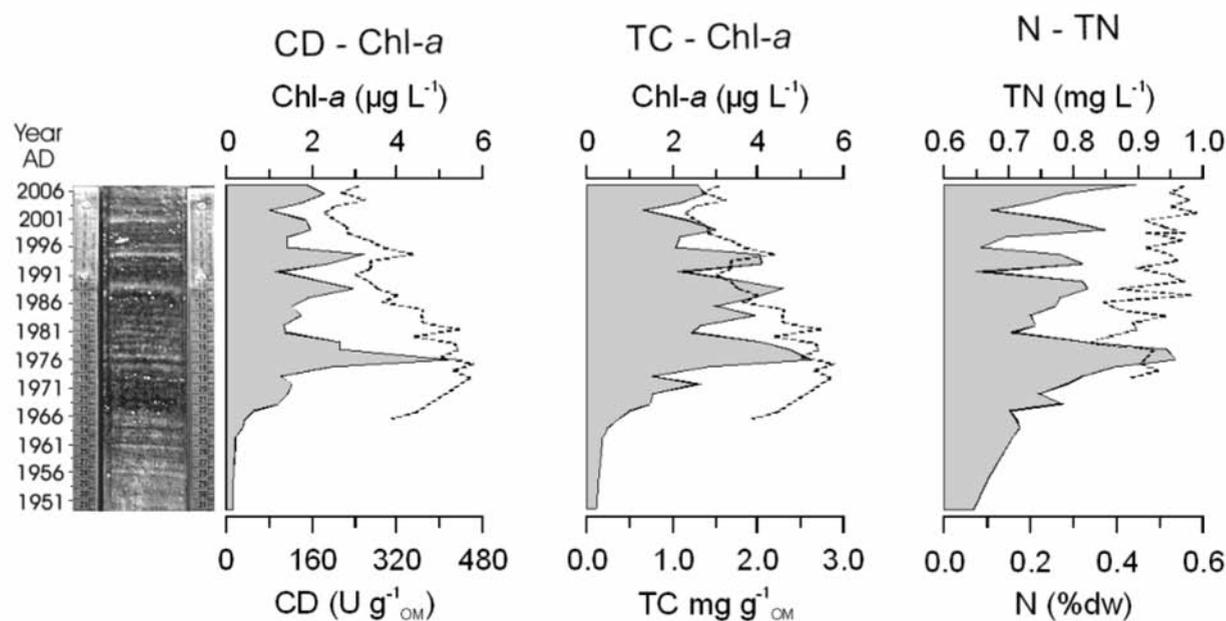


Fig. 7. Comparison between sedimentary total chlorophylls and carotenoids (shade) and long-term data on chlorophyll a in water samples (line) (core LMA06 ex13A/10/2). The comparison between total nitrogen in sediments (shade) and total nitrogen in water samples (whole lake) also is shown.

phyll- α in water samples showed high correlation ($p < 0.01$, Fig. 7). The observed low concentrations (sawlike profiles) coincide with flood events that carried large amounts of detrital materials to the lake and diluted algal material (white layers in the core photo).

From the 1940s to the end of 1950s, the lake was characterized by a phase of low total phosphorus (TP) and high O_2 concentrations (Fig. 8). This was followed by marked eutrophication that peaked in the late 1970s and then an oligotrophication phase. More recently, oligotrophication has been partially reversed by the positive feedback of climate warming on phytoplankton development, in particular its promotion of blooms of cyanobacteria and diatoms (Bertoni et al. 2007; Manca et al. 2007b; Morabito 2007). Total nitrogen concentration started to increase in sediment in the early 1950s. Silica increases occurred first in the mid-1970s and later from 1990 until the youngest core layer (Fig. 7).

Soluble reactive phosphorus (RP) concentrations were less than $10 \mu\text{g L}^{-1}$ before 1960, compared with concentration of up to $30 \mu\text{g L}^{-1}$ at the end of the 70s. These data correlated well with inferred TP (Fig. 8). Since lake restoration through P reduction was initiated in the late 1970s, RP has dropped

rapidly and is presently *ca* $10 \mu\text{g L}^{-1}$ (Fig. 8). TP concentration measured in the euphotic zone (0-20 m) since 1974 shows trajectories similar to those of RP with a recorded concentration maximum of $35 \mu\text{g L}^{-1}$ in 1977-1978 and present values generally between 8 and $10 \mu\text{g L}^{-1}$.

DISCUSSION

During the second half of the 20th Century Lago Maggiore warmed significantly, as did many lakes worldwide. Lago Maggiore is different, however, in that the meteorology of its watershed and many aspects of its limnology have been closely monitored over this roughly 50-year period. Consequently, we have been able to identify relationships between climate warming and the frequency and intensity of storms that carry sediment into the lake, the depth of lake mixing in winter, production of gravity currents and conveyor belt mixing in deep water, biogeochemistry, pollutant activity and changes in biological activity that influence lake productivity and community structure. These changes have been superimposed on the impacts of other anthropogenic stressors, most notably eutrophication, olig-

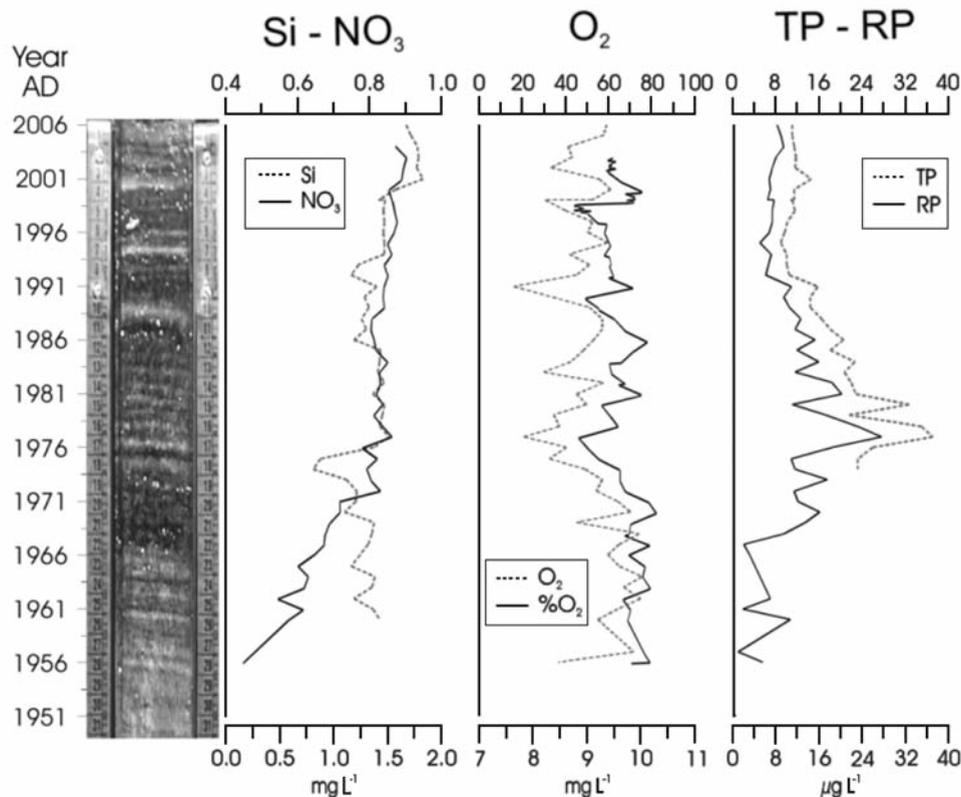


Fig. 8. Long-term data for silica, N-nitrate, dissolved oxygen, total and reactive phosphorus in water samples are plotted against the core LMA06 ex13A/10/2 sedimentary structure. White and black layers correspond to lower and higher P content.

otrophication, DDT, Hg contamination, and fishing practices that have influenced the lake's food web structure. Separating the main effects of climate warming and variables such as nutrient loading from their interactions is a challenge that we just begin to address.

Impacts of climate warming on lake energy budget and mixing dynamics

As a consequence of the climate change currently occurring on this planet, energy exchange between the water masses of large lakes and the overlying atmosphere is different than it once was (Ambrosetti and Barbanti 1999; Livingstone 2003; Glen 2005; Arvola et al. 2010). Modifications are especially marked at middle latitudes such as the Mediterranean region of Europe. The Mediterranean Sea, for example, has experienced a considerable and steady increase in energy content, especially in its deepest layers, over the last 50 years (Bethoux et al. 1990). An ongoing long-term study on deep water warming of Lago Maggiore showed a series of warming and cooling cycles from AD 1887 to present (Carrara et al. 2008). Besides Lago Maggiore, several other deep piedmont lakes in Italy and also north of the Alps (e.g., Lake Geneva; Michalski and Lemmin 1995) are showing increased energy content through time and a trend towards storage of energy at depth due to decreasing frequency of complete water body mixing in winter.

For Lago Maggiore in particular, the temperature of the mixed layer at overturn has increased continuously at a rate of 0.01-0.03°C y⁻¹ (ca 0.8°C increase in the first 10 m) since 1963 (Ambrosetti and Sala 2006; Salmaso et al. 2007). This translates to an annual caloric content increase of 3.942 MJ m⁻² in the whole water mass (Fig. 3). Because temperature influences water density and viscosity, the mixing dynamics of the lake have been altered as well, with consequences for the vertical distribution of oxygen and nutrients. Unexpectedly, we have learned that although mixing through physical convective motion has diminished so that complete homogenization of the water body has become rare, other mechanisms are able to bring oxygen to the deep hypolimnion. These mechanisms are called *hypolimnion erosion* (Ambrosetti et al. 2010). An example is the sinking of cold, oxygenated, highly dense water from mountain tributaries down to the deep hypolimnion (Ambrosetti et al. 1983; Ambrosetti 1990). In addition, we have identified a conveyor belt mechanism that transfers surface water to deepwater layers, radically changing the structure of the hypolimnion (Ambrosetti 1990; Ambrosetti et al. 1979; Ambrosetti and Barbanti 1997).

Due to partial isolation of the hypolimnion from surface processes, seasonal changes in the caloric content of Lago Maggiore's surface waters in recent decades have had little or no influence on waters in the deep hy-

polimnion. Consequently a sort of *climatic memory* reflecting the lake's response to climate forcing over the years between whole lake mixing events has developed. The *climatic memory* facilitates study of relationships between the dynamics of the atmosphere and water mass. In particular, it has recorded increasing variability in energy content due to the longer than seasonal interval between mixing event. The recorded variations are at a similar time scale to the variations in climate that have been recorded. They originate from special hydrometeorological events as well as heat exchange between water masses and are expected to be useful not only in the interpretation of past climate changes but also in the prediction of future developments (Ambrosetti and Barbanti 1999; Ambrosetti et al. 2007; Carrara et al. 2009).

Impacts of climate on floods, erosion and lake sediment deposition

A major change in the limnology of Lago Maggiore during the past 55 years has been elevated sediment accumulation rate. Anthropogenic eutrophication during the '60s and '70s and increased soil erosion through intensified landuse are partly to blame but impacts of climate change on soil erosion rate have been reported throughout Europe (Rose et al. 2011) and likely have been influential in the Lago Maggiore watershed as well. The impact of eutrophication on autochthonous sediment formation is easiest to detect in cores. While layers deposited before mid-20th Century are generally gray and silty, the upper 20 cm of the cores are black and have elevated concentrations of organic matter (LOI), pigments and nutrients (Fig. 4). In general, sediment chemistry profiles for the lake reveal temporal trends similar to those recorded for water chemistry (Figs 7 and 8).

In support of elevated watershed erosion due to land use or climate, we observed a general rise in water column silica concentration over the past two decades, with short-term peaks following extreme precipitation events (G.A Tartari, personal communications; Ambrosetti et al. 2006). Not only has storm frequency and intensity increased coincident with recent climate warming (Tab. 3), but rock weathering is temperature dependent.

Eroded material (driven by heavy precipitation) is transported into the lake and there deposited as detrital layers. In fact, the most distinctive characteristic of the sediments is the presence of whitish detrital laminations that contrast sharply with the otherwise black matrix of the profile's upper 20 cm despite being only millimetres in thickness. These are *turbidites* (Sturm and Matter 1978) deposited during and after short pulses of detrital matter transport triggered by flood events (Mangili et al. 2005). Detrital layers in lake sediment records are valuable proxies for past floods (Kämpf et al. 2011), but detailed microfacies and geochemical data are necessary to distinguish

between major regional floods and local run-off events or slope failures. Based on the available data, layer thickness is not always a direct measure of flood strength. Even the NAO index does not consistently predict flood strength, although the most important floods show a relationship with the winter NAO index. Most significantly correlated with flood strength are precipitation, River Toce discharge and seasonal total inflow ($p < 0.01$, Tab. 2).

Impacts of climate on the middle and upper food web

Some of the ramifications of climate change for Lago Maggiore's biological communities are associated with the increased frequency of severe floods, but the impacts are difficult to quantify. Vollenweider (1956a) in the 1950s and Ambrosetti et al. (1980) in the 1970s made inroads into this task by comparing water chemistry and plankton communities at sites around the lake with different level of water rise. Chlorophyll- α concentration was depressed by as much as 25% in the areas more deeply flooded, probably because both elevated turbidity and increased water depth decreased light availability. Filter feeding zooplankton (*Daphnia hyalina galeata*, *Bosmina coregoni*, *Eudiaptomus padanus* and *Mixodiaptomus laciniatus*) were especially affected, their densities falling over the 10 days they were monitored following flooding. Suspended sediment interferes with food collection by filter feeders (Levine et al. 2005) and thus probably explains this important change in trophic dynamics. Rotifers, which tend to collect particles individually, were less affected.

Other impacts of climate on biological communities are related to the effects of temperature on physiology and thus the metabolism, growth, feeding and life histories of individuals. These impacts can have ripple effects within food webs. For example, de Bernardi and Yøegensen (1998) show that the exergy content of Lago Maggiore's pelagic organisms has fallen with water temperature rise. Distinguishing the impacts of warming from the seemingly larger impacts of eutrophication, oligotrophication and changing levels of fish planktivory is a challenge that we approach primarily through comparison of conditions post-oligotrophication with those before eutrophication occurred (Manca et al. 2007a). Not all variables were adequately studied prior to the 1960s however, so some trend analysis is related to observing post-eutrophication reversals in trends that can be explained only through warming effects. For example, during the lake's mesotrophic phase (1960s and '70s), cladocerans reached peak densities, but had low diversity and the ratio of planktonic/littoral taxa was high (Fig. 6). All three phenomena are common in eutrophic lakes (Jeppesen et al. 2001) and related to the elevated food supply and shrinking of the littoral zone due to reduced transparency. Also distinctive of this period was a large population of *Chy-*

dorus sphaericus, a species unseen in pre-eutrophication samples. The species is normally littoral but is known to attach to filamentous cyanobacteria and thus hitchhike into the pelagic zone (de Bernardi et al. 1990; Manca et al. 1992). Its presence is thus consistent with the records of cyanobacteria blooms during this period. Many biological variables, including the density of cladocerans and ratio of planktonic: littoral species did not respond immediately to phosphorus reduction in the 1970s (Manca et al. 1992), but by 1988 were close enough to initial conditions to look for climatic effects.

Top-down impacts of planktivorous fish on cladocerans is assessed by an established relationship between the zooplankton's population size structure and planktivorous fish abundance. These relationships make it possible to infer the CPUE of planktivorous fish at various times in the past from length measurements made on fossil cladocerans in sediment cores. The resulting data show CPUE peaking strongly in the years immediately after *Coregonus macrophthalmus* introduction to Lago Maggiore in 1949 (Manca et al. 2007a). Fishing records also indicate high growth rates for the stocked fish (Grimaldi 1972). With increased harvesting of the fish, inferred CPUE declined reaching its lowest values between 1973-1982. Especially large size classes of cladocerans species were particularly well represented during this time. When DDT was released into the lake in 1996 forcing a 6-7 year fishing ban (Guzzella et al. 1998), large cladocerans disappeared, indicating an increase in planktivorous fish abundance (Fig. 6, Manca et al. 2007a, b).

Climate warming can affect ecological food chains not only directly by increasing the rates of physiological processes and population growth, but also indirectly by changing habitat use and altering predator-prey interactions. Thus, an additional top-down influence on zooplankton is apparent from increases in *Eubosmina* mucro length and *Daphnia* body size since 1989 (Manca et al. 2007a; Manca et al. 2008): greater grazing pressure from invertebrate predators. *Bythotrephes longimanus* in particular has been abundant during the past two decades (Manca and Ruggiu 1998; Alexander and Hotchkiss 2010). Its mean annual population density increased more than ten fold between 1987 and 1993, a trend correlated with increased values for the North Atlantic Oscillation (NAO) winter index, a measure of winter and spring warmth across Europe (Straile 2002). Since then water temperature has continued to increase and *Bythotrephes* has remained abundant (Manca and DeMott 2009).

Other temperature impacts on zooplankton are apparent in the monitoring and core data for cladocerans. During the warmest year 2003, zooplankton populations, and especially those of *Daphnia*, rose above levels typical of the oligotrophic lake to compare with population abundance during lake mesotrophy. Post-oligotrophication

years before and after 2003 that were significantly cooler had strikingly different zooplankton parameters, those typical of oligotrophy. In general though, the temperature trend has been upward, and cladoceran biomass has followed suit. These observations support the increasingly popular hypothesis that many of the biological impacts of climate warming mimic those of eutrophication (e.g., Schindler 2001, 2009). One reason for greater zooplankton productivity in warm years may be an earlier start to seasonal population growth; laboratory experiments have shown major changes in the reproductive and growth strategies of *Daphnia* along a temperature gradient (Hall and Burns 2002). In addition, the egg ratio of *Daphnia* (E/Nad) was especially high in the warm year of the oligotrophic phase. Normally this variable indicates food availability. Phytoplankton mean annual biomass did not increase in 2003, but the *Daphnia* increase was accompanied by greater water transparency, suggesting that productivity could have increased but been countered by vigorous grazing. Colonial rotifer populations, which had increased as *Daphnia* declined in the early oligotrophication phase, decreased with the 2003 warming event suggesting that they lost their competitive edge (Manca et al. 1994; Manca and Sonvico 1996; Manca et al. 2000; Visconti et al. 2008b).

Where zooplankton response to recent warming differs from its response to eutrophication is in the timing of the annual population maxima; *Daphnia* population densities typically peak in mid-July in Lago Maggiore. There was very little deviation from this pattern over 15 years, but since 1998, the annual peak has shifted to be two months earlier (Manca et al. 2007b; Visconti et al. 2008a). It appears then that a means of distinguishing zooplankton responses related to warming from those due to change in trophic status may be altered population phenology.

Earlier warming and higher average water temperature lead to increasing thermal stability. This results in a longer-lasting dark hypolimnion, in which zooplankton prey may be able to find a refuge from visual predation by fish (Manca et al. 2007b). This change will lead to an earlier increase and longer duration of zooplankton population numerical growth (Manca et al. 2007b). Our findings suggest that the indirect effects of temperature on lake stratification and mixing may influence plankton populations as well. The two most recent highest floods of 1993 and 2000, which occurred after oligotrophication, could be clearly traced in the sediment core profile. In contrast, during peak eutrophication, floods and complete vertical mixing events seem almost undetectable in the sedimentary record. A possible explanation is that eutrophication overruled the lake's natural response to meteorological forcing. That climate signals can be overridden by productivity is becoming clear also from studies

in which palaeo-temperatures are inferred from fossil chironomids (Velle et al. 2005).

In summary, increasing temperature will result in: 1) an earlier onset and a longer duration of the water thermal stratification; 2) an increase in the thickness of the open-water refuge; and 3) an increase in the duration of the presence of the refuge, because of an earlier start time and a later end. These conditions affected the start and duration of the seasonal growth of *Bythotrephes* (phenology) through the release of fish predation pressure (Manca 2011).

Impacts of climate versus phytoplankton and nutrients

Few would contest the assertion that Lago Maggiore's phytoplankton community is sensitive to lake P loading and trophic state. Surveys over the past half-century have consistently shown diatom dominance but the species accounting for most of the biomass have varied from *small Cyclotella* and *Rhizosolenia eriensis*, along with *Fragilaria crotonensis* and *Asterionella formosa* as subdominants in the 1950s (Vollenweider 1956b), to *Tabellaria flocculosa* as a single dominant in the 1960s (Goldman et al. 1968), to *Fragilaria crotonensis* and *Asterionella formosa*, co-dominant in an early 1970s survey. Eutrophic *Stephanodiscus* spp. were present in the latter samples as well. With phosphorus reduction, the phytoplankton replacement series largely reversed, so that *T. flocculosa* reappeared in 1990 and *C. comensis* (and other small centric diatoms) in 1992 up to present (Ruggiu et al. 1998, Fig. 5). Although the trends observed are highly typical of lakes undergoing eutrophication and then allowed to recover, it is not clear that *C. comensis*'s reappearance was totally related to phosphorus removal from sewage. Rühland et al. (2008) report substantial recent increases in *C. comensis* biomass in the palaeolimnological records of over 200 lakes, and argue that climate warming must be a leading cause.

Phosphorus has been identified as the limiting factor for phytoplankton growth in Lago Maggiore. In the early 1960s, the lake's watershed experienced a rapid increase in human population density spurred on by industrial development that increased phosphorus input to the lake dramatically. This led to its rapid transformation from an oligotrophic to a mesoeutrophic water body. Heavy blooms of filamentous cyanobacteria (*Planktothrix rubescens*, mainly, Fig. 5) developed, and a reduction in transparency followed. This sequence is captured in fossil pigment profiles, which show major increases in both the total algae pigment β -carotene and the carotenoid oscillaxanthin, which is largely specific to *P. rubescens* in the lake (Fig. 5). Since 1978-80, P concentrations have declined in Lago Maggiore, partly due to land use change but mostly because the P-content of detergents was reduced and better waste water treatment implemented. In response to P reduction, chlorophyll a concentration has

declined, water transparency has increased and the biodiversity of the phytoplankton community has risen.

As was the case for zooplankton, phytoplankton did not respond immediately to P input reduction. From 1980-1987, epilimnetic phosphorus concentration fell from *ca* 19 to 10-12 $\mu\text{g L}^{-1}$ (Fig. 8), but phytoplankton community structure showed no discernable transformation. This first phase of oligotrophication was followed by a second (1988-1995) characterized by TP stabilization (new steady state concentration about 10 $\mu\text{g L}^{-1}$) and the emergence of biological effects. For phytoplankton, the latter included increased biodiversity, reduced average cell size (mainly due to lower densities of large diatoms and *Planktothrix rubescens*), reduced overall biomass (as represented by biovolume), and a gradual increase in small centric diatoms (Morabito 2007). A third phase is now in play (1995-present), for which TP continues at second-phase levels, but phytoplankton succession has reversed again, so that the re-established oligotrophic species are giving way to meso-eutrophic phase dominants. Most strikingly, non-filamentous cyanobacteria have become problematic again in places (unpublished data).

We believe that the succession underway is related to water temperature elevation and the deepening of the *trophogenic layer* that has resulted (Bertoni et al. 2007). Although data on phytoplankton in Mediterranean lakes remain scarce, the long-term data for Lago Maggiore indicate that the winter NAO index plays a role in the determination of mixing depth and thus in the strength of spring diatoms (Morabito 2007). Similar relationships have been reported for Lake Constance, on the northern side of the Alps (Straile 2000). The Lago Maggiore data also show a strongly negative correlation between spring diatom biomass and summer cyanobacteria biomass, that may be related to the time course of nutrient depletion as well as to the different group requirements for turbulent mixing *versus* quiescent conditions.

Despite phosphorus's return to an earlier level, total nitrogen concentration in Lago Maggiore is at a historic high. This situation, typical of lakes on both sides of the Alps, is related to an increased nitrate burden in atmospheric deposition over the past few decades, as well as reluctance to invest in N reduction when P is viewed as the usual growth limiting nutrient. Although agriculture and livestock breeding contribute some N to the lake, they are very minor N sources compared with atmospheric deposition in this region (Salmaso et al. 2007). Deposition of N at levels considered above critical for alpine and subalpine areas may encourage further eutrophication in lakes where enough excess P is present for phytoplankton to have grown N limited (Rogora et al. 2006). It has been finally shown that amount of nitrogen deposition is related to amount of precipitation (Salmaso et al. 2007).

Climate change and toxic pollutants

Most apparent of the impacts of climate on toxic substances bioavailability in the lake have been those related to the increase of storms and flooding events. A study of DDT dissipation in Lago Maggiore following the industrial spill discovered dramatic increases in concentration during heavy storms due to scouring at the local point source (Guzzella et al. 1998, 2007). POPs and trace metals in the lake are likely affected by similar hydrodynamic events. Temperature probably has impacts on the concentrations of these pollutants as well given its impact on solubility. Moreover, it has been shown that mercury methylation is directly temperature dependent (Hintelmann et al. 1995), while conversion of methyl mercury to elemental mercury is promoted by UV radiation and hindered by DOC (France et al. 2000). In the absence of trophic state response, lakes tend to become increasingly DOC-poor, and thus more transparent, as temperature rises. Overall then, there are many reasons to expect toxic contaminants to be more problematic in the warmer lake of the future.

In addition to climate warming effects due to changes in precipitation patterns, warming temperature may influence pollutant transport to the lake. Re-emission of volatile organic compounds (*e.g.*, PCBs, DDT, HCH, etc.) and metals from contaminated areas of the Lago Maggiore watershed back to the atmosphere has been documented, and shown to be positively related to environment temperatures (Grimalt et al. 2001; Camarero et al. 2009). The volatilized contaminants often are redeposited at cooler sites in the Alps. In fact, snowpacks and glaciers may be important sinks for semivolatile pollutants produced throughout Europe (Kallenborn 2005). Melting of this ice may bring POPs into Lago Maggiore and other lakes. This mechanism, particularly important in other subalpine lakes (*e.g.*, Iseo) has been suggested as the reason for a recent large increase in the DDT content of zebra mussel (*Dreissena polymorpha*) tissue in the lake (Bettinetti et al. 2008) and sediments (Bettinetti et al. 2011).

Need for integration of neo- and palaeolimnological approaches

Climate change, like altered nutrient inputs and fish community manipulation, impacts lakes at multiple levels of organization with ripple effects that proceed at different rates. Traditional limnology, with its point sampling at discrete times, is inappropriate for detection of many of the processes involved, although it offers the opportunity for experimentation and in-depth study of rapid responses. To detect longer-term responses, especially those at a decadal or centennial scale, limnologists need to borrow tools from palaeolimnology. Sediment cores provide a continuous record of community and environmental change that

extends back before the careers of the scientists involved, and that offers the advantage of natural temporal integration of variable values through choice of section thickness. Although Lago Maggiore has an exceptionally long monitoring history, interpretation of the data gathered has been aided tremendously by palaeolimnological analyses. These have both filled in gaps in the sampling record and provided data from before establishment of the Institute of Pallanza. Where there has been overlap in neo- and palaeolimnological data, we normally have found similar trends (*e.g.*, the Cladocera profiles, Fig. 6).

In particular, pigments, subfossil diatoms and Cladocera have successfully inferred lake eutrophication and recovery, and food web alteration due to fluctuations in the strength of fish and pelagic invertebrate predator populations (Marchetto et al. 2004; Manca et al. 2007a). Occasional discrepancies between the environmental conditions inferred from fossil remains and those actually recorded, we attribute to methodological problems (sampling and analyses), but believe could be related as well to recent lake water temperature increases that influence the appropriateness of the transfer functions developed from recent training sets.

Human impacts *versus* climate influence on Lago Maggiore: final remarks

With the time-series data presented above, we have tried to demonstrate that climate as well as nutrient inputs can regulate the lake's food web, although the changes have been at a longer temporal scale. It is clear that today's oligotrophic lake has biological and physical attributes that differ from those of the pre-eutrophication phase, and that continue to evolve. The studies discussed above also provide examples of how climate warming can indirectly affect the population density and phenology of a key invertebrate predator, and hence, the functioning of the pelagic food web.

In several lakes (Schindler 2001; Battarbee et al. 2008), including Lago Maggiore, there is evidence of ecological response to both nutrients and climate. Bottom-up forces drive both community and population responses to eutrophication and eutrophication-like responses to warm years. Changes in abundance and community structure observed with nutrient enrichment appeared fully reversible, although with a delay related to the resilience of the planktonic system (Chorus et al. 2010). Following oligotrophication, a larger interannual variability in population density and biomass of zoo-plankton resulted that appeared to be related to meteorological conditions (Visconti et al. 2008a). This supported the hypothesis that climatic signals are more evident in oligotrophic environments. The more pronounced impact of exceptional meteorological events results in a larger instability of the system (Manca and DeMott 2009). The simultane-

ous impacts of warming and changes in nutrient were distinguishable when seasonal data from contemporary records was analyzed alongside data on changing thermal stratification regime.

Changes in top-down forces (*e.g.*, fish or invertebrate predation) appear to lead to non-reversible changes in Cladocera community structure. Non-reversibility may in fact be related to indirect effects, such as eco-system-mediated responses to warming (Visconti et al. 2008b; Manca and DeMott 2009). Because several factors other than climate change (eutrophication, grazing pressure, fish introduction, fishing ban) have impacted zooplankton, further research and advanced statistical tools are needed to determine the main and interactive effects of each (Adrian et al. 2009).

From these limnological and palaeolimnological studies on Lago Maggiore we may expect that climate change is likely to have a confounding effect on the lake's full recovery from eutrophication. The lake's response to nutrient reduction under the new weather conditions (*e.g.*, warmer and wetter) may be slower than originally envisaged (Battarbee et al. 2008; Chorus et al. 2010). Milder winters (Ambrosetti et al. 2006; Brunetti et al. 2006) have reduced the depth of spring overturn and decreased the frequency of a full homogenization of the water column. This trend may result in oxygen depletion of the deepest water, which would have serious consequences for water chemistry and biology (Mosello et al. 2011). In other words, it seems that the effects of further warming would be similar to those of eutrophication, with a cascading effect over the whole food web (Manca et al. 2007b; Manca and DeMott 2009).

The results summarized here have shown the potential of Lago Maggiore sediments to function as a high-resolution archive of past environments that will enable detailed reconstruction of earlier lake responses to anthropogenic and natural disturbances. Finally, this study illustrates the complexity of ecosystem response to synchronous changes in climate and stresses, anthropogenic or natural, and the difficulty of uncoupling their main effects and many interactions, especially for lakes within populous watersheds (Williamson et al. 2009; Smol 2010).

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