

Longitudinal variability in hydrochemistry and zooplankton community of a large river: a Lagrangian-based approach

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## Abstract

The variability in water quality and zooplankton community structure during downstream transport was investigated in the Po river (Italy) using for the first time a Lagrangian sampling approach.

Two surveys were conducted, one in spring under relatively high discharge levels, and one at low flows in summer. Twelve stations along a 332-km stretch of the river's lowland reach and four major tributaries were sampled. A hydrodynamic modeling system was used to determine water transport time along the river, with a satisfying fit between simulated and observed discharge values. No clear downstream trend in phosphorus and nitrogen concentrations was found.

Conversely, a marked longitudinal decrease in dissolved silica supports the hypothesis of increasing downstream silica limitation during the phytoplankton growing season.

In spring, at low residence time, no apparent plankton growth was observed during downstream transport. In summer, higher temperatures and lower turbulence and turbidity associated with longer residence time stimulated algal growth and in-stream reproduction of fast-growing rotifer taxa, with the gradual downstream development of a truly potamal assemblage and the increase of the ratio of euplanktonic to littoral/epibenthic rotifer taxa. Crustacean zooplankton density was generally low.

The importance of biotic interactions within the zooplankton in driving community abundance and composition appeared to increase in the downstream direction, paralleled by a decrease in the influence of physical forcing. Tributary influence was especially evident where severe anthropogenic alterations of river hydrology and trophic status resulted in enhanced plankton growth, ultimately affecting zooplankton structure in the main river.

## Keywords

Po river, Italy, hydrodynamic forecasting model, space-time variability, hydrochemistry, potamoplankton, community dynamics.

## Introduction

The vast majority of field studies investigating plankton ecology in rivers has generally adopted an Eulerian reference frame, which is based on measuring the flux of objects through or within a spatially bounded area (Doyle & Ensign, 2009). These surveys rely on a traditional sampling design, involving the collection of samples at one or more stations at fixed time intervals, without accounting for water travel time from one station to the next.

An alternative approach is to virtually follow a water parcel as it is advectively transported downstream (Lagrangian reference frame). This method has been used to describe longitudinal changes in water quality and transformation of nutrients and contaminants (Brown *et al.*, 2009, Deutsch *et al.*, 2009; Volkmar *et al.*, 2011; Writer *et al.*, 2011; Coupe *et al.*, 2013) and plankton communities (Weitere & Arndt, 2003; Quiel *et al.*, 2011) in different rivers, as hydrochemical and biological processes occurring during downstream transport can be studied in a more direct way, and apparent plankton growth rates can be estimated.

Relatively few studies have used a Lagrangian approach to investigate the dynamics of the metazoan component of river plankton, mostly in Central and Eastern Europe (de Ruyter van Steveninck *et al.*, 1992; Welker & Walz, 1998; Ietswaart *et al.*, 1999; Zimmermann-Timm *et al.*, 2007; Scherwass *et al.*, 2010; Gruberts *et al.*, 2012; Napiórkowski & Napiórkowska, 2013; Gruberts & Paidere, 2014; Hardenbicker *et al.*, 2015). Most of these studies showed the occurrence of downstream changes in zooplankton abundance and composition in large lowland rivers. These changes have usually been associated with the longer time available to grow and reproduce, although contrasting patterns may emerge as a result of different hydrological conditions, length of the considered river segment, grazing pressure from benthic filter feeders, time of the year and connectivity of the main river channel to the flood plain (de Ruyter van Steveninck *et al.*, 1992; Welker & Walz, 1998; Ietswaart *et al.*, 1999; Scherwass *et al.*, 2010; Hardenbicker *et al.*, 2015).

One of the most important issues in designing a Lagrangian survey is the accurate measurement of river water velocity and, subsequently, the estimation of the timing of sample collection. This task

can be particularly complicated in hydrologically complex and highly variable systems. Drifting devices (freely floating or manned), although widely employed for tracking the movement of oceanic water masses, have rarely been used in river ecology and, more specifically, in Lagrangian surveys (see Gruberts *et al.*, 2012 and references therein). Tracers have also been employed to determine transport times (e.g., Guhr *et al.*, 2003). More often, hydrodynamic model simulations of water transport time are used, for example the QSIM and HYDRAX models for the Elbe river (Zimmermann-Timm *et al.*, 2007; Hardenbicker *et al.*, 2015) and the Rhine Alarm Model (de Ruyter van Steveninck *et al.*, 1992; Ietswaart *et al.*, 1999; Scherwass *et al.*, 2010). These models are fed by real-time *in-situ* hydrological and meteorological data, and predict river flow at various temporal resolutions to which different probability values can be associated.

The primary aim of this work is to investigate the large-scale variability in water quality and zooplankton community abundance and composition during downstream transport in a large, lowland river (Po river, Italy) by means of Lagrangian samplings under different hydrological and seasonal conditions. It is the first time that such an approach is applied to this river.

We hypothesized that water quality and zooplankton abundance/composition should display little or no downstream variability during relatively high spring discharge rates and corresponding low water residence time. On the contrary, under summer low discharge levels, a downstream gradient in hydrochemical and biological variables is expected, due to the longer water residence time enhancing the role of local biogeochemical and ecological processes. More specifically, we expect a longitudinal shift in community structure from an assemblage dominated by littoral/epibenthic taxa to a progressively larger contribution of truly planktonic taxa during downstream transport. The influence of the major tributaries on the spatial variability observed in the Po river is also assumed to be more important in spring, when conditions in the Po are less favourable for the development of an abundant autochthonous community.

This paper largely expands upon a preliminary account of some of the results which have appeared as an extended abstract (Bertani *et al.*, 2014).

## Materials and Methods

### *Study area*

The Po is the main Italian river, both in terms of length (652 km) and catchment surface (70,700 km<sup>2</sup>). The natural meandering course of the river has been radically modified and partially straightened over the centuries, so that the middle and lower reaches are now completely confined within artificial banks, resulting in low hydrological connectivity between the main river channel and floodplain backwaters. At the closing station of Pontelagoscuro, located just upstream of the river delta, the mean annual discharge (period 1918-2003) is 1525 m<sup>3</sup> s<sup>-1</sup> (Syvitski & Kettner, 2007). The annual hydrological regime is strongly influenced by the seasonal pattern of precipitation, land use and water abstractions, with low water levels (down to 200 m<sup>3</sup> s<sup>-1</sup>) usually occurring during summer, and floods (up to over 10,000 m<sup>3</sup> s<sup>-1</sup>) in late fall and spring (Zanchettin *et al.*, 2008). Four left tributaries (Ticino, Adda, Oglio, Mincio) make up about 50% of the total water discharge of the Po, with average discharges of 350, 190, 140 and 60 m<sup>3</sup> s<sup>-1</sup>, respectively. Seventeen million people inhabit the river catchment area, which sustains the largest proportion of the national agricultural and industrial production. High nutrient loads arising from urbanisation and agricultural activities support high levels of primary production in the lowland river section, especially during summer, when chlorophyll-*a* concentrations typically reach values up to 75 µg L<sup>-1</sup> (Rossetti *et al.*, 2009; Viaroli *et al.*, 2013a).

### *Modeling of water transport time*

Water transport time along the river was simulated through the operational forecasting system Po-Flood Early Warning System (Po-FEWS) of the Emilia-Romagna Environmental Protection Agency (ARPA Emilia-Romagna) (Vezzoli *et al.*, 2013). This system was developed and is routinely operated by ARPA Emilia-Romagna to predict flood events in the Po river basin (Ricciardi *et al.*, 2013). It is based on real-time hydro-meteorological data collected by a monitoring

network spread over the whole river basin (588 water level gauges and 1014 rain gauges) and on forecasted meteorological conditions provided by a non-hydrostatic model with lead time of 3 and 15 days. These data feed a coupled rainfall/runoff and hydrodynamic model that generates forecasting scenarios of river discharge and water travel time. Simulations of water velocity along the river under projected meteorological conditions were run two days before each field survey, and results were used to determine the time of sampling at each station. Model performance was assessed by comparing simulated and observed discharge values at six gauging stations along the considered river stretch.

#### *Field sampling and laboratory analyses*

Two surveys were carried out: one in the period 26-30 May, 2010 (spring survey), and one in the period 19-27 August, 2011 (summer survey). In both cases, 12 stations along a 332-km stretch of the Po river lowland reach were sampled (Tab. 1; Fig. 1). The most upstream station (Rea) is located 269 km downstream of the headwaters. The four major tributaries (Ticino, Adda, Oglio, and Mincio) along the considered river stretch were also sampled at their mouths. For abbreviations of sampling and gauging stations used hereafter in the text and figures, see caption of Fig. 1.

Zooplankton and surface water samples were taken from floating pontoons that allowed us to reach the main current in the river channel. Water temperature and electric conductivity (EC) were measured with a multiparametric probe (YSI Model 85). pH was determined with a pH-meter (Radiometer TIM 90), using a GK 2401 C combined electrode (Radiometer). Dissolved oxygen (DO) was measured with the Winkler method (APHA, 1998). Total alkalinity (TA) was determined by potentiometric end-point titration and linearization according to Rodier (1978). Chlorophyll-*a* (Chl-*a*) concentration was determined spectrophotometrically after acetone extraction (Golterman *et al.*, 1978). Suspended particulate matter (SPM) was determined after drying filters at 70°C (APHA, 1998). Standard spectrophotometric analyses were applied for soluble reactive phosphorus (SRP) (Valderrama, 1977), dissolved reactive silica (DRSi) (Golterman *et al.*, 1978) and nitric (NO<sub>3</sub>-N),

nitrous (NO<sub>2</sub>-N), and ammonium nitrogen (NH<sub>4</sub>-N) (APHA, 1998). Dissolved inorganic nitrogen (DIN) was determined as the sum of ammonia, nitrite, and nitrate.

Zooplankton samples were taken by lowering a 15 L bucket into the river; water was then filtered through a 50 µm net until reaching a volume of 60 L. Samples were immediately preserved in a 4% buffered formalin solution. Rotifers and microcrustaceans were identified to the lowest possible taxonomic level, except for bdelloid rotifers, harpacticoids, and copepod juvenile stages, these latter assigned either to nauplii or copepodites, separately for calanoids and cyclopoids. The following references were used for taxa identification: Gurney (1933), Kiefer (1968), Dussart (1969), Dussart & Defaye (1995), Einsle (1996), Karaytug (1999) and Ueda & Reid (2003) for copepods, Margaritora (1985) and Alonso (1996) for cladocerans, Ruttner- Kolisko (1974), Koste (1978), Braioni & Gelmini (1983), Segers (1994, 1995a, 1995b), Segers et al. (1994), Nogrady et al. (1995), De Smet (1996), De Smet & Pourriot (1997) and De Smet & Gibson (2008) for monogonont rotifers. Diploid subitaneous and resting eggs of the dominant large brachionid rotifers (*Brachionus calyciflorus* and *B. quadridentatus*) were counted collectively, because the two species produce eggs of very similar shape and size. Eggs of other rotifer taxa were not enumerated as they were too small to be quantitatively retained by the plankton net. Metazoans other than rotifers, cladocerans, and copepods were also enumerated and grouped into broad taxonomic categories.

#### *Data analysis*

The Wilcoxon signed-rank test for paired samples was used to compare values of physico-chemical variables and Chl-*a* across years and between tributaries and corresponding downstream stations in the Po river (Hardenbicker *et al.*, 2015). Spatio-temporal patterns in zooplankton community structure were explored by means of non-metric multidimensional scaling (NMDS) performed on a Bray-Curtis distance matrix (Legendre & Legendre, 1998). Extremely rare taxa (rotifer and crustacean species reaching in at least one sampling date 1000 ind m<sup>-3</sup> and 500 ind m<sup>-3</sup>, respectively) were excluded from the analysis. Environmental variables with significant (p<0.05)

correlations to either ordination axis are shown on the NMDS biplot. Non-parametric permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) with 999 permutations was performed to test for significant differences in zooplankton community structure across years and between tributaries and corresponding downstream stations in the Po river. Analyses were performed with the software R (R Core Team, 2015) using the package *vegan* (version 2.3-0; Oksanen *et al.*, 2015) for NMDS and PERMANOVA. Correlations between environmental variables and NMDS ordination axes were explored using the *envfit* function in the *vegan* package. This function fits vectors of environmental variables onto the NMDS ordination plot, with the vector length being proportional to the correlation between the variable and the ordination (Oksanen *et al.*, 2015).

The Shannon diversity index (H) was calculated using the package PAST version 1.06 (Hammer *et al.*, 2001).

## Results

### *Hydrology and hydrodynamic model performance*

In early May 2010 a flood occurred, after which river flow steadily decreased until the beginning of sampling. During the spring survey, discharge remained relatively stable, showing only a slight increasing trend (from 1275 to 1451 m<sup>3</sup> s<sup>-1</sup> at PIA, from 1762 to 2038 m<sup>3</sup> s<sup>-1</sup> at BRG\_09, and from 1874 to 2175 m<sup>3</sup> s<sup>-1</sup> at PON\_11) (Fig. 2). The percentage error between simulated and observed discharge values ranged between -5.52% and 0.42% (R<sup>2</sup>=0.99). According to the model, water velocity ranged between 0.72 and 1.15 m s<sup>-1</sup> in different river sections, with an average value of 0.92 m s<sup>-1</sup> along the investigated stretch.

An increase in river discharge was recorded in the first half of August 2011, followed by a gradual decrease in the subsequent days. On August 19, values of 414, 510 and 646 m<sup>3</sup> s<sup>-1</sup> were observed at the gauging stations of PIA, BRG\_09 and PON\_11, respectively. Discharge remained then relatively constant for most of the study period (Fig. 2). The percentage error between simulated



and observed flow values ranged between -6.00% and 22.84% ( $R^2=0.76$ ). Estimated current velocity varied between 0.40 and 0.74 m s<sup>-1</sup> in different river segments, with a mean of 0.57 m s<sup>-1</sup>.

### *Hydrochemistry*

In May 2010, EC had no clear longitudinal trend, although the highest values were recorded downstream of the junction with the OGL river, which showed a maximum of 504  $\mu\text{S cm}^{-1}$ . DO saturation ranged between 82 and 111% at all sites. SPM exhibited a downstream decreasing trend down to a minimum of 45.5 mg L<sup>-1</sup> at BRG\_09, followed by an increase in the lowermost stations. Among the tributaries, OGL was the only one showing SPM levels higher than those recorded in the Po (65.7 mg L<sup>-1</sup>) (Fig. 3).

Nitrate was by far the dominant form of inorganic nitrogen. DIN concentrations in the Po varied between 1401  $\mu\text{g L}^{-1}$  at STA\_06 and 2187  $\mu\text{g L}^{-1}$  at BRG\_09, with the highest values recorded immediately downstream of the OGL confluence. Concentrations in the tributaries were comparable to those recorded in the Po, with the exception of OGL, where a DIN content of 4422  $\mu\text{g L}^{-1}$  was detected. SRP concentrations were between 29 (COR\_03) and 12  $\mu\text{g L}^{-1}$  (RIV\_08) along the Po, while the lowest SRP concentration was recorded at MIN (8  $\mu\text{g L}^{-1}$ ). The DIN:SRP molar ratio was highly variable in the Po (ranging from 136 at COR\_03 to 348 at BGR\_09) and even more so in the tributaries (from 185 at TIC to 507 at OGL). DRSi reached the highest values at the two most upstream Po stations (~2.5 mg L<sup>-1</sup>); at the other stations concentrations ranged between 1.89 mg L<sup>-1</sup> (CRE\_05) and 2.25 mg L<sup>-1</sup> (MOR\_04), while the tributaries exhibited lower values. The molar ratios of DRSi to DIN were largely <1 at all sites. The Chl-*a* content in the Po varied between 2.4  $\mu\text{g L}^{-1}$  (STA\_06) and 6.7  $\mu\text{g L}^{-1}$  (SER\_12), with the most downstream stations generally showing the highest values. Among the tributaries, particularly high Chl-*a* levels occurred at MIN (20.9  $\mu\text{g L}^{-1}$ ) (Fig. 3).

In August 2011, EC decreased along the first four Po stations, followed by a gradual increase along the remaining sites. The lowest tributary EC was measured at TIC (278  $\mu\text{S cm}^{-1}$ ), while at OGL a

value of  $600 \mu\text{S cm}^{-1}$  was observed. DO saturation ranged between 89 and 153% in the Po, with no clear longitudinal trend; the tributaries showed progressively increasing oxygen contents from the uppermost one (77% at TIC) to the most downstream one (125% at MIN). SPM varied between  $18.6 \text{ mg L}^{-1}$  (MOR\_04) and  $41.5 \text{ mg L}^{-1}$  (BRG\_09), with the most downstream stations exhibiting the highest values. The lowest SPM levels were recorded at TIC and ADD ( $6.3$  and  $10.4 \text{ mg L}^{-1}$ , respectively), while OGL showed the highest value ( $52.1 \text{ mg L}^{-1}$ ) (Fig. 3).

Also in the summer survey DIN was composed for the most part by nitrate, which varied between  $1201 \mu\text{g L}^{-1}$  (MOR\_04) and  $2125 \mu\text{g L}^{-1}$  (VIA\_07) in the Po. Particularly high nitrate levels were found at OGL ( $4840 \mu\text{g L}^{-1}$ ).  $\text{N-NO}_2$  and  $\text{N-NH}_4$  were  $\leq 100 \mu\text{g L}^{-1}$  at all sites. SRP concentrations remained  $< 20 \mu\text{g L}^{-1}$  at most of the middle and lower stations, with the exception of SER\_12 that showed a peak of  $28 \mu\text{g L}^{-1}$ . The DIN:SRP ratio varied from 94 (COR\_03) to 1262 (POR\_02) in the Po, and from 166 (MIN) to 264 (OGL) in the tributaries. DRSi had a clear downstream decreasing trend in the Po, from  $3.49 \text{ mg L}^{-1}$  at REA\_01 to  $1.27 \text{ mg L}^{-1}$  at SER\_12; in the tributaries, the highest value was found at OGL ( $2.13 \text{ mg L}^{-1}$ ) and the lowest at MIN ( $0.83 \text{ mg L}^{-1}$ ). The DRSi:DIN ratio in the Po was  $> 1$  only at REA\_01, and the lowest values were found at PON\_11 and SER\_12 ( $\sim 0.37$ ); in the tributaries, the ratio ranged between 0.28 (OGL) and 0.72 (TIC). While no clear trend was evident for Chl-*a* concentrations, the highest values ( $\sim 30 \mu\text{g L}^{-1}$ ) were found at the most downstream stations, except for SER\_12 ( $14.1 \mu\text{g L}^{-1}$ ). With the only exception of MIN ( $22.2 \mu\text{g L}^{-1}$ ), the tributaries had relatively low Chl-*a* concentrations ( $\leq 2.6 \mu\text{g L}^{-1}$ ) (Fig. 3).

Most of the hydrochemical variables showed significant differences across years (Wilcoxon signed-rank test; Temperature:  $Z=0$ ,  $p<0.01$ ; EC:  $Z=1$ ,  $p<0.01$ ; TA:  $Z=1$ ,  $p<0.01$ ; DO saturation:  $Z=4.5$ ,  $p<0.01$ ; SPM:  $Z=130$ ,  $p<0.01$ ; Chl-*a*:  $Z=7$ ,  $p<0.01$ ), with the exception of pH ( $Z=41$ ,  $p=0.18$ ), SRP ( $Z=83$ ,  $p=0.45$ ), DIN ( $Z=73$ ,  $p=0.82$ ), and DRSi ( $Z=56$ ,  $p=0.55$ ). Differences between values of hydrochemical variables measured in the tributaries vs. corresponding downstream Po river stations were not statistically significant (Wilcoxon signed-rank test,  $p>0.05$  in all cases), though this was most likely due to the small sample size ( $n=8$ ) and the fact that differences between tributaries and

Po river had opposite signs, with some tributaries having consistently lower values and some others having higher values than those found in the Po.

### *Zooplankton assemblage*

In the spring survey, a total of 117 taxa were identified: 99 rotifers, 13 cladocerans and 5 copepods. Total zooplankton density fluctuated around 30 ind L<sup>-1</sup> along the first eight stations in the Po, it sharply peaked downstream of the MIN inflow (165 ind L<sup>-1</sup>) and decreased to values around 100 ind L<sup>-1</sup> at the two most downstream sites. Zooplankton densities in the first two tributaries were comparable to those recorded in the uppermost segment of the Po. At OGL total zooplankton abundance reached 65 ind L<sup>-1</sup>, while at MIN a peak of 747 ind L<sup>-1</sup> was detected (Fig. 4).

The NMDS ordination plot showed no clear spatial pattern in Po river community structure upstream of MIN in spring 2010, with the Po river stations grouped into a cluster with no apparent longitudinal structure (Fig. 8). The MIN tributary segregated from the rest of the samples primarily along the first NMDS axis, in the direction of increasing Chl-*a*, pH and DO saturation. The three Po stations downstream of the MIN tributary were somewhat separated from the cluster of upstream Po stations and shifted toward the MIN sample. Overall, no significant differences between community structure in the tributaries vs. corresponding downstream Po river sites were found (PERMANOVA; F=0.69, p=0.64).

Zooplankton density was mainly sustained by rotifers, which made up over 90% of the community at all sites. Loricata taxa constituted 29 to 43% of the rotifer assemblage in the first eight Po stations and at TIC and ADD, while in the two most downstream tributaries and in the last four Po sites their share increased to ~60% (Fig. 7). The ratio of truly planktonic to littoral/epibenthic rotifer taxa showed a gradual downstream increase, from a minimum of 46% at REA\_01 to a maximum of 90% at SER12 (Fig. 7). While at ADD littoral/epibenthic species tended to dominate, with a share of 56%, in the other tributaries planktonic taxa made up 63 to 98% of the whole assemblage (Fig. 7). The percentage composition of the rotifer assemblage in terms of the most abundant taxa remained

quite similar in the first nine stations along the Po. Specifically, bdelloids, *Keratella cochlearis*, *K. cochlearis* f. *tecta*, *Synchaeta* gr. *tremula-oblonga* and *Polyarthra* gr. *vulgaris-dolichoptera* made up a large proportion of the community in the Po sites as well as in the first three tributaries (Fig. 4). At MIN and at the Po stations downstream of its confluence, the share of bdelloids and *Polyarthra* decreased in favour of that of *K. cochlearis* f. *tecta*, *Asplanchna priodonta*, *Brachionus angularis* and *Synchaeta* gr. *stylata-pectinata*. These latter species showed low abundances along most of the river stretch and then sharply peaked at MIN and in the following Po stations (Fig. 4). On the contrary, some littoral/epibenthic taxa showed a clear decreasing pattern in the downstream direction, e.g. *Lecane luna*, *Lepadella patella*, *Trichocerca porcellus* and bdelloid rotifers. Cladocerans never exceeded 1 ind L<sup>-1</sup> in the Po and in the two uppermost tributaries, while slightly higher densities were recorded at OGL and MIN (Fig. 4). The rest of the zooplankton community consisted mainly of copepod larval stages (Fig. 4), with abundances <4 ind L<sup>-1</sup> at all stations except from MIN, where copepods reached 13 ind L<sup>-1</sup>.

The Shannon diversity index ranged between 2.56 (SER\_12) and 3.06 (REA\_01) and it declined in the downstream direction, with the exception of a relatively high value recorded right after the inflow of the OGL river, which had the highest diversity among the tributaries (Fig. 4).

Among other invertebrates, nematodes were the only group found in all samples, with maxima of ~3 ind L<sup>-1</sup> in the first three Po stations and at OGL. Bivalve veligers occurred in the Po downstream of CRE\_05 and at MIN. Dipteran larvae and tardigrades were present in most of the sampled sites, but always with abundances <1 ind L<sup>-1</sup>. Ostracods were rarely found in the Po and with negligible abundances.

In the summer survey, a total of 132 zooplankton taxa were identified: 107 rotifers, 15 cladocerans and 10 copepods. Total zooplankton density showed a gradual increasing downstream trend, with a maximum of 1358 ind L<sup>-1</sup> at PON\_11, followed by a sharp decrease at SER\_12 (241 ind L<sup>-1</sup>).

Among the tributaries, the highest abundances were found at OGL (121 ind L<sup>-1</sup>) and MIN (337 ind L<sup>-1</sup>) (Fig. 5).

Contrary to the spring survey, in summer 2011 the Po river stations showed a strong longitudinal gradient in community structure, with progressively downstream sites ordered along the first NMDS axis in the direction of increasing Chl-*a*, DO, and pH (Fig. 8). The three most upstream tributaries tended to separate along the second NMDS axis, corresponding to increasing conductivity, temperature and DRSi, while the summer MIN sample formed a separate cluster with the spring MIN sample. Substantial differences in community structure across years were confirmed by the PERMANOVA analysis ( $F=11.0$ ,  $p<0.01$ ), while no significant differences between community structure in the tributaries vs. downstream Po river sites were detected (PERMANOVA;  $F=1.56$ ,  $p=0.23$ ).

As in the spring survey, rotifers were the numerically dominant group. Specifically, brachionid rotifers were by far the most abundant taxonomic group (Figs 5 and 6). Spined *Brachionus calyciflorus* largely contributed to the zooplankton peak at PON\_11, with a density of 798 ind L<sup>-1</sup>. At this site, a peak in diploid subitaneous and resting eggs of *B. calyciflorus* and *B. quadridentatus* was also recorded (Fig. 6). Unspined *B. calyciflorus* never exceeded 30 ind L<sup>-1</sup> and it sharply declined in coincidence with the peak in the spined morph. Most brachionid species had remarkably lower abundances in the tributaries than in the Po; on the contrary, at MIN *B. angularis*, *B. forficula*, *K. cochlearis* and *K. cochlearis* f. *tecta* (Fig. 5) reached higher densities than in the Po. Rotifers belonging to the family Synchaetidae, namely *Polyarthra* gr. *vulgaris dolichoptera*, *Synchaeta* gr. *stylata-pectinata* (Fig. 5), and *S.* gr. *tremula-oblonga* also displayed relatively high abundances in the Po, with comparatively low densities in the tributaries. The predator *Asplanchna brightwellii* showed an exponential increase at the lowermost stations (Fig. 5).

Euplanktonic rotifer taxa were generally more abundant than littoral/epibenthic ones, except for TIC, where planktonic taxa accounted for <10%. In the Po, a downstream increasing trend in planktonic taxa was observed, followed by a marked drop at the last station. Loricated rotifers were numerically dominant. The highest percentages (>80%) were found in the lowermost Po stations and at OGL and MIN (Fig. 7).

Microcrustaceans showed relatively low densities in the upper river segment and a sharp increase downstream of the MIN confluence (Fig. 6). In particular, peaks of 16 and 81 ind L<sup>-1</sup> were recorded at MIN for cladocerans (mainly *Moina micrura* and *Diaphanosoma* sp.) and cyclopoid nauplii, respectively. Similarly, cyclopoid adults belonging to *Thermocyclops* sp. reached a maximum of 11 ind L<sup>-1</sup> at MIN, resulting in a density of 3 ind L<sup>-1</sup> at the closest downstream Po station (Fig. 6). Dipteran larvae and nematodes were found in most of the sites; their abundances were higher at the uppermost stations of the Po. An opposite trend was observed for bivalve veligers, which showed the highest densities in the middle and lower section of the river, with a peak 6 ind L<sup>-1</sup> at SER\_12. In the Po, the Shannon diversity index (Fig. 6) showed a clear downstream declining trend paralleled by the increasing dominance of brachionid rotifers; at SER\_12, a marked increase was observed, coinciding with the highest density of *Asplanchna brightwellii* and the decline in most brachionids.

## Discussion

Lagrangian samplings reflect chemical and biological changes of hypothetical water parcels during their downstream transport. The main assumption is that individual water parcels (and therefore dissolved substances and passively advected particles and organisms) travel at the same speed than mean river flow. The presence of retention zones and side arms may slow down portions of the water mass and enhance biogeochemical and biological processes, resulting in an uncoupling between transformation and growth rates and longitudinal transport in the main current (Reckendorfer *et al.*, 1999; Schiemer *et al.*, 2001; Casper & Thorp, 2007). However, in the trait considered in this study, the Po is largely embanked, and river hydrodynamics can be assumed to be only marginally affected by lateral connectivity with the floodplain.

While for the May 2010 survey a very close agreement between observed and simulated discharge values was obtained, in August 2011 actual discharge was somewhat overestimated at the two lowermost stations. This was most likely due to unexpected management activities on the deep sub-

alpine lakes during drought periods that can considerably influence flow variability of the main Po river tributaries (Zanchettin *et al.*, 2008). However, model performance diminished only in the very final segment of the considered river trait, thereby having only limited impact on the overall survey. Contrary to what has been observed in spring, a clear downstream increase in water temperature was detected in August, together with an increase in the concentration of SPM, as it is often reported in the potamal trait of large rivers (Zimmermann-Timm *et al.*, 2007; Scherwass *et al.*, 2010). Lower discharge rates and higher residence time in summer resulted in markedly lower SPM and higher EC and TA when compared to spring values (Fig. 3). Lower turbulence and turbidity, coupled with higher water temperatures, stimulated phytoplankton growth, as indicated by the notably higher summer Chl-*a* concentrations, especially in the lower river segment (Fig. 3). Accordingly, pH and DO saturation were also markedly higher in summer, likely as a consequence of the increased photosynthetic activity. These two variables showed similar spatial variability, which was mainly related to the time of sampling, with the highest values generally recorded in the afternoon or early evening, when primary production was at its peak.

The marked longitudinal decrease in DRSi in summer, in coincidence with the downstream increase in Chl-*a* (Fig. 3), can probably be ascribed to uptake by phytoplankton, which is largely dominated by diatoms (Tavernini *et al.*, 2011). Frequent potential Si limitation, especially during summer diatom blooms, has recently been reported in the Po (Viaroli *et al.*, 2013b). On the contrary, the lack of a clear longitudinal pattern for SRP and DIN in both surveys suggests that even during the phytoplankton growing season these nutrients are unlikely to become limiting, due to extremely high loads deriving from agricultural nonpoint sources in the watershed (Viaroli *et al.*, 2013a).

The four tributaries exhibited contrasting hydrochemical and trophic characteristics (Fig. 3). We did not observe any detectable impact on the Po water quality, probably because of the relatively low discharge levels of the tributaries when compared to those of the Po itself.

Lagrangian surveys have rarely been applied to the investigation of river zooplankton dynamics. In particular, most of the extant work has been carried out on the River Rhine, where different studies

have been performed through the years on a >600-km river trait, considering both the metazoan and the microbial component of the plankton community, as well as phytoplankton (de Ruyter van Steveninck *et al.*, 1992; Ietswaart *et al.*, 1999; Scherwass *et al.*, 2010). Although a clear downstream increase in some of the hydrochemical variables was observed, such as water temperature, conductivity, turbidity, nitrate, phosphate and silicate, chlorophyll-*a* concentration as well as rotifer and microcrustacean abundances showed contrasting longitudinal patterns according to the season and the hydrological conditions. While a significant downstream shift in the taxonomic composition of the phytoplankton assemblage was recorded, relatively less information is available on the composition and diversity of the zooplankton component.

A study conducted on a 500-km trait of the River Elbe focused on the taxonomic composition of the phyto- and zooplankton assemblages, which both increased in density during downstream transport (Zimmermann-Timm *et al.*, 2007). No significant longitudinal change in the rotifer community structure was observed, except for a higher relative abundance of brachionids in the lower parts of the river. Further Lagrangian sampling campaigns performed in the period 2009-2011 (Hardenbicker *et al.*, 2015) in the rivers Rhine and Elbe revealed contrasting patterns in longitudinal development of zooplankton abundances, and opposite impacts of tributaries on plankton biomass in the two rivers.

Other Lagrangian drift experiment performed along the rivers Spree (Welker & Walz, 1998) and Daugava (Gruberts *et al.*, 2012; Gruberts & Paidere, 2014) cannot easily be compared to those reported above, as they were carried out in considerably shorter river traits (21, 61 and 62 km, respectively).

In spring, zooplankton abundance and composition along the Po river did not show significant changes during downstream transport until the confluence of the Mincio river. This confirms our hypothesis that spring flow rates are typically unsuitable for plankton growth in the main channel, as a result of low water residence times, unfavourable physical conditions and low food availability. However, the MIN tributary hosted an exceptionally abundant zooplankton community, and an



increase in total zooplankton density was observed in the Po downstream of the MIN inflow (Fig. 4). As mentioned before, the MIN lower course is highly impounded and regulated, and current is virtually negligible, thus creating favourable conditions for phytoplankton and zooplankton development. As a consequence, despite an average discharge of only  $50 \text{ m}^3 \text{ s}^{-1}$ , the influence of this tributary on the Po river zooplankton was still detectable as far as 100 km downstream of its mouth. Specifically, the community composition recorded downstream of the MIN inflow showed similarities to the assemblage found in the tributary, as indicated by the position of the stations in the NMDS ordination space (Fig. 8). To a lesser extent, OGL seemed to affect the Po river community composition too, as shown by the increase in the share of loricate taxa downstream of its mouth (Fig. 7). This was mainly due to the higher abundances attained by species of the genus *Brachionus* at OGL and later at MIN. In particular, in the MIN river a few taxa made up a large proportion of the rotifer assemblage, resulting in a reduced overall diversity. On the contrary, OGL hosted a more diverse and even assemblage, probably due to the higher turbulence and turbidity that may act as disturbance factors, preventing the development of a structured community with few dominant taxa (De Leo & Ferrari, 1993). As a consequence, a marked increase in community diversity was observed just downstream of the Oglio confluence (Fig. 4).

Diversity in the Po tended to decrease in the downstream direction in both seasons (Figs 4 and 6). This pattern, together with the clear downstream dominance of planktonic taxa (Fig. 7), might be ascribed to the progressive longitudinal decrease in turbulence, resulting in a reduction of re-suspension of organisms from the bottom and more favourable conditions for the development of a truly planktonic community (Zhou *et al.*, 2008). This was especially evident in summer, when halved average current speeds, higher temperatures and primary production, and lower turbulence and turbidity provided suitable conditions for the development of a truly potamal zooplankton assemblage in the main channel (Figs 3, 5 and 6).

The observed summer dominance of brachionid rotifers is a common pattern in large rivers, thanks to the generally better ability of these loricate taxa to feed and grow in the current when compared

to illoricate rotifers (Lair, 2006; Zimmermann-Timm *et al.*, 2007). Accordingly, the gradual downstream increase in abundance of most *Brachionus* and *Keratella* species, and the parallel increase in density of large brachionid subitaneous eggs, suggest that in summer, with current speeds between 0.40 and 0.74 m s<sup>-1</sup>, water residence time was long enough to allow growth and reproduction of fast-growing rotifers in the main stream, although Rzoska (1978) reported that zooplankton reproduction in rivers is rarely observed at flow velocities over 0.4 m s<sup>-1</sup>. The role of tributaries as organism inocula was most likely negligible in this case, as the dominant brachionids in the Po showed markedly lower densities in all major tributaries in summer (Figs 5 and 6).

While the MIN river had no apparent influence on the Po river rotifer assemblage under summer low-flow conditions, it acted as a significant inoculum of microcrustaceans (Fig. 6). The latter, however, do not seem able to sustain high population abundances once they enter the Po river, likely due to their longer development time, higher susceptibility to physical damage during downstream transport and higher vulnerability of their feeding apparatus to suspended solids compared to rotifers (Kirk & Gilbert, 1990; Jack *et al.*, 1993; Sluss *et al.*, 2008).

The unexpected abrupt decrease in density observed in summer at the most downstream Po river station for several dominant rotifer taxa (Figs 5 and 6) might be ascribed to a combination of different factors. Water velocity was negligible at this site (Bertani, pers. comm.), thereby enhancing sinking of non-motile diatoms. This is further supported by the observed decrease in SPM (Fig. 3), and it might explain the marked decline in Chl-*a* when coupled with the downstream decrease in DRSi (Fig. 3). The reduction in available resources may thus have contributed to the observed drop in zooplankton abundance.

Predation by *Asplanchna* may also play a role in shaping the rotifer assemblage in the lower segment of the Po, as suggested by the exponential increase in *Asplanchna* densities in coincidence with the decrease in most rotifer taxa (Fig. 5). Previous studies on gut contents of *A. brightwellii* in the Po confirmed that rotifers made up the largest proportion of the predator's diet (Bertani *et al.*, 2012; 2013). *Asplanchna* was also shown to induce anti-predator morphological changes in *B.*

*calyciflorus* in the river (Bertani *et al.*, 2013). Accordingly, we observed a sharp increase in the spined morphotype paralleled by a decrease in the unspined one in coincidence with the appearance of *Asplanchna* in the lower river trait (Fig. 5). Moreover, the peak in *Brachionus* resting egg production observed just upstream of SER\_12 suggests that density-dependent self-regulation mechanisms in response to crowding might have also contributed to the observed decline in large dominant brachionids (Gilbert, 2003). Finally, a higher grazing pressure from benthic molluscs can also be hypothesized (Hardenbicker *et al.*, 2015), as suggested by the peak of veliger density found in the lower section of the river.

## Conclusions

The Po river and its main tributaries showed high inorganic nutrient levels that are mainly attributable to intensive agricultural and industrial activities in the river basin.

The hydrodynamic model used in this study proved to be capable of simulating Po river flow with a sufficient degree of accuracy to support the design of Lagrangian surveys. This allowed us to directly examine the large-scale longitudinal variability in hydrochemistry and zooplankton community structure during downstream transport in the Po river, including the relative contribution of the major tributaries.

Different seasonal and hydrological conditions resulted in markedly distinct longitudinal patterns in some of the physico-chemical variables and especially in the downstream development of phytoplankton (as determined by Chl-*a*) and zooplankton abundance and composition. In spring, relatively high discharge rates and corresponding low water residence times, coupled with low water temperatures, prevented plankton growth in the main channel. Under summer low flow rates, higher temperatures and a decrease in turbulence and turbidity associated with longer residence times stimulated algal growth and in-stream reproduction of fast-growing rotifer taxa. The importance of biotic interactions within the zooplankton (bottom-up, top-down and self-regulation mechanisms) in driving community abundance and composition may also increase in the

downstream direction, as the impact of physical forcings decreases. The extremely high zooplankton densities found in a severely dammed tributary highlight the impact that anthropogenic alterations of river hydrology can exert on biological communities, with potential repercussions in the downstream systems.

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#### Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1 - Sampling sites and schedule of the Lagrangian surveys. For abbreviations of sampling stations see caption of Fig. 1.

Sampling station	Date of sampling	Hour of sampling	Date of sampling	Hour of sampling
REA_01	May 26, 2010	09:00	August 19, 2011	07:30
TIC	May 26, 2010	11:30	August 19, 2011	10:30
POR_02	May 26, 2010	15:40	August 19, 2011	19:56
COR_03	May 26, 2010	22:45	August 20, 2011	07:17
MOR_04	May 27, 2010	06:00	August 20, 2011	22:49
ADD	May 27, 2010	09:30	August 21, 2011	10:30
CRE_05	May 27, 2010	19:15	August 22, 2011	21:06
STA_06	May 28, 2010	03:00	August 23, 2011	10:33
VIA_07	May 28, 2010	13:50	August 24, 2011	01:23
RIV_08	May 28, 2010	18:10	August 24, 2011	08:11
OGL	May 28, 2010	20:40	August 24, 2011	10:40
BRG_09	May 28, 2010	23:10	August 24, 2011	15:10
MIN	May 29, 2010	09:00	August 24, 2011	17:30
SAC_10	May 29, 2010	05:45	August 25, 2011	00:38
PON_11	May 30, 2010	00:50	August 26, 2011	05:30
SER_12	May 30, 2010	13:20	August 27, 2011	04:30

## Figure captions

Fig. 1- Schematic diagram of the middle reach of the Po river and its main tributaries (in italics) showing the sampling sites. Cumulative distances between sampling sites are also given. The Isola Serafini dam is shown by an open circle. Gauging stations are indicated by arrows. REA\_01: Rea; TIC: Ticino river; POR\_02: Portalbera; SPE: Spessa; COR\_03: Corte Sant' Andrea; PIA: Piacenza; MOR\_04: Mortizza; ADD: Adda river; CRE\_05: Cremona; STA\_06: Stagno; VIA\_07: Viadana; BOR: Boretto; RIV\_08: Riva di Suzzara; OGL: Oglio river; BRG\_09: Borgoforte; MIN: Mincio river; SAC\_10: Sacchetta; PON\_11: Pontelagoscuro; SER\_12: Serravalle.

Fig. 2 - Discharge values ( $\text{m}^3 \text{s}^{-1}$ ) of the Po River in May 2010 (left) and August 2011 (right). Data from only three gauging stations (PIA: Piacenza; BOR: Boretto; PON\_11: Pontelagoscuro) are reported for clarity. Sampling periods are indicated by rectangles.

Fig. 3 - Variation in a) water temperature, b) conductivity, c) pH, d) suspended particulate matter (SPM), e) soluble reactive phosphorus (SRP), f) dissolved inorganic nitrogen (DIN), g) dissolved reactive silica (DRSi) and h) chlorophyll-*a* (Chl-*a*) during the Lagrangian surveys carried out in May 2010 (grey symbols) and August 2011 (black symbols). Square symbols indicate the Po river sampling stations, circles indicate the tributaries.

Fig. 4 - May 2010: density of total zooplankton, selected rotifer taxa and Shannon diversity index calculated for the zooplankton assemblage. Square symbols indicate the Po river sampling stations, circles indicate the tributaries. Densities exceeding the maximum y-axis value are between square brackets.

Fig. 5 - August 2011: density of total zooplankton and selected rotifer taxa calculated for the zooplankton assemblage. Square symbols indicate the Po river sampling stations, circles indicate the tributaries. Densities exceeding the maximum y-axis value are between square brackets.

Fig. 6 - August 2011: density of *B. quadridentatus* f. *brevispinus*, *B. quadridentatus* f. *cluniorbicularis*, resting and subitaneous eggs of *B. calyciflorus* and *B. quadridentatus* (aggregated data), total Cladocera, cyclopoid nauplii, adults of *Thermocyclops* sp. and Shannon diversity index. Square symbols indicate the Po river sampling stations, circles indicate the tributaries.

Fig. 7 - Percentage of euplanktonic (black bars) vs. littoral/epibenthic (grey bars) rotifer taxa (upper panels) and percentage of loricate (black bars) vs. illoricate (grey bars) rotifer taxa (lower panels) recorded at stations along the Po River and in the tributaries (in italics) in May 2010 and August 2011.

Fig. 8 – NMDS ordination biplot of zooplankton communities sampled along the Po river and in four major tributaries in spring 2010 (black) and summer 2011 (grey). Tributaries are indicated by square symbols, and numbers indicate progressively downstream stations and tributaries as follows: 1: REA\_01; 2: TIC; 3: POR\_02; 4: COR\_03; 5: MOR\_04; 6: ADD; 7: CRE\_05; 8: STA\_06; 9: VIA\_07; 10: RIV\_08; 11: OGL; 12: BRG\_09; 13: MIN; 14: SAC\_10; 15: PON\_11; 16: SER\_12. Environmental variables with significant ( $p < 0.05$ ) correlations to either axis are shown in the bottom left corner. Stress: 0.05.