Ann. Limnol. - Int. J. Lim. 50 (2014) 241–247
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DOI: 10.1051/limn/2014017

Available online at:
www.limnology-journal.org

Seasonal evolution of the zooplankton community in two riverine wetlands of the Ticino River (Lombardy, Northern Italy)

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Received 24 September 2013; Accepted 25 June 2014

Abstract – Riverine wetlands are vulnerable ecotonal environments and are often threatened by human activities. A project supported by the Cariplo Foundation, called "Ecological and hydromorphological requalification of wetlands in the Ticino area around Pavia" (South-East Lombardy, Northern Italy), was started in 2010 with the aim of improving the chemical, bio-ecological and hydrological conditions of these wetlands. In line with the aim of this project, we selected two wetlands near the main course of the Ticino River for investigation, one of which is impacted by a combined sewer overflow from the sewerage networks which is always active (San Lanfranco (SL) wetland), whereas the other (Topo (TO) oxbow lake) is less directly impacted by anthropogenic activities. In this paper, we report the ecological situation of these two wetlands before the requalification process by investigating the way the zooplankton assemblages, considered as bio-indicators, respond to anthropogenic pressure. Overall, we collected 19 *taxa*: 4 cladocerans, 1 copepod and 14 rotifers. In the TO oxbow lake, we found a stable community, dominated by the cladoceran *Bosmina longirostris*. By comparison, in the SL wetland we found a pioneer community, dominated by the juvenile stage of the copepod *Thermocyclops dybowskii*. The SL wetland was shown to be most urgently in need of improvement and is therefore first in line for requalification, whereas the TO oxbow lake needs less drastic measures to ensure the long-term ecological functioning of the aquatic environment. After these requalification activities, a systematic zooplankton survey will be carried out to monitor the evolution of the riverine wetlands.

Key words: Microcrustaceans / ponds / trophy / anthropogenic pollution / habitat restoration

Introduction

Riverine wetlands are marginal habitats that can be considered insular biotopes as they are smaller than other neighbouring freshwater ecosystems (Ramsar, 1971). These biotopes have two important roles: from an ecological point of view they can improve environmental variability (Belsare, 1994) and thus increase fauna and flora biodiversity (Habitat Directive 2000/60/EC European community, 2000), and from a hydrological point of view they provide protection from flooding, especially in highly populated areas (Browne *et al*., 1995).

Riverine wetlands have specific ecological dynamics and they are very vulnerable to anthropogenic activities such as habitat destruction, release of toxic materials, introduction of exotic species and eutrophication (Myers, 1997; Bodini *et al*., 2000). Despite their ecological importance, anthropogenic pressures have often isolated these ecosystems from the rest of the environment (Schmitz, 2012).

In 2010, a project called "Ecological and hydromorphological requalification of wetlands in the Ticino area around Pavia" was started with the aim of improving the chemical, bio-ecological and hydrological conditions of wetlands near Pavia (South-East Lombardy, Northern Italy). The first part of the project consisted in performing a census of the wetlands in this area to prioritize the requalification process of these habitats. After that, we selected two small wetlands with different ecological situations, one of which is directly impacted by an active combined sewer overflow from the sewerage networks, the other of which is less impacted by anthropogenic activities.

One of the main communities found in wetlands is zooplankton, some of which can even survive in polluted wastewaters. Zooplankton is usually considered to be a good indicator of the trophic state of the water; however, it is difficult to understand whether a community change is due to biotic intra- or extra-specific interaction or to environmental changes (natural or anthropogenic) (Ferdous and Muktadir, 2009; Chen *et al*., 2010; Jeppesen *et al*., 2011; Kattel, 2012; Bonecker *et al*., 2013; Das *et al*., 2013; Ren *et al*., 2013).

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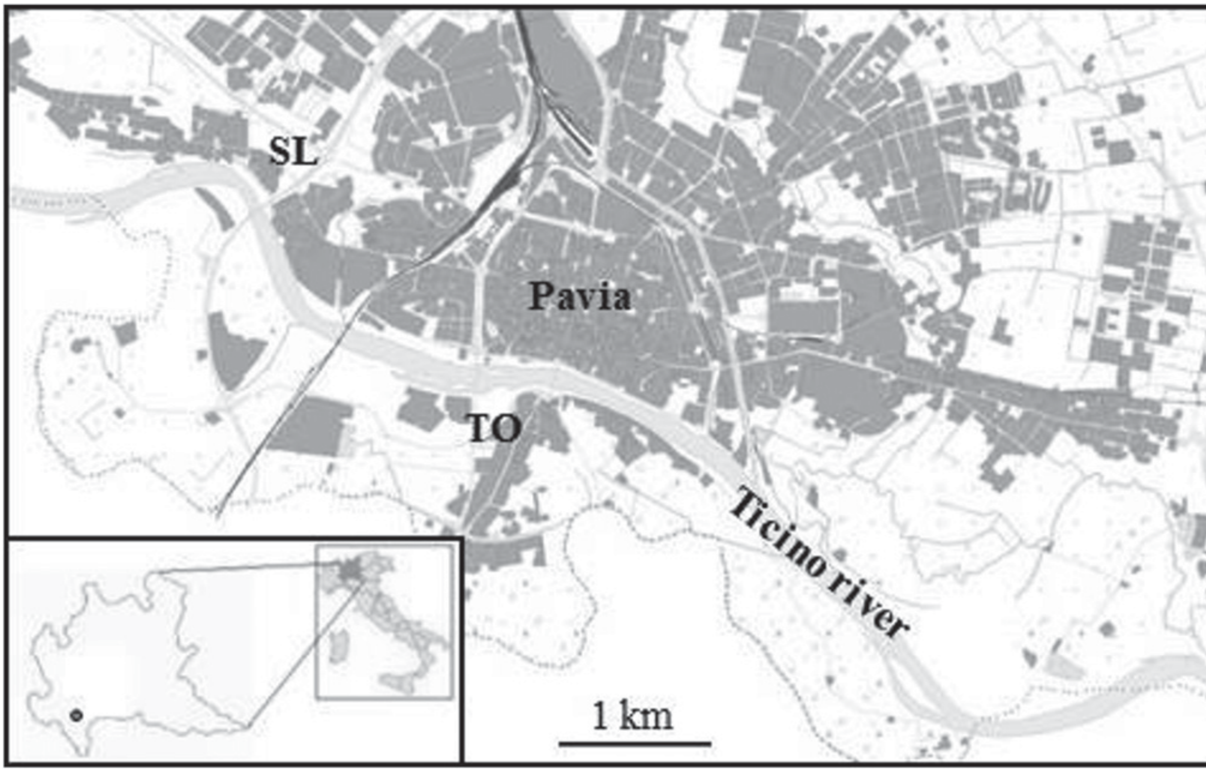

Fig. 1. Study area: "Topo oxbow lake" (TO) and "San Lanfranco wetland" (SL).

In order to understand the possible reaction of the zooplankton communities to constant combined sewer overflow activity, we compared the seasonal evolution in the two selected marginal riverine wetlands characterized by different levels of stress. Furthermore, to better determine the level of pollution in the investigated wetlands, we analysed the physico-chemical parameters of the water.

The results discussed in this paper describe the ecological situation before the requalification process of the wetlands.

Materials and methods

Study area

The lower Ticino River floodplain (South-East Lombardy, Northern Italy), the largest tributary of the left bank of the Po River, is characterized by high anthropogenic pressure resulting from agriculture and industry. Nevertheless, numerous semi-natural areas are present such as ponds, terrace springs, small streams, oxbow lakes and riverine wetlands.

For our study, we selected two riverine wetlands near the main course of the Ticino River: one on the right hydrological side of the river, called "Topo oxbow lake" (hereafter referred to as TO), and the other on the left hydrological side of the river, called "San Lanfranco wetland" (hereafter referred to as SL) (Fig. 1).

TO measures 90 m long and 50 m wide and has a mean depth of 140 cm; it is completely isolated from the Ticino River and receives water through the ground from a perched aquifer. This basin features abundant aquatic vegetation, mainly composed of *Nuphar lutea* and the macro algae *Chara* spp. Moreover, abundant fish fauna is present: although we did not perform a specific fish survey, we recognized various specimens of *Gambusia affinis*, *Cyprinus carpio*, *Lepomis gibbosus* and *Ameiurus* sp.

SL is a narrow, elongated water body which originated from a diversion of a branch of the Ticino River; it measures 400 m long, 20 m wide and has a mean depth of 80 cm. SL is fed by a few springs situated in the upper part of the basin, while a small channel in the lower part leads into the Ticino River.

There is also a combined sewer overflow from the sewerage networks in the upper part of the basin, theoretically active only in the event of rainfall. Unfortunately, a failure in the pumping station causes the recurrent discharge of undiluted urban waste water. Owing to this anthropogenic impact, aquatic plants and fish fauna are absent from the basin and water transparency is limited to a maximum of 50 cm.

Sample collection and data analyses

To investigate the seasonal dynamics of the zooplankton communities, we carried out a fortnightly sampling programme, from June 2011 to August 2012. As both study areas are small, we took samples in the deepest part, at the centre of each wetland, and for each sample we collected four replicates.

We collected zooplankton in a floating plankton net (40 cm Ø × 65 cm length), composed of a rigid hydrodynamic structure and a zooplankton net (100 μm mesh) (Sconfietti and Cantonati, 1990). The zooplankton sampler was towed for 5 m, thus collecting 0.625 m^3 of filtered water; samples were then fixed and taken to the laboratory for identification using Pennak (1953), Dussard (1967), Braioni and Gelmini (1983), Margaritora (1985), Streble and Krauter (1984). The density of zooplankters was obtained following the methodology proposed by Edmonson and Winberg (1971).

During each survey, we recorded water temperature, concentration of dissolved oxygen, pH and water transparency. In addition, once a month while sampling zooplankton, we collected 1 L of water in order to define the concentrations of nitrate ($N–NO_3$), nitrite ($N–NO_2$), ammonia ($N–NH_4$), total nitrogen (N tot) and total phosphorus (P tot). We also defined the biochemical oxygen demand (BOD_5) and the chemical oxygen demand (COD).

We used a photometer NOVA 60 to analyse the principal chemical data; the concentration of some parameters was lower than method sensitivity and was therefore not considered in subsequent analyses (*e.g.*, nitrate data).

We also measured the concentration of the bacterium *Escherichia coli*, considered to be a good indicator of anthropogenic pollution in urbanized areas (Geldreich, 1966).

In order to verify differences between seasons and wetlands, environmental and biotic data were processed through the Kruskal–Wallis test. Furthermore, differences between the two biotic communities were tested by means of an ANOSIM test, and the BIO-ENV procedure was used to identify the environmental variables that best explained variation in the zooplankton community. BIOENV is a non-parametric method that calculates correlation coefficients between site similarity matrices. The biological similarity matrix was compared with environmental similarity matrices and the highest correlation coefficients were reported. Statistical analyses were all performed using the PRIMER 5.0 and MINITAB 15 software packages.

Results

Environmental data

In both ecosystems, the physico-chemical parameters that were recorded described typical seasonal dynamics of riverine wetlands.

In TO, pH values varied between 6.6 and 7.8 and the water was always super saturated (% of dissolved oxygen); COD and BOD_5 values were very low: the former ranged from 8.6 to 55.5 $mg.L^{-1}$, whereas the latter varied from 1.7 to 11.8 $mg.L^{-1}$. The level of nitrogen compounds was also always very low: $N–NH_4$ varied from 0.007 to 0.06 $mg.L^{-1}$, and $N–NO_2$ ranged from 0.006 to 0.08 $mg.L^{-1}$; however, N tot was relatively high, ranging from 0.4 to 2 $mg.L^{-1}$. The concentration of *E. coli* was always 0 UFC/100 mL, except on two occasions when there was a concentration of 300 UFC/100 mL (October 2011) and 400 UFC/100 mL (May 2012).

In SL, the pH value ranged from 6.7 to 9.1 and the percentage of dissolved oxygen was always under saturated. In this basin, the nitrogen compounds, COD and BOD_5 values were always higher than TO. Moreover, in SL, the concentration of *E. coli* was always over 1300 UFC/100 mL, except on a few occasions when the basin was covered by a thin layer of ice. The highest concentration was recorded in July 2011 at 46 000 UFC/100 mL.

The mean values of the environmental parameters collected during the 15-month sampling period showed a noticeable difference between the two riverine wetlands except water temperature, pH and BOD_5 (Table 1).

Zooplankton communities

Overall we collected 19 *taxa*: 4 cladocerans, 1 copepod and 14 rotifers (Table 2). We found 16 species in TO and 17 species in SL. All cladoceran, all copepod and nine rotifer species were present in both wetlands, whereas five rotifer species were only found in one of the two wetlands: *Brachionus falcatus* and *Polyarthra euryptera* in TO, and *Brachionus calyciflorus anuraeiformis*, *B. calyciflorus amphiceros* and *B. quadridentatus f. cluniorbicularis* in SL.

An ANOSIM test showed a slight difference between the two communities in terms of species composition ($R = 0.201$, $P = 0.001$); MDS also supports this hypothesis (Fig. 2).

In the TO community, the proportion of the three main zooplankton groups was similar: 36% cladocerans, 35% rotifers and 29% copepods. The mean density of the zooplankters reached 770 ± 830 $ind.m^{-3}$, and the most abundant species was the cladoceran *B. longirostris*, with a mean density of 231 ± 425.84 $ind.m^{-3}$ per month.

Following the seasonal trend of the community, we found that copepods were the most abundant group in summer 2011. In autumn and winter 2011, we registered a general decrease in zooplankton density: in autumn, the most dominant group was copepods; whereas in winter 2011, rotifers became the most abundant group. In spring 2012, we observed a general zooplankton increase, and the ratio of the three groups was similar to the ratio in autumn 2011, with a dominance of cladocerans; in summer 2012, copepods became the main group (Fig. 3(a)).

In SL, the community was predominantly composed of copepods (56% of the total) and rotifers (39% of the total). The mean density of the zooplankters reached 884 ± 1811 $ind.m^{-3}$.

The community was dominated by the nauplius stage of copepods with a mean density of 233 ± 570.36 $ind.m^{-3}$ per month.

Table 1. The mean values (± SD) and Kruskal–Wallis test results of the environmental parameters collected during the 15-month sampling period in TO and SL wetlands.

Parameters	Mean values (± SD)	Variables	Kruskal–Wallis results	Statistical significant
Dissolved oxygen	TO: 101.5 ± 48%	Wetlands	$H = 19.10$; d.f. = 1; $P = 0.000$	***
	SL: 46.5 ± 44%	Seasons	$H = 6.30$; d.f. = 3; $P = 0.098$	NS
Water temperature	TO: 16.2 ± 7.6 °C	Wetlands	$H = 0.92$; d.f. = 1; $P = 0.336$	NS
	SL: 14.7 ± 7.8 °C	Seasons	$H = 50.74$; d.f. = 3; $P = 0.000$	***
pH	TO: 7.04 ± 0.27	Wetlands	$H = 0.01$; d.f. = 1; $P = 0.929$	NS
	SL: 6.9 ± 1.3	Seasons	$H = 7.25$; d.f. = 3; $P = 0.064$	NS
Transparency	TO: 138.3 ± 25.6 cm	Wetlands	$H = 45.29$; d.f. = 1; $P = 0.000$	***
	SL: 43.6 ± 22.7 cm	Seasons	$H = 2.98$; d.f. = 3; $P = 0.395$	NS
$N–NO_2$	TO: 0.01 ± 0.02 $mg.L^{-1}$	Wetlands	$H = 6.03$; d.f. = 1; $P = 0.014$	**
	SL: 0.07 ± 0.07 $mg.L^{-1}$	Seasons	$H = 0.28$; d.f. = 3; $P = 0.963$	NS
$N–NH_4$	TO: 0.03 ± 0.02 $mg.L^{-1}$	Wetlands	$H = 17.57$; d.f. = 1; $P = 0.000$	***
	SL: 1.13 ± 0.82 $mg.L^{-1}$	Seasons	$H = 0.97$; d.f. = 3; $P = 0.809$	NS
N tot	TO: 0.03 ± 0.02 $mg.L^{-1}$	Wetlands	$H = 6.03$; d.f. = 1; $P = 0.014$	**
	SL: 2.64 ± 1.15 $mg.L^{-1}$	Seasons	$H = 0.28$; d.f. = 3; $P = 0.963$	NS
P tot	TO: 0.03 ± 0.02 $mg.L^{-1}$	Wetlands	$H = 19.14$; d.f. = 1; $P = 0.000$	***
	SL: 0.22 ± 0.11 $mg.L^{-1}$	Seasons	$H = 2.34$; d.f. = 3; $P = 0.505$	NS
BOD_5	TO: 4.12 ± 3.03 $mg.L^{-1}$	Wetlands	$H = 0.41$; d.f. = 1; $P = 0.520$	NS
	SL: 5.05 ± 3.45 $mg.L^{-1}$	Seasons	$H = 5.51$; d.f. = 3; $P = 0.138$	NS
COD	TO: 19.6 ± 12.45 $mg.L^{-1}$	Wetlands	$H = 5.11$; d.f. = 1; $P = 0.024$	*
	SL: 25.19 ± 10.93 $mg.L^{-1}$	Seasons	$H = 2.70$; d.f. = 3; $P = 0.441$	NS

*: $P < 0.05$, **: $P < 0.005$, ***: $P < 0.001$, NS: $P > 0.05$.

Table 2. Presence/absence of zooplankton taxa in TO and SL wetlands.

Taxa/wetlands	TO	SL
Cladocerans		
Bosmina longirostris (Müller, 1776)	x	x
Daphnia longispina (Müller, 1785)	x	x
Moina micrura (Kurtz, 1874)	x	x
Pleuroxus aduncus (Baird, 1843)	x	x
Copepods		
Thermocyclops dybowskii (Lande, 1890)	x	x
Rotifers		
Asplanchna priodonta (Gosse, 1850)	x	x
Brachionus angularis (Gosse, 1851)	x	x
Brachionus calyciflorus (Pallas)	x	x
Brachionus calyciflorus anuraeiformis (Brehm)	–	x
Brachionus calyciflorus amphiceros (Ehrb.)	–	x
Brachionus quadridentatus (Hermann)	x	x
Brachionus quadridentatus f. cluniorbicularis (Skorikov)	–	x
Brachionus falcatus (Zacharias)	x	–
Brachionus patulus (Müller)	x	x
Filinia longiseta (Ehrb.)	x	x
Keratella quadrata (Müller)	x	x
Lecane quadridentata (Ehrb.)	x	x
Platyias quadricornis (Ehrenberg)	x	x
Polyarthra euryptera (Wierzejski)	x	–

In summer 2011, the community was dominated by copepods; in autumn 2011, although the density of all species was very low, there was the same trend as the previous season. In winter 2011, the number of specimens drastically decreased and the most abundant group was rotifers. In spring 2012, we found a general recovery of the community with copepods being the most abundant species. Afterwards, copepods and cladocerans increased their abundance and in summer 2012, we found the same group proportions as in summer 2011 (Fig. 3(b)).

In order to highlight possible differences between the two wetlands (seasons within wetlands), we tested the proportions of the three main groups of their zooplankton communities by means of the Kruskal–Wallis test. If we consider the total number of cladocerans, there is a significant statistical difference between wetlands ($H = 14.50$; d.f. = 1; $P < 0.001$) but not between seasons ($H = 8.17$; d.f. = 4; $P > 0.05$). However, copepods ($H = 23.56$; d.f. = 4; $P < 0.001$) and rotifers ($H = 18.47$; d.f. = 4; $P < 0.005$) showed a significant statistical difference between seasons but not between wetlands (copepods: $H = 0.20$; d.f. = 1; $P > 0.05$; rotifers: $H = 1.60$; d.f. = 1; $P > 0.05$).

When we compared the biotic data with the environmental data of TO using a BIO-ENV analysis, we discovered a very low correlation ($\rho = 0.324$) and that only three variables are required to maximize the matching coefficient: pH, N tot and $N–NO_2$; we performed the same procedure for the SL data and although the correlation was still low ($\rho = 0.481$), it was slightly higher than before. In this case, water temperature and P tot were the most important variables that influence the biotic data.

Discussion

The ecological situation of the two wetlands investigated in this study was found to be different. In TO the water is very transparent but dark-coloured, due to the presence of a rich peat substratum and humic acids which are soluble aromatic organic molecules that result from the

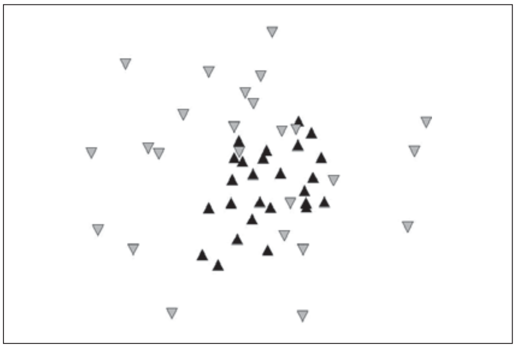

Fig. 2. MDS analysis of the biotic data of TO and SL wetlands. TO (black) and SL (grey).

incomplete decomposition of dead plant and allochthonous debris from riparian vegetation.

Moreover, the presence of aquatic macrophytes also has a positive effect on water transparency because they decrease the re-suspended sediment in the water column and use nutrients instead of phytoplankton (Canfield *et al*., 1984; Jeppesen *et al*., 1990). Furthermore, submerged vegetation might also play a special role for zooplankton as a shelter from predation (Crowder and Cooper, 1982; Gotceitas and Colgan, 1987; Persson, 1993; Burks *et al*., 2002). According to Blindow *et al*. (2000), the extent of this effect depends on the type of vegetation: for example, *Chara* spp., the most dominant green algae in the TO, is particularly suitable as a shelter for zooplankton.

The zooplankton community dynamics in the TO is typical of an undisturbed natural or semi-natural basin: it is dominated by cladocerans and rotifers, jointly comprising 71% of the total. The most abundant species is the cladoceran *B. longirostris*, a cosmopolitan filter feeder, typically found in eutrophic water. In our case study, the main factor that is likely to have influenced the reduction of the abundance of large cladocerans (*i.e*., *Daphnia* spp.) in favour of small ones (such as *B. longirostris*), could be the presence of abundant fish fauna, as suggested by various authors (Hurlbert *et al*., 1972; Hurlbert and Mulla, 1981; Irvine *et al*., 1989).

Considering environmental and biotic data collected during this study and following the classification proposed by Margalef (1983), TO could be classified as a mesotrophic and non-impacted riverine wetland which tends towards a natural eutrophic stage, even though it is problematic to assess the trophic level of riverine wetlands through chemical parameter boundaries found in literature because the values usually refer to lakes.

The other riverine wetland that we studied (SL) is directly linked to the Ticino River; the presence of active sewage in this basin influences its chemical status and water transparency. The differences in water chemical values recorded in these two shallow basins are undoubtedly due to the frequent discharge of sewage into the SL basin. The high organic charge from the sewage quite often caused strongly under saturated dissolved oxygen levels, especially during summer months, and also caused

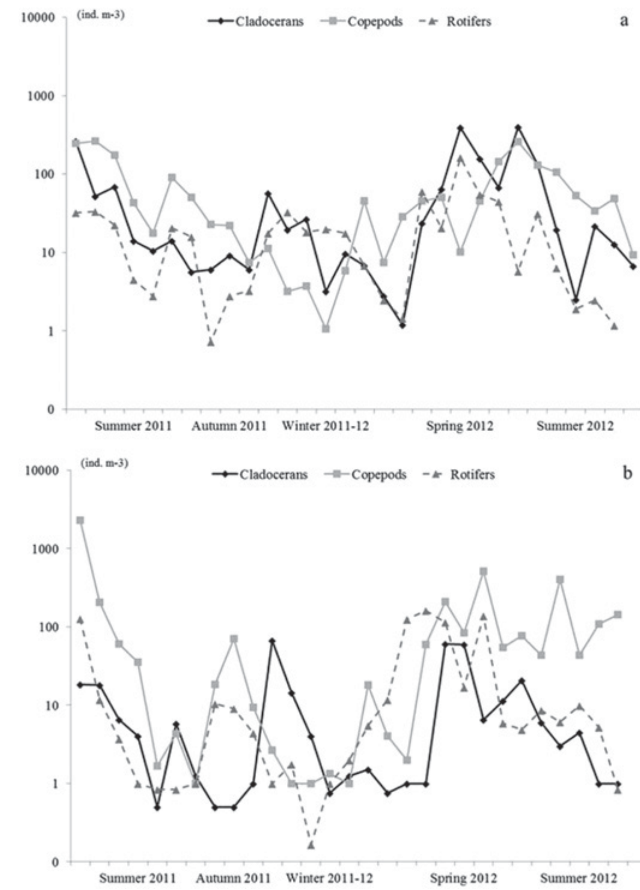

Fig. 3. Seasonal trend (log. scale, ind. m^{-3}) of the three main zooplankton groups (cladocerans, copepods and rotifers) collected during the 15-month survey in TO (a) and SL (b).

high concentration of all recorded nitrogen compounds ($N–NO_2$, $N–NH_4$ and N tot), high values of COD, BOD_5 and *E. coli*.

Despite the anthropogenic pressure and a complete absence of submerged vegetation, the zooplankton community in the SL is composed of the same pool of species that we detected in the TO (see Table 2), but the dominant species was the juvenile form of the copepod *T. dybowskii*, a widespread copepod which generally inhabits small water bodies (Maier, 1990); high abundance of this species could be interpreted as a sign of eutrophic conditions (Sousa *et al*., 2008). In spring 2012, we also observed a demographic explosion of β-mesosaprobic rotifer species (*e.g*., *Asplanchna priodonta*, *Brachionus angularis* and *B. calyciflorus*) which became the most abundant group of the community (Sládeček, 1983).

Zooplankton assemblages with a dominance of juvenile copepods, very few adults, and a low number of other species, could be the result of the sewage's effect because most of the specimens die at a young age, and only a few manage to reproduce. These characteristics are typical of pioneer community, which usually colonize unstable environment (Allan, 1976; Matsumura-Tundisi *et al*., 1990; Sampaio *et al*., 2002; Illyová and Pastuchová, 2012; Vad *et al*., 2012).

Pandey and Verma (2004) and Sahib (2004) observed that the zooplankton community is directly correlated with abiotic factors. During our analyses, we found that three (P tot, N tot and N–NO_2) out of the five environmental variables that have the greatest influence on the zooplankton community are directly linked to the wet-weather discharges; whereas the other two variables (water temperature and pH) are mainly linked to seasonal variation.

In small biotopes, such as riverine wetlands, the presence of any extent of anthropogenic impact immediately influences the equilibrium of such vulnerable ecosystems and prejudices their relevant ecological role.

Therefore, in accordance with the aim of the requalification project supported by the Cariplo Foundation, SL is first in line to be improved. The first step will be to repair the wet-weather discharge to reduce the direct anthropogenic impact; the second step will be to dig the upper part of the basin to form a deeper area that will aerate the whole pond; finally, the rest of the basin will be implanted with an autochthonous reed bed that will act as a biofilter to purify the occasional input of wet-weather discharge before it enters the Ticino River. Therefore, these restoration activities could lead to the development of a more stable zooplankton community. By comparison, TO needs a less drastic requalification to ensure the long-term ecological functioning of the aquatic environment: the bottom of the basin will be slightly excavated with the aim of rejuvenating its physical habitat.

Both of these wetlands need to be improved, not only due to their proximity to an urbanized area but also to increase their value from historical, social and ecological points of view.

Acknowledgements. This study was partly developed within a Cariplo Foundation project in collaboration with Municipality of Pavia, Park of Lombardy in the Ticino Valley, AIPO (Interregional Agency for the PO River) and the University of Pavia. We would like to thank Prof. Italo Venzaghi for his support during chemical analyses and Charlotte Buckmaster for revising the English text.

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