Chloride balance in freshwater system of a highly anthropized subalpine area: load and source quantification through a watershed approach

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Key Points:

- A chloride increase was detected over twenty-five years in a lake of the mid-latitudes, not related to the discharge of inflows.
- The increased anthropization in the watershed is likely the main cause of the chloride increase in the lake.
- Road deicing salt is the main source of chloride in the watershed, released only in the winter season.

Abstract

Recent studies have highlighted an increase of chloride in many lakes worldwide, with negative effects on chemical and physical properties of inland waters and freshwater biota. In this study, we assessed the long-term trend (1993−2017) of chloride in Lake Iseo, located in the mid-latitudes, and analyzed its relationship with discharge data of the inflows and hydrological and hydroclimatic changes. Additionally, we performed a specific annual survey, collecting water samples at a biweekly frequency in tributaries, calculating chloride load, and comparing the result with the chloride increase observed in lacustrine water. The combined use of GIS and multivariate analyses allowed identification of possible sources of chloride and their seasonal dynamic within the mixed-land-use watershed: impervious surface and deicing salt, population and sewage effluents, livestock and rainwater. Their potential contribution to total mass balance was assessed, cross-checking the results between chloride source evaluation and load estimation. Chloride load from wastewater treatment plants played an important role, albeit we highlighted that the main source was the road deicing salt, with a primarily winter contribution. The increased anthropization in the watershed was likely the main cause of the chloride enhancement. This study demonstrates that the problem of salinization can also affect lakes located in the mid-latitude areas and provides a reproducible method for the identification and quantification of the different sources. Our results will help to better understand the potential sources of salinity in rivers that may reveal processes controlling the salinization of freshwater systems, with implications for future management practices.

1 Introduction

Many long-term studies have reported a human-induced salinization process in lakes worldwide and a sharp increase of chloride, which is a good proxy for salinity as it is a highly conservative and soluble ion (Dugan, Summers, et al., 2017; Rogora et al., 2015). Elevated salt concentrations represent a concerning ecological issue and exert an adverse effect on freshwater ecosystems, affecting the physical, chemical, and biological features of surface water (Hintz et al., 2017; Thunqvist, 2004). Indeed, an increased chloride concentration can condition the acid neutralizing capacity of the water and can change the density gradient in lakes, with subsequent incomplete or delayed circulation, increasing the mobilization and bioavailability of metals and other contaminants, and can negatively affect freshwater biota in several ways, potentially threatening ecosystem services (Cañedo-Argüelles et al., 2013; Dugan, Summers, et al., 2017; Jones et al., 2017; Schuler & Relyea, 2018; Thunqvist, 2004). A high chloride concentration may also cause infrastructure corrosion, damage to vegetation, and the contamination of drinking water supplies (Lax et al., 2017).

Chloride can enter water bodies from both natural and anthropogenic sources. Natural chloride sources are principally related to atmospheric deposition and rock weathering. However, human activities play a major role and the recent increases in chloride concentrations are thought to be human-caused (Hubbart et al., 2017; Müller & Gächter, 2012b; Panno et al., 2006). Road deicing salt has been widely recognized as one of the major sources of chloride in streams, rivers, and lakes, especially across north temperate climates in North America and Europe (Dugan, Bartlett, et al., 2017; Novotny et al., 2008). The main agent used is sodium chloride (NaCl), which is highly soluble and has a moderate cost (Novotny et al., 2008; Rogora et al., 2015). However, a huge percentage of the applied salt reaches surface water bodies via runoff: while sodium (Na^+) is

trapped in the soil by the cationic exchange, chloride is a conservative ion and remains in freshwater or passes down to groundwater (Lax et al., 2017; Rogora et al., 2015).
Besides deicing salt, another important anthropogenic source of chloride is wastewater, due to, among others, treated and/or untreated sewage, human wastes, garbage disposal wastes, and water softening (Dugan, Summers, et al., 2017; Panno et al., 2006; Rogora et al., 2015). Chloride is not removed during the conventional sewage treatment to an appreciable extent; therefore, treated waters can discharge a similar chloride load as untreated waters. Moreover, some treated water could have higher chloride loads, since certain chemical treatment practices add chloride salts (e.g., $AgCl_3$, $FeCl_3$) to effluents to remove suspended particles and enhance the phosphorus removal process (Hubbart et al., 2017; Rogora et al., 2015). Fertilizers, livestock waste, industrial effluents, and solid waste incineration constitute further chloride sources (Dugan, Summers, et al., 2017; W. R. Kelly et al., 2012). In general, the literature has reported widely the strict relationship between chloride load to aquatic ecosystems and watershed land use practices (Hubbart et al., 2017; Lax et al., 2017; Müller & Gächter, 2012b). Thus, understanding the various set of chloride-related pressures in a mixed-land-use watershed could represent a complex challenge, but, at the same time, this is the only way to put in place a suitable management strategy. Indeed, the identification of contamination sources is unavoidable to mitigate water contamination problems (Hubbart et al., 2017; Panno et al., 2006).
Even if the sources that can cause this process can vary widely depending on the regional- and watershed-scale, the salt pollution is a concern in many freshwater basins worldwide, to the point where it has been described as a "salinization syndrome on a continental scale" (Kaushal et al., 2018). Moreover, salinization acts coupled with pre-existing stressors, resulting in new combined effects. It is reported that salinization and climate change might have synergistic effects that weaken vertical mixing dynamics and seasonal replenishment of deep-water oxygen in lakes (Lind et al., 2018). Monitoring and management standards are still lacking and there is a need to increase society's awareness of the problem, to avoid reaching levels of concern regarding salt pollution. Indeed, even if the sources of salinization could be eliminated immediately, salts can remain in freshwater environments for years or decades (Schuler et al., 2018). Thus, studies that allow the identification and quantification of possible sources and that provide an estimation of the external loading of chloride from the catchment are particularly valuable.
The enhancement of chloride concentration has been reported in many freshwater ecosystems of Po River basin (Northern Italy), one of the most populated and heavily impacted areas in Europe, in particular in the deep South-Alpine lakes (i.e. lakes Maggiore, Como, Iseo, Garda), whose water represents the 85% of the national freshwater reservoir and are widely used for irrigation purposes and as drinking sources (Rogora et al., 2015). Thus, a chloride increase in these basins could have important ecological and socio-economic consequences. To address this matter, the overarching goals of the current work are to a) determine the amount of chloride that reaches one of these basins (Lake Iseo as a reference case) carried by the different tributaries, and its increase over time, and b) identify the possible sources of this anion, quantifying their respective contributions, and the possible cause of the increase. These two objectives were pursued through a sequential approach:

a) Identification of the possible relationship among the long-term time series of chloride concentration recorded in Lake Iseo and hydrological variables (e.g., enhanced rainfall, increased discharge);
b) Estimation of the mass balance of chloride in Lake Iseo;

c) Estimation of the percentage apportionment of the possible chloride sources in Lake Iseo watershed;
d) Analysis of the temporal evolution of the main sources within the watershed during the last 20 years.

2 Materials and Methods

2.1. Study area

The Oglio River, a left-side tributary of Po River, with a length of 280 km and a basin of 6650 km^2, is the main inflow and outflow of Lake Iseo. The Oglio River watershed is usually divided into an "upper" (upstream of Lake Iseo, delimited by the Adamello glacier to the north and by Lake Iseo to the south,) and "lower" (downstream of Lake Iseo) part. This study concerns the major part of the Lake Iseo watershed (thereafter "Lake Iseo watershed"), which includes the upper part of Oglio River watershed and the watershed of Borlezza Stream, which is the other main tributary of Lake Iseo (Fig. 1). The basin altitude ranges from 3000 m a.s.l. to 186 m a.s.l., with an average altitude of 1429 m a.s.l. The major part of the watercourses presents a short path and high slope. The climate of the study area is temperate continental, with a mean temperature of 13 °C in the hilly zone, 10 °C in the pre-alpine area and 6 °C in the alpine area. The mean annual precipitation is around 1500–2500 mm (Fig. S1a). Snowfall is frequent in the late-winter period, especially above 800 m a.s.l. (CMSB, 2007). The substrate is characterized by igneous rocks in the northern area of the valley, sedimentary rocks (mainly sandstones, conglomerates, limestones, and dolostones) in the southern portion, and metamorphic rocks (mainly mica schists) that characterize the central portion (CMSB, 2007). The main aquifer system is located at the valley floor of Oglio River and is composed of alluvial, fluvioglacial and lacustrine deposits. This aquifer is unconfined and the groundwater table depth is around 10−15 m bgl. The Oglio River can be losing water (mainly in its northern stretches), or gaining water, and thus collecting water from the aquifer. Groundwater circulation is short due to the limited extent of the system (i.e., a shallow system, limited to the valley floor, with a short lateral extent).

Lake Iseo is a deep south-Alpine lake, the fifth-largest Italian lake in terms of volume (7.6 km^3), with a surface area of about 62 km^2, located around 175 km far from the sea. A great drainage basin characterizes this lake; the ratio of catchment to surface area is about 30. The maximum and mean depths are 258 m and 124 m, respectively. The lake is included in the LTER network (Site LTER_EU_IT_008—"Southern Alpine Lakes"; http://www.lter-europe.net) (Leoni, Marti, et al., 2014; Minella et al., 2016). The level of the water surface of the lake is controlled by a dam built in 1933 at the outflow of the Oglio river. The dam allows a fine regulation of outflows that satisfy the irrigation water needs, accumulating water in winter and spring and releasing it in the growing season (summer), while maintaining the environmental base-flow of the Oglio River downstream. The in-lake oscillation ranges between a minimum of −0.3 m and a maximum of 1.1 m, referred to as the zero point (185.13 m a.s.l.); this excursion (i.e., 1.4 m) corresponds to 85.4 Mm^3 (Consorzio dell'Oglio, 2017; Pilotti et al., 2013).

Lake Iseo is a naturally oligotrophic water body; however, over the past 40 years, the increase in nutrient loadings brought the lake to a meso-eutrophic condition, with an average concentration of total phosphorus of 80–90 $\mu g\ P\ L^{-1}$ (Leoni, Marti, et al., 2014). Conductivity and pH along the water column range among 254.2–299.6 $\mu S\ cm^{-1}$ and 7.2–8.2, respectively. Over the past 35 years, the thermal regime of Lake Iseo abruptly changed, with a less frequent complete winter mixing, which occurred only in 2005 and 2006 during harsh and windy winters (Leoni et al., 2019; Pareeth et al., 2017; Rogora et al., 2018). Studies demonstrated that winter large-scale circulation

atmospheric patterns control a chain of linked causal factors, affecting the winter air temperature, spring water temperature, and the resulting water vertical-mixing depth and epilimnetic concentration of total phosphorus, structure and dynamics of planktonic communities, and pelagic food web (Bettinetti et al., 2012; Leoni, 2017; Leoni, Patelli, et al., 2018; Nava et al., 2017).
This study focused on the three main inflows of Lake Iseo (i.e., Oglio River 'RC', Borlezza Stream 'B', and ex-Italsider Canal 'C', see Fig. 1), as they are likely to summarize the load discharged in the lake watershed. Furthermore, we considered the wastewater plant located at the village of Costa Volpino (Fig. 1), whose effluent directly discharges into Lake Iseo.
The study area is mainly designated as forested and seminatural lands. Indeed, based on the Corine Land Cover classification map (Regione Lombardia, 2018), the percentage distribution of the different land uses is as follows (Fig. S2): forest and seminatural areas (83.6%), agricultural areas (9.2%, of which only 13.7% represent permanent crops and arable land), artificial surfaces (3.8%), water bodies (3.2%) and wetlands (0.2%).

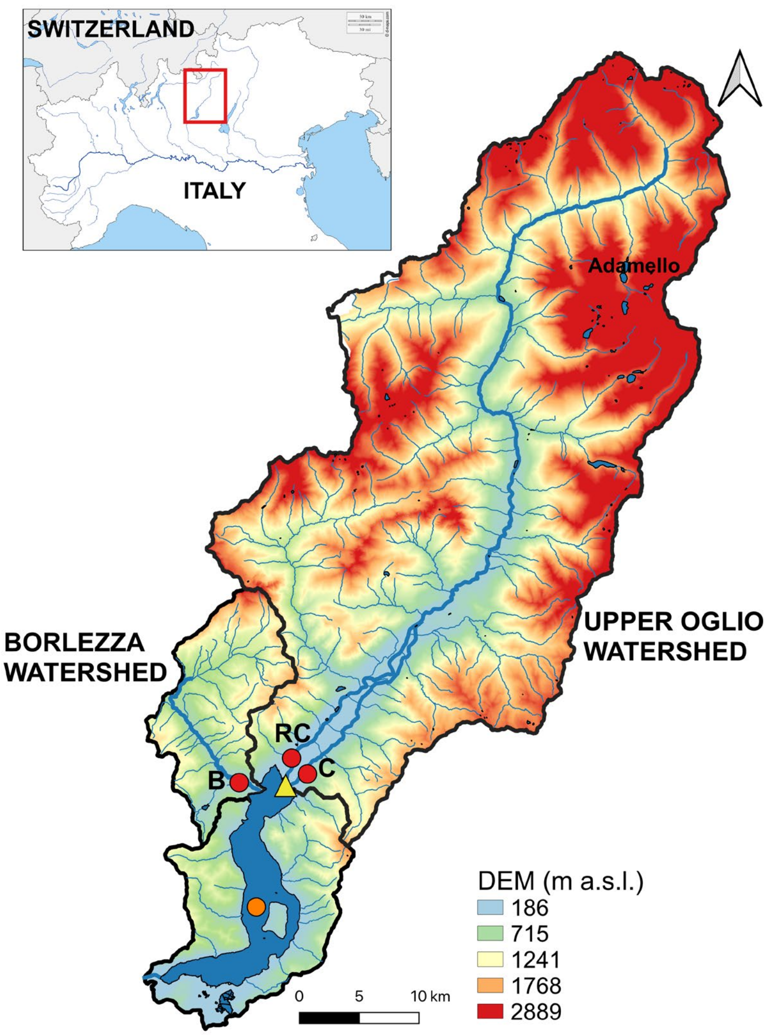

Figure 1. Lake Iseo watershed with the different sub-watersheds delimitated (upper Oglio and Borlezza watershed). The red dots indicate the sample sites with the acronyms of the different stations. 'B' Borlezza Stream, 'RC' Oglio River, 'C' ex-Italsider Canal. The orange dot indicates the location of the sampling site in Lake Iseo and the yellow triangle the location of the wastewater plant of Costa Volpino.

2.1 Data

2.1.1 Legacy data

Discharge data of the three main inflows and the main outflow of the lake were analyzed. Concerning the outflow, the long-term time series of mean daily discharge, calculated through the continuity equation, were provided by Consorzio dell'Oglio, that is the organization who manages the Lake Iseo dam. These data cover the period from 1993 to 2017 (Consorzio dell'Oglio, 2017). Concerning the inflows, gauges of hydroelectric plants located on the Borlezza stream and the ex-Italsider canal provided high-frequency discharge data, with a sub-daily frequency of 1 and 5 minutes, respectively, from May 2016 to April 2017. The discharge of Oglio River was calculated as the difference between the overall discharge into Lake Iseo, calculated by Consorzio dell'Oglio, and the mean daily discharge of Borlezza stream and ex-Italsider Canal.

All data were quality assured/quality controlled (QA/QC), with a graphical inspection of all data to identify data gaps, anomalies and potential pitfalls in the dataset.

Mean daily rainfall (mm) was measured by ARPA Lombardia at the stations of Aprica, Capo di Ponte, Costa Volpino, Darfo Boario Terme, Monno and Pisogne (ARPA Lombardia, 2017). Meanwhile, a reference value of chloride concentration in rainwater equal to 0.6 mg L^{-1} was taken from the previous work by Rotiroti et al. (2019).

The wastewater treatment plant of Costa Volpino supplied scattered values about chloride concentration and discharge of the plant effluent during the study period (May 2016 to April 2017). Land use information about the Lake Iseo watershed at three different times in the basin development (i.e. 1980, 1999, 2015) were provided by Regione Lombardia (2018). These years were chosen considering the availability of information and the better cover for the period throughout the chemical and hydrological parameters have been measured. The number of inhabitants and livestock data in the watershed were supplied by Italian National Institute of Statistics (ISTAT, years 1981, 1991, 2001, 2011), which censes the Italian population every ten years (Table 1).

Table 1. Data Employed in the Present Study and their Sources.

Data typology	Data source
Rainfall amount	Arpa Lombardia, 2018
Chloride concentration of rainfall	Rotiroti et al., 2019
River Oglio discharge	Consorzio dell'Oglio, 2018
Stream Borlezza discharge	Hydroelectric plant company, personal communication, 2018
Canal ex-Italsider discharge	Hydroelectric plant, company, personal communication, 2018
Wastewater plant chloride concentration	Wastewater plant company, personal communication, 2018
Wastewater plant discharge	Wastewater plant company, personal communication, 2018
Chloride concentration in Lake Iseo	This study
Anion/cation and nutrient concentration of River Oglio	This study
Anion/cation and nutrient concentration of Stream Borlezza	This study

Anion/cation and nutrient concentration of Canal ex-Italsider	This study
Number of inhabitants in Lake Iseo watershed	ISTAT, 1981, 1991, 2001, 2011
Livestock units in Lake Iseo watershed	ISTAT, 1981, 1991, 2001, 2011
Road network in Lake Iseo watershed	Regione Lombardia, 2018
Land use map of Lake Iseo watershed	Regione Lombardia, 2018
Tourism data	Provincia di Bergamo, 2017; Provincia di Brescia, 2017

2.1.2 Field data

Lake chloride concentration was analyzed within the monitoring programme of Lake Iseo from 1993 to 2017. Samples of water were taken in the deepest point of the lake (45°43'11"N, 10°03'46"E, LTER) from 10 different depths along the water column (0, 10, 20, 30, 50, 75, 100, 150, 200, 250 m), with a frequency variable between 25 and 30 days. Chloride concentration was determined using ion chromatography (Thermo ScientificTM DionexTM, Waltham, MA, USA) (Leoni et al., 2019; Leoni, Garibaldi, et al., 2014; Leoni, Nava, et al., 2018; Marti et al., 2015). The average Cl^- concentrations of each water layer were obtained from the discrete concentrations, weighted by the volume of the respective water layers. Both internal and external controls are routinely applied to ensure the quality of the data. Furthermore, the laboratories involved in long-term studies on the deep south-Alpine lakes (i.e., LTER Network), in which Lake Iseo is involved, regularly performed intercalibration exercises since the 1980s, both in the frame of national and European projects and through ad hoc field and laboratory intercomparisons (e.g. Rogora et al., 2015).

We realized a specific chemical monitoring survey between May 2016 and April 2017 at three sampling points, located in the three main inflows of Lake Iseo (Fig. 1). We performed a grab sampling from each location every two weeks, collecting a total of 99 samples and analyzing, for each one, eight different chemical parameters: total phosphorus (TP), chloride (Cl^-), sodium (Na^+), potassium (K^+), magnesium (Mg^+), calcium (Ca^{2+}), sulphate (SO_4^{2-}), nitrate (NO_3^-). The analyses were performed with spectrophotometry for total phosphorus and ion chromatography for the anions and cations, using standard methods in accordance with APHA-AWWA-WEF (APHA/AWWA/WEF, 2012).

2.2 Data analyses

2.2.1 Time series analysis

To analyze the different time series for both hydroclimate variables within the watershed and lake chemical parameters a "Seasonal and Trend decomposition using Loess" (STL) was performed (Cleveland et al., 1990). Time series generally consists of the following: T_t, a trend component, which represents an upward or downward movement over the time horizon; S_t, a seasonal component, which is a repetitive pattern over time; and I_t, an irregular component, remaining after the other components have been removed. The STL is an iterative non-parametric procedure that repeatedly uses a locally weighted regression (LOESS) smoother to refine and improve estimates of the S_t and T_t components. At the end of the STL process, the seasonal and trend components are extracted from the data series (Cristina et al., 2016). The analysis was performed on the time series of the overall discharges in Lake Iseo and on the time series of the

lake chloride concentration recorded in the whole water column (0–250 m) and in the superficial layers (0–10 m).
Additional statistical tests, well-suited to time series analysis, were performed, such as Mann Kendall Test, to identify the presence of a temporal trend, Sen's slope, to quantify the slope of the trend, and cross-correlation techniques, to test the presence of a correlation between two variables (Montgomery et al., 2011; Yue et al., 2002).

2.2.2 Multivariate analysis, load estimation and source apportionment

The relationship among the chemical parameters collected through the field activity (May 2016–April 2017) was analyzed using principal component analysis (PCA) based on the correlation matrix. We performed separate analyses for the three tributaries, selecting eight different chemical parameters: TP, Cl^-, Na^+, K^+, Mg^+, Ca^{2+}, SO_4^{2-}, NO_3^-. Thirty-three observations were analyzed for each tributary. All data were centered and scaled to allow comparison among parameters. Only those components with eigenvalues higher or equal to one were considered as significant components (Kaiser, 1958).
The scattered chloride concentration and the high-frequency discharge data of the three inflows and the outflow of Lake Iseo allowed the estimation of the incoming and outcoming chloride load and, by difference of these two, the chloride load in the lake. The load estimation was performed through the R package 'RiverLoad' that we previously developed (Nava et al., 2019). This software allowed the estimation of the load from May 2016 to April 2017 (365 days) with seven different algorithms, whose results were then compared (for an extensive explanation of the different methods see Nava et al., 2019). The load estimation was performed with seven (M1-M7) of nine methods available in RiverLoad. Indeed, the regression methods (M8, M9) were not suitable as the relationship between discharge and concentration was weak and consequently only the averaging methods (M1–M6) and the ratio estimators (M7) were applied. Concerning the outflow, its chloride concentration was considered equal to the value recorded in the superficial layer of Lake Iseo after a period of validation (monthly samples for one year) of this assumption (Rotiroti et al., 2019). The selection of the more suitable algorithm for the load estimation was done as follows: the outcoming load was subtracted from the total incoming load and divided by the lake water volume (7.62 km^3), obtaining the annual chloride increase in the lake (mg L^{-1} $year^{-1}$) for the period May 2016 to April 2017; this value was compared with the increase of chloride concentration obtained from the Sen's slope of the monthly measurements in the lake for the same period; the method ensuring the best fit between these two figures was selected as the most suitable. 'RiverLoad' was not applied to the effluent of the wastewater treatment plant at Costa Volpino, due to the limited data available; its chloride load was estimated as the product of scattered discharge and concentration data..
The apportionment of the different sources of chloride was estimated through both literature and measured data. From the knowledge of the studied system and the evaluation of the scientific literature, we selected four possible sources of chloride for the study area: a) road deicing salt, b) wastewater (from both residents and tourists), c) livestock and d) rainfall. The contribution of agriculture and industrial activities is negligible, as only few industrial activities and permanent crops are present in the study area (Fig. S2). The contribution of road deicing salt was calculated considering the total surface area of the road network within the watershed, the amount of salt scattered for squared meters and the number of days in which road salt was applied. The latter was estimated through meteo-climatic data, considering the days in which the mean temperature was below 0 °C. Through the information provided by the different municipalities located in the

watershed, we considered a mean distribution of NaCl equal to 20 g m^{-2}. The population data include both permanent residents and tourists, the latter being almost equally distributed throughout the year. The contribution of the wastewater source was calculated by multiplying the number of total inhabitants by a reference value of a per-capita chloride contribution from sewage treatment plants equal to 8 kg Cl^- (Müller & Gächter, 2012b; Rogora et al., 2015). Manure applied to cultivated land and the weathering of soil contribute to chloride transfer from soil to surface waters, as animal waste contains elevated concentrations of Cl^- (W. R. Kelly et al., 2012; Müller & Gächter, 2012b). Livestock units (LSU) were estimated to allow easy comparison among the species; then, a mean contribution of 42 kg Cl^- $year^{-1}$ per LSU was applied (Müller & Gächter, 2012b). Finally, the rainfall contribution on the entire catchment area was evaluated considering the rainwater amount reported by ARPA Lombardia (ARPA Lombardia, 2017) and the rainwater concentration of chloride of 0.6 mg L^{-1}, reported in the previous work by Rotiroti et al. (2019).The percentage apportionment of the different sources was estimated for the whole year (May 2016−April 2017), the winter months (December−March), and the remaining period of the year (April−November).

A qualitative analysis of the evolution of the watershed over the last 25 years (from 1993−2017) was performed to understand the changes that occurred which might have caused the enhancement of chloride reported in Lake Iseo. The information obtained through the specific survey performed was employed as a starting point for the interpretation and assessment of the chloride sources evolution along the time horizon. Not all the data were available for the same period of time; however, the most appropriate data was collected and, in some cases, an interpolation procedure was performed.

Statistical analyses and figures were produced using different packages (base packages and “ggplot2”, “stlplus”, “factoextra”, “RiverLoad”) in R 3.4.1. (Hafen, 2016; Kassambara & Mundt, 2016; Nava et al., 2019; Wickham, 2009).

3 Results

3.1 Time series analysis of chloride in Lake Iseo and hydrological variables

Lake Iseo experienced increasing chloride concentration throughout the entire water column (0-250 m) from 1993 to 2017 (Fig. 2a), showing a monotone positive trend along the time horizon, confirming what was previously reported by Rogora et al. (2015). The Mann Kendall test highlighted a significant positive trend for Cl^- ($p < 0.001$), with a slope of 0.03 mg L^{-1} $year^{-1}$ and an increase over the 25 considered years of about 33%. Data concerning chloride concentrations between 0 and 10 m of depth are reported (Fig. 2b), as this layer responds to the variations of the hydrological parameters and watershed discharge more quickly than the entire water column (Hogg et al., 2013). Besides the presence of a positive long-term trend, a seasonal component could be highlighted. Indeed, the chloride concentration in the superficial layers increased within the season from September to April each year, despite a reduction in February, with the peaks recorded in January and April. A decrease was recorded between May and August, with the trough occurring in August. This seasonal pattern was not present taking into account the whole water column, from 0 to 250 m (Fig. 2a).

Figure 2c shows the decomposition plot of the overall Lake Iseo inflow discharge (m^3 s^{-1}) recorded on a daily basis from 1993 to 2017. The data, representative of the total inflow of the three main tributaries, showed great variability, with a minimum and maximum value equal to 8.5 m^3 s^{-1} and 655.8 m^3 s^{-1}, respectively, and an average value of 54.5 ± 39.5 (mean ± SD) m^3 s^{-1}. The seasonal component showed two annual peaks, one in May and the other in November; the lowest values

were recorded in February and September. A monotonic positive trend could not be highlighted, and the Mann Kendall test was not significant. The remainder component had a great amplitude.
The STL analysis performed on precipitation data did not show, as previously reported for inflow, any significant trend and the seasonal component had low importance (Fig. S1a). Instead, the seasonal component was relevant in the air temperature time series in which great amplitude was highlighted; similarly, no monotonic trend could be found (Fig. S1b).
Although the inflow did not display a positive monotonic trend over time, consistent variations could be observed in its time series. Discharge was related to rainfall, as highlighted by the cross-correlation analysis. Indeed, this analysis performed on daily rainfall and inflow data showed a high correlation. When considering the Costa Volpino rain station (Fig. S3a), the highest value of correlation was reported at lag 0 ($r = 0.45$, $p < 0.05$) and lag -1 ($r = 0.47$, $p < 0.05$); whereas taking into account precipitation occurred at Edolo (Fig. S3b), the highest correlation could be found at lag -1 ($r = 0.51$, $p < 0.05$), with a value of $r = 0.46$ ($p < 0.05$) at lag 0. Significant values were reported until more than 10 lags in both cases.

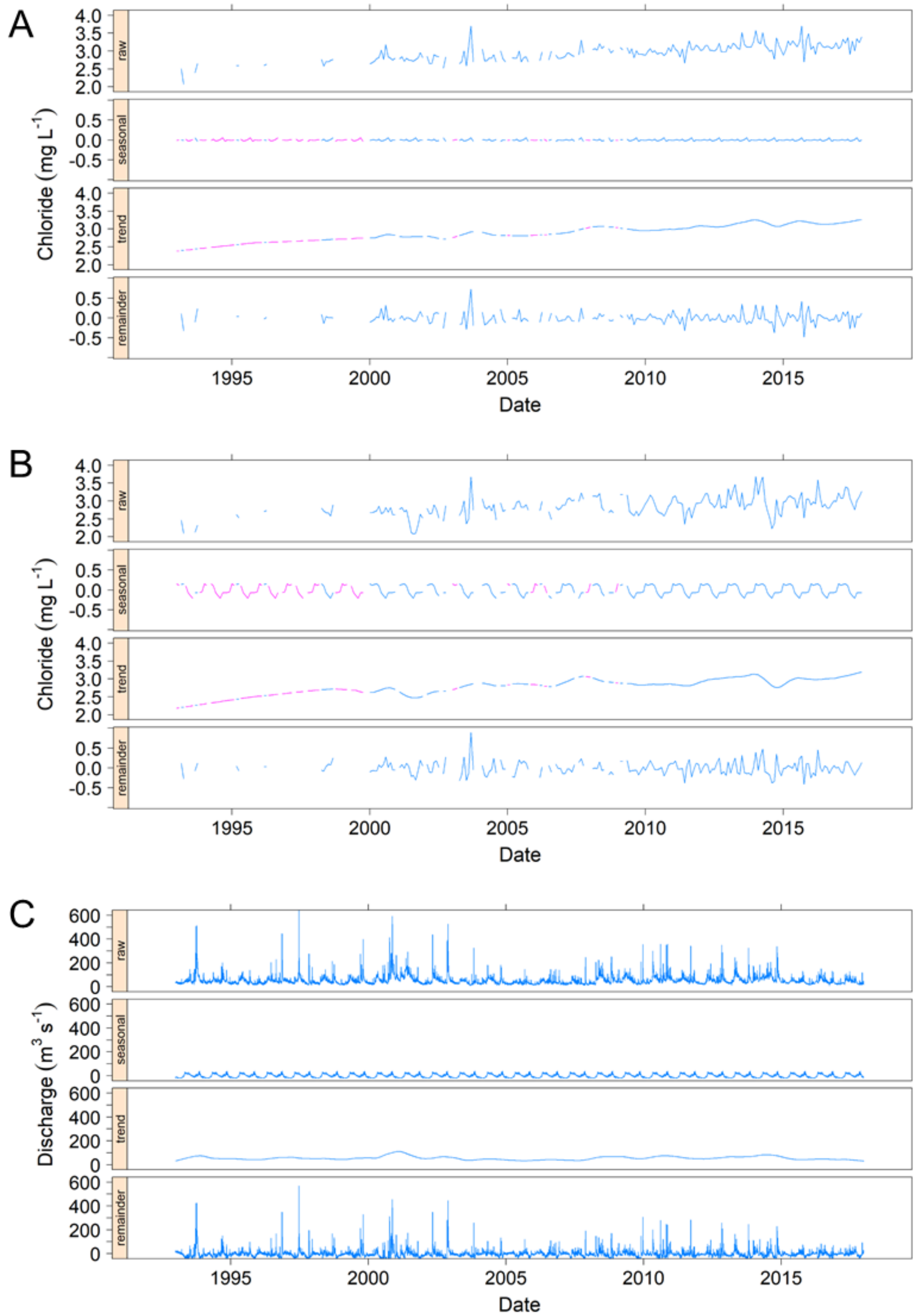

Figure 2. Seasonal and Trend decomposition using Loess (STL) performed on (a) monthly chloride concentration data (mg L^{-1}) collected from 0 m to 250 m; (b) monthly chloride concentration data (mg L^{-1}) collected from 0 m to 10 m; (c) daily inflow data (m^3 s^{-1}) from 1993 to 2017 in Lake Iseo. The original time series is displayed in the top panel ("raw"), the seasonal, the trend and the irregular ("remainder") components in the lower panels. Please note the different scales along the vertical axis. Purple points indicate missing data that have been interpolated.

3.2.Seasonal and annual dynamics of chloride and other compounds in the watershed

The change in concentration over time, from May 2016 to April 2017, in the three main tributaries of Lake Iseo is reported in Figure 3. The three monitoring sites showed important differences in the values of the various compounds analyzed. The lowest concentrations were recorded for almost all the compounds for the ex-Italsider Canal. Instead, high values were highlighted in the Borlezza Stream. Chloride and sodium showed comparable trends, also with similar values, and a comparable pattern was also highlighted for potassium (Fig. S4). The concentrations were fairly constant throughout the monitoring period, except for the increase recorded during the late winter (i.e., February and March).

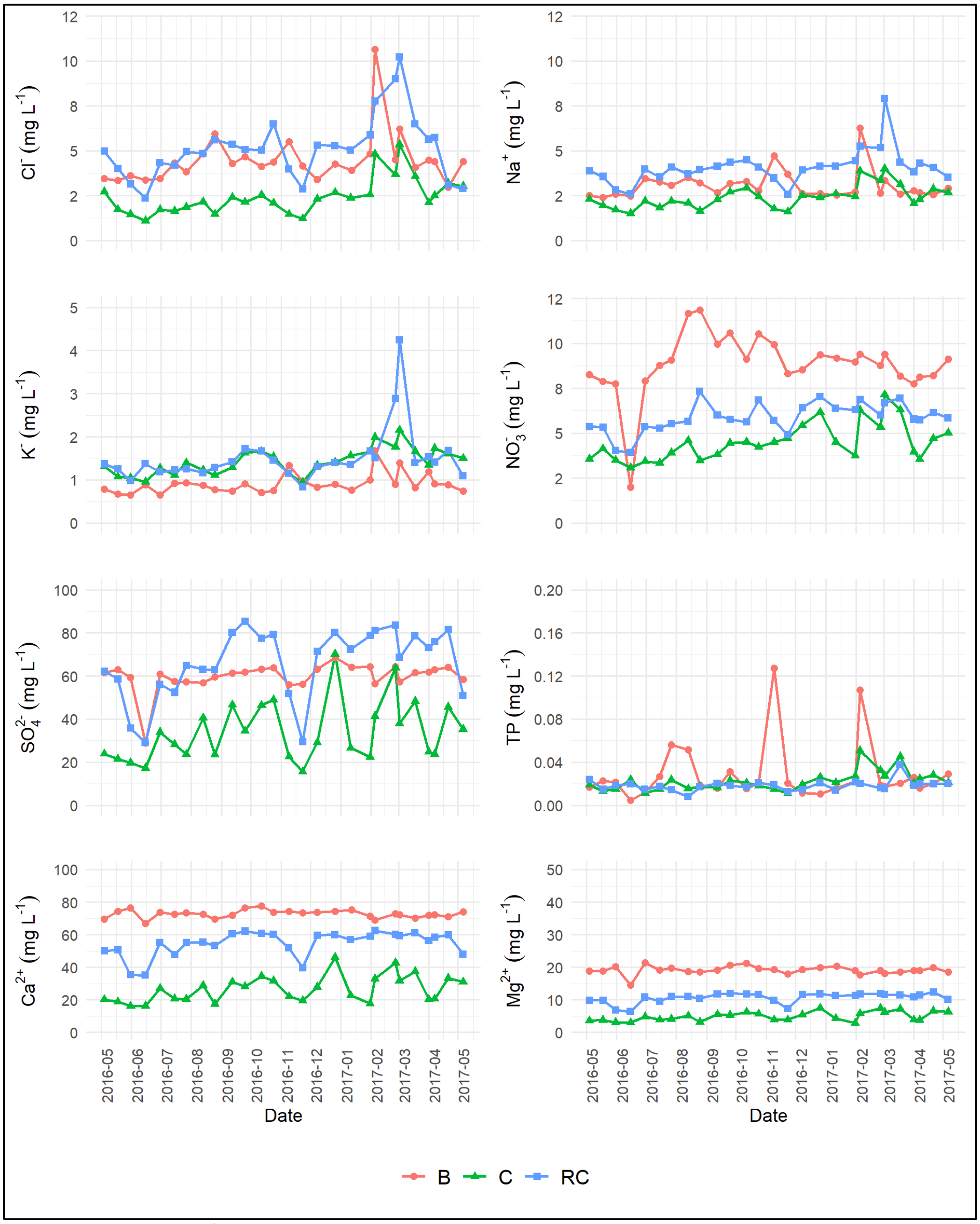

Figure 3. Concentration (mg L^{-1}) of chloride, sodium, potassium, nitrates, sulfates, total phosphorus, calcium and magnesium recorded in the main tributaries of Lake Iseo from May 2016 to April 2017. 'B' Borlezza Stream, 'C' ex-Italsider Canal, 'RC' Oglio River. 'Cl^-' stands for chloride, 'Na^+' sodium, 'K^-' potassium, 'NO_3^-' nitrate, 'SO_4^{2-}' sulphate, 'TP' total phosphorus, 'Ca^{2+}' calcium, 'Mg^{2+}' magnesium.

3.3.Multivariate analyses of concentration data of the lake inflows

Results of the PCA are reported in the scores and loadings plot of Figure 4 and in the loading table (Table S1). Figure 4a showed the results for the Oglio River. On the basis of the Kaiser criterion, only the first two components were selected as significant; these two components explain 81.4% of the total variance of the observations (PC1 63.9%; PC2 17.5%). A strong correlation could be highlighted between chloride and sodium, and to a lesser extent to potassium. The samples related to these loadings were mainly collected during the winter months. Conversely, spring, summer, and fall samples were linked to the remaining loadings, especially nitrates, sulphates and calcium.

The same observations can be reported for the Borlezza Stream, where the first two components were significant and represented the 79.1% of the total variance (PC1 44.2%; PC2 34.9%; Fig. 4b). This trend could not be highlighted for the ex-Italsider Canal, as the winter samples were spread in the plane defined by the first two components (total variance 88.8%; PC1 73.5%; PC2 15.3%). However, the samples collected in summer were mainly distributed near the loadings of nitrates, sulphates, and calcium, as previously highlighted for Oglio River (Fig. 4c).

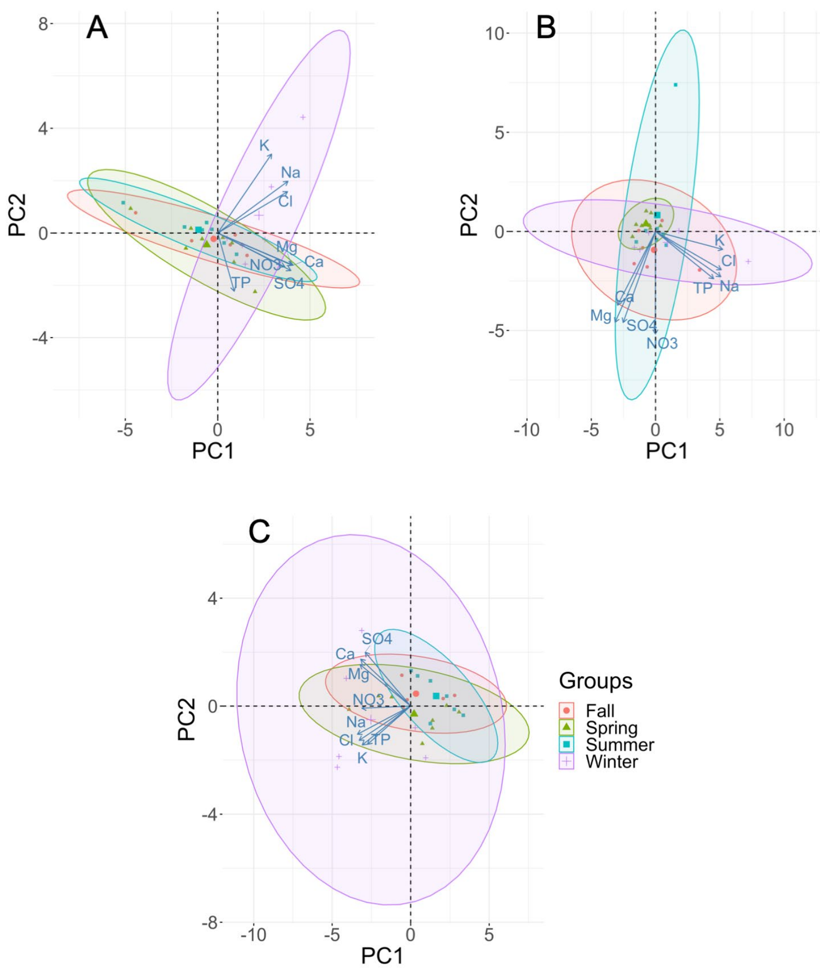

Figure 4. Score plot of the Principal Component Analysis (PCA) performed on the chemical parameters collected in (a) Oglio River; (b) Borlezza stream; (c) ex-Italsider Canal between May 2016 and April 2017. Samples are grouped ("Groups") by different seasons (confidence level 0.95). See Fig. 3 for the abbreviations.

3.4.Discharge data and load estimation

The discharge data of the three main inflows showed large variation (Fig. 5). The major contribution to the total inflow was from the Oglio River, which accounted for 54% on average with a mean discharge of around 23.9 $m^3 s^{-1}$ (median 18.9 $m^3 s^{-1}$). The ex-Italsider Canal displayed a similar value with a mean of 20.2 $m^3 s^{-1}$ (median 19.3 $m^3 s^{-1}$) and a mean contribution of around 44%. Finally, the Borlezza stream only accounted for the 2% of the total inflow, with a mean discharge of 0.8 $m^3 s^{-1}$.

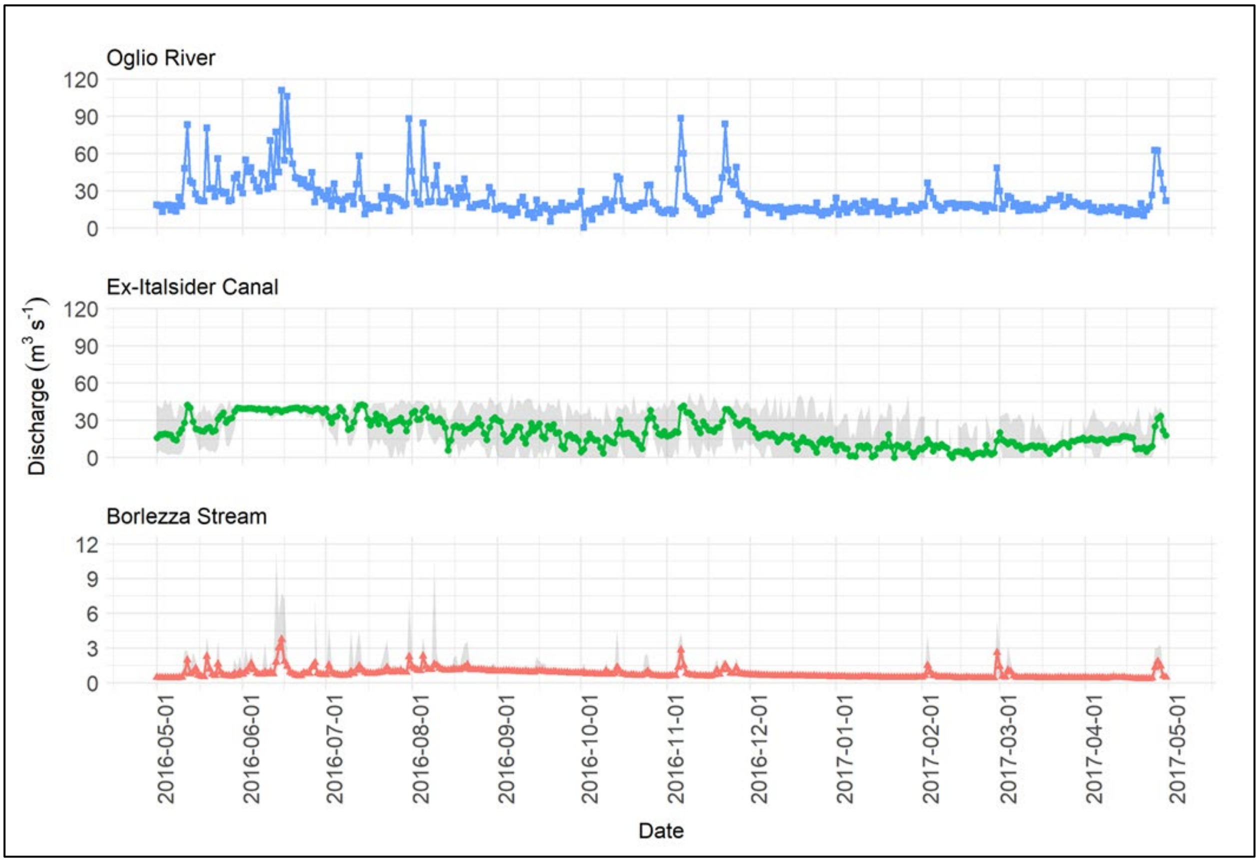

Figure 5. Mean daily discharge data ($m^3 s^{-1}$) of the three main tributaries of Lake Iseo. Please note the different scale for the Borlezza Stream. The grey band represents the daily minimum and maximum value recorded for ex-Italsider Canal and Borlezza Stream, for which high-frequency data, with a sub-daily resolution, were available.

Table S2 reports the results of the chloride load estimation for the sum of the inflows and the outflow and the derived increase of the chloride concentration in Lake Iseo for the all methods considered in RiverLoad (see Sect. 2.2.2). The calculated lake increase from the in-lake monthly sampling was equal to 0.11 mg L^{-1} $year^{-1}$ for the considered period (95% CI [0.087, 0.139]). The method used in RiverLoad that best fit with the value of the increase recorded in the lake is the Beale ratio estimation (M7). This is also one of the most precautionary, with a load value of 5099 tons of chloride for the 365 days considered. This value is distributed among the different inflows in the following way: 3317 tons from Oglio river (65%), 1225 tons from ex-Italsider Canal (24%), and 196 tons from the Borlezza Stream (4%). The contribution of the wastewater treatment plant of Costa Volpino, among the same period, was equal to 361 tons (7%).

With the same algorithm, the mean monthly load carried by the three main inflows was also estimated (Fig. 6). The chloride load showed the maximum value during March (583 tons), whereas the minimum value was recorded in January (284 tons). A slight increase could be highlighted from June to August, followed by a sharp decrease.

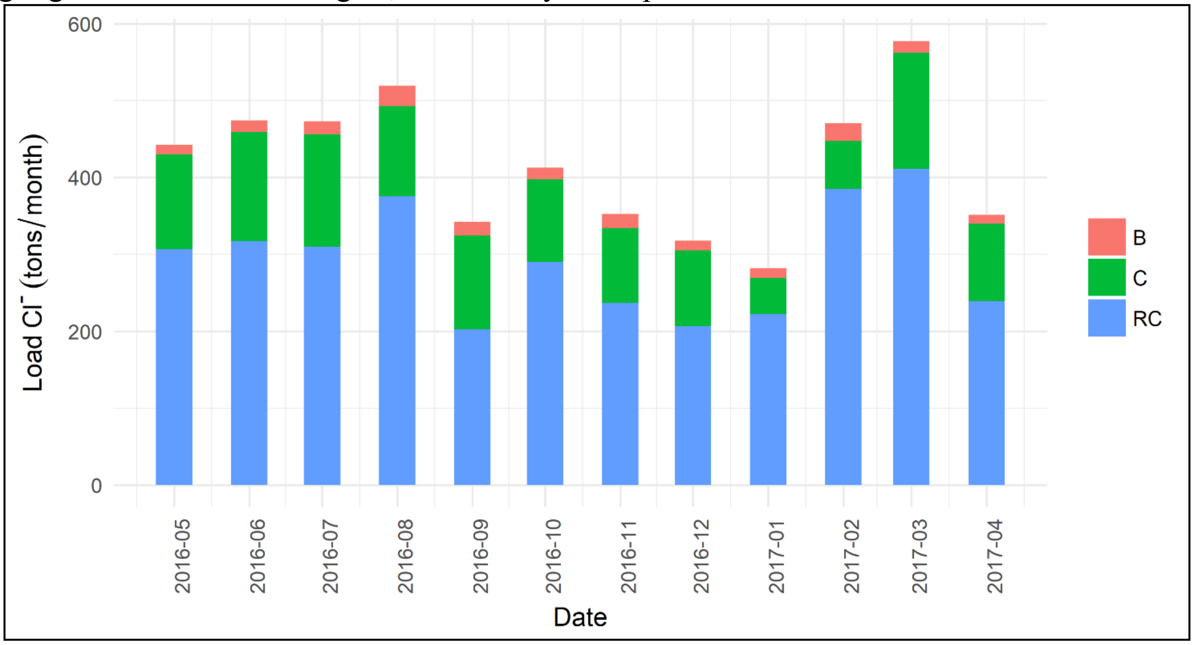

Figure 6. Chloride load in the different months estimated with the R package 'RiverLoad' from May 2016 to April 2017 for the different tributaries: Oglio River 'RC', Borlezza Stream 'B', ex-Italsider Canal 'C'.

3.5.Chloride source apportionment

The contribution of road deicing salts over the reference year of this study (from May 2016 to April 2017) is estimated to be 1657 tons. This value is the product of the road surface area (Fig. S5), the amount of salt scattered (20 g m^{-2}) and the number of days in which road salt was applied.

The contribution of wastewater is estimated to be 1131 tons, calculated by multiplying the total population (residents) in the watershed (141,391 inhabitants; Fig. S6) by the per-capita value of 8 kg. The contribution of tourists is small compared to that of residents, accounting for just 22 tons.

The contribution of livestock is estimated to be 734 tons. This figure is the product of the total number of LSU in the study area (17,475 LSU; Fig. S7) and the mean chloride contribution for each LSU (42 kg $year^{-1}$). The contribution of rainwater is estimated to be 1037 tons, calculated as the product of the annual cumulative rainwater in the study area and the reference value of chloride concentration in rainwater of 0.6 mg L^{-1}.

The sum of all these contributions is 4581 tons, that is in good agreement with the value calculated as the sum of the chloride load of all the three inflows of the lake and the wastewater treatment plant (Sect. 3.4). The difference between these two figures is small (10.1%). The comparison of the data obtained through these two different sets of information (i.e., overall source contribution and estimated load) facilitated a crosschecking operation. The deviation recorded between the two values can be likely considered as uncertainty/error in the estimation or some minor unaccounted chloride sources in the source identification and apportionment. The calculation of the percentage contribution of each source over the total load calculated from the load estimation of the main inflows (5099 tons) leads to the following figures (Fig. 7): 33% for road deicing salt, 23% for wastewater, 20% for rainwater, 14% for livestock and 10% for other unaccounted sources/error of the estimation.

Road deicing salt and livestock are mainly seasonal sources of chloride with a winter and a spring-summer contribution, respectively. Consequently, the data concerning chloride sources were further elaborated to obtain the apportionment restricted to the winter months (December–March) and the remaining period of the year (April–November), recalculating the data regarding rainfall and population for those months. We considered road deicing salt as concentrated during the winter months, and livestock only present between April and November. During the winter months, the road deicing salt accounted for two thirds of chloride, representing 71% of the total. Wastewater only contributed to the 16%, followed by rainfall (6%), with a remaining 7%. For the remaining period of the year, wastewater and livestock gave a similar chloride contribution, accounting for 28% and 27% respectively. Rainfall played the major role, contributing for 33%. The remainder was equal to 12% (Fig. 7).

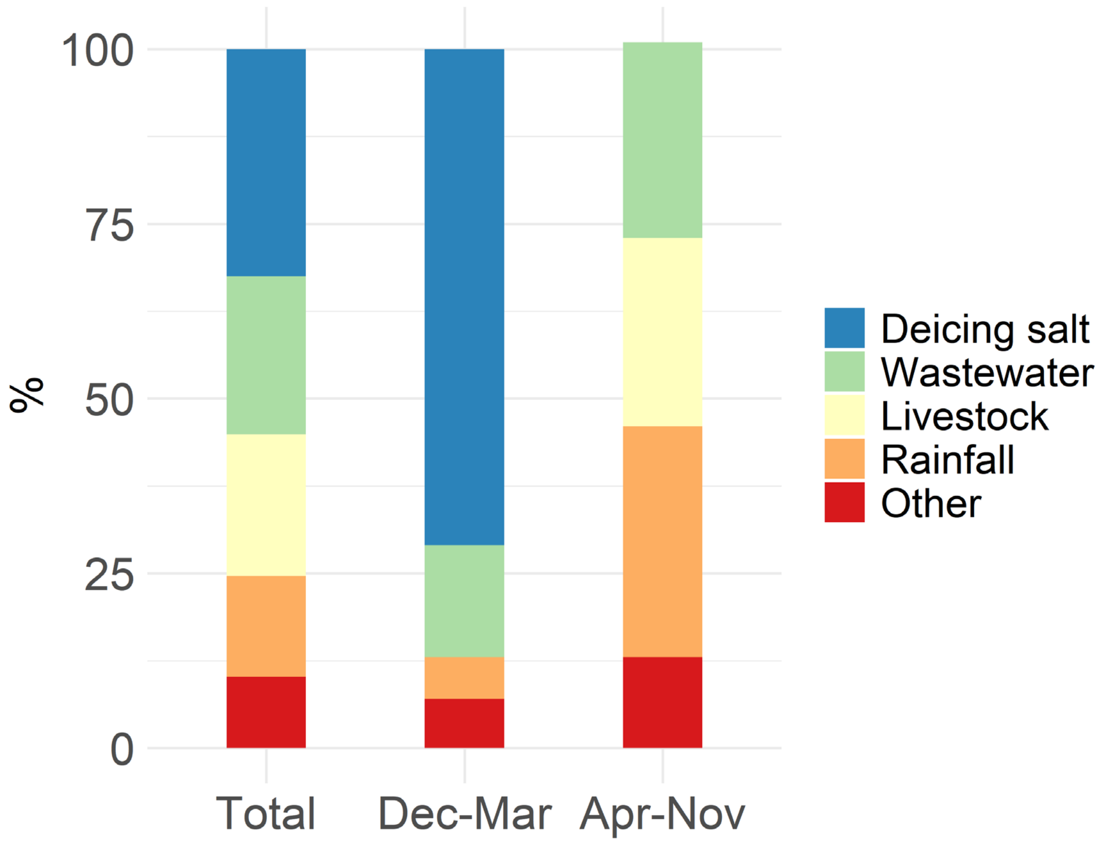

Figure 7. The percentage apportionment of the different sources to the chloride load in the whole year ("Total"), the winter months from December to March ("Dec-Mar"), and the remaining period of the year ("Apr-Nov").

3.6.Long-term evolution of chloride sources

A general increase in population was detected over the last ~25 years, with the number of inhabitants going from 133,186 in 1993 to 141,391 in 2017. The analysis of the land-use maps highlights the changes that occurred in the Lake Iseo watershed: urban areas clearly increased throughout the considered period, spreading mainly close to the Oglio River's course; forested and seminatural areas had a slight increase; and agricultural areas showed a decrease. The increase over time of both the population and the urban areas infers an increase of the wastewater contribution to the total chloride load. Historical information about the road network extension was available only in relation to major streets, and consequently, only an indicative idea can be provided by this analysis. In 1993 the road extension was equal to 1.5 km^2, compared to 2.4 km^2 in 2017, with a difference of about 0.9 km^2. This suggests a likely increase of road salt contribution to total chloride load. Conversely, livestock units showed a slight decrease over the considered period, with a change from 17,902 LSU in 1993 to 17,475 LSU in 2017, resulting in a difference of 427 LSU. Rainfall data did not display significant changes in the considered period, as can be seen from the STL analyses performed on precipitation data (Figure S2).

4 Discussion

4.1. Sources of chloride

The estimation of the apportionment of the different sources pointed that deicing salts are, over the whole year, the major source of chloride in the basin. Many studies performed in lakes of both North-Europe (Müller & Gächter, 2012a; Thunqvist, 2004), and North-America (Corsi et al., 2015; Dugan, Summers, et al., 2017; Godwin et al., 2003; Novotny et al., 2008; Swinton et al., 2015) found results similar to ours, showing that deicing salts represented the main source of chloride. However, the contribution in percentage of these salts to the total amount of chloride load may differ depending on site-specific conditions (e.g., amount of salt applied per kilometer of roads, climatic conditions, urbanization and road extension, private application etc.). Our results (33% for the whole year and 71% considering only the winter season) were in the range reported in literature, even if substantial differences can be highlighted when comparing different systems due to local factors that could influence the importance of the sources (Lax et al., 2017).

The evolution of the chloride load over the different months (Fig. 6) evidenced that the chloride load was not equally distributed throughout the year. We recorded an increase in the winter months, especially in February and March, that can be linked to the road deicing salt source. Moreover, the PCA performed on the concentration data of Oglio River and Borlezza Stream clearly highlighted the relationship between chloride and sodium during the winter months, confirming the origin of this amount of chloride.

It is also possible to see an increase in the chloride load from June to August (Fig. 6). This increase was less pronounced but more prolonged compared to the one measured during the winter months. In this case, it is likely that this increase recorded during the summer months was related to animal husbandry. Indeed, grazing season in Val Camonica generally starts at the end of May and last until the beginning of October, when the livestock is moved back to the lower valleys.

Given these considerations, the calculated percentage apportionment of the different sources over the whole year represented an average condition. Road deicing salt and livestock have a strong seasonal variation. Focusing the attention on the winter months, the percentages of the apportionment changed considerably and the chloride input was mainly concentrated in a limited period of time. The fact that the largest chloride source is operating during a limited period of time has important implications from an ecosystem perspective. Indeed, this means that the concentration is higher during this specific period, as highlighted in the seasonal component of Lake Iseo, with detrimental effects for different taxa (see Section 4.4.).

4.2 Origin of the chloride increase

Our analysis of the long-term time series highlighted the stationarity of inflow data, as there is not an increasing trend throughout the investigated period (see section 3.1.). Consequently, it is possible to exclude that the increase of chloride concentration seen in Lake Iseo can be related to long-term inflow changes, as the temporal evolution of these parameters is not comparable. With an unvaried amount of discharge along the time horizon, the chloride increase has to be linked to the increase of point and non-point sources within the watershed, which is thoroughly investigated below. The increased chloride concentration could not be imputable to livestock activities, as the number of livestock units slightly diminished over recent decades. The resident population, on the contrary, grew and the area increasingly developed as a destination for tourism. Along with the enhanced number of inhabitants, the contribution of chloride from wastewater sources may have increased over recent decades. Finally, the contribution from road deicing salts likely increased over the last decades for the following reasons. Firstly, as the data showed an enhancement of population and of tourism, the application rate could have increased as an attempt to maintain more ice-free conditions; secondly, the density of impervious area per urban area unit could have increased, thereby increasing the need for road salt (Corsi et al., 2015). Finally, even if ongoing climate changes have resulted in warmer winters, supposedly leading to a minor use of road deicing salt, the co-occurring development and strengthening of road infrastructures could have caused an increased amount of salt being scattered. The data collected do not allow a quantitative evaluation and consequently we can only formulate some reasonable assumptions. Thus, considering all points raised, we speculate that the increase of population and the related infrastructures and services (anthropization) in the Lake Iseo watershed from the 1990s to 2017 are likely the main cause of the recorded increase of chloride.

4.3. Alternative mechanisms

The increase of temperature due to global warming could increase the evaporation of water from the lake, thus being a cause of the chloride concentration increase seen in Lake Iseo, as reported in other case studies (Bonte & Zwolsman, 2010). However, the possible role of evaporation from the surface of the lake in increasing the salt concentrations in lake water can be considered negligible, since stable water isotopes data from previous studies (Rotiroti et al., 2019; Zanotti et al., 2019) evidenced that Lake Iseo undergoes little evaporation. The isotopic composition of Lake Iseo in February 2016 ($\delta^{18}O = -9.7‰$ and $\delta^{2}H = -65.9‰$) aligned with the local meteoric water line (LMWL) for northern Italy ($\delta^{2}H = 7.71\ \delta^{18}O + 9.40$), as reported by Longinelli and Selmo (2003), indicating no evaporation, and the evaporation occurred in late summer ($\delta^{18}O = -9.5‰$ and $\delta^{2}H = -64.5‰$ for the September 2016 sample) is estimated, through the Gonfiantini (1986) equation, to be only around 1%.

An alternative explanation to justify the increase of chloride load seen in the summer season could be the gaining by the rivers of groundwater that brings the delayed winter peak of concentrations due to leaching of road deicing salts, as reported in other parts of the world (V. R. Kelly et al., 2008, 2019). However, this seems not to be the case for the present study area since a) rivers and streams are losing water in many parts of the area (especially in their upstream stretches), and b) the main aquifer system is restricted to the Oglio River valley bottom, thus having a limited lateral and vertical extent, that generates short groundwater flow-paths and thus short residence times, which are incompatible with a delay of around 6 months (from winter to summer).

4.4. Ecological Implications

Although the concentration reported is by now below European guideline levels for drinking water (250 mg L^{-1}), the increase in chloride concentrations may be indicative of pronounced and growing human impacts on water quality of the Lake Iseo-Oglio River system (Winter et al., 2011). Moreover, the enhancement of chloride is of concern as sublethal effects of salt remain widely unexplored and different studies have highlighted that taxa changes can occur at concentrations below the threshold of several national guidelines (Kotalik et al., 2017; Rogora et al., 2015; Wallace & Biastoch, 2016). Indeed, it is reported that chloride negatively affects species at all trophic levels, from biofilm to fish, and negative impacts have also been highlighted at the community-level (Hintz & Relyea, 2019). For instance, some evidence suggest as road salt can have a stimulating effect on phytoplankton abundance (Hintz & Relyea, 2019). This can have detrimental effects in Lake Iseo, where the phytoplankton biomass is already high due to eutrophication issues (Leoni et al., 2019). Moreover, changes in the occurrence of various diatoms or phytoplankton species can take place with increased chloride concentration, and this may correspond to stoichiometric changes of algal food resources, with consequences for zooplankton growth and therefore for the entire pelagic community in Lake Iseo. Besides this, there is neither awareness of this problem, as less toxic pollutants such as chloride, can also be widespread, but the magnitude of their effects are often unknown, owing to reduced research attention (Schuler et al., 2018).

4.5. Methodological considerations

In this research, the estimations of riverine chloride load to Lake Iseo for the period from May 2016 to April 2017 were consistent with the value of increase recorded from our data in Lake Iseo. This facilitated the validation of our results. The accuracy of the load estimation, in general, depends on many factors, such as the frequency of sampling, the length of the estimation period, the watershed size of the catchment, the behavior of contaminants, and human activities (Quilbé et al., 2006). Our monitoring programme, based on a fortnightly frequency, could produce, in theory, biased and non-accurate results. However, the crosscheck of the results obtained with those of the lake monitoring made us confident about the method selected and, consequently, the result obtained. Moreover, the application of different methods on the same data set was a way to obtain more information about the reliability of the estimation, as suggested by Quilbè et al. (2006). To the best of our knowledge, previous studies did not adopt this systematical approach and, generally, the estimation was performed by applying only one algorithm. Consequently, the approach adopted in the present study seems to be more accurate. Furthermore, the method selected (the so-called Beale ratio estimator) was shown to be quite accurate and precise in previous studies with a bimonthly sampling frequency where 'real' load data were available (Nava et al., 2019).

5 Conclusions

The present work evaluated the chloride increase detected in a deep lake, located at the mid-latitudes, focusing the attention on the identification and quantification of the possible sources of chloride.

Our main findings are as follows:

- The increase of chloride concentration in Lake Iseo was not linked to variation in discharge along the time horizon, as comparable trends among these long-term time series were not present; consequently, the causes of this increase were researched in point and non-point sources;
- The annual input of chloride was high, with a value of 5099 tons over the considered period, and the main source was represented by road deicing salt, which was followed by the contribution of wastewater effluents. The good agreement between monitored chloride input by tributaries, the increase in the lake's concentration, and the overall contribution of

the different sources indicated that this study captured all relevant sources of chloride to the lake and has given a robust result;

- The chloride source apportionment varied widely between seasons, as some sources (i.e., road deicing salt and livestock) display substantial seasonal variation; consequently, a considerable amount of chloride was discharged in Lake Iseo in a short period of time;
- The chloride increase reported in Lake Iseo was likely to be linked to the growing anthropization in its watershed.

Our data does not indicate at what point specifically the chloride concentration started to rise, but it is likely that the abundance of this anion will continue to increase; consequently, there may be different repercussions for Lake Iseo in the future. The consequences can be relevant not only for the Lake Iseo ecosystem but also for the water that flows out from the lake and reaches the so-called "lower-basin", in which these waters are widely used for irrigation purposes.

Our findings demonstrate that the problem of salinization can affect lakes located at the mid-latitudes, providing at the same time a reproducible approach, which can be adapted to identify and quantify the various chloride sources. Even if the causes of chloride increase can vary based on geographic distribution, this worldwide issue is importantly affecting the deep lakes, which represents a valuable water source. Since salinization can deeply influence the quality of water (Estévez et al., 2018), it is of paramount importance to identify the various anthropogenic sources of chloride and define management plans, aiming to reduce chloride load and avoid reaching the extent of problems already highlighted in higher latitudes.

Acknowledgments, Samples, and Data

We thank Consorzio dell'Oglio for providing useful data and information. We are also grateful to ARPA Lombardia and the hydroelectric plants companies for providing streamflow data. We would also like to show our gratitude to the students who helped collecting samples of water for the analysis of the different ions. If the paper will be accepted, the data will be available at: https://osf.io/?view_only=

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