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Fiorenza G. Margaritora<sup>a</sup>; Emanuela Cherubini<sup>a</sup>; Diego Copetti<sup>b</sup>; Elena Legnani<sup>b</sup>; Marco Seminara<sup>a</sup>; Gianni Tartari<sup>b</sup>; Daria Vagaggini<sup>a</sup>

<sup>a</sup> Department of Animal and Human Biology, University 'La Sapienza', V.le dell'Università, Rome <sup>b</sup> IRSA-CNR Brugherio, Monza

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## Recent trophic changes in Lake Pusiano (northern Italy) with particular reference to the influence of hydrodynamics on the zooplankton community

FIORENZA G. MARGARITORA\*†, EMANUELA CHERUBINI†, DIEGO COPETTI‡, ELENA LEGNANI‡, MARCO SEMINARA†, GIANNI TARTARI‡ and DARIA VAGAGGINI†

†Department of Animal and Human Biology, University 'La Sapienza', V.le dell'Università, 32 00185, Rome

‡IRSA-CNR Brugherio, Monza

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Lake Pusiano (northern Italy) has been subjected for more than 20 yr to increasing eutrophication which caused hypereutrophic conditions in the mid-1980s. Due to the introduction of a sewage-treatment network in 1986, the total P concentration in the lake water dropped from  $185 \mu\text{g l}^{-1}$  in 1985 to  $74 \mu\text{g l}^{-1}$  in 2003. Although there was a reduction in nutrient load, cyanobacteria blooms were observed from 1994 onwards. The aim of this study was to compare physical and chemical parameters and the zooplankton community between two investigation periods (February–December 2002 and January 2003–February 2004) that were separated by an extreme flood event in November 2001 affecting the total plankton community. From the analysis of the data sampled in 2002–2003, an increase in transparency (from 3.5 m to 5.7 m) and a decrease in chlorophyll *a* concentration (from  $6 \mu\text{g l}^{-1}$  to  $1.5 \mu\text{g l}^{-1}$ ), both measured during the circulation period (December–February), were recorded. Within the phytoplankton, a shift from cyanobacteria to chlorophytes was detected. The zooplankton community changed in the composition of species, that is a higher number of species known to be less tolerant to toxic cyanobacteria were observed. The results emphasise that the flood event at the end of 2002 accelerated the process of reoligotrophication in the lake and contributed to an increased diversification of the zooplankton community. The generally increasing frequency of occurrence of flooding events following heavy rainfalls might be considered a major factor in regulating plankton community composition in the future.

*Keywords:* Zooplankton; Trophic changes; Flood impact

### 1. Introduction

Lake Pusiano (Italy:  $45^{\circ} 48' 08''$  N;  $09^{\circ} 16' 3''$  E) is the largest lake among the Brianza Lakes (surface area  $5.3 \text{ km}^2$ , maximum depth 24 m) and is situated between the two southern arms of Lake Como (figure 1). For more than 30 yr, the lake constituted the object of several limnological studies [1–8]. In 1954, the lake showed the first symptoms of eutrophication derived

\*Corresponding author. Email: fiorenza.margaritora@uniroma1.it

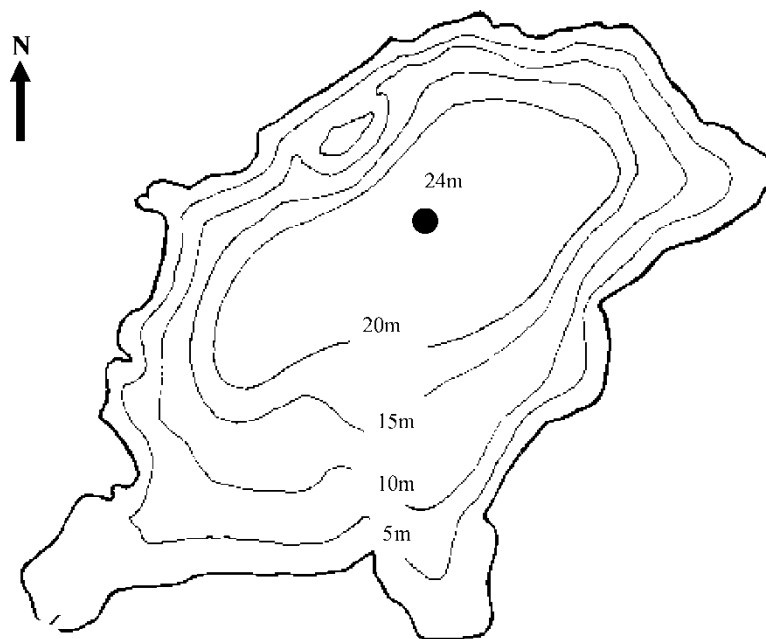


Figure 1. Sketch map of Lake Pusiano. The black dot indicates the centre-lake sampling station.

from organic and industrial pollution, with a hypolimnetic oxygen deficit and oversaturated surface waters [1]. Since 1972 [2], the lake entered a phase of hypereutrophy with total hypolimnetic anoxia and hydrogen sulphide produced at the sediment level. In the 1970s and 1980s, the construction of waste-water treatment plants led to a progressive reduction in total phosphorus concentrations (TP) in the lake waters, down to a value three times smaller in 2002 than in 1985 [9]. In the same period, starting from 1994, blooms of toxic cyanobacteria were observed [10]. In the above-mentioned historical phase, the zooplankton also showed changes, mostly in qualitative composition, with an apparent reduction in species number. These biocoenoses typically react to the alterations of the environment and, by this reaction, indicate lake stress and water-quality deterioration [11–13]. Adequate knowledge of the changes in the zooplankton community composition over time provides a valid tool for predictive managing and recovery interventions.

In this regard, we carried out the present research, from March 2002 to January 2004, in the course of a wider intensive monitoring program on hydrochemical parameters supported and developed by IRSA-CNR of Brugherio (MI) and the Centre for Water Research of Perth (Australia). In November and December 2002, at the end of the first year of the study, a huge inundation caused a volume of water renewal equivalent to the whole metalimnetic layer [9], reducing the theoretical water renewal time to one-tenth. This phenomenon produced significant changes in the lake, and we consider it to be a true disturbance creating two different phases, allowing zooplankton reactions to be observed in the short term.

## 2. Materials and methods

The present study is based on zooplankton samples collected at least monthly from March 2002 to January 2004. Because of the flood occurring in November–December 2002, we

have divided the investigation period into two parts (pre-flood and post-flood, indicated in the graphs and from here onwards as the first and second period), lasting from March 2002 to December 2002 and from January 2003 to January 2004, respectively. The samples collected on a monthly basis were intensified during the periods with algal blooms (April, May, and June 2002). Sampling was carried out in a centre-lake station, corresponding to the point of maximum depth of the lake, using a 74  $\mu\text{m}$  plankton net. Vertical hauls were collected from a depth of 20 m to the surface, and we collected further samples from the epilimnion and from the depth corresponding to the border of the lower metalimnion during the stratification period.

Samples were pooled and preserved in 5% formalin and then poured inside Imhoff sedimentation cones of 1000  $\text{cm}^3$  volume. Aliquots of sedimented zooplankton were resuspended in 150  $\text{cm}^3$  ( $>5 \text{ cm}^3$  biovolume) or 100  $\text{cm}^3$  ( $<5 \text{ cm}^3$  biovolume) of water. Diluted samples were sorted in seven representative subsamples of 1  $\text{cm}^3$  volume in the laboratory, and the organisms counted and identified under an Utermöhl inverted microscope to calculate densities as described previously [14, 15]. For each taxon, we recorded the developmental stages (nauplii, copepodites or juveniles, and adults) and all taxa were identified down to the lowest possible taxonomic level according to previous studies [16–19].

To characterize the community's species composition, we used the evenness index [20] and the Shannon–Weaver diversity index [21]. A multiparametric probe (Idronaut Ocean Seven Mod 401, lake version) was used to determine pH, temperature, dissolved oxygen, and chlorophyll *a* concentrations at short depth intervals across the water column.

Transparency was measured with a Secchi disk. A summary of chemical methods applied during the study is reported in [10].

### 3. Results

#### 3.1 Physical and chemical data

Lake Pusiano is a warm monomictic lake, with stable thermal stratification lasting from April to November, a surface temperature from 20 to 25 °C (maximum in August), and a winter homothermal temperature of 5–7 °C.

The mean annual transparency (figure 2) in the first period was 3.3 m, and in the second period this parameter increased to 4.5 m, with a peak of 9.5 m during the circulation (February 2004).

The highest pH (8.91) was measured during the summer algal blooms (June–July 2002) in the epi- and metalimnion, as a result of the intense photosynthetic activity, while during

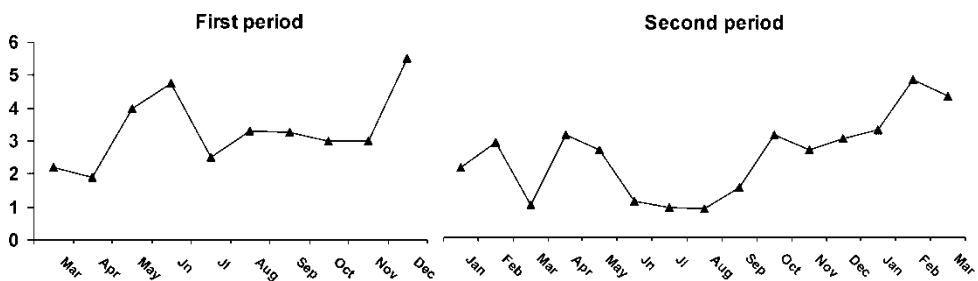


Figure 2. Graphs of transparency for Lake Pusiano during the two study periods.

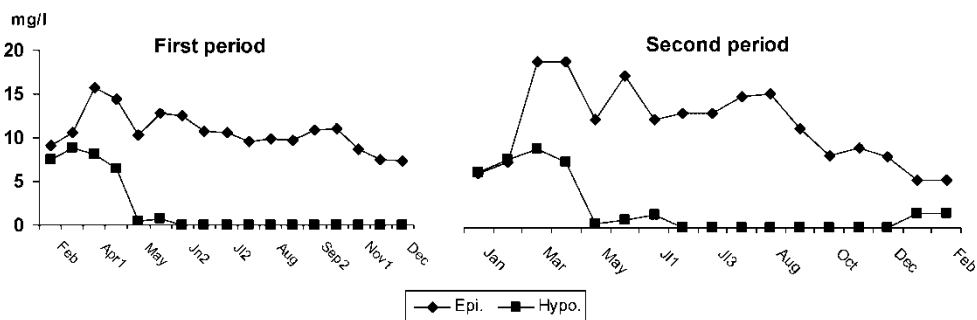


Figure 3. Graphs of dissolved epilimnetic and hypolimnetic oxygen concentrations in Lake Pusiano during the two study periods.

circulation (December–February) a pH of 8.00 was recorded, as a consequence of the high buffer capacity due to alkalinity values ( $2\text{--}3\text{ meq l}^{-1}$ ).

From April to November 2002, the dissolved oxygen concentration showed epilimnetic maxima (often with supersaturation) coinciding with peaks in the summer algal productivity; during the same period, the hypolimnion was anoxic. The dissolved oxygen concentration in the hypolimnion showed lower values in the second homothermal period with respect to the first, with higher values in February 2002 ( $7.50\text{ mg l}^{-1}$ ) than in February 2003 ( $5.97\text{ mg l}^{-1}$ ), and even almost a total deficit on the bottom in February 2004 ( $1.20\text{ mg l}^{-1}$ ; figure 3).

The TP concentration (figure 4) in the water column showed its maximum values on the lake bottom during winter (December 2002:  $479\text{ }\mu\text{g l}^{-1}$ ; December 2003:  $569\text{ }\mu\text{g l}^{-1}$ ), while the minima occurred during summer and autumnal months within the surface layers (late July 2002:  $7\text{ }\mu\text{g l}^{-1}$ ; November 2003:  $9\text{ }\mu\text{g l}^{-1}$ ).

Total nitrogen (TN, figure 5) reached peak values of  $4\text{ mg l}^{-1}$  in both periods before late winter overturn at a depth close to the bottom, whereas the lowest values were reached within the surface layers during the 2003 summer period around  $0.5\text{ mg l}^{-1}$ .

The seasonal variation of chlorophyll *a* concentrations was characterized by an intense spring bloom during both study periods (figure 6). During the first period, the phytoplankton population was dominated by cyanobacteria. The minimum of chlorophyll *a* occurred in the flood months, and after this event cyanobacteria severely decreased in abundance, and chlorophyta increased [8] without any significant difference in chlorophyll *a* concentration.

### 3.2 Zooplankton

During the first period, the mean zooplankton total density was  $83\ 148\text{ ind m}^{-3}$ , while in the second period, this increased to  $382\ 771\text{ ind m}^{-3}$ . In the first period, the community mainly

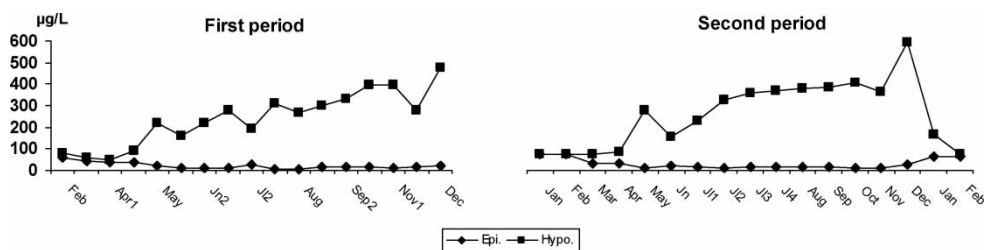


Figure 4. Graphs of total epilimnetic and hypolimnetic phosphorus concentrations in Lake Pusiano during the two study periods.

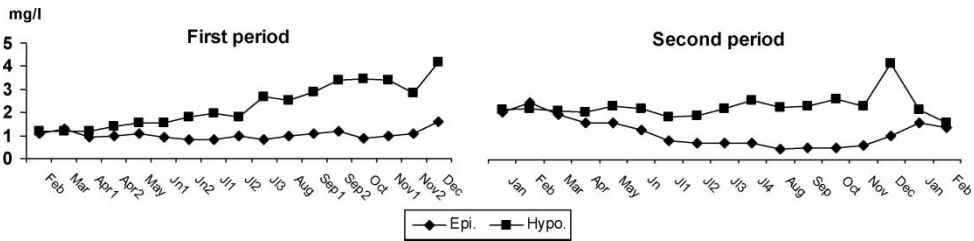


Figure 5. Graphs of total epilimnetic and hypolimnetic nitrogen concentrations in Lake Pusiano during the two study periods.

comprised rotifers, with mean densities of  $70\,876\text{ ind m}^{-3}$  (75% of the whole assemblage), followed by copepods with  $18\,800\text{ ind m}^{-3}$  (19.9%) and cladocerans with  $4535\text{ ind m}^{-3}$  (4.8%). In the second period, there was a diminishing mean density of rotifers, dropping to  $45\,948\text{ ind m}^{-3}$  (59%), while copepods increased to  $25\,478\text{ ind m}^{-3}$  (33%). Cladocerans also increased but to a lesser extent, with  $5552\text{ ind m}^{-3}$  (7.2%, figure 7). Among rotifers, after the flood we recorded a decrease in the mean densities of *Kellicottia longispina* (from  $23\,012$  to  $3673\text{ ind m}^{-3}$ ), an increase in *Conochilus unicornis* (from  $3801$  to  $10\,674\text{ ind m}^{-3}$ ), and the appearance of two new species, albeit in limited numbers: *Trichocerca chattoni* and *Ascomorpha saltans* (figure 8).

Among copepods, *Thermocyclops hyalinus* and *Mesocyclops leukartii* reached density peaks in spring months ( $11\,542\text{ ind m}^{-3}$  and  $5603\text{ ind m}^{-3}$  in April 2003, respectively) and outnumbered the other species all summer long. Conversely, in winter *Cyclops abyssorum* reached its peak with  $3370\text{ ind m}^{-3}$  (February 2002) and  $2977\text{ ind m}^{-3}$  (January 2004). The diaptomid *Eudiaptomus gracilis* was less abundant but regularly detected and reached peak densities in spring months (maxima of  $2153\text{ ind m}^{-3}$  in April 2002 and  $2907\text{ ind m}^{-3}$  in April 2003; figure 9).

The dominant taxa among cladocerans exhibited the highest numbers in spring: *Daphnia hyalina* ( $21\,021\text{ ind m}^{-3}$  and  $18\,788\text{ ind m}^{-3}$  in April 2002 and 2003, respectively) and *Bosmina longirostris*, which, unlike the first, showed a marked density decrease in the months following the flood (from  $13\,017\text{ ind m}^{-3}$  in April 2002 to  $2612\text{ ind m}^{-3}$  in April 2003). After this event, two new species were found: *Ceriodaphnia quadrangula hamata* (maximum density  $800\text{ ind m}^{-3}$  in April 2003) and *Chydorus sphaericus* ( $93\text{ ind m}^{-3}$  in October 2003), while *Diaphanosoma brachyurum* increased remarkably in density (from  $843\text{ ind m}^{-3}$  in September 2002 to  $7376\text{ ind m}^{-3}$  in August 2003; figure 10). An increased percentage of juveniles on the total of cladocerans was observed in the second period (figure 11). The most important invertebrate predators in the zooplankton community were represented by the cladoceran *Leptodora*

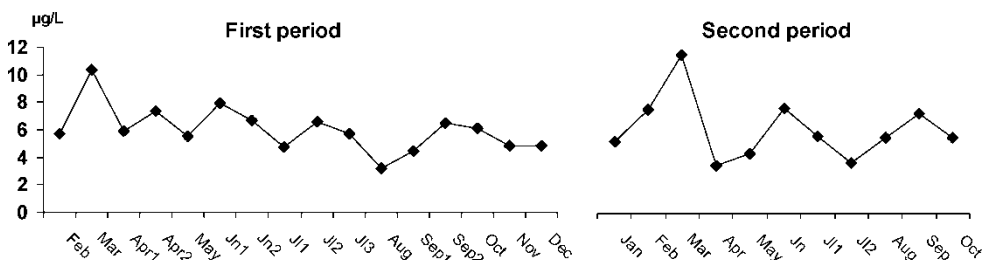


Figure 6. Graphs of chlorophyll *a* concentrations in Lake Pusiano during the two study periods. Values are expressed as integrated samples along the water column.

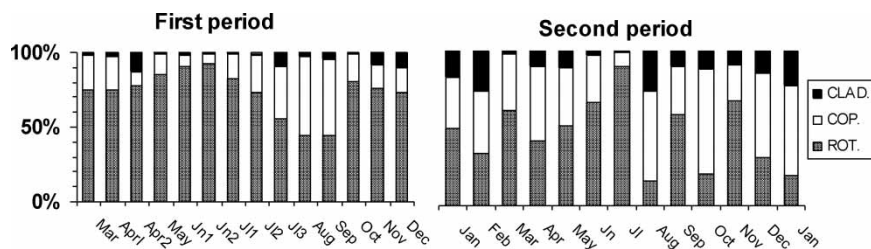


Figure 7. Percentages of the three main zooplanktonic groups in Lake Pusiano during the two study periods.

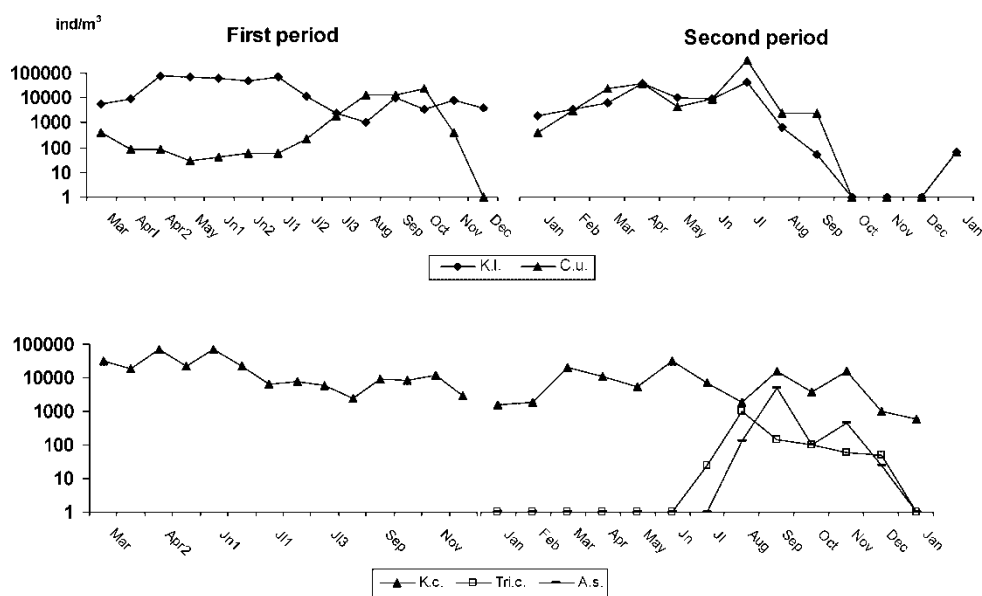


Figure 8. Graphs of densities of the most relevant rotifer taxa in Lake Pusiano during the two study periods. For abbreviations, see table 1. Values are expressed in logarithmic scale.

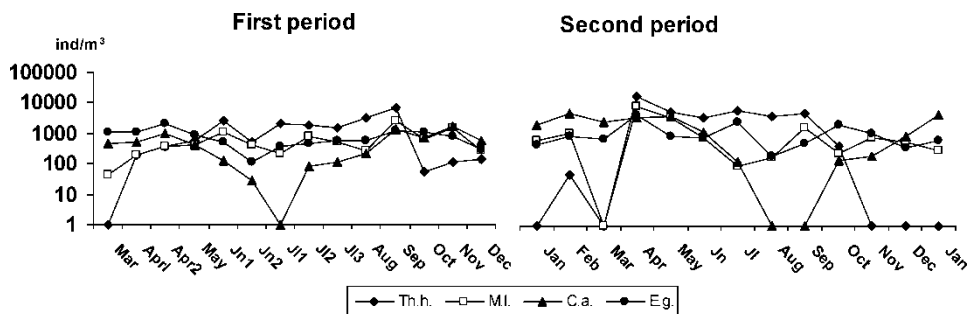


Figure 9. Graphs of densities of the most relevant copepod taxa in Lake Pusiano during the two study periods. For abbreviations, see table 1. Values are expressed in logarithmic scale.

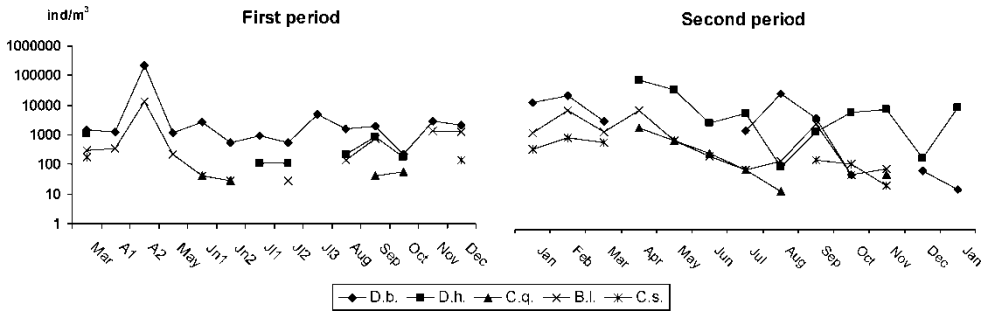


Figure 10. Graphs of densities of the most relevant cladoceran taxa in Lake Pusiano during the two study periods. For abbreviations, see table 1. Values are expressed in logarithmic scale.

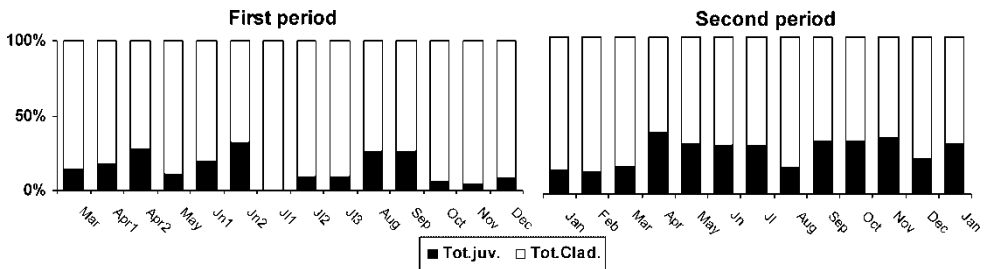


Figure 11. Percentage of juveniles (black bars) and adults (white bars) of cladocerans in Lake Pusiano during the two study periods.

*kindtii* and the phantom midge larva *Chaoborus flavicans*, a benthic organism with a wide migrating ability into open and epilimnetic waters (figure 12). In the first period, *Chaoborus flavicans* showed maximum densities in the upper layer (0–10 m depth, 684 ind m<sup>-3</sup>) during summer, while in the second period it was practically absent from surface layers and was mainly collected during the fall and at greater depths (239 ind m<sup>-3</sup>). On the contrary, *Leptodora kindtii* increased its mean density in the second period, showing a peak density of 749 ind m<sup>-3</sup> in July 2003.

The species richness of zooplankton community (table 1) increased in the second period with respect to the first (from 17 to 21 species), because of the occurrence of two new rotifer

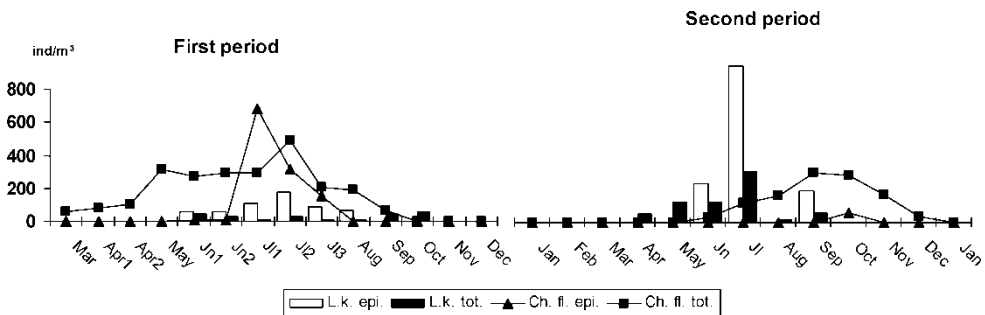


Figure 12. Combined graphs and histograms illustrating the occurrence of the main invertebrate predators in Lake Pusiano during the two study periods in vertical samples of different extension (epi.: epilimnion; tot.: the entire water column).



Table 1. Synopsis of past and present lists of zooplanktonic taxa from Lake Pusiano.

References Years of study	Keys	1 1954	1 1967	2 1977	3 1988/89	Present study 2002	Present study 2003/04
<b>Rotatoria</b>	ROT.						
<i>Asplanchna priodonta</i> Gosse	–	•	•	•	•	•	•
<i>Keratella cochlearis</i> Ruttner-Kolisko	K.c.	•	•	•	•	•	•
<i>K. c. f. hispida</i> (Lauterborn)	–	•	•				
<i>K. c. f. tecta</i> (Lauterborn)	–	•	•				
<i>K. quadrata</i> (O.F.M.)	–	•	•	•	•	•	•
<i>Kellicottia longispina</i> (Kellicott)	K.l.	•	•	•	•	•	•
<i>Brachionus angularis</i> Gosse	–		•				
<i>Brachionus</i> spp.	–		•	•	•		
<i>Anuraeopsis fissa</i> (Gosse)	–	•	•				
<i>Trichocerca birostris</i> (Minkiewicz)	–	•					
<i>T. capucina</i> Wierzejski & Zackarias	–	•	•		•		
<i>T. cylindrica</i> (Imhof)	–		•				
<i>T. chattoni</i> (De Beauchamp)	Tri.c.						•
<i>Trichocerca</i> spp.	–			•			
<i>Ascomorpha saltans</i> Bartsch	A.s.						•
<i>A. ovalis</i> (Bergendahl)	–	•	•	•			
<i>Gastropus hyptopus</i> (Ehrenberg)	–	•					
<i>Polyarthra euryptera</i> (Wierzejski)	–	•	•				
<i>P. vulgaris-dolichoptera</i> Ruttner-Kolisko	–	•	•			•	•
<i>Polyarthra</i> sp.	–			•	•		
<i>Synchaeta pectinata</i> Ehrenberg	–	•	•	•	•	•	•
<i>Filinia terminalis</i> (Plate)	–	•	•	•	•	•	•
<i>Pompholix complanata</i> Gosse	–	•			•		
<i>Conochilus unicornis</i> (Rousselet)	C.u.	•		•	•	•	•
<i>Collotheca</i> sp.	–		•				
<b>Cladocera</b>	CLAD.						
<i>Diaphanosoma brachyurum</i> (Liévin)	D.b.	•	•	•	•	•	•
<i>Daphnia hyalina</i> Leydig	D.h.	•	•	•	•	•	•
<i>Ceriodaphnia pulchella</i> Sars	–	•	•	•			
<i>C. quadrangula hamata</i> (O.F.M.)	C.q.						•
<i>Eubosmina longispina</i> (Leydig)	–			•			
<i>Bosmina longirostris</i> (O.F.M.)	B.l.	•	•	•	•	•	•
<i>Scapholeberis mucronata</i> (O.F.M.)	–		•				
<i>Leptodora kindtii</i> (Focke)	L.k.		•	•	•	•	•
<i>Chydorus sphaericus</i> (O.F.M.)	C.s.						•
<b>Copepoda</b>	Cop.						
<i>Mesocyclops leuckarti</i> (Klaus)	M.l.		•	•	•	•	•
<i>Cyclops abyssorum</i> Sars	C.a.	•	•	•	•	•	•
<i>C. vicinus</i> Ulianine	–				•		
<i>Thermocyclops hyalinus</i> (Fischer)	Th.h.	•	•	•	•	•	•
<i>Eudiaptomus gracilis</i> (Kiefer)	E.g.					•	•
<b>Diptera</b>	–						
<i>Chaoborus flavicans</i> (Meigen)	Cha.f.	•	•	•	•	•	•
<b>Total number of taxa</b>		24	27	21	20	17	21

Note: Numbers indicate references as follows: 1 = Bonomi *et al.* [1]; 2 = De Bernardi *et al.* [3]; 3 = Chiaudani and Premazzi [5].

and two cladoceran species. Reasonably high evenness values both before and after the flood (0.71 and 0.74, respectively) indicated the absence of a distinct dominance in the community. Evenness was related to the Shannon diversity (figure 13), which remained high during the whole study period (means of 2.8 and 2.77 in the first and in the second period, respectively). The variability in Shannon diversity increased during the second study period, *i.e.* the diversity value decreased from 3.3 in April 2003 to 2.0 in August 2003.

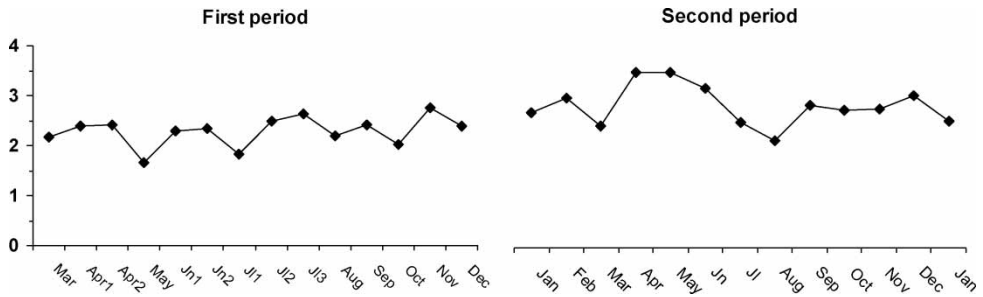


Figure 13. Graphs of the Shannon diversity index in Lake Pusiano during the two study periods.

#### 4. Discussion and conclusions

Interesting changes were observed in Lake Pusiano when comparing this study with previous studies and the pre-flood with the post-flood period. The increase in transparency during the second study period is probably a result of the sewage treatment started in the middle of 1980s, and has been favoured by the washout effect of the flood of November 2002. Coinciding with sewage treatment, TP concentrations have been reduced during the last decade [6], but phytoplankton have not changed accordingly, i.e. cyanobacterial dominance was continually observed during the 1990s. This phenomenon has been observed in lakes that have been dominated by cyanobacteria, particularly *Planktothrix rubescens* [22, 23]. In this study, the process of reoligotrophication has been favoured by washout, decreasing cyanobacteria that dominated during the first period (*P. rubescens* is typically a slow-growing species that cannot exist in systems with a short water-renewal time), and chlorophytes increased during the second period after the washout [10]. Notably, the total phytoplankton biovolume as indicated by chlorophyll *a* was not altered but remained constant during the entire study period.

In spite of the above reported shift inside the phytoplankton assemblages, the lake seemed to be characterized by similar trophic conditions, the annual average chlorophyll *a* concentrations being  $6.03 \mu\text{g l}^{-1}$  in 2002 and  $6.12 \mu\text{g l}^{-1}$  in 2003.

The changes in phytoplankton composition probably contributed to the observed changes in the zooplankton. From the 1950s and 1960s onwards (table 1), a reduction in species richness was observed mainly due to the vanishing of rotifers and cladoceran species coinciding with a general increase in trophic conditions. In this study, the number of zooplankton species increased following the flooding during the second period due to the appearance of taxa known to occur under more oligotrophic conditions: *Kellicottia longispina* and *Ceriodaphnia quadrangula hamata* [24]. *C. quadrangula hamata* has been shown to be linked to a chlorophyte-dominated phytoplankton and to a microparticle-sized seston [25, 26]. In contrast, *C. pulchella*, was observed to occur at higher trophic levels [18]. The first record of *Eudiaptomus gracilis*, a species quite rare in Italy, corresponds to this recent oligotrophication phases of Lake Pusiano [27]. In addition, physiological and behavioural adaptations to the toxic cyanobacterium *Planktothrix rubescens* may have contributed to the coexistence of *E. gracilis*, *Daphnia hyalina*, and *Cyclops abyssorum*. Some authors have reported the ability of *Eudiaptomus gracilis* to selectively exclude *P. rubescens* from food, and for *Daphnia hyalina* and *Cyclops abyssorum* a tolerance to toxic microcystin [28, 29].

Following the flood event, a decrease in rotifers and an increase in cladocerans was observed. In general, rotifers are favoured over cladocerans in lotic systems because of their shorter generation times [30]. On the other hand, rotifers are generally favoured by the dominance of cyanobacteria, which may explain the decrease in rotifers numbers as observed in this

study [31]. The density of *K. longispina*, very abundant in the first period and with respect to the previous surveys, showed a drastic decrease in the second period, perhaps connected to the increase in allochthonous organic charge, but surely driven also by the above-mentioned washout phenomenon. Conversely, the washout seems likely to have been the first factor driving the increase in *C. unicornis*, as this taxon is a well-known colloidal suspension, bacterium, and seston feeder [16].

The most significant variations induced by the flood were observed among cladocerans. The reproduction rate of *Daphnia hyalina* and *Bosmina longirostris* increased due to improved food conditions, as shown by the increased proportion of juveniles with respect to adult cladocerans. In addition, the increase in individual numbers of *Diaphanosoma brachyurum* and the appearance of *C. quadrangula hamata* and *C. sphaericus* contributed to the highest species richness observed during the second study period.

Among the invertebrate predators, *Chaoborus flavicans* dominated over *Leptodora kindti* during the first study period. The abundance of *Chaoborus* in the epilimnetic layers might have induced a partial food overlap of both invertebrate predators resulting in competitive repression and possibly predation of juveniles *Leptodora* by *Chaoborus*. The increased transparency after the flood might have forced *Chaoborus* down to deeper layers due to the predation by visual predators, resulting in decreased competition between *Leptodora* and *Chaoborus* and higher *Leptodora* individual numbers.

The tendency of species richness to increase with reoligotrophication is in accordance with observations on other lakes of different origins undergoing a slow improvement process after the reduction in external nutrient loading (*i.e.* the volcanic Lake Nemi [32]). This is in line with ecological theory [33].

In conclusion, although Lake Pusiano is still considered to be eutrophic, the increase in transparency and the shift from a cyanobacteria-dominated phytoplankton to a chlorophyta-dominated phytoplankton contributed to an increased diversification of the zooplankton community, resembling less eutrophic conditions. The flood event at the end of 2002 accelerated this process of general reoligotrophication, and this observation contributes to our understanding of the response of plankton communities under changing environmental conditions.

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