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Temporal variation in benthic macroinvertebrate community from impaired streams

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ACRONYMS

AIPo	Agenzia Interregionale per il fiume Po
ANOVA	Analysis Of Variance
ARPA	Agenzia Regionale di Protezione Ambientale
CABIN	Canadian Aquatic Biomonitoring Network
CARAVAGGIO	Core Assessment of River hAbitat VAlue and hydromorphological cOndition
CE01	Cedar Creek
CEFI	Canadian Ecological Flow Index
CV	Coefficient of Variation
DI01	Dingman Creek
EPT	Ephemeroptera, Plecoptera, Trichoptera
FBI	Family Biotic Index
GL05	North Thames River
GOLD	Gastropoda, OLigochaeta, Diptera
HMS	Habitat Modification Score
HQA	Habitat Quality Assessment
HSD	Honest Significant Difference
IRSA-CNR	Istituto di Ricerca Sulle Acque - Centro Nazionale di Ricerca
LUIcara	Land Use Index
ME01	Medway Creek
nMDS	non-Metric Multidimensional Scaling
NW01	South Thames River
OPWQMN	Ontario Provincial Water Quality Monitoring Network
OX01	Oxbow Creek
PCoA	Principal Coordinate Analysis
PERMANOVA	Permutational Analysis Of Variance
PLS	Partial Least Squares
RE01	Reynolds Creek
ST05	Stoney Creek

STAR_ICM	Intercalibration Common Metric STAR
UTRB	Upper Thames River Basin
VIP	Variance Importance in the Projection
WFD	Water Framework Directive

ABSTRACT

During the last century global population has experienced immense growth leading to huge changes in land use planning to cope with its own sustentation. More in detail, world population has shifted from an agriculture-based economy to an industrial society, which has pushed the population to move from rural to urban areas. The development of urban areas has led to changes in the physical structure of the environment (i.e. water bodies and surrounding area) being responsible for water quality changes by diffuse and point pollution and alterations in hydrological features such as flow magnitude and frequency. As a consequence of the physical and chemical alterations, instream community structure and composition has been altered and, hence, the ecological integrity of rivers has been jeopardized.

Despite efforts to restore the natural state and functioning of the river systems there is still a lack of knowledge on three questions that I sought to explain in this dissertation: (i) is the variation of macroinvertebrate community inherent to the impairment of the river or is there a natural fluctuations that guides long-term variation?; (ii) how do rivers respond to restoration activities when biological communities may already be adapted to such impaired conditions?; and (iii) which are the most successful restoration measures at improving the biological condition of the river.

To answer these questions I studied impaired river systems in Canada and Italy. Interannual variability of macroinvertebrate community from eight Canadian rivers, representing a gradient of anthropogenic water quality pressures and variable hydrological regimes, were studied over a period of 20 years, focusing on the relationship between water quality, hydrologic variables and sampling features. In Italy the process of restoration of an urban river was followed over a period of 3 years studying the relationship between environmental variables and macroinvertebrate community, focusing on the hydromorphological improvements.

Results of the Partial Least Square (PLS) Regressions on data from the long-term study demonstrated that the benthic community assemblage was not driven by any of the

measured environmental variables (i.e. water quality, hydrologic variables, sampling features), while at a short-term benthic community responded to water quality and hydrometric features, but did not show significant responses to restoration measures. The temporal stability of the studied benthic communities to variations in environmental and anthropogenic conditions may be reflective of the limited pool of tolerant taxa within these systems.

RESUMEN

Durante el siglo pasado, la población mundial ha sufrido un crecimiento inmenso que ha llevado a grandes cambios en la planificación del uso del suelo para hacer frente a su propia sustentación. Más concretamente, la población mundial ha pasado de una economía basada en la agricultura a una sociedad industrial, lo que ha llevado a la población a trasladarse de las zonas rurales a las urbanas. El desarrollo de áreas urbanas ha aportado cambios en la estructura física del ambiente (es decir, a los cuerpos de agua y áreas aledañas) responsables de los cambios en la calidad del agua por contaminación difusa y puntual y alteraciones en las características hidrológicas tales como la magnitud y frecuencia de los caudales. Como consecuencia de las alteraciones físicas y químicas, se ha alterado la estructura y composición de la comunidad acuática y, por lo tanto, se ha puesto en peligro la integridad ecológica de los ríos.

A pesar de todos los esfuerzos para restaurar el estado natural y el funcionamiento de los sistemas fluviales, todavía existe una brecha de conocimiento sobre las tres preguntas que se intentan explicar en esta tesis: (i) ¿es la variación de la comunidad de macroinvertebrados inherente al deterioro del río o hay un ruido natural que guía la variación a largo plazo?; (ii) ¿cómo responden los ríos a las actividades de restauración si las comunidades biológicas ya están adaptadas a tales condiciones deterioradas? y (iii) ¿cuáles son las medidas de restauración más eficientes para mejorar la condición biológica del río?

Para responder a estas preguntas, se analizaron diferentes sistemas deteriorados en Canadá e Italia. La variabilidad interanual de la comunidad de macroinvertebrados de ocho ríos canadienses, que representan un gradiente de presiones antropogénicas de la calidad del agua y regímenes hidrológicos variables, se estudió durante un período de 20 años, centrándose en la relación entre la calidad del agua, variables hidrológicas y características de muestreo. Mientras que en Italia se siguió el proceso de restauración de un río urbano durante un período de 3 años estudiando la relación entre las variables ambientales y la comunidad de macroinvertebrados, centrándose en las mejoras hidromorfológicas.

Los resultados de las regresiones parciales de mínimos cuadrados (PLS) en el estudio a largo plazo demostraron que el ensamble de la comunidad bentónica no fue impulsado por ninguna de las variables predictivas medidas (es decir, calidad del agua, variables hidrológicas, características de muestreo), mientras que a corto plazo la comunidad bentónica respondió a la calidad del agua y a las características hidrométricas, pero no mostró respuestas significativas a las medidas de restauración. La estabilidad temporal a las variaciones en las condiciones ambientales y antropogénicas de las comunidades bentónicas estudiadas puede verse reflejado en el limitado grupo de taxones tolerantes dentro de estos sistemas.

CHAPTER 1. GENERAL INTRODUCTION

1.1. EFFECTS OF HUMAN ACTIVITIES IN AQUATIC ECOSYSTEM

As recorded by the United Nations (UN 2017), world population has consistently increased during the last century, changing from 2.5 to 7.5 billion. This huge population growth has come together with a rapid change in land use with urban areas expanding exponentially. In fact, the rural population has been decreasing since 1950 and as a consequence the urban population has increased in number. Indeed, in 1950 the urban population accounted for the 30% of the worldwide population, while in 2014 it accounted for the 54%, and it is expected to increase up to 66% in 2050 (UN 2018). Hence, **the increase in size population and the economic growth demand entails changes in regional planning and management, both directly affecting land use patterns.**

Due to the changes caused to the environment by human activities (e.g. urbanization, intensification of agriculture, loss of riparian forest) the physical structure of the environment is altered (Allan 2004), leading to changes in habitat structure and availability (Barton and Farmer 1997, Jowett et al. 1990, Miltner and Rankin 1998, Prowse et al. 2011, Qiu et al. 2007, Riseng et al. 2011). The reduction of the naturally vegetated surfaces and the increase of impervious surfaces (Anderson 1970, Graf 1977, Seaburn 1969, Zhang et al. 2010) can cause greater amounts of runoff (Seaburn 1969) and flooding during storm events (Anderson 1970, Schwartz and Herricks 2007). Moreover, a reduction of water infiltration in the terrain (Graf 1977) can alter sedimentation rates in watercourses (i.e. increase in fine sediment deposition) (Hogg and Norris 1991, Wood and Armitage 1997).

Anthropogenic activities (e.g. urbanization, intensification of agriculture, etc.) are the main cause of the degradation of the ecological integrity of rivers in terms of water quality, physical structure and hydrological aspects (Allan 2004, Lenat 1993, Lenat and Barbour 1990, Paul and Meyer 2001). Because of changes in land use, water quality of freshwater environments is compromised (Allan 2004, Konrad and Booth 2002, 2005, Stepenuck et al. 2002) and nutrient composition and availability altered by diffuse and point pollution(Friberg et al. 2003, Yates et al. 2007). Land use changes also lead to alterations of flood magnitude and frequency, changes that are attributable to the increasing intensity of runoff events, the increasing frequency of high flows and daily variation in the stream flow (Anderson 1970, Konrad and Booth 2002, 2005, Richter et al. 2003, Stepenuck et al. 2002).

Main stressors of urbanization in streams and rivers **are changes in hydromorphology (e.g. artificialization of channel and banks) and changes in water quality by spills and diffuse pollution,** which compromise the ecological status of rivers (Allan 2004, Schwartz and Herrick 2007). Past studies have linked the effects of different amounts of urbanization to changes in physical habitat (Fernández et al. 2011, Jowett and Duncan 1990, Richter et al. 2003) available to macroinvertebrate communities (Hogg and Norris 1991), taking into account the alterations in flood frequency and magnitude (Anderson 1970, Konrad and Booth 2002, 2005, Stepenuck et al. 2002) and water quality (Konrad and Booth 2002, 2005, Stepenuck et al. 2002). **Such alterations might change habitat availability (Prowse et al. 2011), promote displacement of organisms (Gibbins et al. 2001, Waters 1972) and affect well-being of biota (Bilotta and Brazier 2008, Newcombe 1994, Palmer et al. 1995).**

Some stressors of agricultural activities in streams and rivers **are changes in hydromorphology on the surrounding area (e.g. deforestation, soil compaction, increase of impervious surfaces) and on the aquatic ecosystem (e.g. the elimination of the riparian area, stream bank erosion, damming for irrigation).** These hydromorphological alterations of the surrounding area mainly decrease the permeability of soils and increase runoff water, which can have detrimental effects on the aquatic ecosystem by increasing the transport of sediments and pollutants to waterways (Stoate et al. 2009, Zia et al. 2013). Soil compaction increases the impermeability of soils, decreasing the rate of infiltration and thus negatively affecting groundwater recharge (Stoate et al. 2009, Tilman 1999, Zia et al. 2013). In addition, changes in the impermeability of the soil surface can increase the intensity of runoff events, which entails a rise in the transport of fine sediments to the water courses (Stoate et al. 2009, Zia et al. 2013). The increase of total suspended and dissolved solids in water leads to siltation events on the riverbed, with subsequent detrimental consequences in spawning areas for fish (Zia et al. 2013). Examples of direct hydromorphological effects on the watercourses are damming for irrigation, which can lead to the loss and fragmentation of the aquatic and terrestrial habitats, favouring the proliferation of exotic species and changing water quality, hydrologic and sediment dynamics (Stoate et al. 2009).

Other important stressors to the aquatic habitat **due to the agriculture are changes in water quality by point and non-point source pollution of nutrients** (Moore and Palmer 2005, Pierce and Yates 2017, Tilman 1999). Activities such as livestock farming and cultivations contribute negatively to the water quality by the use or presence of fertilizers, pesticides and manure, containing high concentrations of phosphorous and nitrogen compounds, heavy metals and pathogens (Moore an Palmer 2005, Stoate et al. 2009, Zia et al. 2013). Coupled with increases in surface runoff, these detrimental compounds can reach the water courses and alter nutrient cycling, water quality and thus, the instream biological community (Harding et al. 1998, Stoate et al. 2009). The increase of nutrients such as nitrogen and phosphorous compounds causes eutrophication, which leads to a cascade reaction in which stream community and structure is simplified, water quality declines, primary production increases and negative effects on fish and macroinvertebrates occur (Stoate et al. 2009, Tilman 1999, Zia et al. 2013). **Long term issues on the biological community (fish and macroinvertebrates) of the agricultural land use have been also explored by some authors, which have concluded that after a long period (30 years) of sustained agriculture, biotic community is so deeply altered that decades are needed for the recovery of the community (Harding et al. 1998).**

1.2. RIVER RESTORATION

Aquatic systems are some of the most modified ecosystems onearth due to anthropogenic activities (Fenoglio and Bo 2009). Fortunately, during last decades environmental sensibility has grown and restoration projects have got a foothold in the management plans. **Historically**, the main **reason for river restoration was aesthetics** but, due to the increasing recognition of the importance of environment health and the progress in the scientific field, different legislations were born to cope with this goal (Stanford et al. 2017). Initially the methods used for river restoration were mainly focused on the modification of the channel morphology but a broader approach in which the "riverscape", described by Stanford et al. (2017) as the ensemble of river and its catchment basin and the interaction between the aquatic and terrestrial ecosystems, is considered are now used. **Thanks to this holistic view of the river ecosystems several river ecosystems all over the world have been subjected to different processes of restoration in order to improve morphological and hydrological conditions, water quality, and biodiversity** (Bernhardt et al. 2005, Fenoglio and Bo 2009, Wohl et al. 2015).

Direct and indirect sources of pollution due to human activities have been one of the main causes of the impairment of water quality in river ecosystems. Indeed, following the industrial revolution and last centuries peak growth in urbanization water bodies were severely degraded in many areas. It has just been during the last decades that, thanks to different legislations that entered into force, water quality has generally improved mainly by eliminating point and diffuse pollution (Bernhardt et al. 2005, Fenoglio and Bo 2009). Another anthropogenic alteration that still persists in several river ecosystems is **channelization**. Embankment of rivers causes the disappearance of habitats, the homogenization of the channel and the destruction of riparian habitats (Bernhardt et al. 2005, Fenoglio and Bo 2009). Several countries have solved this problem by renaturalizing the banks, the refurbishment of the river sinuosity, the elimination of transverse structures and reconstruction of riffle/pool sequences (Bernhardt et al. 2005, Fenoglio and Bo 2009). Another problem derived from human activity and, in to alesser extent, climate change, is the **diminution of water flow, which is associated with serious ecological changes in rivers** (Armanini et al. 2015, Fenoglio and Bo 2009). The urgent need to protect aquatic ecosystems has led to the creation of new guidelines to protect **environmental flows** in different countries during the last decades (Armanini et al. 2015, Fenoglio and Bo 2009). The most common methods for environmental flow assessment have evolved from simpler methods, such as historic flow methods and hydraulic rating, to more complete methods, such as habitat simulations and holistic frameworks (see review by Armanini et al. 2015). Consequently, different approaches have been applied in different countries to model the most suitable measure of environmental flow. In some cases, the improvement of water quality, channel reconfiguration and diversification of habitats and flows (among other measures) have not been enough to improve biological diversity. In these cases, specific measures regarding the biota have been taken, such as the **protection and repopulation of specific species or the reintroduction of autochthonous ones** (usually done with ichthyofauna) (Bernhardt et al. 2005, Fenoglio and Bo 2009).

1.3. BENTHIC MACROINVERTEBRATES AS ECOLOGICAL INDICATORS

All the above-mentioned physical and chemical alterations entail instream community structure and composition changes, with fish and macroinvertebrates the organisms most studied in the matter (e.g. Allan 2004, Hogg and Norris 1991, Lenat and Crawford 1994, Marzin et al. 2012, Paul and Meyer 2001, Seaburn 1969, Wood and Armitage 1997). **Specifically, freshwater benthic macroinvertebrates** have been extensively used as a tool for ecological assessment due to their easy sampling techniques, cost-effectiveness, sensitivity and efficiency for detecting human pressures and environmental stressors (e.g. Allan 2004, Jackson and Füreder 2006, Marzin et al. 2012). In fact, **macroinvertebrate monitoring has been used as a suitable tool for water quality and habitat assessment as they are directly influenced by bank stability, fine sediment, temperature, presence of pollutants, etc. (Carter et al. 2017), but changes in the community can also come from naturally occurring environmental effects that provide the community structure with an intrinsic variation** (Resh et al. 2013, Wiley et al. 1997).

Naturally occurring environmental factors can be divided into two categories depending on the timing of their consequences in the biota: within years and among years. Hence, changes in the biological community can be driven by short term factors including rainfall, temperature and food availability, or by annual and inter-year variability in climatic features such as precipitation and temperature. The biological variability can also be driven by extreme events, such as prolonged droughts and major floods, and multi-year cycles phenomena, such as El Niño and La Niña (Resh et al. 2013).

As a consequence of the anthropogenic activities organisms might be displaced downstream (Gibbins et al. 2001, Waters 1972), their respiratory organs might be damaged (Bilotta and Brazier 2008, Newcombe 1994, Palmer et al. 1995) and attributes such as mobility, size and reproduction might be altered (Palmer et al. 1995). Community composition and structure is also compromised, with richness and diversity metrics being widely described in the literature and confirming that impaired streams present a poor total diversity with taxa adapted to those adverse conditions (Lenat and Crawford 1994, Paul and Meyer 2001, Seaburn 1969). Other studies (Lenat 1993, Lenat and Barbour 1990, Paul and Meyer 2001) have shown how the community was affected by different land uses, showing a gradient from high to low taxa richness from forested to agricultural and urbanized streams, where dominant taxa varied from sensitive (EPT families) to tolerant species (e.g. Chironomidae), respectively.

A major goal of applied ecological research is to understand how temporal variability in environmental conditions drives freshwater biological assemblage (Resh et al. 1988). Indeed, understanding the inter-annual patterns of biological variability is a critical step in the development of sustainable management strategies (e.g. water abstraction management, discharge consents, or riparian land management) that can incorporate the potential effects of environmental changes (Reynoldson et al. 2001, Clarke et al. 2003, Hannah et al. 2004, 2007, Armanini et al. 2014).Environmental fluctuations occur normally in nature as disturbances at a predictable range. These disturbances including climate, geology, topography and hydrological pathways, prompting successional processes in the correspondent biotic community, which show a natural background variability that allow organisms to adjust to the new conditions after a disturbance (Winterbourn 1997). This property of adjustment is currently named in literature as resistance or resilience and is related to disturbance-specific endurances or avoidance strategies (Herskovitz and Gasith 2013, Robinson 2012). M**ore challenging is how this variability affects communities from impaired streams,** a fact that can obscure the effects of the environmental factors (Buffagni et al. 2004, Holling 1973, Winner et al. 1980). Unfortunately, **these type of studies have been usually carried out for management purposes and limited data is available in the peer-reviewed literature** (Jackson and Füreder 2006).

While short time series may not identify the ability of macroinvertebrates to recover from disturbances (Collier 2008, Death et al. 1995, Dodds et al. 2012, Gibbins et al. 2001, Hildrew et al. 1994, Palmer et al. 1995, Resh et al. 2013, Townsend et al. 1987), long-term datasets can detect the temporal variation and impact of both anthropogenic alterations or natural extreme events on freshwater macroinvertebrates (Jackson and Füreder 2006, Resh et al. 2013). They can also identify subsequent community recovery over several generations (Jackson and Füreder 2006, Resh et al. 2013, Trexler et al. 2005). Consequently, long-term studies can predict changes in freshwater community by identifying: 1) fluctuations in extremely variable systems, 2) cumulative effects of stressors, 3) rare events, 4) multi-year cycles, and 5) changes that affect multiple generations (Dodds et al. 2012, Resh et a. 2013). A disadvantage of short term studies is that they can misrepresent macroinvertebrates recovery from disturbances because the community requires a longer time to recover than the available time series (Collier 2008, Death et al. 1995, Dodds et al. 2012, Gibbins et al. 2001, Hildrew et al. 1994, Palmer et al. 1995, Townsend et al. 1987). However, **despite the importance of long-term ecological datasets, studies including extensive paired spatial and temporal data are still limited** (although see exceptions: Buffagni et al. 2009, Jackson and Füreder 2006, Statzner et al. 1994) due to funding constraints, personnel and institutional changes, inaccessibility of data or continuity of the data (Jackson and Füreder 2006, Monk et al. 2006, 2008, Reid et al. 2006).

1.4. GENERAL OBJECTIVE OF THE THESIS AND STRUCTURE

In this research, examined the inter-annual variability in macroinvertebrate communities for various rivers impaired by poor water quality. **The main objective of the research was to discern between the macroinvertebrate responses to natural**

environmental variation and to anthropogenic activities by studying different river systems. The objective of the thesis can be subdivided into two goals: (i) the characterization of long-term variation and the study of the inter-annual variability in environmental and benthic macroinvertebrate community structure; and (ii) the detection of the main environmental factors that best drive macroinvertebrate variation.

The dissertation is subdivided into 4 chapters. Chapter 1 provides background information on the consequences to the aquatic environment from land use changes and introduces river restoration as a solution to cope with this problem, focusing on the importance of long-term data. Chapter 2 examines long-term trends in macroinvertebrate and environmental variables in various impaired streams, and how environmental variables affect the biological community. Chapter 3 present a case study of a restoration plan of an urban river and associated temporal trends in the benthic macroinvertebrate community. In addition a group of hydromorphological variables is studied in order to look into the capacity of the variables to detect the restoration and to quantify the hydrological alteration after the intervention. Chapter 4 summarizes the overall findings of the research, caveats and recommendations, and future research needs.

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CHAPTER 2. TEMPORAL VARIATION IN BENTHIC MACROINVERTEBRATE COMMUNITY

2.1. INTRODUCTION

Anthropogenic activities are the main cause of the degradation of the ecological integrity of rivers in terms of water quality, physical structure and hydrological aspects (Allan 2004, Lenat 1993, Lenat and Barbour 1990, Paul and Meyer 2001). In fact, anthropogenic activities are responsible for changes in water quality (Allan 2004, Konrad and Booth 2002, 2005, Stepenuck et al. 2002) and nutrient composition (Friberg et al. 2003, Yates et al. 2007) by diffuse and point pollution into freshwater environments. In addition, human activities cause changes in the physical structure of the environment, including changes in structure and composition of land use (e.g. urbanization, intensification of agriculture, loss of riparian forest, hydrological alterations) (Allan 2004), as well as reshaping habitat and habitat structure and availability (Barton and Farmer 1997, Jowett et al. 1990, Miltner and Rankin 1998, Prowse et al. 2011, Qiu et al. 2007, Riseng et al. 2011). Associated with land use changes are alterations of hydrological conditions such as flood magnitude and frequency are usually altered, increasing runoff and frequency of high flows and daily variation in streamflow (Anderson 1970, Konrad and Booth 2002, 2005, Stepenuck et al. 2002, Richter et al. 2003). As a consequence of these hydrological changes, sedimentation rate is altered (i.e. increments in fine sediment deposition) (Hogg and Norris 1991, Wood and Armitage 1997).

In agriculturally dominated environments, several stressors, such as sediment, nutrients, and pesticides are common sources of impact for stream ecosystems (Friberg et al. 2003,Yates et al. 2007). Several studies have shown the direct effects of changes in water quality parameters on the benthic community either by focusing on spatial data (e.g. Buffagni et al. 2008) or on causal assessment within experimental sites (e.g. Culp et al. 2014), but few studies have analyzed long-term data to understand the importance of long-term variation of water quality on the benthic community (e.g. Löfgren 2014),particularly while assessing other potentially co-varying factors, such as hydrology.

Long-term ecological researches of aquatic ecosystems are still rare (although see exceptions: Jackson and Füreder 2006, Statzner et al. 1994), even though increasing attention on retrieving ecological longer datasets is evident. Short-term spatial stability of benthic communities in reference and impaired streams has been widely studied across different environmental gradients under both taxonomic and functional approaches (see review of Menezes et al. 2010). Under these studies, taxonomic composition was highly variable (Barbour et al. 1995, 1996) and the different degrees of taxonomic variability on the community responded to environmental conditions while the functional composition of the community was stable in time (Charvet et al. 2000, Li et al. 2001, Statzner et al. 2001). These results suggest that **variability of the benthic community is accompanied by internal noise due to the natural variability of the system** (Vannote et al. 1980).

Hence, a number of questions are raised: **(i) If environmental stressors were constant over time, would the benthic macroinvertebrate community present any sign of cumulative effect?;and (ii) Is the temporal pattern of benthic macroinvertebrates associated with variations in environmental stress?** If we assume that the environmental conditions of the studied sites remain stable over time (Vannote et al. 1980), it is important to understand the provenience of variation of the benthic community (Collier 2008). Hence, if environmental stress is not the main driver of the community variability, then the natural range of variation can obscure the real condition of the river. Environmental fluctuations occur normally in nature as disturbances at a predictable range, and the correspondent benthic community are predicted to show a natural background variability that will allow the organisms to adjust to the new conditions after a disturbance (Winterbourn 1997). Rivers in cold regions are usually subjected to extreme, natural, seasonal hydrological variation, often dominated by a heavy influence of ice (i.e. snow melt and freeze-up conditions; Monk et al. 2011, Peters et al. 2014), which drives the variability in the annual hydrological regime (Bonsal et al. 2006, Burn et al. 2008). However, when the seasonal fluctuations become stronger, presenting higher inter-annual changes in the hydrological conditions, direct effects on the timing of macroinvertebrate life cycles may occur, altering community dynamics across the years (Bradley and Ormerod 2001, Brown et al. 2011, Huusko et al. 2007, Kamler1965, Monk et al. 2006, Poff and Allan 1995, Stickler et al. 2007, Winterbourn 1997). In this regard, several studies have explored the influence of hydrological flows on the aquatic community (e.g. Brown et al. 2011, Huusko et al. 2007, Kamler 1965, Monk et al. 2006, Poff and Allan 1995, Stickler et al. 2007). However, to date, few studies have used long-term paired biological-hydrological data to quantify the relationship between the variation in the composition and structure of benthic macroinvertebrate communities and hydrological events (e.g. Peters et al. 2012).

2.2. MAIN OBJECTIVES

Quantifying the inter-annual patterns of the variability in the benthic macroinvertebrate community is a critical step in the development of sustainable management strategies (e.g. water abstraction management, discharge consents, riparian land management) that can incorporate the potential impact of climate change (Armanini et al. 2014, Clarke et al. 2003, Hanna et al. 2004, 2007, Reynoldson et al. 2001). Here Iexamined the inter-annual variability in macroinvertebrate communities in relation to long-term water quality, hydrological data and sampling features for eight river sites in southern Ontario, Canada, that are subjected to a natural high hydrological variability and are impaired by poor water quality. The main objectives of this chapter were to:

(i) Characterize long-term variation and study the inter-annual variability in benthic macroinvertebrate community structure and assemblage, described by community persistence, compositional stability, and eight common bioassessment metrics. By addressing this objective answered the following question**: is the community showing any pattern or is it changing stochastically in time?**

(ii) Characterize long-term variation in environmental variables (water quality and hydrological variables). **Are there any temporal trends on the environmental variables, which could affect the biota?**

(iii) Examine the associations between water quality and hydrological events and long-term variation in the benthic community. **Which are the environmental drivers in the variation of the macroinvertebrate community?**

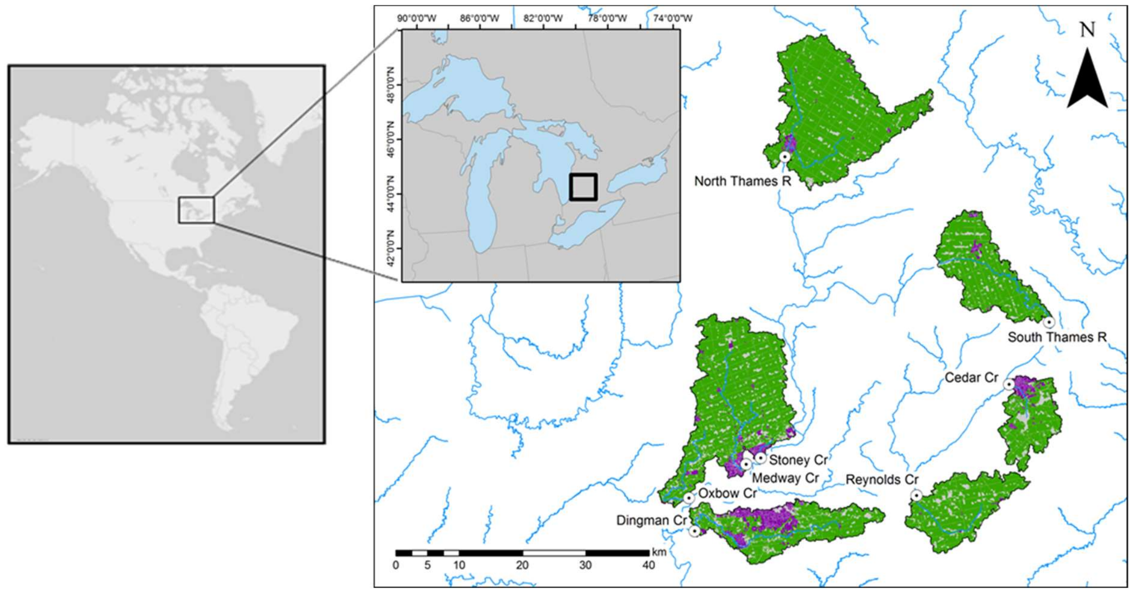
By studying a long temporal period I predicted that I would observe an association between water chemistry and the benthic community assemblage, which should be adapted to the impaired conditions of the rivers. Results of my study can assist in establishing an ecological baseline and provide a framework for future research and ecological management.

2.3. METHODOLOGY

2.3.1.STUDY AREA

My study was conducted in the Upper Thames River Basin (UTRB) in southwestern Ontario, Canada (Fig. 1). The UTRB is characterized by a temperate climate and glacial deposits of tills, sand, and clays. Land cover in the basin is dominated by anthropogenic land use, especially intensive agricultural and urbanized areas, which account for an average of 76 and 8% of the 3,420Km^2 watershed, respectively (Maaskant and Quinlan 2017). The watershed is home to a population of about 472,000 inhabitants (Maaskant and Quinlan 2017). 36% of the watercourses are natural while 64% are modified by channelization or burial (Maaskant and Quinlan 2017). Streams in the watershed suffer from excess nutrients mainly due to non-point sources, increasing loads of phosphorous and sediment in the river system (Nürnberg and LaZerte 2015). Point sources have also lead to important pollution in the area but recent studies on the watershed claim a significant reduction on pollution spills (mainly chemical, fuel and sewage spills) from 670 reported spills in 2010 to 390 in 2015 (Maaskant and Quinlan 2017). Long-term mean annual flows (from 2011 to 2015) ranged from 2.8 $m^3 s^{-1}$ at tributary subwatersheds to 13.6 $m^3 s^{-1}$ at main subwatersheds (Maaskant and Quinlan 2017). From the 28 subwatersheds that constitute the UTRB, 8 sites form different subwatersheds were chosen for our study due to data availability (Fig. 1).

Figure 1. Dominant land uses and location of the Upper Thames River Basin study area (southwestern Ontario, Canada). *Green: agricultural land use; Purple: urban land use; Light grey: other uses. White circles (at the outlet of each subwatershed): Upper Thames River Basin (UTRCA) biomonitoring and Environment Canada hydrologic monitoring stations.Map created with ArcGIS® software by Esri®.*



Data for our study were collected from eight streams with drainage areas ranging from 37 to 315 km^2 between 1997 and 2016 (Table 1). Compared to the overall land use in the whole watershed, the drainage area of the majority of the study sites was occupied by higher proportions of agricultural land use (values higher than 76%). At the same time, the study sites that presented significant urban land use showed higher values than the overall proportions on the whole watershed (values higher than 8%). In particular, Cedar, Dingman and Stoney Creek catchments had more than 10% of urban land cover. Four of the streams had run of the river dams located upstream of the sampling site. Landscape characteristics were extracted from Maaskant and Quinlan (2007). The data for these subwatersheds were obtained by GIS (Geographic Information System) and SOLRIS (Southern Ontario Land Use Resource Information System) by experts from the Upper Thames River Conservation Authority. Regional land use datasets were only available for 2011 and 2017, but since land use did not change significantly over time and values were similar to the previous 5 years, data were associated with each site as a constant value (Table 2).

Table 1. Landscape characteristics (drainage area, land cover, dam presence) of the eight rivers of the Upper Thames River Basin (southern Ontario, Canada).

River	Latitude	Longitude	Drainage area (km^2)	Urban land cover (%)	Agricultural land cover (%)	Forest cover (%)	Dam present
Cedar Creek	43.122	-80.7515	87.75	10. 0	70. 6	3. 8	yes
Dingman Creek	42.9341	-81.3513	148.59	15. 5	64. 0	8. 7	no
North Thames River	43.4504	-81.2068	315.4	1. 3	90. 4	3. 1	yes
Medway Creek	42.966	-81.4180	85.66	3. 8	82. 4	6. 6	yes
South Thames River	43.2153	-80.6919	148.85	0. 6	84. 8	5. 7	no
Oxbow Creek	43.0137	-81.2804	203.19	1. 8	85. 3	5. 0	yes
Reynolds Creek	42.9816	-80.9546	145.14	2. 4	79. 6	9. 6	no
Stoney Creek	43.022	-81.2534	37.33	14. 4	62. 8	7. 2	no

Note: Subwatershed characteristics drawn from Maaskant and Quinlan (2007). North Thames River includes both Whirl and North Mitchell UTRCA subwatersheds.

2.3.2. DATA COLLECTION

Environmental data

Water chemistry data were obtained from the Ontario Provincial Water Quality Monitoring Network (OPWQMN) database (http://www.ontario.ca/environment-and-energy/provincial-stream-water-quality-monitoring-network). Water quality samples were generally collected monthly under ambient flow conditions during the open water period for each year from 2004 to 2014 (Table 2). Medway Creek had a longer dataset extending from 1997 to 2004. Data from 2006 was missing from Reynolds Creek. We calculated annual averages of conductivity, nitrate, and total phosphorus samples. Information about total nitrogen and phosphates was also gathered but due to the lack of records for the whole study period at Medway Creek, we decided not to include these two parameters in the analysis. Likewise, the 5 to 7 samples available for most sites, for most years was deemed insufficient to generate meaningful annual averages of water temperature for the sampling sites. Annual averages of the three analyzed water quality parameters were calculated based on all water samples available from the 12 months preceding each benthic sampling event, generally from June to May.

Table 2. Co-occurrence of hydrological, chemical and biological data on the eight rivers of the Upper Thames River Basin (southern Ontario, Canada).

	1997	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
Cedar Creek	○ ●	○ ●	○ ●	○ ●	○ ●	○ ●	○ ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ ●	○ ●
Dingman Creek	●	○ ●	○ ●	○ ●	○ ●	○ ●	○ ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ ●	
North Thames River	○ ●	○ ●	○ ●	○ ●	○ ●	○ ●	○ ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ ●	○
Medway Creek	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ ●	○
South Thames River	○ ●	○ ●	○ ●	○ ●	○ ●	○ ●	○ ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ ●	○
Oxbow Creek		○	○	○	○	○	○ ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ ●	○
Reynolds Creek		○	○	○	○	○	○ ●	○ x ●	○ x ●	○ ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ ●	○ ●
Stoney Creek							○ ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ x ●	○ ●	○

Notes: ○ Benthos x Chemistry ● Hydrology

Hydrological variability of northern rivers has been well documented within Canada (Monk et al. 2011). Due to its geography, rivers in Canada are subjected to extreme, natural, seasonal variation including a heavy influence of ice (Monk et al. 2011, Peters et al. 2014). Seasonal variations, often dominated by snow melt and freeze-up conditions, drive variability in the annual hydrological regime (Bonsal et al. 2006, Burn et al. 2008). Therefore, it is critical to incorporate variability in hydrological data with patterns in long-term biological data (Trexler et al. 2005). Hence, hydrologic stations on all sites continuously monitored discharge and were maintained year round. Hydrologic data were extracted for the eight study sites from the HYDAT database (http://www.ec.gc.ca/rhc-wsc/default.asp?lang=En&n=9018B5EC-1) using HEC-DSSVue v2.0.1 (Environment Canada, Burlington, Ontario UACEH 2009). Hydrological variables were calculated based on the Canadian Hydrological Indicators of Change (CHIC; Monk et al. 2012, Peters et al. 2014), which yield information on measures with key links to ecological processes (Table 3). The variables were calculated for the ice-influenced and open water periods. The ice-influenced period encompassed from December 1st of the year preceding sampling to last day with ice present while the open water period extended from the last day of ice to the biological sampling date for each year. Data were available from 1997 to 2015 at Cedar, Dingman and Medway Creek sites as well as the North and South Thames River sites (Table 2). The remaining three sites, Oxbow, Reynolds and Stoney Creeks, had data from 2003 through 2015. Ice-influenced flow variables described the spring freshet and the peak flow event of the ice-influenced period as well as the date these events occurred on. Open water variables described the magnitude and date of the peak flow event occurring between spring freshet and benthic sampling. Hydrologic variables were selected based on evidence that these hydrologic events are ecologically relevant (Monk et al. 2012, Peters et al. 2014). All flow magnitudes were presented as discharge (m^3s^{-1}) and variables representing dates were given as numerical values representing the hydrologic day of the year, where October 1st is Day 1 and September 30th of the following calendar year is Day 365 (Monk et al. 2012).

Table 3. Relationship between ice-influenced and open water period hydrological variables and ecological processes. *Extracted from Peters et al. 2014.*

Ice-influenced period variables	**Example of ecological importance**
Spring freshet initiation date	Freshet represents key flows that structure aquatic habitat availability and channel morphology through substrate scour and ice jam-associated flooding
Flow magnitude on day of spring freshet initiation	Freshet represents key flows that structure aquatic habitat availability and channel morphology through substrate scour and ice jam-associated flooding
Date of peak water level during the ice-influenced period	Related to the connectivity of lateral and channel habitats
Flow magnitude on day of ice-influenced peak water level	Related to the connectivity of lateral and channel habitats
Open water period variables	**Example of ecological importance**
Date of 1-day minimum open-water flow	Timing of short-term extreme low-flow conditions can influence aquatic spawning
Date of 1-day maximum open-water flow	Timing of short-term extreme high-flow conditions can influence ecological processes cued to water availability

Benthic macroinvertebrate sampling

Benthic macroinvertebrate data were collected annually in the same reach as the hydrological and water quality data, from 1997 to 2016, except for Stoney Creek where data was only available from 2003 (Table 2). Benthic samples were collected in late May or early June by Upper Thames River Conservation Authority staff using a modified Canadian Aquatic Biomonitoring Network protocol (CABIN; Reynoldson et al. 2007). The modified CABIN protocol used a three-minute travelling kick with a D-frame net of 500µm mesh. Samples were initially preserved in 10% formalin solution prior to being transferred to 70% ethanol. Collected samples were processed in the lab using a gridded pan and a fixed count-subsampling process where invertebrates from randomly selected grid cells were removed until a minimum count had been achieved. The required fixed minimum count has varied over the 20-year sample collection history. Samples collected prior to

2000 were subsampled to 100 individuals, whereas samples were subsampled to 200 and subsequently 300 through the early 2000's to present. The required fixed minimum count (i.e. subsampling protocol) was considered in the analyses as a variable in order to assess the impact of changes in the sub-sampling protocol on the temporal and spatial variability of the benthic community.

Individual taxa were identified to family-level in order to avoid rarities of isolated species between sampling sites, resulting in a total of 61 taxa being collected over the study time period. Eight structural metrics were calculated on an annual basis for each sample to assess differences in community composition (Table 4), except for the similarity indices (i.e. Jaccard and Bray-Curtis) that were calculated between consecutive years(Bray and Curtis 1957, Jaccard 1902).

Table 4. List of biological metrics computed for the analysis of the community composition of the eight rivers of the Upper Thames River Basin (southern Ontario, Canada).

Name	Description	Reference
Berger-Parker dominance index	Measure of taxa dominance based on the proportional abundance of the most abundant taxon	(Berger and Parker 1970)
Bray-Curtis dissimilarity index	Values used to assess compositional stability calculated across consecutive years for all sites based on relative abundance	(Bray and Curtis 1957)
Canadian Ecological Flow Index (CEFI)	Measure to assess ecological responses to hydrological alterations	(Armanini et al. 2014)
Hilsenhoff Family Biotic Index (FBI)	Index based on the tolerance of family taxa to organic pollution	(Hilsenhoff 1982, 1987)
Jaccard similarity index	Values used to assess community persistence calculated across consecutive years for all sites based on presence/absence of taxa	(Jaccard 1902)
Taxa richness	Total number of taxa in a sample	(Hering et al. 2004)
% of Chironomidae	Percentage of Chironomidae taxa in the sample	(Hering et al. 2004)

Table 4. Continuation.

Name	Description	Reference
% of EPT families	Percentage of Ephemeroptera, Plecoptera and Trichoptera taxa in the sample	(Hering et al. 2004)

2.3.3. DATA ANALYSIS

Assessment of biological and environmental variability

In order to summarize the biological attributes and the environmental data a descriptive analysis was performed. The environmental data that was included was the water chemistry and the hydrological variables derived from the ice-influenced and open-water periods. It produced a statistical table of means and measures of dispersion, i.e. standard deviation (SD) and coefficient of variation (CV). The analysis was done using the *vegan* package (Oksanen et al. 2009) with R cran (R Development Core Team 2010).

A two-factor permutational analysis of variance (PERMANOVA; Anderson 2001) was used to detect differences between the biological communities in terms of community composition (i.e. presence/absence and relative abundance). The PERMANOVA considered Years (20 years, 1997-2016) and Site (8 sites).The analysis was done using the *vegan* package (Oksanen et al. 2009) using 999 permutationswith R cran (R Development Core Team 2010).

A non-parametric regional Mann-Kendall test (Kendall 1975, Mann 1945) was used to assess the presence of monotonic trends in the biological and environmental time series. The variable of land use was not studied under this approach since, as above-mentioned, the data was stable during the whole study period. Consequently, trend analyses were conducted for each individual parameter of water chemistry and hydrological variables for each individual site to detect within-site variation using the longest dataset available. For the purposes of this study, I defined a “trend” as a long-term linear change in the mean as indicated by the Mann-Kendall analyses. Trends were retained statistically significant if $p< 0.1$, under the null hypothesis, H_0, that biological and environmental data were identically distributed. Theil-Sen’s slope was also calculated as part of the analysis, indicating the linear rate of change (calculated as the median of all slopes), where negative values indicated decreasing trends and positive values indicated increasing trends. The analyses were done using the *rkt* package (Marchetto 2015) with R cran (R Development Core Team 2010).

Environment to biota associations

Partial Least Squares (PLS) regressions (Legendre and Legendre 1998) were used to assess the relationships between the calculated benthic metrics and the three groups of environmental parameters (i.e. water quality parameters, ice-influenced and open-water variables). PLS is a well-tested approach to identify relationships among dependent and independent variables in time series analysis (see Kalela-Brundin 1990, Smoliak et al. 2010) and it is also suitable to test whether variables show significant temporal trends (Kinnard et al. 2011). Through this analysis, I was able to describe multiple ecological response variables simultaneously, and to explore data and patterns while avoiding multicollinearity issues among environmental indices (Bougeard et al. 2011, Eriksson et al. 1995, Olden and Poff 2003).

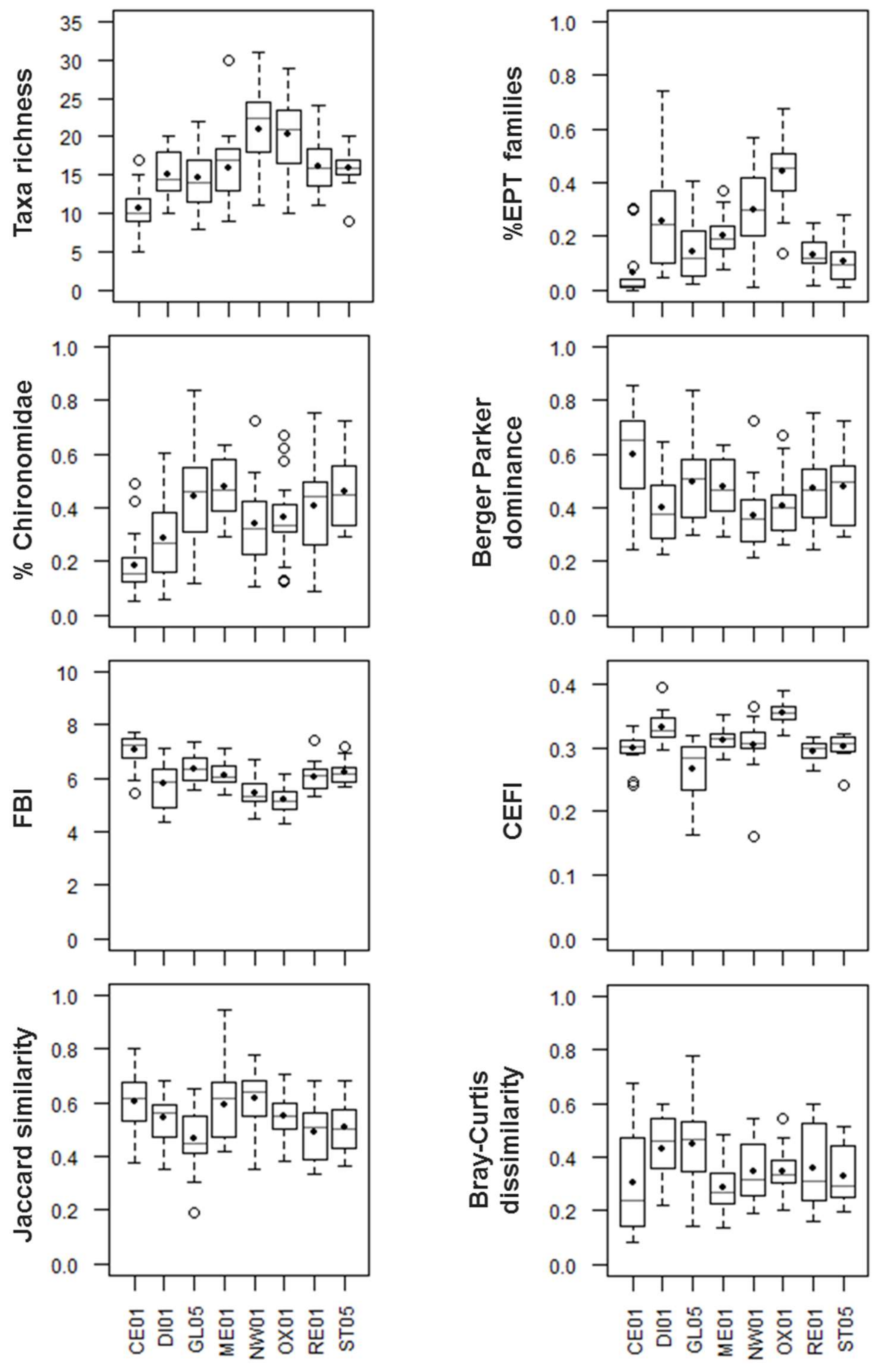
PLS regressions were completed on each site individually using the biological metrics as response variables, and environmental variables as predictors. Years included in the analysis were restricted to ones with a complete set of response and predictor variables. Consequently models tested relationships for 11 years of complete data, excepting Reynolds Creek, which had only 10 years of data in the PLS regression (Table 2).The performance of the PLS models was expressed in terms of the cross-validated explained variances of the environmental variables (r^2_x), cross-validated explained variances of the biological community (r^2_y) and predictive ability of the model (Q^2_y). PLS models were considered significant when $Q^2_y > 0.097$ and to have a good predictive capacity when $r^2_y > 0.5$, according to Trap et al. (2013). A 10-foldcross-validation method, iterated 999 times, was used to select the number of significant components through the calculation of the Q^2_y. The analyses were computed using the *pls* package (Mevik and Wehrens 2007) with R cran (R Development Core Team 2010).

2.4. RESULTS

2.4.1. ASSESSMENT OF BIOLOGICAL VARIABILITY

The descriptive analysis of the benthic macroinvertebrate assemblage resulted in a series of information that is shown in Fig. 2 - 4 and in Annex A. Benthic macroinvertebrate assemblages had an average richness of 10.6 to 21 families across the eight sampled sites with Oxbow Creek and the South Thames River having the greatest richness (Fig. 2 and Annex A). Within-site inter-annual variability of taxa richness as measured by the coefficient of variation (CV) was between 0.2 and 0.3 for all sites except the Stoney Creek site which had a CV of 0.16. Assemblages were generally dominated by EPT taxa (mean range = 10.6 - 64.6%) and Chironomids (18.5 - 45.8%). However, the relative abundance of % EPT varied widely (CV >0.5) among years at five of the sites (Cedar Creek, North Thames River, Medway Creek, Reynolds Creek and Stoney Creek). Percentage of Chironomids was less variable with a maximum coefficient of variation of 0.61 at Cedar Creek. Mean and variance of the Berger-Parker dominance metric was comparable to % Chironomidae at all sites, but the Cedar Creek and Dingman Creek sites, suggesting taxa other than EPT and Chironomids were dominant at these two sites. FBI and CEFI scores exhibited the smallest amount of variability of all calculated metrics with the largest CV of both these metrics across all sites being 0.09. Mean CEFI scores ranged from 2.7 to 3.5 indicating that all sites supported a community adapted to moderate variations in flow conditions. Six of the eight sites had mean FBI scores between 5.51 and 7.5 indicating these sites were scored Fair to Fairly Poor in terms of tolerance to organic enrichment. The remaining two sites, Oxbow Creek and the South Thames River, had mean FBI scores within the upper end of the Good category (4.51 to 5.50).

Figure 2. Inter-annual variation of biological metrics describing benthic macroinvertebrate community composition for 8 rivers in the Upper Thames River Basin(southern Ontario, Canada). *Solid black lines represent median values, solid grey lines represent mean values, boxes represent the 25th-75th percentile range, bars represent the range while circles represent outlier values. CE01 = Cedar Creek, DI01 = Dingman Creek, GL05 = North Thames River, ME01 = Medway Creek, NW01 = South Thames River, OX01 = Oxbow Creek, RE01 = Reynolds Creek, ST05 = Stoney Creek.*



Among-site differences in biological assemblage were similar among the eight study sites over the course of the study period (Fig. 2). Mean community persistence, represented by Jaccard similarity index varied from a maximum of 62% at the South Thames River site to a minimum of 49% at the North Thames River Site. Similarly, mean compositional stability, as represented by Bray-Curtis dissimilarity, indicated that the North Thames River site had the greatest between year change in composition with a mean dissimilarity of 45% whereas Medway Creek exhibited the smallest mean dissimilarity. Furthermore, there was only moderate variation over the course of the study period with CV varying between 0.2 and 0.4 for most sites for both persistence and stability. An exception was Cedar Creek, which had a CV of 0.6 for stability. However, the overall variance was not completely representative of the range of values exhibited by several of the sites. For example, Jaccard similarity exceeded 90% between two consecutive years at the Medway Creek site whereas similarity was below 20% between two consecutive years at the North Thames River site. The North Thames River site also had maximum and minimum Bray-Curtis dissimilarity values that deviated about 40% from the mean value for the site. Similarly, the Cedar Creek site had a dissimilarity value that was nearly 50% greater than the site's mean value.

Community persistence and compositional stability showed a high temporal variability (i.e. inter-annuality by pairs of years) at each site (Fig. 3 and 4). North Thames River presented the lowest values of Jaccard similarity index of all sites, between 1996 and 1997, and also the highest values of Bray-Curtis dissimilarity index of all sites, between 2000 and 2001, suggesting a high species turnover and great variability in the community structure, respectively. Generally, sites seem to show a cyclicality over the whole period in both Jaccard and Bray-Curtis indices, although Stoney Creek seem to exhibit a slightly different pattern during the first years in which it was monitored (2003-2007), characterized by low values of Jaccard and high Bray-Curtis compared to the rest of the sampled period (Fig. 3 and 4).

Figure 3. Inter-annual variation of Jaccard similarity index describing community persistence in terms of presence/absence for 8 rivers in the Upper Thames River Basin (southern Ontario, Canada). *CE01 = Cedar Creek, DI01 = Dingman Creek, GL05 = North Thames River, ME01 = Medway Creek, NW01 = South Thames River, OX01 = Oxbow Creek, RE01 = Reynolds Creek, ST05 = Stoney Creek.*

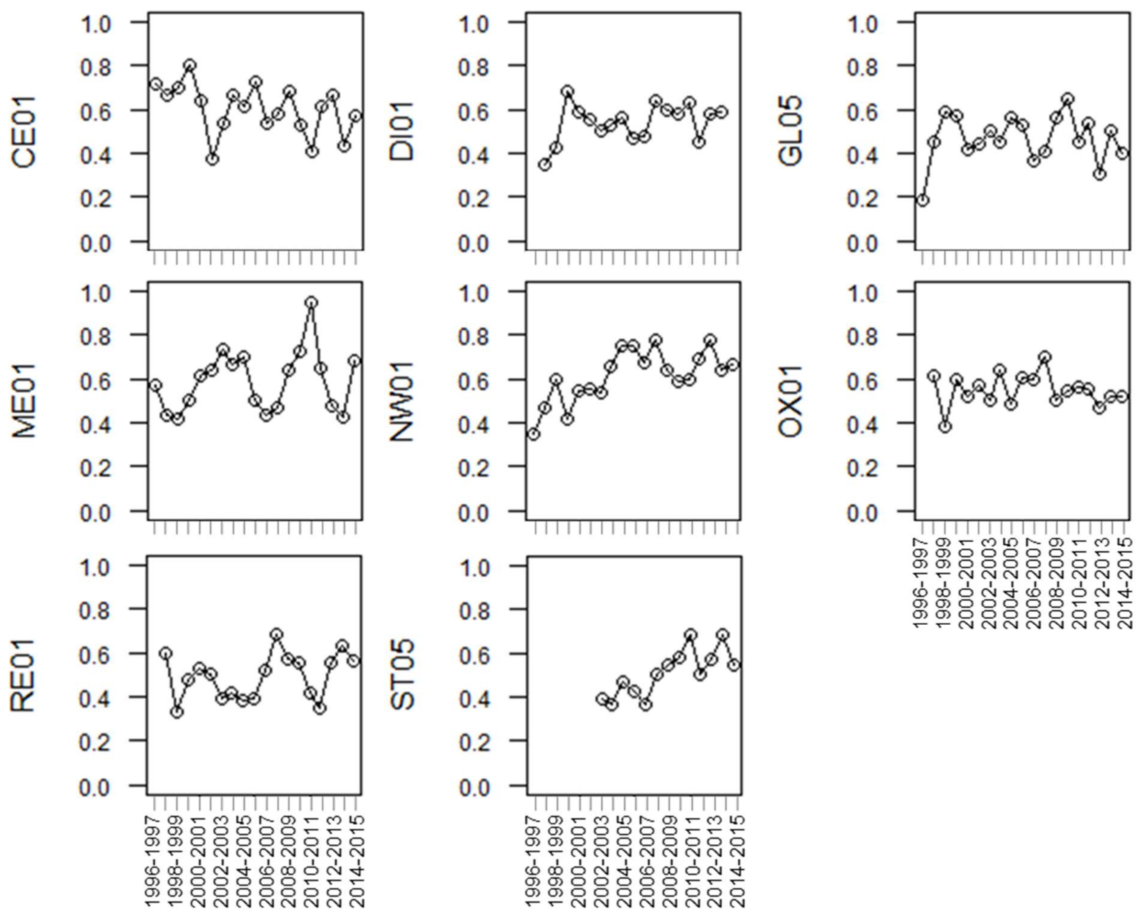
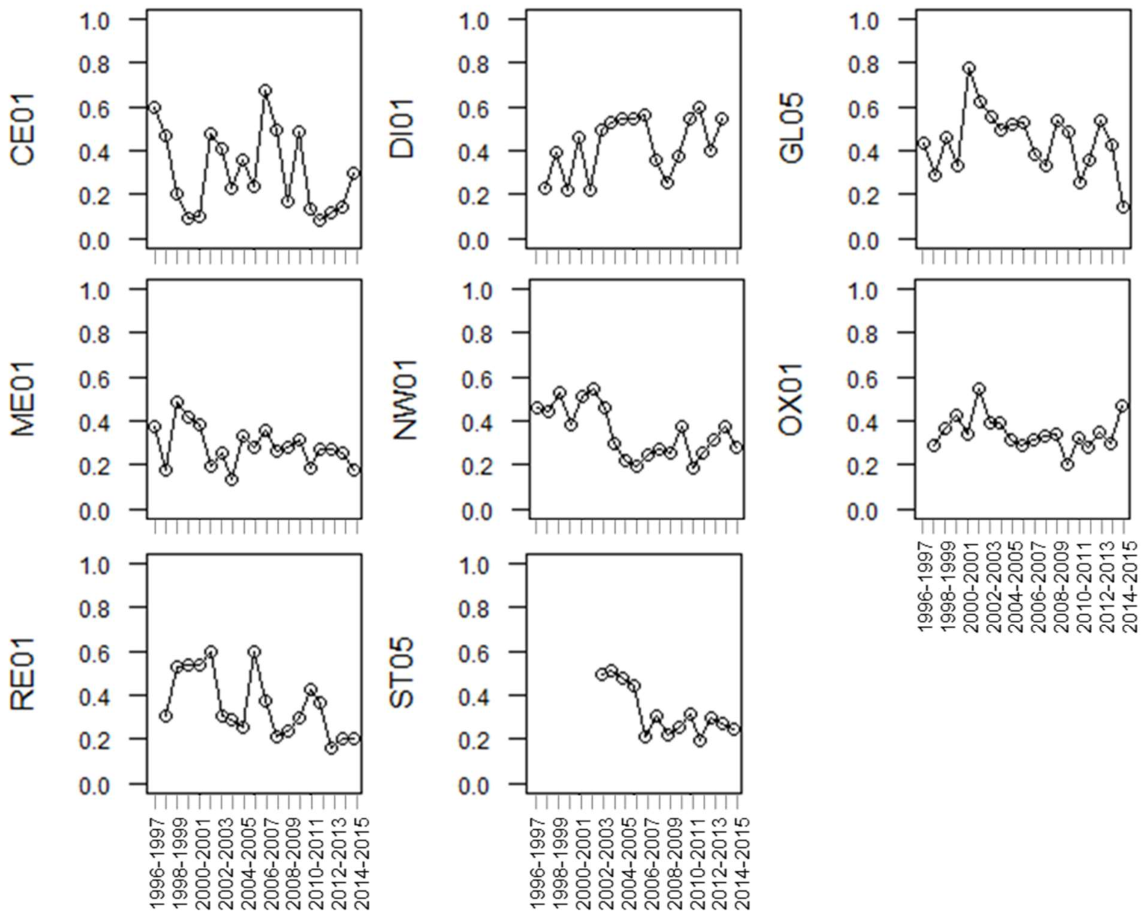


Figure 4. Inter-annual variation of Bray-Curtis dissimilarity index describing community compositional stability in terms of relative abundance for 8 rivers in the Upper Thames River Basin (southern Ontario, Canada).*CE01 = Cedar Creek, DI01 = Dingman Creek, GL05 = North Thames River, ME01 = Medway Creek, NW01 = South Thames River, OX01 = Oxbow Creek, RE01 = Reynolds Creek, ST05 = Stoney Creek.*



The two-factor PERMANOVA analysis comparing taxonomic composition, expressed as both presence/absence and relative abundance, based on Years and Site. The largest portion of the variance observed was due to spatial variability (partial R^2 = 0.32 and R^2 = 0.38, p = 0.001, for presence/absence and relative abundance respectively), while the portion of variance explained by Year (Interannual variability) was small (partial R^2 = 0.06 and 0.01 for both biological descriptors, p = 0.001). The spatial component interacted significantly with the interannual variability (partial R^2 = 0.04) but only for the relative abundance of taxa. Overall, the low amount of variance explained by interannual variability indicates considerable overlap in taxa composition between years.

Regional Mann-Kendall tests indicated that five of the eight calculated biological metrics demonstrated a statistically significant ($p < 0.1$) monotonic trend over the course of the study for at least one of the eight study sites (Table 5 and Annex A). Of the 15 significant trends four occurred at the South Thames River site, three at the Reynolds Creek site, two at each of the Cedar Creek, Medway Creek, and Stoney Creek sites and one at the Dingman and Oxbow Creek sites. Taxa richness showed the strongest trends (all slopes greater than 0.3) through time with five of the sites having positive trends suggesting increased in the sampled taxa richness. The remaining trends had substantially weaker trends with slopes less than 0.25.

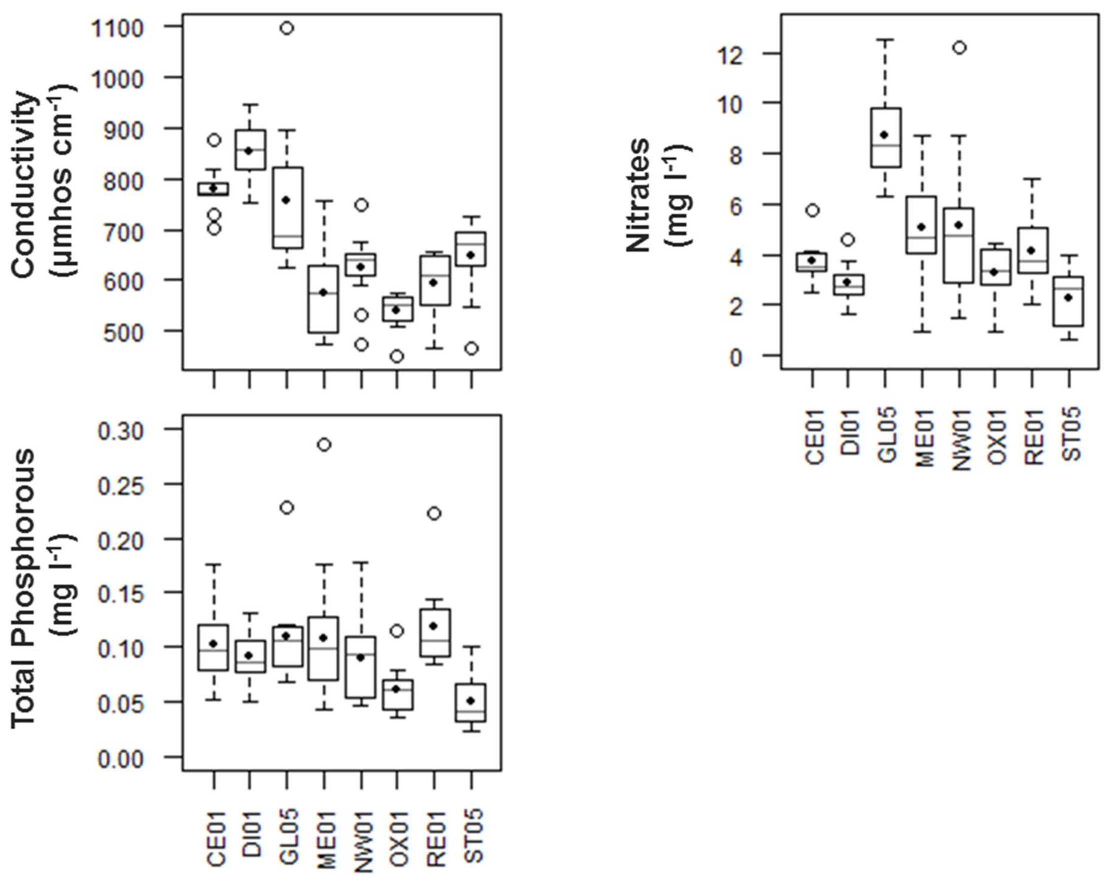
Table 5. Significant trends detected by Regional Mann-Kendall tests for biological and environmental variables measured at 8 different rivers in the Upper Thames River Basin (southern Ontario, Canada).

Biological parameters	**CE01**	**DI01**	**GL05**	**ME01**	**NW01**	**0X01**	**RE01**	**ST05**
Taxa richness	+			+	+	+	+	
% of EPT families								
% of Chironomidae							-	
Berger–Parker dominance index								
Hilsenhoff Family Biotic Index					-			
Canadian Ecological Flow Index								
Jaccard similarity index	-				+			+
Bray-Curtis dissimilarity index		+		-	-		-	-
Water quality parameters	**CE01**	**DI01**	**GL05**	**ME01**	**NW01**	**0X01**	**RE01**	**ST05**
Conductivity (µmhos cm^{-1})								
Nitrates (mg l^{-1})		-		-				
Total phosphorous (mg l^{-1})	-			-				-
Hydrological variables	**CE01**	**DI01**	**GL05**	**ME01**	**NW01**	**0X01**	**RE01**	**ST05**
Spring freshet initiation date								
Flow magnitude on day of spring freshet initiation (m^3s^{-1})								
Date of peak water level								
Flow magnitude on day of peak water level (m^3s^{-1})								
Date of 1-day maximum open-water flow								
1-day maximum open-water flow magnitude (m^3s^{-1})								

2.4.2. ASSESSMENT OF ENVIRONMENTAL VARIABILITY

The descriptive analysis of the environmental variables resulted in a series of information that was shown in Fig. 2 - 4 and in Annex B. Analysis of annual means of the water quality parameters indicated water quality varied substantially among sites (Fig. 5 and Annex B). Mean annual conductivity was greatest at the Dingman Creek site showing conductivity values that were on average more than 300 µmhoscm^{-1} larger than Oxbow Creek, the site with the lowest mean annual average. Within site variation in mean annual conductivity, as measured by CV's, was greatest at the North Thames River site and smallest at the Cedar Creek site. Overall, conductivity was less variable over time within sites (all CVs < 0.20) than it was the concentration of the measured nutrients (all CVs > 0.20). Mean annual average nitrate concentrations were greatest at the North Thames River site exceeding 8.5 mgl^{-1}. The smallest mean annual average nitrate concentration (2.3 mgl^{-1}) was observed at the Stoney Creek site. However, the Stoney Creek site also exhibited substantial variability in mean annual nitrate concentration (CV > 0.5). Maximum and minimum mean annual nitrate concentrations were often 2 to 3 fold different from the mean annual average and the maximum exceeded 12 mgl^{-1} at the North and South Thames River sites. Within site variability in annual mean nitrate and total phosphorus concentrations were generally comparable (CVs within 0.2). Mean annual average total phosphorous concentrations also varied less among sites than did nitrate concentrations with average total phosphorous concentrations being within 0.07 mgl^{-1} for all sites.

Figure 5. Variability of water quality variables for 8 rivers in the Upper Thames River Basin (southern Ontario, Canada).*Solid black lines represent median values, solid grey lines represent mean values, boxes represent the 25th-75th percentile range, bars represent the range while circles represent outlier values. CE01 = Cedar Creek, DI01 = Dingman Creek, GL05 = North Thames River, ME01 = Medway Creek, NW01 = South Thames River, OX01 = Oxbow Creek, RE01 = Reynolds Creek, ST05 = Stoney Creek.*

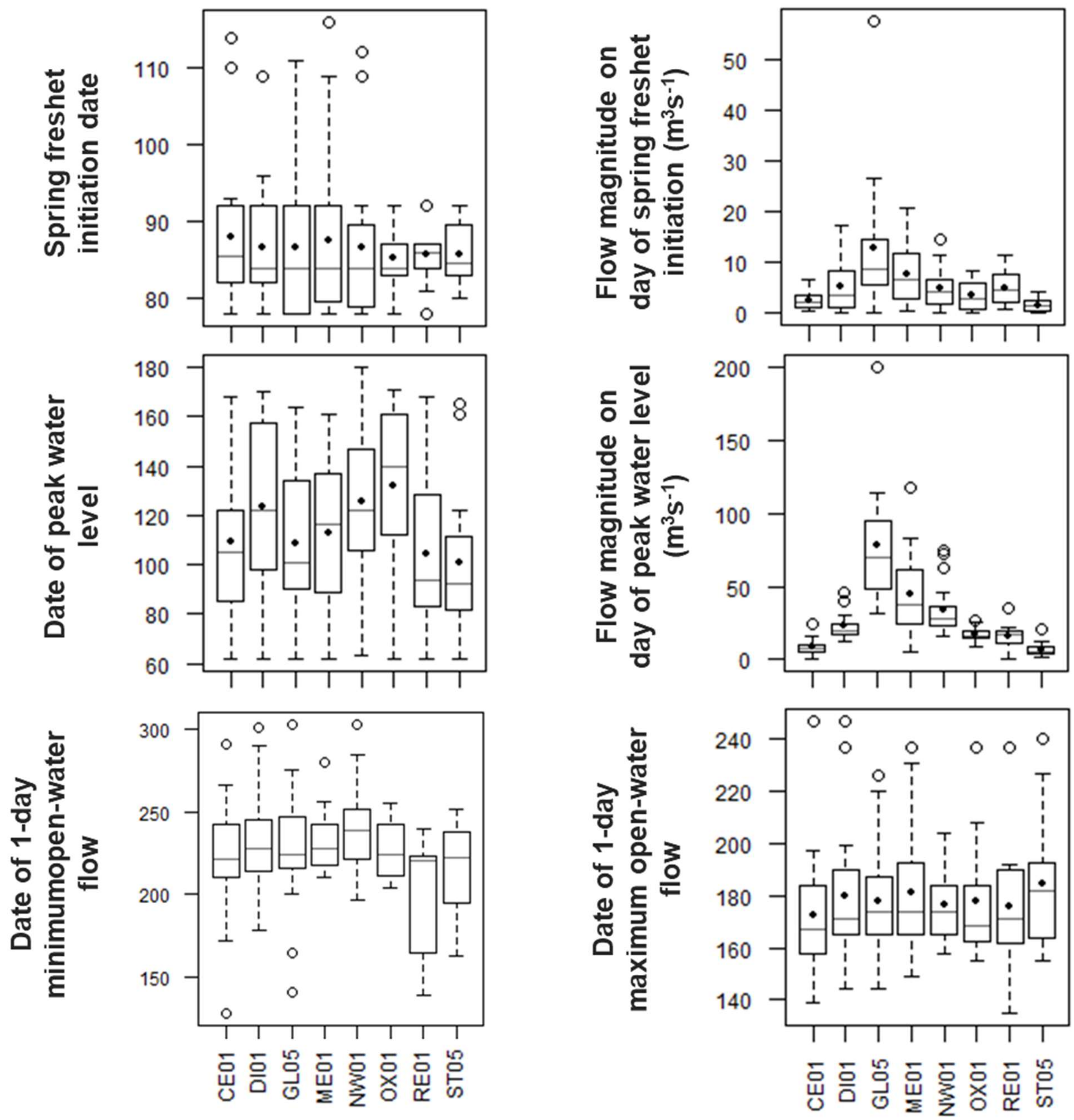


Descriptive statistics describing the hydrologic variables (Fig. 6 and Annex B) revealed substantial inter-annual variability in the date of spring freshet and peak flows. Spring freshet occurred on average around the 86th day (max = 88, min = 85) of each year for all of the 8 study sites. However, maximum annual values were as late as the 115th day. Oxbow, Reynolds and Stoney Creek sites did not, however, display these late spring freshet dates but rather showed reduced inter-annual variation in the initiation of spring freshet. In contrast, the date of peak water level during the ice-influenced period exhibited comparable ranges of dates for all eight sites. Furthermore, the average date of peak water level in the ice-influenced period was more variable among sites being earliest at Reynolds and Stoney Creek (105^{th} and 101^{st} days respectively) and latest at Oxbow Creek (131^{st} day). The date of maximum flow was less variable among sites within the open water period with all sites, except the Stoney Creek site, having a mean date within two weeks of the 225^{th} day. The range of dates of maximum flow in the open water period was, however, greater for all sites than observed for the ice influenced period with most sites having a range over at least 80 days. Flow magnitudes at spring freshet as well as peak flow in ice-influenced and open water periods showed similar patterns in terms of within site inter-annual variability. The North Thames River site showed the largest range of peak flows at 50 m^3s^{-1}, 200 m^3s^{-1} and 150 m^3s^{-1} for spring freshet, ice-influenced and open water periods, respectively. In contrast, Cedar and Stoney Creek exhibited comparatively small ranges of inter-annual variation in peak flow. Differences in means of peak flow at spring freshet were less than 5 m^3s^{-1} for all sites and with the exception of the North Thames River were within 30 m^3s^{-1} for peak flows in the ice-influence and open water periods.

Mann Kendall tests revealed five statistically significant ($p< 0.1$) trends for water quality (Table 5 and Annex B). Decreasing trends were observed for nitrate at the Dingman and Medway Creek sites, whereas decreasing trends for total phosphorous were detected at Cedar, Medway and Stoney Creeks. The slopes for total phosphorous were substantially lower (two orders of magnitude) than for nitrate. No trends were observed for any of the hydrologic variables.

Generally, we did not find any apparent connection between the lack and/or presence of Mann-Kendall trends in environmental and biological data with the landscape characteristics (i.e. land cover and presence of dams) of the study sites.

Figure 6. Variability of hydrological variables for 8 rivers in the Upper Thames River Basin (southern Ontario, Canada). *Solid black lines represent median values, solid grey lines represent mean values, boxes represent the 25th-75th percentile range, bars represent the range while circles represent outlier values. CE01 = Cedar Creek, DI01 = Dingman Creek, GL05 = North Thames River, ME01 = Medway Creek, NW01 = South Thames River, OX01 = Oxbow Creek, RE01 = Reynolds Creek, ST05 = Stoney Creek.*



2.4.3. ENVIRONMENT TO BIOTA ASSOCIATIONS

PLS regressions associating the calculated biological metrics with the environmental variables (i.e. water quality parameters and ice-influenced and open-water variables) for each of the eight sites showed that between 12% and 27% of the variance was captured in the dependent variables and between 12% and 47% was captured in the predictor variables. However, the cross-validated Q^2_y values were below the threshold of 0.097 indicating that temporal variation in the environmental and sampling variables was not predictive of inter-annual variation in the biological metrics for any of the sites (Table 6). Based on this finding we removed the water chemistry variables from the analysis to increase the length of record for each site by at least 2 years and up to 8 years for each site, however, cross-validated Q^2_y values remained below the threshold for all sites.

Table 6. Results of the PLS regression relating the biological metrics (y) and the environmental variables (x). *Q^2_y = Ability of the PLS model to predict the y-score (cross-validated); r^2_x = Cumulative explained variation of the x data (environmental variables); r^2_y = Cumulative explained variation of the y data (biological metrics).*

	PLS model parameters		
	r^2_y	r^2_x	Q^2_y
Cedar Creek	12.79%	47.07%	-0.011
Dingman Creek	24.91%	25.89%	-0.002
North Thames River	25.88%	24.81%	-0.010
Medway Creek	26.61%	12.39%	-0.271
South Thames River	22.68%	34.20%	0.014
Oxbow Creek	21.65%	19.62%	-0.286
Reynolds Creek	23.64%	27.32%	-0.170
Stoney Creek	19.63%	20.92%	-0.201

2.5. DISCUSSION

Although there are numerous long-term studies that have been performed in order to understand the temporal variability of the benthic communities in reference (unimpacted) sites (e.g. Füreder et al. 2002, Verdonschot 2009), there is still a lack of long-term studies directed to streams impaired by anthropogenic activities (Allan 2004, Hynes 1971). Hence, further studies taking into account impaired streams were needed to detect the underlying causes of changes in the biological community. Under our initial assumption that impairment of disturbed sites might obscure the effects of environmental conditions, in this study we focused in the identification and interpretation of long-term variability and the presence of relationships between environmental conditions and biological community as well as the importance of long-term variation in water chemistry and hydrological features.

Stochastic temporal pattern of the biological community

The two similarity indices used to characterize temporal variability in the structure of the macroinvertebrate community, Jaccard similarity and Bray-Curtis dissimilarity indices, accounted for the consistency of taxa presence/absence over time (community persistence; Bradley and Ormerod 2001, Collier 2008, Connel and Sousa 1983, Holling 1973) and for the dissimilarity of taxa abundance over time (compositional stability; Collier 2008, Milner et al. 2006, Scarsbrook 2002), respectively. The average values found in the studied sites, i.e. more than 55% of taxa occurring in consecutive years and 36% inter-annual dissimilarity on the relative abundance, were comparable to similar past studies in impaired streams (Bradley and Ormerod 2001, Collier 2008) suggesting that the community presents a stochastic pattern in a 20 year period. In addition, composition stability showed low variability (in terms of standard deviation) as compared to impacted streams studies (Marchant and Dean 2014). The differences observed between our findings and those from unimpaired streams (Milner et al. 2006,Robinson et al. 2000, Winterbourn 1997), mainly showing wide ranges in persistence and stability, suggest that impairment of streams may reduce inter-annual persistence (Collier 2008, Winterbourn 1997).

Unlike persistence and stability, the majority of the common bioassessment community metrics exhibited minimal temporal variability and few long-term trends were detected. The lack of variation between years may be an indication of variability inherent to impaired streams (Hunsaker 1990, Rosenberg and Plan 1999).

Taxon richness, a metric known to be sensitive to disturbances (Robinson et al. 2000), was found to be relatively low suggesting impoverished benthic communities as expected at this streams exposed to land use pressures (Buffagni et al. 2004). The fact that the study sites are (i) subjected to extreme, natural, seasonal hydrological variation and (ii) that suffer from high disturbance conditions, might provide a habitat template that favors low-diversity macroinvertebrate communities, as comparable with Wang et al. (2013) impaired streams research.

The reduced pool of taxa adapted to such conditions of fair to fairly-poor water quality reflected the impairment of the study streams. Indeed, metrics of dominance (Berger-Parker index) and tolerance (Hilsenhoff Family Biotic Index) reflected such impaired conditions and remained relatively stable throughout the study period despite the high rates of turnover and moderate compositional stability.

The two compositionally based metrics, % of Chironomidae and % of EPT families had two and four fold larger than the other CVs tested metrics, respectively, suggesting that taxa form these groups fluctuated more through time. As such, it is likely that changes in presence and abundance of these taxa contributed most to observed patterns in persistence and stability.

Overall, results in my long-term study highlight the substantial variability observed in community structure and found only a few directional trends in taxa richness and similarity indices. Therefore, the lack of monotonic trends in the inter-annual variation of the biological common metrics during the 20 years study period (1997-2016) suggests that the macroinvertebrate community showed stochastic temporal patterns in the study sites.

Stable environmental patterns

Studies that aim to detect long-term environmental trends are uncommon due to the lack of continuous monitoring data of spanning periods greater than 20 years (Jackson and Füreder 2006, Zhang et al. 2001) with the same sampling techniques. Although we have detected some trends in the water quality data, we were not able to find clear patterns between sites of such increase in nutrients within an 11-year window. In fact, according to authors such as Burt (1994) and Burt et al. (2008) greater periods must be used in order to detect long-term trends in environmental data.

Recorded historical data reveal that increments in temperature and changes in precipitation regime in Southern Canada (especially during the spring period; Beck et al. 2005, Bonsal et al. 2000, Hershkovitz and Gasith 2013,Zhang et al. 2001) are the cause of shifts in timing and duration of the different flow events, and the reason of the increase in frequency and magnitude of extreme weather conditions (Hershkovitz and Gasith 2013, Zhang et al. 2001). Although the relationship between precipitation and the hydrologic regime is dependent upon the region and sampling season, characteristics of the catchment area, etc., some authors have studied it under a long-term basis (see

Kunkel et al. 1999, Whitfield and Cannon 2000). In my study, I aimed to detect these type of changes in climate conditions by adding flood-related hydrological variables, but I did not find any significant trends in the data. The lack of long-term trends in the environmental data agrees with the long-term research done by Zhang et al. (2001) in Ontario; although their study period preceded to ours (1947-1996), the length of the study was sufficiently big to assume that the environmental trends could follow similar patterns until the present. One thing to note from Zhang et al. (2001) study is that Ontario streams showed the weakest trends of the studied regions in Canada suggesting that this area is less affected by changes in temperature and precipitation regime than other regions in the country. This past finding may explain why trends in the specific hydrologic variables were absent in our study.

Lack of relationship between environmental data and biota

Different authors have studied the relationship between abiotic factors showing how flow alteration (magnitude, intensity and/or duration) modifies water quality by modifying the physical habitat (Jowett and Duncan1990, Richter et al. 2003), or by altering water temperature or nutrient concentration (Fischer 2004, Prowse et al. 2011). However, as mentioned above, few studies have analyzed long-term data to understand the importance of long-term variation in water quality on the benthic community (e.g. Löfgren et al. 2014) while assessing other potentially co-varying factors, such as hydrology.

Long-term studies allow detection of underlying trends in the ecological conditions associated with subtle but persistent environmental changes (Jackson and Füreder 2006). However, despite detecting long-term trends in some of the environmental data, our study found no evidence that directional change in environmental conditions was associated with inter-annual variation in benthic community structure. Other long-term studies on impaired streams support the lack of relationship between flow characteristics and compositional stability despite the high variability of the hydrology in the area as comparable to our studied streams (Marchant and Dean 2014). The apparent insensitivity of the studied benthic communities to long-term variations in environmental conditions may be reflective of a limited pool of taxa that can tolerate the persistent and intense anthropogenic pressures the streams in my study were exposed to. Other past studies have found macroinvertebrate communities influenced by medium to intense anthropogenic pressures have an impoverished community composition (Buffagni et al. 2004) and are usually populated by pollution tolerant taxa (Winner et al. 1980). Thus, it may be expected that a macroinvertebrate community adapted to impaired conditions would demonstrate more resistance to variability in environmental conditions (Holling 1973). Regardless of the mechanism, the lack of strong relationships between the stochastic, yet stable, behavior of the benthic community indicates that the observed rates of species turnover may be the result of species substitution, where functionally similar taxa are replacing each other over time (Archaimbault et al. 2005, Petchey and Gaston 2006 and Poff et al. 2006), as opposed to a deterministic response to persistent, directional changes in environmental conditions (Bady et al. 2005, Bêche et al. 2006,Menezes et al. 2010, Statzner and Bêche 2010, Tomanova et al. 2008).

2.5.1. CAVEATS AND LIMITATIONS

Time problem: Disconnection between (biological and abiotic) sampling events

An explanation that may account for the lack of association found between benthic macroinvertebrate community structure and abiotic conditions is the occurrence of disconnected sampling events between them. The difference between the timing of hydrological monitored events and the biological monitoring might have allowed the recovery of the benthic community and, thus, might have hindered the identification of direct relationships between abiotic and biotic data (Wang et al. 2013). In addition, the short life-cycles of the benthic macroinvertebrates as well as the rapid response and recovery to perturbations might have complicated the detection of changes in community structure from preceding environmental conditions occurring over larger time scales (Jackson and Füreder 2006, Monk et al. 2008).

Difficulty of creating an accurate annual representativeness of the benthic community status

It has been widely studied that benthic composition is variable within the year and that the biological metrics incorporate seasonality (Hilsenhoff 1982,Lawrence et al. 2010, Lenat and Barbour 1990, Šporka et al. 2006 and Welte and Campbell 2003), which make it difficult to accurately represent benthic community status with a small number of samples. For solving the problem of seasonality some authors suggest sampling during spring as being the season with the most diversity (Šporka et al. 2006, Welte and Campbell 2003) or the application of corrective factors for seasonality as the developer of the FBI (Hilsenhoff 1982) proposed. Of the attributes that we have used for our study, EPT families are dependent on temperature and precipitation (Brittain 1974, 1983, Radford and Hartland-Rowe 1971,Welteand Campbell 2003) but some authors claim that changes within the year at a family level are not significant due to species substitution (Lawrence et al. 2010).

Subsampling: an additional source of variability

By adding features related to the biological monitoring, i.e. total counts and benthic sampling date, into the analyses I suggest that they could have masked the relationship between biological and environmental factors. As a matter of fact, different studies have already arisen some controversy regarding the total count of macroinvertebrates in determining the best suitable sampling protocols (see Doberstein et al. 2000), because different sizes of counts (mainly subsampling protocols based in lower minimum counts, i.e. 100 organisms), might lead to a loss in valuable information (Resh 1979, Courtemanch 1996, Vinson and Hawkins 1996). In my study, it appears that the biological monitoring protocols have introduced an additional source of variability into the benthic data, which could have been a determinant in creating the apparent stochastic variability in the data inhibiting my ability to detect a clear relationship with the tested biological metrics.

2.6. CONCLUSIONS

1) The high variability of the similarity indices (Jaccard and Bray-Curtis) reflected a stochastic temporal pattern in a 20 year period. More specifically, impairment reduced the inter-annual community persistence.

2) Common bioassessment metrics presented low temporal variability. The reduced pool of taxa reflected the impaired conditions of the streams.

3) No clear trends in 11-year window in water quality data and flood related hydrological variables, maybe due to the short length period of the data. The results were comparable with long-term researches in Ontario.

4) Water quality, hydrological variables and sampling features played a minor role in shaping the sampled benthic communities, possibly due to the impoverished community composition. It may be that the underlying cause of the long-term stochastic changes in benthic taxa composition is species substitution based on turnover of functionality.

5) The difference between the timing of hydrological monitored events and the biological monitoring might have allowed the recovery of the benthic community and, thus, might have hindered identification of direct relationships between abiotic and biotic data.

6) The high seasonality of the biological composition (e.g. in terms of % EPT families and % Chironomidae) might have hindered the creation of an accurate annual representativeness of the benthic community status.

7) Subsampling variable might have introduced an additional source of variability into the benthic data, being determinant for creating a stochastic environment and for finding a clear relationship with the tested biological metrics.

2.7. CONCLUSIONES

1) La elevada variación de los índices de semejanza (Jaccard y Bray-Curtis) reflejaron un patrón temporal estocástico durante un periodo de estudio de 20 años. En concreto, el

hecho de ser un ambiente desfavorable redujo la persistencia interanual de la comunidad.

2) Las métricas comunes de evaluación biológica presentaron una variación temporal baja. El reducido grupo de taxones reflejaron las condiciones desfavorables de los ríos.

3) No se encontraron lineas de tendencia en 11 años de estudio en la calidad del agua ni en las variables hidrológicas relacionadas con el caudal, probablemente debido a la corta duración del periodo de datos. Los resultados fueron comparables con los de investigaciones a largo plazo realizados en Ontario.

4) La calidad del agua y las variables hidrológicas jugaron un papel menor en la configuración de las comunidades bentónicas muestreadas probablemente debido a la composición empobrecida de la comunidad. Podría parecer que las causas subyacentes de los cambios estocásticos a largo plazo en la composición de la comunidad bentónica responden a una sustitución de especies basada en el reemplazo de la funcionalidad.

5) El espacio de tiempo copmrendido entre el muestreo de los eventos hidrológicos y los biológicos puede haber permitido la recuperación de la comunidad bentónica y, por tanto, puede haber entorpecido la identificación de relaciones directas entre los datos abióticos y bióticos.

6) La elevada estacionalidad de la composición biológica (por ejemplo en términos de % de familias de EPT y de quironómidos) puede haber dificutado la creación de una representación anual precisa que reflejara el estado real de la comunidad bentónica.

7) La variable submuestreo puede haber introducido una fuente adicional de variación en los datos bentológicos, siendo determinante para la creación de un ambiente estocástico y para encontrar una clara relación con las métricas biológicas analizadas.

2.8. REFERENCES

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ANNEX A. ASSESSMENT OF BIOLOGICAL VARIABILITY

Table 7. Statistical properties for biological metrics measured in 8 rivers in the Upper Thames River Basin (southern Ontario, Canada). *Community persistence and compositional stability are based on inter-annual values while the remaining indices are based on annual values.CE01 = Cedar Creek, DI01 = Dingman Creek, GL05 = North Thames River, ME01 = Medway Creek, NW01 = South Thames River, OX01 = Oxbow Creek, RE01 = Reynolds Creek, ST05 = Stoney Creek.*

		Jaccard similarity index	Bray-Curtis dissimilarity index	Taxa richness	Berger-Parker dominance index	% of EPT families	% of Chironomidae	Canadian Ecological Flow Index	Hilsenhoff Family Biotic Index
CE01	Mean	0.603	0.305	10.6	0.598	0.065	0.185	0.3	7.052
	SD	0.113	0.186	2.909	0.176	0.104	0.113	0.023	0.633
	CV	0.187	0.61	0.274	0.294	1.612	0.612	0.078	0.090
DI01	Mean	0.542	0.429	15.111	0.402	0.259	0.285	0.333	5.775
	SD	0.085	0.133	3.123	0.131	0.182	0.144	0.023	0.866
	CV	0.157	0.309	0.207	0.325	0.706	0.506	0.07	0.150
GL05	Mean	0.468	0.447	14.55	0.495	0.142	0.445	0.268	6.359
	SD	0.109	0.145	3.634	0.143	0.103	0.165	0.046	0.523
	CV	0.233	0.324	0.25	0.288	0.728	0.37	0.171	0.082
ME01	Mean	0.591	0.286	16	0.477	0.205	0.477	0.312	6.126
	SD	0.14	0.091	4.668	0.11	0.078	0.11	0.017	0.442
	CV	0.236	0.317	0.292	0.23	0.38	0.23	0.056	0.072

Table 7. Continuation.

		Jaccard similarity index	Bray-Curtis dissimilarity index	Taxa richness	Berger-Parker dominance index	% of EPT families	% of Chironomidae	Canadian Ecological Flow Index	Hilsenhoff Family Biotic Index
NW01	**Mean**	0.615	0.349	21	0.369	0.302	0.338	0.305	5.432
	SD	0.118	0.115	5.161	0.124	0.144	0.147	0.041	0.565
	CV	0.191	0.33	0.246	0.336	0.479	0.436	0.133	0.104
OX01	**Mean**	0.55	0.349	20.263	0.409	0.44	0.364	0.354	5.191
	SD	0.074	0.077	5.516	0.114	0.144	0.148	0.019	0.549
	CV	0.134	0.22	0.272	0.278	0.327	0.407	0.055	0.106
RE01	**Mean**	0.493	0.357	16.211	0.472	0.131	0.406	0.295	6.054
	SD	0.102	0.145	3.645	0.14	0.067	0.176	0.016	0.538
	CV	0.207	0.406	0.225	0.296	0.516	0.434	0.053	0.089
ST05	**Mean**	0.511	0.327	15.857	0.477	0.106	0.458	0.303	6.213
	SD	0.106	0.114	2.627	0.134	0.08	0.141	0.021	0.441
	CV	0.208	0.349	0.166	0.28	0.749	0.307	0.069	0.071

Table 8. Significant trends detected by Regional Mann-Kendall tests for biological metrics measured at 8 different rivers in the Upper Thames River Basin (southern Ontario, Canada).*RKT-slope derived from the significant (p-value < 0.01) Mann-Kendall tests. K-score in brackets.CE01 = Cedar Creek, DI01 = Dingman Creek, GL05 = North Thames River, ME01 = Medway Creek, NW01 = South Thames River, OX01 = Oxbow Creek, RE01 = Reynolds Creek, ST05 = Stoney Creek.*

Biological parameters	**CE01**	**DI01**	**GL05**	**ME01**	**NW01**	**0X01**	**RE01**	**ST05**
Taxa richness	0.273 (83)			0.444 (102)	0.631 (74)	0.500 (50)	0.333 (54)	
% of EPT families								
% of Chironomidae							-0.005 (-56)	
Berger–Parker dominance index								
Hilsenhoff Family Biotic Index					-0.004 (-56)			
Canadian Ecological Flow Index								
Jaccard similarity index	-0.009 (-56)				0.014 (80)			0.023 (47)
Bray-Curtis dissimilarity index		0.012 (50)		-0.008 (-53)	-0.013 (-59)		-0.014 (-67)	-0.023 (-38)

ANNEX B. ASSESSMENT OF ENVIRONMENTAL VARIABILITY

Table 9. Statistical properties for environmental variables measured in 8 rivers in the Upper Thames River Basin(southern Ontario, Canada). *CE01 = Cedar Creek, DI01 = Dingman Creek, GL05 = North Thames River, ME01 = Medway Creek, NW01 = South Thames River, OX01 = Oxbow Creek, RE01 = Reynolds Creek, ST05 = Stoney Creek.*

		Conductivity (μmhos cm^{-1})	**Nitrates** (mg l^{-1})	**Total phosphorous** (mg l^{-1})	**Spring freshet initiation date**	**Flow magnitude on day of spring freshet initiation**	**Date of peak water level**	**Flow magnitude on day of peak water level**	**Date of 1-day maximum open-water flow**	**1-day maximum open-water flow magnitude**
	Mean	778.293	3.703	0.102	88	2.353	109.611	8.637	172.444	10.393
CE01	**SD**	45.059	0.826	0.037	9.726	1.593	33.5	5.517	24.749	6.423
	CV	0.058	0.223	0.364	0.111	0.677	0.306	0.639	0.144	0.618
	Mean	853.893	2.847	0.092	86.529	5.315	123.412	23.406	180.059	17.316
DI01	**SD**	62.555	0.81	0.024	7.859	5.22	33.871	8.732	27.296	10.288
	CV	0.073	0.284	0.259	0.091	0.982	0.274	0.373	0.152	0.594
	Mean	755.663	8.691	0.109	86.684	12.96	108.474	77.689	178.158	71.395
GL05	**SD**	147.317	1.834	0.044	9.877	12.91	32.21	39.414	20.785	35.713
	CV	0.195	0.211	0.406	0.114	0.996	0.297	0.507	0.117	0.5

Table 9. Continuation.

		Conductivity (μmhos cm^{-1})	Nitrates (mg l^{-1})	Total phosphorous (mg l^{-1})	Spring freshet initiation date	Flow magnitude on day of spring freshet initiation	Date of peak water level	Flow magnitude on day of peak water level	Date of 1-day maximum open-water flow	1-day maximum open-water flow magnitude
	Mean	576.405	5.043	0.108	87.421	7.687	112.789	45.319	181.368	30.858
ME01	**SD**	77.747	1.942	0.058	10.548	6.028	30.66	29.162	24.003	14.082
	CV	0.135	0.385	0.542	0.121	0.784	0.272	0.643	0.132	0.456
	Mean	624.898	5.114	0.09	86.579	4.999	125.526	34.363	176.211	27.758
NW01	**SD**	72.88	3.09	0.041	9.731	4.094	32.277	17.32	13.002	15.663
	CV	0.117	0.604	0.454	0.112	0.819	0.257	0.504	0.074	0.564
	Mean	540.036	3.232	0.061	85.154	3.481	131.75	17.375	178	14.009
OX01	**SD**	37.501	1.101	0.023	4.862	2.934	35.742	5.142	23.584	4.523
	CV	0.069	0.341	0.371	0.057	0.843	0.271	0.296	0.132	0.323
	Mean	593.385	4.109	0.119	85.692	4.972	104.615	16.013	176.154	18.648
RE01	**SD**	62.241	1.648	0.041	4.328	3.385	31.703	8.383	24.785	8.113
	CV	0.105	0.401	0.347	0.051	0.681	0.303	0.523	0.141	0.435

Table 9. Continuation.

		Conductivity (µmhos cm^{-1})	Nitrates (mg l^{-1})	Total phosphorous (mg l^{-1})	Spring freshet initiation date	Flow magnitude on day of spring freshet initiation	Date of peak water level	Flow magnitude on day of peak water level	Date of 1-day maximum open-water flow	1-day maximum open-water flow magnitude
	Mean	647.597	2.271	0.05	85.75	1.572	100.75	7.112	184.25	6.648
ST05	**SD**	78.704	1.163	0.027	4.245	1.308	32.947	5.434	26.461	5.302
	CV	0.122	0.512	0.535	0.05	0.833	0.327	0.764	0.144	0.798

Table 10. Significant trends detected by Regional Mann-Kendall tests for environmental variables measured at 8 different rivers in the Upper Thames River Basin (southern Ontario, Canada). *RKT-slope derived from the significant (p-value < 0.01) Mann-Kendall tests. K-score in brackets. CE01 = Cedar Creek, DI01 = Dingman Creek, GL05 = North Thames River, ME01 = Medway Creek, NW01 = South Thames River, OX01 = Oxbow Creek, RE01 = Reynolds Creek, ST05 = Stoney Creek.*

Water quality parameters	**CE01**	**DI01**	**GL05**	**ME01**	**NW01**	**0X01**	**RE01**	**ST05**
Conductivity (μmhos cm^{-1})								
Nitrates (mg l^{-1})		-0.164 (-27)		-0.254 (-54)				
Total phosphorous (mg l^{-1})	-0.006 (-29)			-0.007 (-85)				-0.007 (-37)
Hydrological variables	**CE01**	**DI01**	**GL05**	**ME01**	**NW01**	**0X01**	**RE01**	**ST05**
Spring freshet initiation date								
Flow magnitude on day of spring freshet initiation								
Date of peak water level								
Flow magnitude on day of peak water level								
Date of 1-day maximum open-water flow								
1-day maximum open-water flow magnitude								

CHAPTER 3. ASSESSMENT OF A QUALITATIVE RESTORATION OF AN URBAN STREAM

3.1. INTRODUCTION

Global population has consistently increased during the last century, growing from 2.5 to 7.5 billion (UN 2017). This rapid population growth has been associated with the expansion of urban areas. The process of urbanization has had a negative impact on rivers by replacing naturally vegetated surfaces with impervious surfaces (Anderson 1970, Graf 1977,Seaburn 1969, Zhang et al. 2010) leading to a decreased infiltration of precipitation (Graf 1977) and increased runoff, with subsequent changes in hydrologic regime, including increased flooding during high flow events (Anderson 1970, Schwartz and Herricks 2007). As a consequence, the shifts in hydrologic regime in streams and rivers lead to alterations in channel hydromorphology. Management responses to channel alterations have included engineering of channel bed and banks to reduce erosion resulting from increased flows.

Changes in channel hydromorphology along with impaired water quality due to increased point and diffuse pollution have been shown to compromise the ecological status of rivers worldwide (Allan 2004, Schwartz and Herrick 2007). Therefore, different directives entered into force in order to safeguard aquatic habitats. Specifically in Europe, the incorporation of the Water Framework Directive (WDF 2000/6/CE) required that all water bodies achieved good status of water bodies by 2015 (and posterior extension to 2027), according to both biological, hydromorphological and physico-chemical criteria. Despite the goal of the WFD to safeguard inland water habitats, the last decade has seen a decline in the quality of such habitats, mainly due to stakeholders lacking the resources to implement required measures. In response, the RESTORE EU LIFE+ Project (http://www.restorerivers.eu) was developed with the aim of developing a network of knowledge on good practices in river restoration activities between policy makers, river basin planners, practitioners and experts (Mant and Elbourne, 2012). Consequently, restoration activities in urban rivers have substantially increased and, indeed, to date there have been nearly 1600 river ecosystem restoration projects completed across Europe (Belletti et al. 2015, Fernández 2011, Smith et al. 2014). Such projects aimed to (i) restore the natural state and functioning of the river system in support of the catchment area, flow regime, riparian and instream habitat, water quality, biodiversity or other elements (e.g. recreation, aesthetics or education); and (ii) to enhance habitat and landscape, as well as reduce floodplain risk, by changing features from riverbed and banks, channel, floodplain and river corridor (Speed et al. 2016).There has been, however, limited monitoring and reporting on the success of these projects. Moreover, there is a particular lack of simultaneous reporting on the impacts/improvements of restoration actions on both hydromorphological and biological conditions. The few existing results of restoration projects, which followed a before-after design, showed improvements on the invertebrate community, although not much more information is known about which feature of the community was improved or how the community was monitored. In Italy, similarly to the RESTORE EU LIFE+ Project, the equivalent Contratto di Fiume (http://www.contrattidifiume.it) aims to protect the fluvial environment by the requalification of rivers in order to reach a good quality status of water quality, reduce flood risk and promote ecosystem services. Thanks to this new agreement several rivers have been rehabilitated at a catchment scale. Still, the effectiveness of the restoration projects have not yet been assessed.

In 2006 a restoration project in the Seveso River in Northern Italy was designed for the rehabilitation of the whole sub-basin with the aim of reducing water pollution and hydraulic risk. As part of this project two restoration pilot subprojects were undertaken in two reaches of the Seveso River, which flows through the Parco Nord Milano within the city of Cormano (Milan, Italy). The goals of this small restoration was flood protection and habitat improvement, since past channel engineering projects had hardened the rivers banks with concrete retaining walls, narrowed the bankfull width and increased stream velocities, bed erosion and flooding during high flow events. Restoration activities included the elimination of concrete walls, reshaping and revegetation of banks, and diversification of instream habitats. Main restoration activities were focused on the introduction of bioengineering elements such as wooden crib repellents, palisade of stone blocks, block ramps, etc., differentially applied for the two reaches. Here I report on an assessment of how these pilot sub-projects affected the hydromorphological conditions and the resident benthic macroinvertebrate community as a measure of the success of the restoration activities. The assessment was conducted by comparing both restoration reaches with an adjacent upstream control reach just few meters upstream. Results of the assessment will be used to inform future restoration activities in the Seveso River basin and stream restoration practices in general.

3.2. MAIN OBJECTIVES

As it has been mentioned before, the interpretation of the outcomes resulting from restoration processes is still in an early phase at a scientific level, since restoration projects have usually followed a managerial approach. In addition, the effects of the restoration activities are difficult to interpret due to the lack of standardized methods to evaluate the improvements (sometimes minimal) of the measures in already and continuously impaired ecosystems. Most of the measures that are used to restored aquatic ecosystems include changes in the hydromorphology of the river systems and, consequently, the detection of suitable indices that can accurately assess the restoration process from a biological point of view is a challenge. More challenging is the development of suitable hydromorphological indices that could detect benthic variability in restoration projects that had focused only in the in-stream component of the aquatic ecosystem, while leaving intact other sources of impairment such as point and diffuse pollution. Consequently, I examined the inter-annual variability in macroinvertebrate communities in relation to water quality, hydromorphological indices and hydrometric level for an urban stream impaired by poor water quality in northern Italy, subjected to a restoration project . The main objectives of this chapter were to:

(i) Hydromorphological assessment of the study area before and after the restoration process. **Has the habitat actually changed after the restoration? Can I identify temporal trends?**

(ii) Characterize spatial and temporal variability in benthic macroinvertebrate community structure and assemblage, described by presence/absence and relative abundance, as well as five common bioassessment metrics. **Is the community showing any pattern or is it changing stochastically in time?**

(iii) Examine to which extent environmental variables and hydromorphological characteristics affect benthic community after a restoration process. **What are the drivers of variation in the macroinvertebrate community? Can we identify any hydromorphological indices that reflect changes in the biological community after a restoration process?**

3.3. METHODOLOGY

3.3.1. STUDY AREA

The 52km long Seveso River drains over 930km^2 of the Padanian alluvial plain, located in the region of Lombardia in northern Italy (Fig. 7). The Seveso River basin has been subjected to extensive land use change throughout the twentieth century as urbanization of former agricultural lands has increased urban cover from 5% to 68%. The population of the area has also shown a nearly 100-fold increase, growing from 25,500 inhabitants in mid-late nineteenth century to 209,000 in 2001 (Clerici 2015). Changes in land use have degraded the water quality by direct and diffuse contamination and have also changed the hydromorphology of the river, leading to the Seveso River being considered one of the most impaired rivers in the region (Detti et al. 2014).

Prior to restoration activities in 2012 the segment of the Seveso River flowing through the Parco Nord Milano was characterized by vertically resectioned banks, homogeneity in the microhabitats and an absence of meanders. A *Control* reach was established upstream of the reach to be restored (Restored Site). Restoration activities of Seveso River were accomplished between 2012 and 2014 over about 600 linear meters of river channel. The channel engineering techniques used differed between the upper and lower portions of the restored reach (Table 11). Thus, following completion of restoration activities the Restored area was divided into 2 different sampling reaches, *Restored 1* (upper reach) and *Restored 2* (lower reach) (Fig. 8).

Figure 7. Map showing the location of the restoration area (black dot) in the Seveso River Watershed (grey shadow) in Lombardia Region (a).

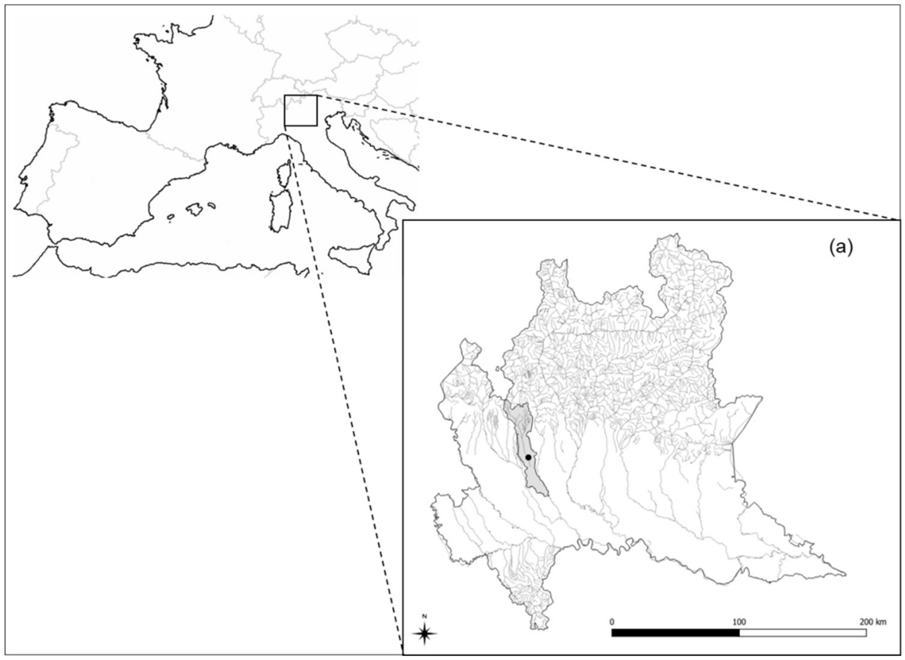


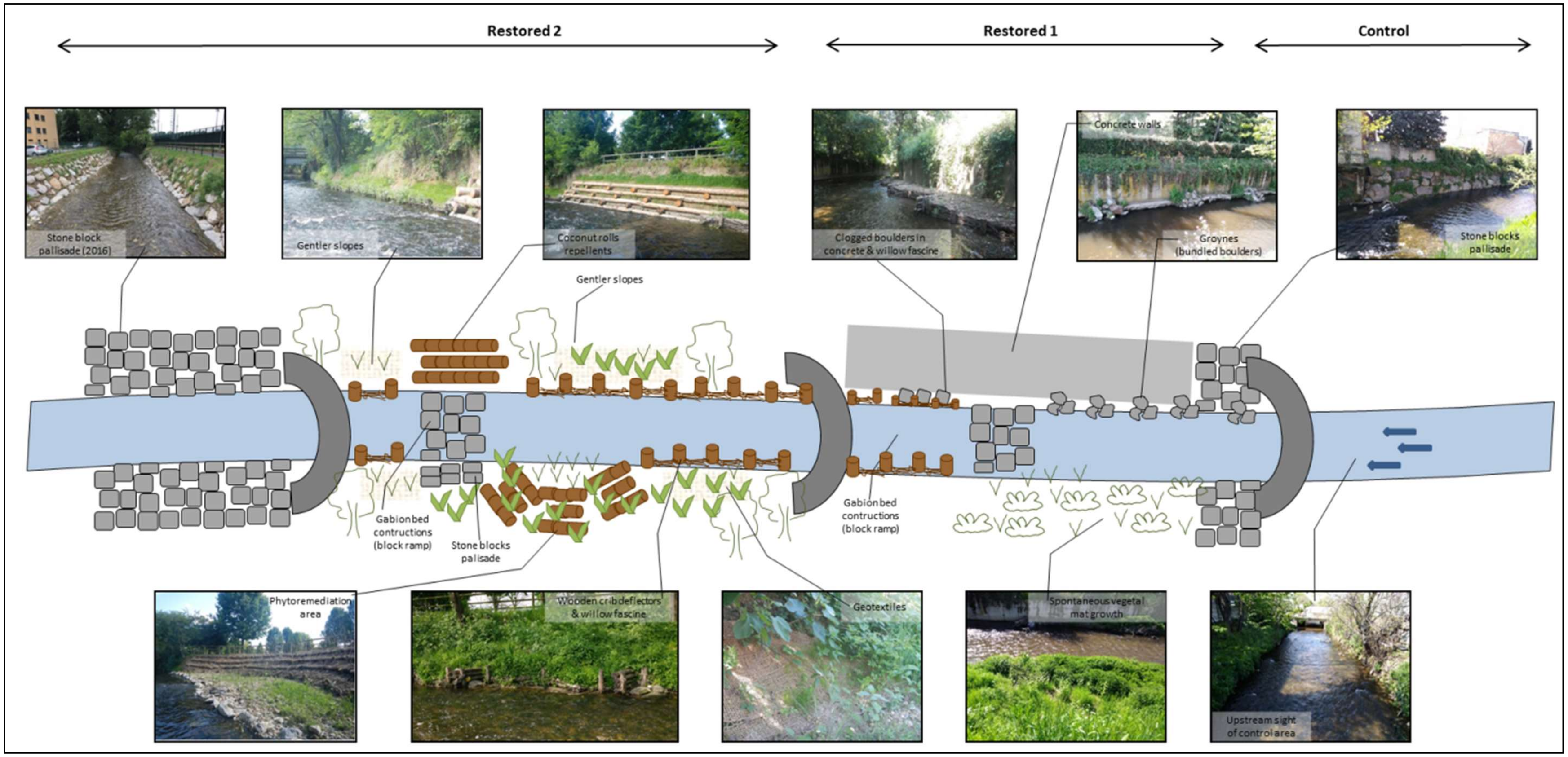
Table 11. Restoration measures and objectives of each measure, divided by reach.

Reaches	Bank/Bed	Measures	Objectives
1	Right bank	Clogged boulders in concrete (inclined groynes made with bundled boulders)	- Reinforcement at the foot of the foundation
1	Right bank	Inclined groynes made with bundled boulder	- Redirection of the flow and transformation of the flow energy - Meandering of the channel - Enhance stream habitat heterogeneity - Creation of berms and riffle/pool areas - Bedding out of the vegetation in slow water areas
2	Right bank	Coconut repellents	- Block bank erosion - Secure banks
2	Left bank	Creation of a phytoremediation area (wooden crib repellents on the bank, and area filled with plant land and planting culms and rhizomes)	- Improve water quality - Improve diversity
2	Both banks	Revegetation of banks by the settlement of geotextiles	- Secure banks - Stabilize slopes - Provide shadow to the channel, decreasing water temperature, maintenance of soil moisture and slow down surface runoff

Table 11. Continuation.

Reaches	Bank/Bed	Measures	Objectives
1 & 2	Both banks	Willow fascines (living branches bundled together)	- Trap sediments - Protection against erosion at the foot of the foundation
1 & 2	Both banks	Wooden deflectors	- Redirection of the flow and transformation of the flow energy - Meandering of the channel - Enhance stream habitat heterogeneity - Creation of berms and riffle/pool areas - Bedding out of the vegetation in slow water areas
1 & 2	River bed	Gabion bed constructions (block ramps)	- Consolidation of river bed - Creation of areas favourable for the oxygenation of water
1 & 2	Both banks	Elimination of concrete walls	- Reshaping of banks - Creation of gentler slopes
1 & 2	Both banks	Stone block palisade (repellents made of stone blocks joined by steel rope)	- Block bank erosion - Secure banks

Figure 8. Seveso River restoration scheme.



3.3.2. DATA COLLECTION

Pre-restoration data was collected at the *Control* reach and in the combined areas of the restored reaches in 2012. Restoration actions were assessed at both *Restored* and the *Control* reaches by monitoring hydromorphology, water quality and benthic macroinvertebrate communities in 2014, 2015 and 2016. All sampling campaigns were undertaken in the autumn season.

Hydromorphological characterization

River hydromorphology was assessed using CARAVAGGIO (Core Assessment of River hAbitat Value and hydromorphological cOndition) method (Buffagni et al. 2005, 2013). This protocol characterizes: (i) the channel (e.g. substrate, flow types, deposition features, vegetation), (ii) the riverbanks (e.g. land use, slope, material, vegetation complexity, bank modifications such as reinforcements or embankments), and (iii) the riparian corridor for a 500m length of river channel (e.g. land use, width of vegetation strip, structure of vegetation). Data are collected along transects located every 50m for a total of ten transects. In order to obtain a higher resolution of the hydromorphological modifications, I adapted the length of the studied section to 250m (10 spot-checks located every 25m), as proposed by Buffagni et al. (2005, 2013).

Data collected using the CARAVAGGIO approach was applied to calculate three indices: Habitat Modification Score (HMS), Land Use Index (LUIcara) and Habitat Quality Assessment (HQA). The Habitat Modification Score index (HMS) evaluates morphological alteration because of artificial structures (Buffagni et al. 2010,Raven et al. 1998a). Structures are classified as transversal (structures that occupy the whole width of the channel such as bridges, weirs, fords, culverts) or lateral (structures affecting the banks such as deflectors and groynes). The value of HMS can range from 0 to 91. Larger scores indicate greater morphological alteration of the river (Buffagni et al. 2010; Table 12). The Land Use Index (LUIcara) describes land use in the riparian corridor (Erba et al. 2015). The index is calculated based on land use characteristics on the bank face and banktop, as well as land use extending 50m from the banktop (Buffagni et al. 2010). Structure of the vegetation on bank face and within 1m of the banktop is also assessed. The value of LUIcara can range from 0 to 39.2. Larger scores indicate greater amounts of anthropogenic land use (Table 12). The Habitat Quality Assessment index (HQA) quantifies the diversity and quality of stream habitats through assessment of substrate and flow types, channel and banks characteristics, and riparian vegetation structure (Buffagni et al. 2010, Raven et al. 1998a). Larger index scores indicate greater habitat diversity and quality (Buffagni et al. 2010). The classification scales varies between 6 different macrotypes of river (i.e. Alps, Apennines, low diversified Apennines, temporal Mediterranean rivers, small lowland streams, and other rivers), because the expected habitat diversity is not the same for all rivers. We applied the small lowland streams classification as our macrotype and thus the maximum observable HQA score was 77 (Table 12).

Table 12. Quality levels and corresponding assessment scores of three hydromorphological indices derived from CARAVAGGIO method (Buffagni et al. 2005, 2013). *HMS = Habitat Modification Score, LUIcara= Land Use index, HQA = Habitat Quality Assessment. For the HQA score thresholds are for small lowland streams. Adapted from Buffagni et al. 2010.*

Quality level	HMS	LUIcara	HQA
Excellent	0 - 6	0.00 – 2.00	42 - 77
Good	7 - 18	2.01 - 7.50	34 - 41
Moderate	19 - 42	7.51 – 15.00	26 - 33
Poor	43 - 72	15.01 – 30.00	18 - 25
Bad	73 - 91	30.00 – 39.20	0 - 17

Benthic macroinvertebrate sampling

Benthic macroinvertebrates were collected following the multi-habitat proportional protocol for wadeable rivers (Italian Decree DM 260/2010, EC 2000). The method consists of ten replicate samples collected using a Surber sampler ($0.05m^2$) with 500µm net mesh for total area of $0.5m^2$ at each sampling reach and preserved in 90% denatured ethyl alcohol solution. Individual replicates were collected across all habitats in relation to proportional abundance of all microhabitats (e.g. megalithal, mesolithal, microlithal, xylal and artificial) and flow types (e.g. chute, broken standing waves and smooth flow). All collected individuals from each sample were counted and identified to family level. A total of 32 taxa were used for the data analysis.

Environmental data

Water samples were collected as grab samples and taken to the laboratory for the analyses on concentrations of total phosphorous as $\mu g l^{-1}$ P_t (EPA 3005 A 1992 + EPA 6010C 2007), ammonia as $mg l^{-1}$ $N\text{-}NH_4$ (APAT CNR IRSA 4030 A1 Man 29 2003) and nitrates as $mg l^{-1} N\text{-}NO_3$ (APAT CNR IRSA 4020 Man 29 2003) in order to fulfil the Italian Legislative Decree n. 152 from April 3rd 2006. All samples were collected at approximately the same daily time, around 5 p.m., and taken immediately to the laboratory for the analysis.

Hourly hydrometric levels (in centimetres) of the Seveso River were supplied by the environmental protection agency (ARPA Lombardia). Data was collected by an automatic gauge station 5km upstream of the study reach. The reference level (hydrometric level zero) was fixed at 164.9m.a.s.l. as according to the regional basin agency AIPo (Agenzia Interregionale per il fiume Po). Therefore, a positive value of the hydrometric level indicated an increment in the water level and a negative value shows a decrease in the water level. Average and maximum hydrometric level of the previous three months were calculated as indicators summer flows for each sampling campaign. Daily average hydrometric level for each sampling day was also calculated.

3.3.3. DATA ANALYSIS

Hydromorphological assessment

In order to summarize the hydromorphological features, a descriptive figure was generated showing the temporal variability of the indices at each site. To go in depth, analyses of variance (ANOVA) were used to detect differences between hydromorphological indices between sites (*Control* and *Restored1*; *Control* and *Restored 2*; *Restored 1* and *Restored 2*) after the restoration activities were completed (i.e. from 2014 to 2016). Hence, nine different one-way ANOVAs, one for each hydromorphological variable, were performed. Whenever the differences between *Control* and *Restored* reaches were significant (p< 0.05) the success of the hydromorphological indices to detect restoration measures was achieved. At the same time, whenever the differences between *Restored 1* and *2* reaches were significant (p< 0.05) different approaches of restoration were detected. Analyses were computed with the *vegan* package (Oksanen et al. 2009) with R cran (R Development Core Team 2010).

Assessment of biological variability

Benthic communities were assessed using presence/absence and relative abundance descriptions of community composition, as well as five common bioassessment metrics (Table 13).In one hand, Jaccard's and Bray-Curtis similarity indices (Bray-Curtis 1957, Jaccard 1902) were used to calculate presence/absence and relative abundance data, respectively. In the other hand, the common bioassessment metrics that were included in the study were Shannon-Wiener diversity index, the number and proportional abundance of EPT families, ratio between EPT and Chironomidae individuals and 1-GOLD. Shannon-Wiener diversity index, number and percentage of EPT families, and 1-GOLD are metrics (among others) included in the calculation of the European STAR_ICM index (IRSA-CNR 2007) and are used for the valuation of habitat alterations (Buffagni et al. 2016). The ratio between EPT and Chironomidae (EPT/Chironomidae) individuals has been shown to be sensitive to riparian vegetation (Corbi and Trivinho-Strixino 2008). Moreover, EPT/Chironomidae provides information about microhabitat types as Chironomidae are often proportionally more abundant in streams with finer substrates (Frenzel 1996). All metrics except EPT/Chironomidae were calculated using the MacrOper.ICM assessment software (AQEM/STAR Ecological River Classification System; http://www.eur-star.at), a tool to permute the abovementioned European STAR_ICM index (Buffagni and Erba 2007).

Table 13. Selected biological metrics for the present study.

Name	Description	Reference
Shannon-Wiener diversity index	$D_{S-W} = -\sum_{i=1}^{i}\left(\frac{n_i}{A}\right) \cdot ln\left(\frac{n_i}{A}\right)$	(Hering et al. 2004,Böhmer et al. 2004)
Number of EPT families	Σ Families of Ephemeroptera, Plecoptera and Trichoptera	(Böhmer et al. 2004, Ofenböck et al. 2004)
% EPT families	Percent composition of Ephemeroptera, Plecoptera and Trichoptera families	(Plafkin et al. 1989)
EPT/Chironomidae	Σ of individuals classified as Ephemeroptera, Plecoptera and Trichoptera / Σ of individuals classified as Chironomidae	(Plafkin et al. 1989)
1-GOLD	1 - (Relative abundance of Gastropoda, Oligochaeta and Diptera)	(Pinto et al. 2004)

To ensure the comparability between sites during the study, an initial analysis on the biological community before the restoration project was done. Since only 2012 was being compared, *Restored 1* was excluded from the analysis due to lack of biological data. Hence, to identify significant (p< 0.05) spatial differences among communities from *Control* and *Restored 2* the variation of the community composition (i.e. presence/absence and relative abundance) was addressed under a one-factor permutational analysis of variance (PERMANOVA; Anderson 2001) using 999 permutations, while the variation of the common bioassessment metrics (i.e. number and percentage of EPT families, EPT/Chironomidae, Shannon-Wiener diversity index and 1-GOLD) was addressed under a one-way analysis of variance (ANOVA).

A second two-factor PERMANOVA was run to identify significant (p< 0.05) temporal and spatial differences among communities in terms of presence/absence and relative abundance, considering Years (3 years, from 2014 to 2016), Site (3 sites, *Control*, *Restored 1* and *Restored 2*), and its interaction (Years*Site) using 999 permutations. In addition, two non-metric multidimensional scaling (nMDS) ordination were performed to visualize those differences. Analyses were computed in the *vegan* package (Oksanen et al. 2009) with R cran (R Development Core Team 2010).

Additional ANOVAs were used to detect differences between common bioassessment metrics between sites (*Control* and *Restored1*; *Control* and *Restored 2*; *Restored 1* and *Restored 2*) after the restoration activities were completed (i.e. from 2014 to 2016). Hence, fifteen different one-way ANOVAs, one for each bioassessment metric, were performed. Whenever the differences between *Control* and *Restored* reaches were significant (p< 0.05) changes due to the restoration measures could be hypothesised. At the same time, whenever the differences between *Restored 1* and *2* reaches were significant (p< 0.05) different approaches of restoration were detected, as per hydromorphological indices. Analyses were computed with the *vegan* package (Oksanen et al. 2009) with R cran (R Development Core Team 2010).

Environment to biota associations

In order to have a broader view of the temporal variation in our study area, the environmental features (i.e. water quality and hydrometric levels) were explored by comparing data coming from (i) *Control* and *Restored 2* in the case of the water quality, and (ii) data coming from an upstream gauge in the case of the hydrometric level.

Then a Partial Least Squares (PLS) regression (Legendre and Legendre 1998) was used to assess relationships between the calculated benthic metrics (i.e. Shannon-Wiener diversity index, number and percentage of EPT families, EPT/Chironomidae and 1-GOLD) and the measured environmental variables, including water quality parameters, hydromorphological variables and hydrometric levels. PLS is a well-tested approach to identify relationships among dependent and independent variables in time series analysis (see Kalela-Brundin 1990, Smoliak et al. 2010). PLS is also suitable to test whether variables show significant temporal trends (Kinnard et al. 2011), while avoiding multicollinearity issues among environmental indices (Bougeard et al. 2011, Eriksson et al., 1995, Olden and Poff, 2003). The regression took into account both Restored reaches (*Restored 1*, *Restored 2*) during the “After” restoration period (from 2014 to 2016), in order to identify environmental variables that most predicted variation in the bioassessment metrics. The analyses were done using the *plsdepot* package (Sanchez and Sanchez 2012) with R cran (R Development Core Team 2010).

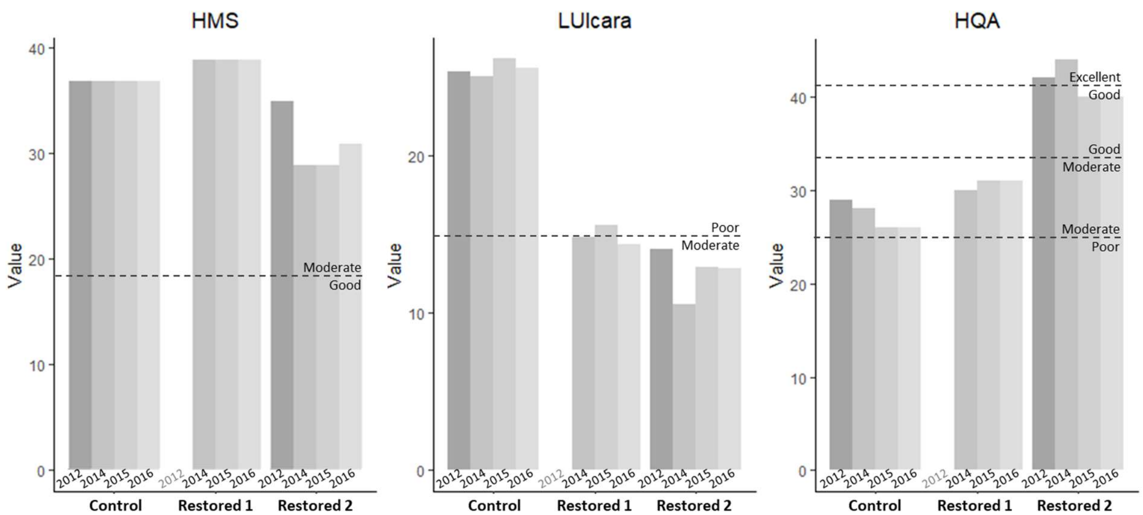
The performance of the PLS model was expressed in terms of the cross-validated explained variances of the environmental variables (r^2_X), cross-validated explained variances of the biological community *(r^2_Y)* and predictive ability of the model (Q^2_Y). PLS models were considered significant when $Q^2_Y > 0.0975$ (sensu Abdi 2010, Trap et al. 2013). A 999 permutation ten-fold cross-validation method was chosen to select the number of significant components through the calculation of the Q^2_Y. The method subsetted the data into 10 equal segments, using one random subset as validation data and the remaining 9 as training data. Variance Importance in the Projection (VIP) values indicating information about the relevance of the environmental variables to the PLS model taking into account the biological variance explained by each latent variable, were calculated at a $p < 0.05$ level (Wold, 1995). VIP scores greater than 1 for each single predictor variable were considered to have a strong predictive power on the PLS model (Pearce and Yates 2017). In addition to the single VIP scores, standardize coefficients indicating the direction of the predicted association were also calculated.

3.4. RESULTS

3.4.1. HYDROMORPHOLOGICAL ASSESSMENT

A descriptive histogram showing the temporal variability of the hydromorphological indices was done, including the three sites. Thus, as shown in figure 9, all reaches were classified as moderate quality level by the HMS, although *Restored 2* was at least 7 points as an average smaller than the other two reaches in all years. The values of HMS in *Control* and *Restored 1* were stable during all the study period, while in *Restored 2*the value was stable except in 2016 that the downstream end of the reach was subjected to additional restoration measures to secure banks by the substitution of vertical banks with stone block palisade. LUIcara at *Control* was classified as poor quality level, whereas both *Restored* sites were classified as moderate quality level as an average. HQA at *Control* and *Restored 1* sites were both classified as moderate quality level although *Restored 1* was near the category maximum and *Control* near to the minimum. In contrast, *Restored 2* which was classified as moderate and good quality level by HQA.

Figure 9. Temporal variability of hydromorphological indices at the three sites included in the study. *HMS = Habitat Modification Score; LUIcara = Land Use Index; HQA = Habitat Quality Assessment.*



One-way ANOVAs, performed to statistically identify the differences on the hydromorphological metrics of *Control* and both *Restored* sites during the period after the restoration, yielded significant differences ($p < 0.05$) among all three sites for the three hydromorphological variables(Table 14). Consequently, the hydromorphological indices were capable of detecting the changes occurred after the restoration activities at both sites and of differentiating the restoration treatment between sites.

Table 14. Hydromorphological assessment between reaches in the period after the restoration activities from 2014 to 2016. *Results obtained by one-way ANOVA. Significant differences (p-value < 0.05) between reaches in italics. DF = Degrees of Freedom; SS = Sum of Squares; MS = Mean of Squares; F = F value; p = p value.*

	HMS														
	Control* Vs. *Restored1					***Control* Vs. *Restored2***					***Restored1* Vs. *Restored2***				
	DF	**SS**	**MS**	**F**	***p***	**DF**	**SS**	**MS**	**F**	**p**	**DF**	**SS**	**MS**	**F**	***p***
Site	*1*	*68.57*	*68.57*	*2.01E+31*	*0.00*	*1*	*720.00*	*720.00*	*234.00*	*0.00*	*1*	*1097.00*	*1097.00*	*310.90*	*0.00*
Residuals	68	0.00	0.00			78	240.00	3.10			68	240.00	3.50		
	LUIcara														
	DF	**SS**	**MS**	**F**	***p***	**DF**	**SS**	**MS**	**F**	**p**	**DF**	**SS**	**MS**	**F**	***p***
Site	*1*	*1932.50*	*1932.50*	*8826.00*	*0.00*	*1*	*3358.00*	*3358.00*	*3578.00*	*0.00*	*1*	*93.80*	*93.80*	*86.52*	*0.00*
Residuals	68	14.90	0.20			78	73.00	1.00			68	73.72	1.08		
	HQA														
	DF	**SS**	**MS**	**F**	***p***	**DF**	**SS**	**MS**	**F**	**p**	**DF**	**SS**	**MS**	**F**	***p***
Site	*1*	*200.12*	*200.12*	*183.50*	*0.00*	*1*	*4061.00*	*4061.00*	*1785.00*	*0.00*	*1*	*2011.00*	*2011.90*	*1173.00*	*0.00*
Residuals	68	74.17	1.09			78	178.00	2.00			68	116.70	1.70		

3.4.2. ASSESSMENT OF BIOLOGICAL VARIABILITY

In order to test the comparability between the biological communities of the sites, an initial analysis of the biota was performed taking into account the biological status before the restoration took place. Due to the lack of data from 2012 in *Restored 1*, only *Control* and *Restored 2* were considered in these analyses. Hence, the one-factor PERMANOVAs between *Control* and *Restored 2* showed no significant differences ($p > 0.05$) in the biological composition of the two reaches prior to restoration in 2012, in terms of both presence/absence and relative abundance (Table 15). It also showed that Site explained 11% and 4% of the variance in presence/absence and relative abundance descriptors, respectively.

Table 15. Comparison of the biological composition between Control and Restored 2 sites before the restoration took place. *Results obtained by PERMANOVA. DF = Degrees of Freedom; SS = Sum of Squares; MS = Mean of Squares; F = F value; R^2 = Explained variation; p = p value.*

			Presence / Absence			
	DF	**SS**	**MS**	**F**	**R^2**	***p***
Site	1	0.18	0.18	2.31	0.11	0.08
Residuals	18	1.44	0.08		0.89	
Total	19	1.62			1.00	
			Relative abundance			
	DF	**SS**	**MS**	**F**	**R^2**	***p***
Site	1	0.05	0.05	0.71	0.04	0.40
Residuals	18	1.39	0.08		0.96	
Total	19	1.45			1.00	

One-way ANOVAs between *Control* and *Restored 2* in 2012 showed no significant differences between sites for all the common bioassessment metrics except for 1-GOLD that showed significant differences ($p < 0.05$) with F = 6.56 (Table 16). Accordingly to these results we can assume that the biological communities at both sites, *Control* and *Restored 2*, and by extension *Restored 1*, were comparable in terms of biological composition and structure, and consequently, *Control* site can be used as a true Control for the comparison between sites after the restoration.

Table 16. Comparison of the common bioassessment metrics between Control and Restored 2 sites before the restoration took place. *Results obtained by one-way ANOVA. Significant differences (p-value < 0.05) between reaches in italics. DF = Degrees of Freedom; SS = Sum of Squares; MS = Mean of Squares; F = F value; p = p - value.*

			Shannon-Wienerdiversityindex		
	DF	**SS**	**MS**	**F**	***p***
(Intercept)	1	9.01	9.01	166.32	0.00
Site	1	0.06	0.06	1.15	0.30
Residuals	18	0.98	0.05		
			Number of EPT families		
	DF	**SS**	**MS**	**F**	***p***
(Intercept)	1	8.45	8.45	23.40	0.00
Site	1	0.05	0.05	0.14	0.71
Residuals	18	6.50	0.36		
			% of EPT families		
	DF	**SS**	**MS**	**F**	***p***
(Intercept)	1	0.48	0.48	23.71	0.00
Site	1	0.00	0.00	0.11	0.75
Residuals	18	0.37	0.02		
			EPT / Chironomidae		
	DF	**SS**	**MS**	**F**	***p***
(Intercept)	1	0.01	0.01	12.22	0.00
Site	1	0.00	0.00	1.31	0.27
Residuals	18	0.01	0.00		
			1-GOLD		
	DF	**SS**	**MS**	**F**	***p***
(Intercept)	1	0.00	0.00	17.32	0.00
Site	*1*	*0.00*	*0.00*	*6.56*	*0.02*
Residuals	18	0.00	0.00		

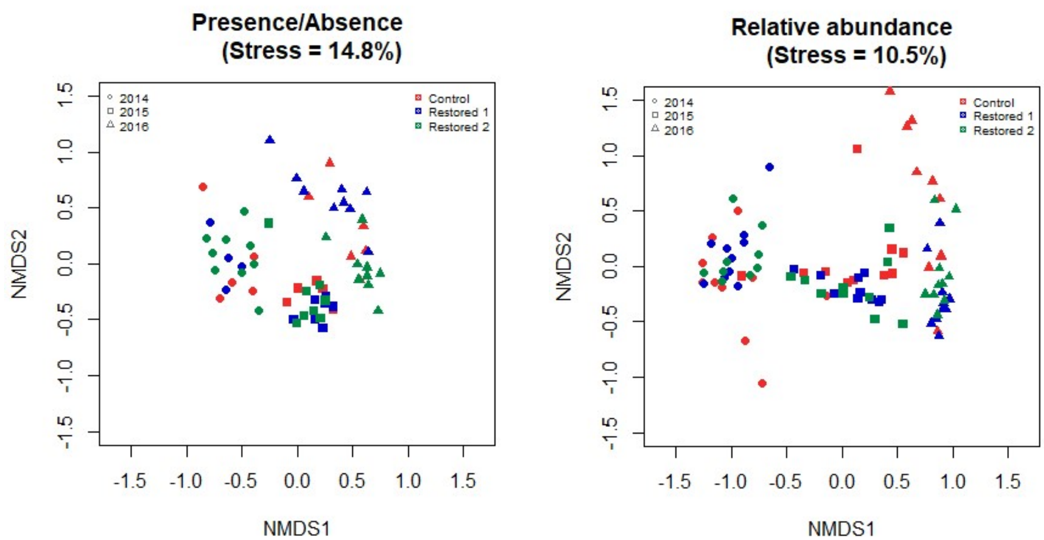
The two-factor PERMANOVA between *Control* and both *Restored* reaches performed during the after period and based on Year and Site identified as significant (p< 0.05) only the interaction between site and year for relative abundance although the variance associated with the interaction was low (R^2 = 0.034; Table 17). In contrast, Year explained 44% and 49% of the variance in presence/absence and relative abundance descriptions, respectively. Site was also significant for presence/absence and relative abundance but explained a limited amount of variation (3% and 3.6%, respectively).

Table 17.Comparison of the biological composition between Control and Restored sites after the restoration (from 2014 to 2016).*Results obtained by two-factor PERMANOVA. Significant differences (p-value < 0.05) between reaches in italics.DF = Degrees of Freedom; SS = Sum of Squares; MS = Mean of Squares; F = F value; R^2 = Explained variation; p = p value.*

	Presence/Absence					
	DF	**SS**	**MS**	**F**	**R^2**	***p***
Site	*2*	*0.32*	*0.16*	*2.26*	*0.03*	*0.05*
Year	*1*	*4.98*	*4.98*	*70.54*	*0.44*	*0.00*
Site*Year	2	0.18	0.09	1.25	0.02	0.28
Residuals	84	5.94	0.07		0.52	
Total	89	11.42			1.00	
	Relative abundance					
	DF	**SS**	**MS**	**F**	**R^2**	***p***
Site	*2*	*0.70*	*0.35*	*3.40*	*0.04*	*0.01*
Year	*1*	*9.67*	*9.67*	*93.72*	*0.49*	*0.00*
Site*Year	*2*	*0.66*	*0.33*	*3.22*	*0.03*	*0.01*
Residuals	84	8.66	0.10		0.44	
Total	89	19.70			1.00	

The nMDS ordinations performed with presence/absence and relative abundance data during the period after the restoration were stable (Fig. 10). Both ordinations indicated that changes are occurring between years and sites but it seems that relative abundance is changing differently during the last sampled year (i.e. 2016) in which *Control* site is significantly different from *Restored* sites.

Figure 10. Non-metric multidimensional scaling (nMDS) ordination of invertebrate community composition for the period after the restoration (from 2014 to 2016) for all sites.



One-way ANOVAs between *Control* and *Restored 1, Control* and *Restored 2,* and *Restored 1* and *2*during the after period (2014-2016) showed no significant differences ($p > 0.05$) between sites for all the common bioassessment metrics (Table 18). Accordingly to these results we can hypothesize that none of the five common bioassessment metrics that have been used for the detection of the restoration were able of identify the changes as it did the community composition descriptors (i.e. presence/absence and relative abundance).

Table 18. Comparison of the common bioassessment metrics between different sites after the restoration. *Results obtained by one-way ANOVA. Significant differences (p-value < 0.05) between reaches in italics. DF = Degrees of Freedom; SS = Sum of Squares; MS = Mean of Squares; F = F value; p = p - value.*

		Control* Vs. *Restored 1				***Control* Vs. *Restored 2***				***Restored 1* Vs. *Restored 2***			
						Shannon-Wiener diversity index							
	DF	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***
(Intercept)	1	*59.85*	*59.85*	*345.33*	*0.00*	*63.57*	*63.57*	*383.23*	*0.00*	*61.05*	*61.05*	*414.34*	*0.00*
Site	1	0.03	0.03	0.147	0.702	0.01	0.01	0.036	0.851	0.06	0.06	0.381	0.54
Residuals	58	10.05	0.17			9.62	0.17			8.55	0.15		
						Number of EPT families							
	DF	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***
(Intercept)	1	*173.4*	*173.4*	*413.31*	*0.00*	*176.82*	*176.82*	*286.73*	*0.00*	*190.82*	*190.82*	*323.92*	*0.00*
Site	1	0.27	0.27	0.636	0.429	0.42	0.42	0.676	0.414	0.02	0.02	0.028	0.867
Residuals	58	24.33	0.42			35.77	0.62			34.17	0.59		
						% of EPT families							
	DF	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***
(Intercept)	1	*6.255*	*6.255*	*246.7*	*0.00*	*5.203*	*5.203*	*307.51*	*0.00*	*5.855*	*5.855*	*251.51*	*0.00*
Site	1	0.019	0.019	0.759	0.387	0.007	0.007	0.39	0.535	0.048	0.048	2.078	0.155
Residuals	58	1.471	0.025			0.981	0.017			1.35	0.023		

Table 18. Continuation.

		Control* Vs. *Restored 1				***Control* Vs. *Restored 2***				***Restored 1* Vs. *Restored 2***			
		EPT / Chironomidae											
	DF	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***
(Intercept)	1	*50.67*	*50.67*	*17.608*	*0.00*	*70.99*	*70.99*	*16.29*	*0.00*	*55.94*	*55.94*	*18.048*	*0.00*
Site	1	0.9	0.9	0.311	0.579	0.13	0.13	0.03	0.8633	1.71	1.71	0.552	0.461
Residuals	58	166.9	2.88			252.72	4.36			179.77	3.1		
		1-GOLD											
	DF	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***
(Intercept)	1	*10.105*	*10.105*	*94.384*	*0.00*	*9.398*	*9.398*	*97.972*	*0.00*	*13.162*	*13.162*	*110.94*	*0.00*
Site	1	0.316	0.316	2.954	0.091	0.202	0.202	2.103	0.152	0.013	0.013	0.108	0.744
Residuals	58	6.21	0.107			5.564	0.096			6.881	0.119		

3.4.3. ENVIRONMENT TO BIOTA ASSOCIATIONS

Analysis of the water quality parameters indicated that water quality did not vary substantially between sites, although water quality did vary among years (Table 19). Total phosphorous seemed to have a decreasing trend, presenting its largest concentrations during 2012 and 2014 and showing an important decrease during the last two years. Ammonia concentration showed considerable variability between Before and After the restoration activities. Nitrate concentration was highly variable among years and no trend seem to be detected.

Table 19. Water quality parameters measured at* Control *and* Restored 2 *sites Before (2012) and After the restoration activities, in Seveso river at its pass through Cormano (Milan, Italy).

	Control				Restored 2			
Water quality parameters	**2012**	**2014**	**2015**	**2016**	**2012**	**2014**	**2015**	**2016**
Total phosphorous (μgL^{-1} P_t)	1400	1900	500	700	1300	1800	500	700
Ammonia ($\mu gL^{-1}N\text{-}NH_4$)	10.00	0.63	0.28	0.40	9.1	2.41	0.52	0.34
Nitrate ($\mu gL^{-1}N\text{-}NO_3$)	1.30	9.60	2.81	8.80	0.9	9.1	2.8	8.34

The average hydrometric level of the summer months was negative for all years, except 2014. However, maximum hydrometric level during summer was less than 2 cm different between 2012 and 2014. The Maximum summer level in 2012 and 2014 was more than 70 and 50 cm higher than for 2015 and 2016, respectively. Water level on the day prior to sampling was negative in all years with the greatest departure from the standard-level in 2016 (Table 20).

PLS regression associating biological metrics to environmental predictors generated a significant model (Q^2_y = 0.311) that explained 52% of the variation in predictor variables (r^2_x) and 34% of the variation in response variables (r^2_y). The association between predictor (i.e. environmental and hydromorphological features) and response variables (i.e. biological metrics) was depicted in a biplot showing the scores and loadings from the PLS regression (Table 21, Fig. 11). Predictor variables that showed strong predictive power (i.e. VIP > 1) in the model projection were, as per water quality parameters, the concentration of total phosphorous (1.42) and ammonia (1.15); as per hydrometric level, average summer level (1.42) and maximum summer level (1.41). None of the hydromorphological indices were associated with the biological metrics.

Table 20. Hydrometric levels measured Before (2012) and After the restoration activities, in Seveso river at its pass through Cormano (Milan, Italy).

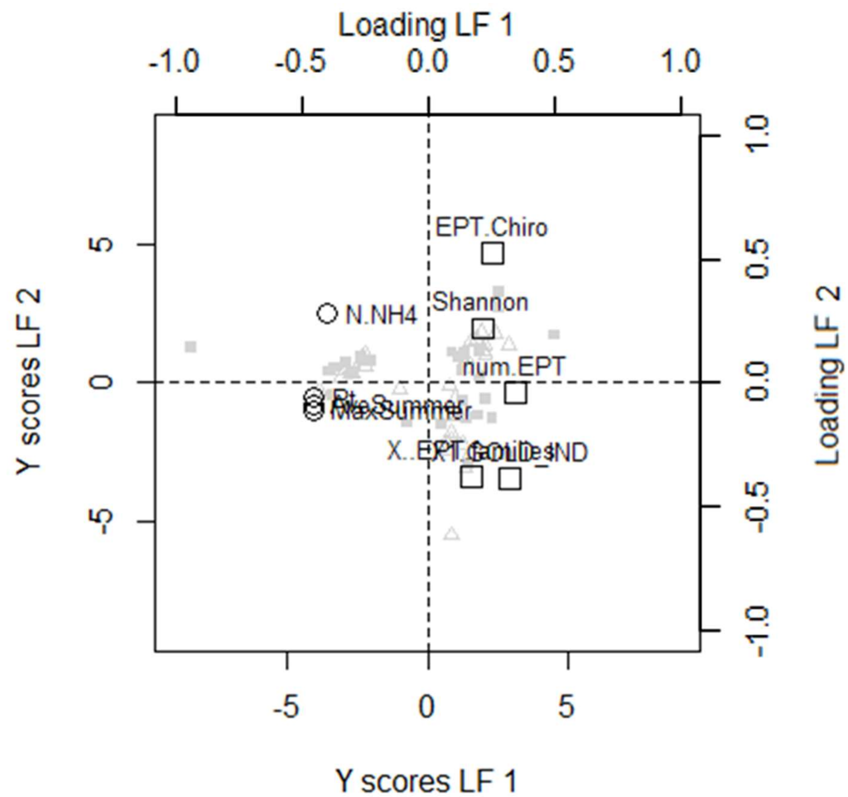
Hydrometric levels *	**2012**	**2014**	**2015**	**2016**
Average summer (cm)	-6.90	8.65	-5.62	-6.40
St. Dev.	10.14	22.43	12.74	15.14
Maximum summer (cm)	228.70	229.80	156.20	177.50
St. Dev.	10.14	22.43	12.74	15.14
Average day (cm)	-1.71	-4.57	-4.38	-10.77
St. Dev.	8.54	1.07	0.78	1.11

* Hydrometric level have been measured upstream the study area and hence, is the same for all sampled sites.

Table 21. Variance Importance in the Projection (VIP) values and standardized coefficients of predictor variables of the PLS regression model. *(*) Indicates significant predictor variables (i.e. VIP > 1) in the model projection.*

Water quality parameters	**VIP (t1)**	**Shannon**	**# EPT**	**% EPT**	**EPT/Chiro**	**1-GOLD**
Total phosphorous (µg/L P_t)	1.420*	3.60	0.98	-4.48	-1.13	1.06
Ammonia (mg/L $N\text{-}NH_4$)	1.150*	0.93	-0.03	-1.27	-0.74	0.29
Nitrate (mg/L $N\text{-}NO_3$)	0.977	-0.80	-0.27	1.40	0.20	0.18
Hydromorphological variables	**VIP (t1)**	**Shannon**	**# EPT**	**% EPT**	**EPT/Chiro**	**1-GOLD**
HMS	0.123	0.10	-0.04	-0.45	0.09	0.09
LUIcara	0.664	2.60	0.53	-2.62	-1.25	0.23
HQA	0.218	2.04	0.41	-2.37	-0.62	0.14
Hydrometric levels	**VIP (t1)**	**Shannon**	**# EPT**	**% EPT**	**EPT/Chiro**	**1-GOLD**
Summer average	1.418*	5.08	1.45	-6.21	-1.72	-0.50
Summer maximum	1.409*	-8.13	-2.67	9.08	1.98	-1.41
Daily average	0.455	1.09	0.46	-1.35	-1.15	0.68

Figure 11. Scores and loadings biplot for the partial least squares (PLS) regression analysis of the common bioassessment metrics (Shannon diversity, number and percentage of EPT families, EPT/Chiro and 1-GOLD) as influenced by environmental variables considered to be important in the model (VIP > 1). *Y-scores of individual samples per monitoring campaign on both latent factors (LF) are represented on the primary axes and are denoted by grey shapes, where individual shapes represent a single study site. Variable loadings are represented on the secondary axes and show the association between predictor variables (i.e. environmental variables: circles), and response variables (i.e. common bioassessment metrics: squares). Predictor variables situated closer to the trend line and further from the origin are considered more influential in the model. Likewise, the position of predictor variables in reference to the response variables indicates the direction of association. VIP = variable influence on projection.*



3.5. DISCUSSION

In-stream physical restoration aims to improve the state of the aquatic community by modifying channel features and river banks at a *site* scale (Belletti et al. 2015, Lake 2001, Miller et al. 2010), assuming that the status of the biological community is limited by physical habitat heterogeneity (Miller et al. 2010). Following this approach and taking into account the high degree of urbanization of the basin, the main goal of the present project was to enhance habitat quality, being aware that the total naturalization of the river was not possible. Consequently, in order to reduce the adverse effects of resectioning and reinforcement, the restoration efforts focused on widening the channel and increasing the number of meanders, promoting point bars and stimulating instream biodiversity (Raven et al. 1998c).

The improvement of the habitat was depended on the restoration techniques

The effectiveness and performance of restoration measures depends on different factors: (i) scientific rigor with which they have been settled; (ii) the design of the project depending on the type of river, area, goals, etc.; and (iii) the combined effects of the different measures together (Lake 2001, Palmer 2008, Roni et al. 2008,Speed et al. 2016). Under these assumptions, the variation between the two restored reaches of our study indicated that **improvement in hydromorphological conditions was dependent on the bioengineering techniques applied.** There is an extensive literature on the effectiveness of restoration techniques (see review from Roni et al. 2008) that shows how instream habitat restoration activities, such as wood and boulder structures addition, can improve instream habitat. However, the failure rate of these structures is significantly variable (e.g. failure rate in North American studies ranged between 0 and 85%). These studies claimed that restoration failure is mainly due to the materials used for the construction of instream structures or the stream type among other reasons, but the use of more natural materials have led to improvements in the habitat such as increments of more than 50% in pool frequency and depth, woody debris, habitat heterogeneity and complexity, spawning gravel, and sediment and organic matter retention (Roni et al. 2008). In fact, in our study *Restored 1* showed higher values of HMS, penalized by the presence of concrete banks and numerous lateral structures added, while *Restored 2* presented a higher diversity and quality of stream habitats thanks to the less impacting bioengineering structures. Similarly, European studies on low-gradient channelized streams subjected to restoration have recorded an increase in habitat complexity, in depth, flow and substrate heterogeneity, channel morphology and organic matter retention (Roni et al. 2008). Overall, our assessment of the **hydromorphological indices** calculated during the whole monitoring period **indicated that the restoration activities**

reduced anthropogenic impacts in the riparian corridor and diversified instream habitats, mainly by increasing the physical habitat heterogeneity in the *Restored* reaches compared to the *Control*.

Common bioassessment metrics: Lack of sensitivity to differentiate between sites

In our study, common bioassessment metrics used for analysis of the community have already been used for the assessment of habitat alterations (Buffagni et al. 2016, Corbi and Trivinho-Strixino 2008, Frenzel 1996), Moreover, past studies have measured the responses of these biological metrics to habitat diversification or degradation (AQEM Consortium 2002, Buffagni et al. 2016, Pinto et al. 2004,Tavzes et al. 2006). For example, EPT-related metrics have been often used as indicators of stream quality due to their negative response to the decrease in habitat diversification, and more in particular to the variety in flow velocity and the stream bed diversity (i.e. increasing deposition of organic substrate or absence of coarse material) (AQEM Consortium 2002). Tavzes et al. (2006) suggested that EPT/Chironomidae responded negatively to habitat degradation, showing a habitat template that favours low-diversity macroinvertebrate community (Wang et al. 2013). Another example of biological metrics used as habitat descriptive are Chironomids and Oligochaeta, considered tolerant colonisers of fine sediments (AQEM Consortium 2002). Finally, other studies have focused in tolerant taxa, as is Gastropods, Oligochaeta and Diptera (1-GOLD), claiming a negative relationship with habitat degradation (Buffagni et al. 2016, Pinto et al. 2004).

One of the challenges of my study was to understand the temporal and spatial variability of the biological assemblage in impaired streams after restoration activities. **Indeed, my results showed that spatial and temporal variability in benthic community was significant after the restoration, with temporal variation being largest source of variation in terms of community composition (i.e. presence/absence and relative abundance).** The interaction between temporal and spatial variation in benthic community whilst statistically significant, was smaller than the variation between sites, suggesting that although the benthic community changed in time, the underlying differences between restored and unrestored sites were still noticeable. Moreover, the temporal variation that was detected while studying the persistence (i.e.

presence/absence; Collier 2008, , Bradley and Ormerod 2001, Holling 1973) and stability (i.e. relative abundance; Collier 2008, Connel and Sousa 1983, Milner et al. 2006, Scarsbrook 2002) of the macroinvertebrate community showed how the community was still changing and adapting to the new condition by the fall of 2016. Hence, long term monitoring is likely necessary to more clearly identify recovery from disturbances (Allan 2004, Collier 2008, Dodds et al. 2012, Jackson and Füreder 2006).

Although the hydromorphological indices and community composition metrics have demonstrated their capacity to differentiate between a *Control* site and a *Restored* sites, I cannot conclude the same for the common bioassessment metrics that I applied. **Of all the biological metrics use to assess ecological effects of the hydromorphological changes (AQEM Consortium 2002, Buffagni et al. 2016, Pinto et al. 2004, Tavzes et al. 2006), only 1-GOLD was different between sites (*Control* and *Restored 2*) after the restoration.** 1-GOLD, a metric that expresses the inverse of the relative abundance of taxa commonly considered as colonizers (Pinto et al. 2004), might suggest that Restored 2 presents events of substitution of colonizers by more specialized taxa (Buffagni et al. 2016, Pinto et al. 2004) and, consequently, we can hypothesize that *Restored 2* shows a slower degradation than the *Control* reach.**Overall, the inability of the common bioassessment metrics to discriminate between sites before and after the restoration process might be due to their seasonal variation,** as widely studied by other authors (Azrina et al. 2006, Šporka et al. 2006, Welte and Campbell 2003). Although our study was designed to avoid such seasonality by monitoring only during fall, metrics based on EPT families and Shannon-Wiener diversity index are mainly dependent on temperature and precipitation (Azrina et al. 2006, Brittain1974, 1983, Radford and Hartland-Rowe 1971, Welte and Campbell 2003) and some authors suggest sampling during spring as being the most diverse season (Šporka et al. 2006, Welte and Campbell 2003).

Weak predictive capacity of the hydromorphological variables on the macroinvertebrate response

Hydromorphological variables have shown to be effective in differentiating the different types of restoration measures although they had not shown a strong predictive capacity on the macroinvertebrate community response. In fact, the group of environmental variables that most predicted the changes in the common bioassessment metrics was the group of water quality parameters with a total sum of significant VIP of 3.714, followed by the group of hydrometric levels with a total sum of significant VIP of 3.544. **The high importance of the variables related to water quality and hydrology as predictors of macroinvertebrate community variability resides on the impaired conditions of the river**. As we know, Seveso river is a typical channelized urban stream, impaired by a poor water quality; consequently, benthic macroinvertebrate community, adapted to such conditions suffers from the “Urban Stream Syndrome”, presenting a low diverse community constituted by a pool of taxa tolerant to organic pollution (Komínková 2012) which prevents biological recovery from restoration (Roni et al. 2008). The lack of sensitivity of the macroinvertebrates to this restoration project might be also due to a deficient knowledge of the general situation of the area in terms of limiting factors for biotic production (Roni et al. 2008), since the possible source population in the surrounding area to promote recolonization of restored sites could be limited by the low quality habitat of the catchment area (Sundermann et al. 2011).The problem of the lack of source populations might remain invariable after the restoration activities that we have studied since the restoration project has been carried out in a small reach and not at a catchment scale, and hence, macroinvertebrate community is not able to recolonize new areas (Sundermann et al. 2011). In fact, the modifications that have been carried out during the project have provided just instream modifications, i.e. introducing artificial and natural elements into the active stream channel, limiting the final effects of the overall restoration of the river (Roni et al. 2008).

Not only the restoration project took place at an instream level but probably the objectives of the restoration activities were not designed specifically to improve the benthic macroinvertebrate community but only physical habitat. Indeed, most restoration projects addressed under an instream approach have been deigned to cope with fish community rehabilitation but few to improve macroinvertebrate community, and overall not many of them are included in peer-reviewed literature (see Roni et al. 2008). Differently to the fish community, findings taking into account the improvement of macroinvertebrate after restoration projects show highly variable results, being the following the major findings: (i) increase in abundance, in some functional groups, or in diversity; and (ii) no differences at all after the restoration activity (Roni et al. 2008). The latter finding from this literature review (Roni et al. 2008) coincide with our results: no significant evidence of benthic macroinvertebrate improvement (or minimal improvement on benthic macroinvertebrate) due to the restoration activities was found. In other words, the biological metrics were not sensitive to the hydromorphological variables used for the assessment of the restoration, highlighting the fact that the project was not designed to improve benthic community habitat.

3.5.1. CAVEATS AND LIMITATIONS

Tools for project assessment and limited time span

A major goal of our study was to identify variables that were able to assess the project performance. It is difficult to find these variables in literature (Miller et al. 2010) but thanks to the three hydromorphological indices (LUIcara, HMS, HQA) derived from the CARAVAGGIO approach we were able to detect and quantify the restoration efforts. More challenging was the identification of suitable biological features to evaluate the performance in such a short time frame; not an easy task as some authors have already demonstrated (Muotka et al. 2002, Yount and Niemi 1990). Some of the bioengineering techniques that have been used in this study (e.g. vegetation of banks, addition of groynes, creation of riffle-pool areas, meandrification of the river channel) have contributed positively to the improvement of the hydromorphological status and, consequently, to the ecological status of the river as supported by other authors (Caruso and Downs 2007, Donat 1995, Swartz and Herricks 2007). **However, the highly modified physical conditions that affect Seveso river, mainly simplified streambed structure, channelization and absence of vegetated banks ("urban stream syndrome"; Komínková 2012), together with the impaired conditions on water quality from urban and industrial pollution play against a fully recovery in a short time scale** (Detti et al. 2014, Donat 1995, Swartz and Herricks 2007, Yount and Niemi 1990). The apparent slow recovery of the macroinvertebrate community in terms of composition (i.e. presence/absence and relative abundance) and the apparent insensitivity in terms of structure (i.e. common bioassessment metrics) is indicative of the impoverished community composition of a stream with a high degree of anthropogenic pressure with taxa adapted to high levels of organic pollution (Buffagni et al. 2004, Winner et al. 1980).

Although channelization removal seem to be one of the most powerful tools to achieve good ecological status (Komínková, 2012), recovery time could range between 1 to 5 years depending on the extension and intensity of the physical modifications (Yount and Niemi 1990). In addition, it has been found that the impairment on water quality could slow down the improvement of the ecological status (Buffagni et al. 2004, Holling 1973, Winner et al. 1980), reaching up to 50 years of recovery time in heavily modified water bodies (Yount and Niemi 1990). Therefore, due to the massive physical modification to which Seveso River has been subjected, the election of good bioengineering techniques is crucial for the success of the restoration (Donat 1995). The fact that the main stressors (i.e. point and diffuse organic pollution) still persisted after the restoration activities, and that the biological community did not greatly change, in terms of community composition, following the restoration activities demonstrate a low capacity of the benthic community to recover (Sundermann et al. 2011). Further elimination of point sources of pollution is necessary for the mitigation of urban development impacts (Caruso and Downs 2007).

3.6. CONCLUSIONS

1) Improvement in hydromorphological conditions was dependent on the bioengineering techniques applied.

2) The selected hydromorphological indices indicated that the restoration activities reduced the anthropogenic impacts in the riparian corridor and diversified instream habitats, mainly by increasing the physical habitat heterogeneity.

3)The highly modified physical conditions that affect Seveso river together with the impaired water quality from urban and industrial pollution play against a full recovery in a short time scale.

4) Restoration activities led to a significant positive change in the benthic community in terms of spatial and temporal variability.

5) Only the 1-GOLD metric had the capacity to discern between *Control* and *Restored* sites, suggesting that the improvement of the habitat quality lead to a substitution of colonizers by more specialized taxa.

6) The lack of differentiation capacity between sites of the common bioassessment metrics used in the study might be due to their seasonal variation.

7) Weak predictive capacity of the hydromorphological variables on the macroinvertebrate response. The impaired conditions of the river seemed to hide the positive effects of the restoration.

8) "Urban stream syndrome" of Seveso river play against a fully recovery in a short time scale.

3.7. CONCLUSIONES

1) La mejora de las condiciones hidromorfológicas depende de las técnicas de bioingeniería que fueron aplicadas.

2) Los índices hidromorfológicos elegidos indicaron que las actividades de restauración redujeron los impactos antrópicos del corredor ripario y diversificaron los habitats del río, principalmente incrementando la heterogeneiddad física del habitat.

3) Las condiciones de elevada modificación física que afectan al río Seveso, junto con las condiciones desfavorables de la calidad del agua frovenientes de una contaminación industrial y urbana, juegan en contra de una recuperación a corto plazo.

4) Las actividades de restauración han llevado a cambios positivos de la comunidad bentónica en términos de variabilidad espacial y temporal.

5) Solo la métrica 1-GOLD mostró capacidad para diferenciar entre los sitios de Control y Restaurados, sugiriendo que la mejora de la calidad del habitat llevó a la sustitución de los colonizadores por taxones más especializados.

6) La falta de una clara capacidad de diferenciación entre sitios por parte de las métricas comunes de bioevaluación usadas en el estudio puede deberse a su variación estacional.

7) Capacidad predictive débil por parte de las variables hidromorfológicas en la respuesta de los macroinvertebrados. Las condiciones desfavorables del río parecen esconder los efectos positivos de la restauración.

8) El "síndrome del río urbano" del Seveso juega en contra de una recuperación a corto plazo.

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CHAPTER 4. FINAL CONSIDERATIONS

4.1. IMPLICATIONS FOR BIOMONITORING. FUTURE DIRECTIONS

During the last century the world has suffer a rapid growth in population that has led to the exodus of people from rural to urban areas, leading to the transformation of land use patterns. These changes in land use have transformed the landscape with rivers being one of the ecosystems bearing much of the brunt in terms of human caused impairment. Different measures have been proposed in order to cope with the increasing habitat modification of river ecosystems. Such measures, regulated by different legislations all over the world, have achieved the aim of restoring and rehabilitating, at least, part of the aquatic ecosystems at different levels from instream to catchment scale. However, due to riverscape modifications, hydrology, water quality and biology have been compromised to the point that biological communities are now adapted to such impaired conditions, which might obscure the real status of the river ecosystem. In impaired habitats like these, the temporal variability of the communities rely on trait substitution instead of taxa substitution and, thus, bioassessment metrics commonly used for quality assessment are not able to detect changes. More specifically, in our studies resulted difficult to find the best set of environmental drivers that best explain the variability on the benthic community and how to measure and quantify it. Probably due to the fact that all study sites were impaired by poor water quality and during the study period it did not change, theimprovement of the macroinvertebrate community obscured the long-term interannual variability of the community and the results of the restoration in both studies.

The lack of association between temporal variation in the biota and environmental variables during the study period has implications for biomonitoring, since the general assumption commonly made by riverine ecological assessment systems is that community variance can be largely explained through environmental variables (Humphrey et al. 2000). Rather results of my study suggest that both **water quality and hydrological variables played a minor role in shaping the sampled benthic communities,** despite the extensive environmental dataset used to describe the environmental state of the study area. Furthermore, some of the selected variables were devoted to this particular region (e.g. ice-influenced and open-water period variables for the Canadian study sites) and have been proven to detect measurable effects on the riverine habitat.

Short-term spatial stability of benthic communities in reference and impaired streams has been widely studied across different environmental gradients under both taxonomic and functional approaches (see review of Menezes et al. 2010). Under these studies, taxonomic composition was highly variable between reference sites (Barbour et al.1996) and was not always strongly associated with environmental conditions. In contrast, functional composition of the community has been observed to be more stable across sites (Charvet et al. 2000, Statzner et al. 2001, Culp et al. 2011, Statzner et al. 1997, Bêche et al. 2006), suggesting that variability of the benthic community is surrounded by an internal noise due to the natural (inherent) variability of the system (Vannote et al. 1980). It is usually assumed that river ecosystems in reference (unimpaired) conditions show certain stability in time (Vannote et al. 1980), but long-term studies are still necessary to elucidate any patterns of variability in the benthic community. Quite obviously the identification of long-term and inter-annual pattern of variability is crucial in understanding potential spatial effects of climate change (Reynoldson et al. 2001, Clarke et al. 2003, Daufresne et al. 2007, Hanna et al. 2004, 2007, Armanini et al. 2014). Consequently, our study findings suggest that **stochastic species substitutions may be as, or more, important to within site variability of benthic community composition at impaired sites than environmental variables.** While taxa substitution occurs normally in disturbed streams (Bradt et al. 1999) the underlying causes of such turnover are difficult to determine in these situations, generating uncertainty regarding community capacity to buffer disturbances in impaired ecosystems (Collier 2008, Holling 1973, Winterbourn 1997). Thus, the strong inherent background variability embedded in the community might be a challenge to understand underlying drivers of temporal variation and to deal with the uncertainties in river management.

In addition, the lack of relationship between environmental factors and the benthic community composition in my study streams had strong effects on the perception of community status and highlights a potential problem with the statistical stability of traditional ecological assessment methods for impacted rivers. The fact that specific biological metrics are driven by temporal variability and not exclusively by environmental factors, obscures the purpose of the bioassessment of sites characterized by communities that follow a stochastic pattern as the one studied in this research. In fact, some authors have presented a significant seasonal variability of some common bioassessment metrics in reference sites of the same area of our study site, especially in taxa richness and Family Biotic Index (Linke et al. 1999). A solution to this problem might be the use of specific biological metrics that account for the actual status and inherent biological variability of the community such as trait-based metrics that are less sensitive to taxonomic substitutions through time. **Trait-based approaches could help in identifying stressors responsible for specific patterns of ecological degradation, and could be good for detecting stressors in impaired streams. The occurrence of specific traits linked to resistance to a given stressor can enhance the identification of the drivers of change.**

The results gathered during both of my studies emphasize the **need for selecting instream habitat restoration activities after a series of measures that include first the improvement of water quality and hydrology, and second the improvement of the habitat at a bigger scale (riparian and floodplain habitat)** (Shields et al. 1995, Roni et al. 2008). In addition, I support a stronger emphasis on **the establishment of the amount and drivers of temporal stability in community structure, particularly within impaired streams, as part of riverine bioassessment protocol development**. Once such variability is better understood, then a number of approaches can be used to integrate and reduce the effects of such variability in the ecological assessment (see Resh et al. 2013), including: (i) selection of a subset of reference samples in order to compare sites with similar environmental conditions; (ii) adjustments of the sampling strategy to avoid sampling in hydrological or climatic periods that might enhance the variability sources; and (iii) derivation of correction factors as proposed by Buffagni et al. (2013) to limit the influence of specific natural sources of variability, such as the lentic-lotic character, on biological metrics.

4.2. IMPLICACIONES PARA EL BIOMONIOREO. FUTURAS DIRECCIONES

Durante el ultimo siglo el mundo ha sufrido un rápido crecimiento de la población que ha llevado al éxodo de la gente de zonas rurales a urbanas, lo que ha conllevado la transformación del uso del territorio. Estos cambios en el uso del territorio han transformado el paisaje siendo los ríos los ecosistemas más danados y modificados. Durante la historia se han llevado a cabo diferentes medidas para hacer frente a al aumento de modificaciones de los ecosistemas riparios. Tales medidads, reguladas por diferentes legislaciones en todo el mundo, han conseguido alcanzar el objetivo de restaurar y rehabilitar, al menos, parte de los ecosistemas ecuáticos a diferentes niveles, a scala de río o de cuenca.Sin embargo, debido a las modificaciones del paisaje ripario, hidrología, calidad del agua y biología se han puesto en peligro hasta el punto de que las comunidades biológicas están adaptadas a tales niveles de deterioro que pueden oscurecer el estado real del ecosistema ripario. En condiciones desfavorables como estas, la variabilidad temporal de las comunidades recae en la sustitución de rasgos biológicos en lugar de en la sustitución de especies y, por tanto, las métricas usadas normalmente para la evaluación de la calidad no son capaces de detectar cambios en el ecosistema. Específicamente, en nuestros estudios resultó difícil encontrar el mejor grupo de variables ambientales motoras de la variabilidad de la comunidad bentónica y cómo medirla y cuantificarla. Probablemente debido al hecho de que todos los sitios de estudio estuvieran deteriorados por una calidad muy baja del agua y que ésta no cambió a lo largo de todo el periodo de estudio, la mejora de la comunidad escondió la variabilidad interanual de la comunidad a largo plazo y los resultados de la restauración.

La falta de relación entre la variación temporal de la biota y de las variables ambientales durante el periodo de estudio tiene implicaciones para el biomonitoreo, ya que la asunción general que se hace en los sistemas de evaluación ecologica de ríos es que la variabilidad de la comunidad puede ser mayormente explicada a través de las variables ambientales (Humphrey et al. 2000).Sin embargo, los resultados de nuestros studios sugieren que la calidad del agua y las variables hidrológicas juegan un papel menor en la configuración de las comunidades bentónicas, a pesar de que el conjunto de datos ambientales usados para el estudio recogieran una información muy valiosa para describir el estado ambiental del area de estudio, y a pesar de que algunas de las variables seleccionadas estuvieran asignadas a una región en particular (por ejemplo, las variables influenciadas or el hielo y el periodo abierto en la zona Canadiense) y que hubieran demostrado sus efectos medibles en habitats riparios.

La estabilidad espacial a corto plazo de las comunidades bentónicas en ríos de referencia y deteriorados han sido ampliamente estudiados bajo diferentes fradientes ambientales y bajo diferentes enfoques, taxonómicos y funcionales (leer la revisión de Menezes et al. 2010).Bajo estos estudios, la composición taxonómica fue altamnete variable entre sitios de referencia (Barbour et al. 1996) y los diferentes grados de variabildad taxonómica de la comunidad respondieron a condiciones ambientales, mientras que la composición funcional de la comunidad fue estable entre sitios (Charvet et al. 2000, Statzner et al. 2001, Culp et al. 2011, Statzner et al. 1997, Bêche et al. 2006), sugiriendo que la variabilidad de la comunidad bentónica está rodeada por un ruido interno debido a la variabilidad natural (intrínseca) del sistema (Vannote et al. 1980).Normalmente se assume que los ecosistemas riparios en condiciones de referencia muestran cierta estabilidad en el tiempo (Vannote et al. 1980), pero todavía son necesarios más estudios a largo plazo para dilucidar los patrones de variabilidad de la comunidad bentónica. Parce obvio que la identificación de patrones de variabilidad interanual y a largo plazo son críticos para entender los efectos espaciales potenciales del cambio climático (Reynoldson et al. 2001, Clarke et al. 2003, Daufresne et al. 2007, Hanna et al. 2004, 2007, Armanini et al. 2014).Como consecuencia, los hayazos de nuestro estudio sugieren que las sustituciones estocásticas de especies pueden ser tan o más importantes para la variación de la composición de la comunidad bentónica que la variables ambientales en sitios deteriorados. Mientras que la sustitución de species ocurre normalmente en ríos deteriorados (Bradt et al. 1999), las causas subyacentes de tal rotación son difíciles de determinar en estas situaciones, generando una cierta incertidumbre en cuanto a la capacidad de la comunidad para amortiguar las perturbaciones en ecosiistemas deteriorados (Collier 2008, Holling 1973, Winterbourn 1997).Por tanto, la fuerte variabilidad intrínseca de fondo que envuelve a la comunidad puede ser un reto para entender el motor subyacente de la variación temporal y para hacer frente a las incertidumbres en la gestión de ríos.

Además, la falta de relación entre factores ambientales y la composición de la comunidad en nuestros ríos estudiados tiene una fuerte influencia en la percepción del estado de la comunidad y enfatiza un problema potencial con la estabilidad estadística de los métodos tradicionales de gestión ecológica para ríos deteriorados. El hecho de que métricas biológicas específicas estén influenciadas por una variabilidad temporal y no exclusivamente por factores ambientales, complica el objetivo del la gestión biológica de sitios caracterizados por tener comunidades que siguen un patrón estocástico como el etudiado en esta investigación. De hecho, algunos autores han presentado la variabilidad estacional de algunas de las métricas biológicas comunmente usadas en la gestión de la calidad en sitios de referencia en la misma area de nuestro estudio, especialmente en la riqueza de especies y en el Family Biotic Index (Linke et al. 1999).Una possible solución a este problema puede ser el uso de métricas biológicas específicas que justifiquen el estado actual y la variabilidad biológica intrínseca de la comunidad como las métricas basadas en rasgos biológicos, que son menos sensibles a las sustituciones de especies en el tiempo. Los enfoques basados en los rasgos biológicos pueden ayudar a identificar los agentes estresantes responsables de los patrones específicos del degrado ecológico, y pueden ser buenos para detectar los agentes estresantes en sistemas ya deteriorados. La ocurrencia de rasgos específicos ligados a la resistencia de un factor estresante concreto puede fomentar la identificación del motor del cambio.

Los resultados obtenidos durante nuestros estudios enfatizan la necesidad de programar actividades de restauración en ríos solo después de haber llevado a cabo una serie de medidas que incluyan primero la mejora de la calidad del agua y de la hidrología, y segundo la mejora del habitat a una escala mayor (habitat ripario y llanura de inundación) (Shields et al. 1995, Roni et al. 2008).Asimismo, insistimos en un mayor énfasis en el establecimiento de la cantidad y naturaleza de los motores de la estabilidad temporal de la estructura biológica, particularmente en ríos deteriorados, como parte del desarrollo del protocolo de gestión biológica. Una vez que la variabilidad está bien estudiada, entonces numerosos enfoques podrán llevarse a cabo para integrar y reducir los efectos de tal variabilidad en la gestión ecológica (ver Resh et al. 2013), incluyendo: (i) la selección de un subconjunto de muestras de referencia para poder comparar los sitios con condiciones ambientales similares; (ii) ajustes en la estrategia de muestreo para evitar el muestreo en periodos hidrológicos o climáticos desfavorables que aumenten las fuentes de variación; y (iii) la derivación de factores de corrección como propuesto por Buffagni et al. (2013) para limitar la influencia de fuentes específicas naturales de variabilidad, como el carácter léntico-lótico, en las métricas biológicas.

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