Environmental Gradients and Ecological Processes as Constraints in the Distribution Patterns of Freshwater Biological Communities

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Environmental Gradients and Ecological Processes as Constraints in the Distribution Patterns of Freshwater Biological Communities

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1. Introduction

1.1 Limiting factors approach

In the past decades, impressive literature has developed, relating species densities to environmental variables in freshwater ecosystems.

Notwithstanding the huge (and increasing) quantity of literature available about the topic, our capacity to properly set meaningful models to explain most of the variability of data is far from ideal. This proves true both in the study of ecological processes *sensu lato* and in the applied research made to predict the effects of human activities that can create impacts on freshwater ecosystems.

Different statistical and analytical methods try to relate habitat availability and some characteristics of biological communities such as density, species richness, abundance and diversity. However, methods based on models testing hypotheses about central tendency (e.g. ANOVA) or central responses (e.g. ordinary least square regression, PCA, GAM, GLM) set aside the concurring role of other unmeasured factors (Austin 2007).

Every homogeneous group of organisms have a range of tolerance to any given factor, and some factors are more critical than others (Gordon *et al.* 2004). In fact, the concept of niche as "a possible environmental state permitting the species to exist indefinitely" (Hutchinson 1957) is a *n*-hypervolume involving *n* environmental gradients and, thus, variables. Each of them can influence the relationships among the distribution of organisms and a single environmental gradient and inflating variability.

In some cases, looking at the mean response can lead to assess biased or uninformative relationships (Lancaster & Downes 2010). Thus, it is often very difficult to disentangle the

different sources of variation, of natural or anthropogenic origin, in the organism density, abundance and diversity along gradients. From the ecological point of view, testing hypotheses about the environmental gradients as limiting factors or constraints on the density of organisms could be more informative than testing them about "average" responses (Downes 2010). One possible way to do that is to use quantile regression.

The papers of Cade & Noon (2003); Henning et al. (2005); Schooley & Wiens (2005); Lancaster & Belyea (2006); Allen & Vaughn (2010); Doll (2011); Schmidt et al. (2012); Campbell & McIntosh (2013) provide a strong case for the use of quantile regression for modelling the responses of species to environmental variations.

1.2 Habitat based model

Habitat-based models have been widely used to define a relationship between instream flow and habitat availability for various species of fish (Ayllón et al. 2010; Gore et al. 1991; Maddock 1999) and sometimes macroinvertebrate (Gore, Crawford & Addison 1998; Gore, Layzer & Mead 2001; Rosenfeld & Ptolemy 2012).

This kind of models is basically composed of a hydraulic simulation and of suitability curves of species on the basis of which it is possible to define a relationship between the flow and the availability of habitat for aquatic organisms. The conceptual basis of habitat based models is ecological, in fact it is well known that aquatic organisms do not occupy any environment regardless of hydraulics inside the river, but they show strong preferences for certain values of the hydromorphological parameters such as water depth, current velocity, substrate dimension and composition (Van Liefferinge *et al.* 2005; Dolédec *et al.* 2007).

When the flow rate varies, also the depth, the current velocity and often the type of substrate that is gained/lost from the river change and, consequently, the habitat availability for the reference species in the stretch of river changes.

The relationship of physical habitat to aquatic organism densities assumes that the production of benefits is limited by the availability of physical habitat. This assumption is not always true. Production may be limited by water quality (Kail, Arle & Jähnig 2012; Morrissey *et al.* 2013), by the activities of man (Fjeldstad *et al.* 2012; Hansen & Hayes 2012) or by events and conditions occurring at a temporal and spatial scale beyond the scope of the model application (Bonada *et al.* 2008; Menge *et al.* 2011; Comte *et al.* 2013). In essentially all situations, physical habitat is a necessary, but not sufficient, condition for production and survival of aquatic organisms. Thus, habitat based models results may best be viewed as indicators of population potential in systems where the habitat conditions described by the model are major population constraints.

In this perspective the use of density-environment relationships that show the limiting effects of the habitat characteristics and not the average effects on the same variables seems more adequate.

Habitat-based association models could be used in order to understand ecological dynamics and to predict changes in biological communities, especially when human-induced alterations are involved (Muñoz-Mas *et al.* 2012; Schmalz *et al.* 2014). They can also be used in the prediction of the effect of climate and land-use change on the biological community.

1.3 Outline of the thesis

The hypothesis that I tested in my research was that, often, the relationship among distribution of organisms and

environmental gradients can be successfully evaluated as a limiting factor using the proper analytical methods. Hence, the analyses were carried out using quantile regression to assess fish and macroinvertebrate distribution patterns along environmental gradients.

This approach was used in the analyses of field data belonging to different habitat, (rivers, streams and lakes) and different biological communities (macroinvertebrates and fishes).

Later I showed possible applications, using such relationships within habitat based association models to determine environmental flows and to predict the effect of future climate change.

In particular, in Chapter 2 the link between lakes macroinvertebrate community characteristics and their habitat were studied. Twenty-one variables that represent chemical, physical and morphological characteristics of nine different lakes were measured. Using quantile regression to evaluate limiting responses, we selected the variables that best explain the number of taxa present and the diversity of a site.

Chapter 3 shows the way quantile regression can be used to describe the limiting action of both physical habitat characteristics and other environmental variables, such as water quality and pH, on macroinvertebrate density and diversity.

This study was conducted in rivers and streams belonging to two countries: Italy and Finland. This kind of relationships can contribute to the definition of bioassessment indices and can be used within habitat based models.

In Chapter 4 a new approach for the identification of environmental flows summing up the limiting factor approach, the basic ecological relationships and the use of appropriate

spatial scale for different organisms was developed. Potential suitability curves for different life stages of brown trout were realized and used together with those produced previously for macroinvertebrates, in order to define environmental flows. This is one of the few examples available in the literature that shows how to consider together different biological communities, at different trophic levels, within a habitat based model.

In Chapter 5 the impact of potential climate change on the hydrological and ecological status of the alpine stretch of Serio river was investigated. A hydrological model was used to mimic the river hydrological regime in different conditions. Flow driven seasonal habitat availability assessment at river scale was carried out. Future (until 2100) hydrological cycle was then projected; present and prospective habitat availability for fish and macroinvertebrates was investigated.

1.4 References:

Allen, D.C. & Vaughn, C.C. (2010) Complex hydraulic and substrate variables limit freshwater mussel species richness and abundance. *Journal of the North American Benthological Society*, **29**, 383–394.

Austin, M. (2007) Species distribution models and ecological theory: A critical assessment and some possible new approaches. *Ecological Modelling*, **200**, 1–19.

Bonada, N., Rieradevall, M., Dallas, H., Davis, J., Day, J., Figueroa, R., Resh, V.H. & Prat, N. (2008) Multi-scale assessment of macroinvertebrate richness and composition in Mediterranean-climate rivers. *Freshwater Biology*, **53**, 772–788.

Cade, B.S. & Noon, B.R. (2003) A gentle introduction to quantile regression for ecologists. *Frontiers in Ecology and the Environment*, **1**, 412–420.

Campbell, R.E. & McIntosh, A.R. (2013) Do isolation and local habitat jointly limit the structure of stream invertebrate assemblages? *Freshwater Biology*, **58**, 128–141.

Comte, L., Buisson, L., Daufresne, M. & Grenouillet, G. (2013) Climate-induced changes in the distribution of freshwater fish: observed and predicted trends. *Freshwater Biology*, **58**, 625–639.

Dolédec, S., Lamouroux, N., Fuchs, U. & Mérigoux, S. (2007) Modelling the hydraulic preferences of benthic macroinvertebrates in small European streams. *Freshwater Biology*, **52**, 145–164.

Doll, J.C. (2011) Predicting biological impairment from habitat assessments. *Environmental monitoring and assessment*, **182**, 259–277.

Downes, B. (2010) Back to the future: little-used tools and principles of scientific inference can help disentangle effects of multiple stressors on freshwater ecosystems. *Freshwater Biology*, **55**, 60–79.

Fjeldstad, H.P., Barlaup, B.T., Stickler, M., Gabrielsen, S.E. & Alfredsen, K. (2012) Removal of weirs and the influence on physical habitat for salmonids in a Norwegian river. *River Research and Applications*, **28**, 753–763.

Gordon, N.D., McMahon, T.H., Finlayson, B.L., Gippel, C.J. & Nathan, R.J. (2004) *Stream Hydrology*, 2nd ed. John Wiley & Sons, Ltd.

Gore, J., Crawford, D. & Addison, D. (1998) An analysis of artificial riffles and enhancement of benthic community diversity by physical habitat simulation (PHABSIM) and

direct observation. *Regulated Rivers: Research & Management*, **14**, 69–77.

Gore, J.A., Layzer, J.B. & Mead, J. (2001) Macroinvertebrate instream flow studies after 20 years: a role in stream management and restoration. *Regulated Rivers: Research & Management*, **17**, 527–542.

Hansen, J. & Hayes, D. (2012) Long-Term implications of dam removal for macroinvertebrate communities in Michigan and Wisconsin Rivers, United States. *River Research and Applications*, **28**, 1540–1550.

Henning, K., Estrup, H. & Schröder, H. (2005) Rejecting the mean: Estimating the response of fen plant species to environmental factors by non-linear quantile regression. *Journal of Vegetation Science*, **16**, 373–382.

Hutchinson, G. (1957) Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, **22**, 415–427.

Kail, J., Arle, J. & Jähnig, S. (2012) Limiting factors and thresholds for macroinvertebrate assemblages in European rivers: Empirical evidence from three datasets on water quality, catchment urbanization, and river restoration. *Ecological Indicators*, **18**, 63–72.

Lancaster, J. & Belyea, L. (2006) Defining the limits to local density: alternative views of abundance–environment relationships. *Freshwater Biology*, **51**, 783–796.

Lancaster, J. & Downes, B.J. (2010) Linking the hydraulic world of individual organisms to ecological processes: putting ecology into ecohydraulics. *River Research and Applications*, **26**, 385–403.

Van Liefferinge, C., Seeuws, P., Meire, P. & Verheyen, R.F. (2005) Microhabitat use and preferences of the endangered Cottus gobio in the River Voer , Belgium. *Journal of Fish Biology*, **67**, 897–909.

Menge, B. a., Gouhier, T.C., Freidenburg, T. & Lubchenco, J. (2011) Linking long-term, large-scale climatic and environmental variability to patterns of marine invertebrate recruitment: Toward explaining "unexplained" variation. *Journal of Experimental Marine Biology and Ecology*, **400**, 236–249.

Morrissey, C. a., Boldt, A., Mapstone, A., Newton, J. & Ormerod, S.J. (2013) Stable isotopes as indicators of wastewater effects on the macroinvertebrates of urban rivers. *Hydrobiologia*, **700**, 231–244.

Muñoz-Mas, R., Martínez-Capel, F., Schneider, M. & Mouton, A.M. (2012) Assessment of brown trout habitat suitability in the Jucar River Basin (SPAIN): comparison of data-driven approaches with fuzzy-logic models and univariate suitability curves. *The Science of the total environment*, **440**, 123–131.

Rosenfeld, J. & Ptolemy, R. (2012) Modelling available habitat versus available energy flux: do PHABSIM applications that neglect prey abundance underestimate optimal flows for juvenile salmonids? *Canadian Journal of Fisheries and Aquatic Sciences*, **69**, 1920–1934.

Schmalz, B., Kuemmerlen, M., Kiesel, J., Cai, Q., Jähnig, S. & Fohrer, N.(2014) Impacts of land use changes on hydrological components and macroinvertebrate distributions in the Poyang lake area. *Ecohydrology*. DOI: 10.1002/eco.1569

Schmidt, T.S., Clements, W.H. & Cade, B.S. (2012) Estimating risks to aquatic life using quantile regression. *Freshwater Science*, **31**, 709–723.

Schooley, R. & Wiens, J. (2005) Spatial ecology of cactus bugs: area constraints and patch connectivity. *Ecology*, **86**, 1627–1639.

2. Quantile regression analysis as predictive for lake macroinvertebrate biodiversity

Abstract

In Italy, the Water Framework Directive introduced major changes to water management, by making water resources more efficient and enforceable, so, this study aims to highlight some of the potential implications of its implementation for lake management. In this respect, the Life+ INHABIT project was crucial in launching the monitoring plan of lake macroinvertebrates, standardized at national level.

Quantile regression analysis was focused on nine lakes located in two Italian regions (Piedmont and Sardinia). The studied lakes cover a wide trophic spectrum, from oligotrophy to hyper-trophy. In particular, all Sardinians lakes had higher trophic conditions than Piedmontese lakes, reaching only a meso-eutrophic state. The lakes were sampled through the national standardised protocol taking three replicates for each of the three recognized lake zones.

The study lakes showed high chemical variability with conductivity varying between 53 and 561 µS/cm, pH between 6.5 and 9.1, and alkalinity between 14 and 398 mg/L. The bottom sediments were characterized by fine sand (range 51-99%), followed by silt (0.6-35%) and clay (0 – 28%). Lake Habitat Survey was also applied and the results of its synthetic Indices highlighted higher values in natural lakes and lower in the reservoirs. In all the lakes, macroinvertebrates mainly consisted of Dipteran chironomids and oligochaetes characterized by relative abundance up to 80% and >90%, respectively.

In order to link macroinvertebrate community characteristics to their habitat we measured twenty-one variables that represent chemical, physical and morphological characteristics of the

environment and for each variable the range of variation was provided to clarify the domain of application. Using quantile regression to evaluate limiting responses, we selected sampling depth and oxygen percent saturation (oxygen content) as the two variables that best explain the number of taxa present and the diversity of a site. The authors also stress the importance of the combination of selected variables in structuring lacustrine macroinvertebrate communities. We provide models able to predict the potential of community diversity as a function of environmental characteristics. All of this evidence, can help water managers in deciding to initially invest resources in those lakes where the biological communities were expected to be impaired and to identify which lakes could be in pristine or in near-pristine conditions.

Keywords: macroinvertebrates; taxonomy-based metrics; lakes; reservoirs; quantile regression; oxygen

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2.1 Introduction

Since 2000, the Water Framework Directive (WFD) has been adopted and implemented by the different Member States of the European Union (EU, 2000). The main objective of the WFD is to establish a framework for the protection of all types of water in an attempt to achieve good chemical and ecological status by 2015, recently extended to 2020, ensuring in the meanwhile, the balance of aquatic ecosystems by preventing their deterioration and adapting nature conservation policies with the sustainable use of natural resources.

In Italy, the WFD introduced major changes to water management (Law Decree n.152/2006; Ministerial Decree n. 56/2009), by making water resources more efficient and enforceable, within this context, this study aims to highlight some of the potential implications of its implementation for lake management. In this respect, the Life+ INHABIT project was crucial in launching the monitoring plan of lake macroinvertebrates, standardized at national level (Boggero et al., 2013), and in allowing a first collection of biological information about historically known lakes and those with unavailable information.

The study focused on nine lakes belonging to two different Italian ecoregions (Alpine and Mediterranean), considering both natural lakes and reservoirs of different lake-types, characterized by a large trophic gradient (from oligotrophy to hyper-eutrophy), being especially important for their Regions both from the administrative and the eco-touristic point of view, and representing different uses of the water resources (i.e. hydroelectric generation and potable use). Four Biological Quality Elements (EQB: phytoplankton, macrophytes, macroinvertebrates and fish) were analysed in the framework of the project, and in the present work the relationships

between macroinvertebrates and environmental conditions are presented and discussed.

It is generally known that many factors control the macroinvertebrates abundance, distribution and productivity, both in shallow and in deep lakes, such as: oxygen content, substrate composition, sampling depth, temperature, nutrient availability, basin characteristics, chemical parameters and their variations, morphology of the basin. Each of these factors, in addition to ecological processes, can influence the relationships between the biological population and a single environmental gradient inflating variability.

The importance of oxygen content for macroinvertebrates was recognised for the first time by Thienemann (1925), whose researches led to the development of sensitive and tolerant species lists capable to separate the environments in different trophic categories (Brinkhurst 1974). Over time, many researchers have stressed the importance of water chemistry, trophic conditions and substrate type in influencing taxa richness and abundances (e.g. Abraham et al., 1999; Brodersen et al., 1998; Friday, 1987; Heino, 2000; Jeffries, 1991; Rasmussen & Lindegaard, 1988; Timm and Mols, 2012).

In Italy, in the last decade, a Benthic Quality Index was created to evidence eutrophication pressure impacts on macroinvertebrates (Rossaro *et al.* 2012), but its use implies a thorough knowledge of the taxonomy of Dipterans chironomids and oligochaetes, the two main groups inhabiting lakes. Therefore, became highly necessary to find supporting ways, useful to the authorities responsible for monitoring and to water managers, making them capable of a more rapid bio-assessment discriminating between lakes in good and in bad quality conditions.

Commonly, statistical analyses are focused on the determination of a central response model among biological

population characteristics and environmental gradients (Weatherhead & James 2001), these methods set aside the concurring role of other unmeasured factors (Austin 2007), such as competition for space, predation and stochasticity, which control the abundance, distribution and productivity of macroinvertebrates.

In accordance with what was said so far, quantile regression (Koenker & Bassett 1978), was acknowledged to be capable of addressing the differential effects of a factor on various quantiles of a response variable not recognizing only a relationship between a central tendency of a factor and a response variable. Thus it can be used to identify and evaluate ecological limiting factors (Cade & Noon 2003).

Moreover quantile regression allows to predict not only the more probable values of the studied biological metric, but also the maximum or minimum values that could be expected in environmental conditions comparable to the ones used for the model fitting (Cade & Noon 2003; Doll 2011).

The papers of Cade & Noon (2003); Henning, Estrup & Schröder (2005); Schooley & Wiens (2005); Lancaster & Belyea (2006); Allen & Vaughn (2010); Doll (2011); Schmidt, Clements & Cade (2012); Campbell & McIntosh (2013); Fornaroli *et al.* (2015) provide a strong case for the use of quantile regression for modelling species environmental responses.

The focus of the present work will thus be the ranking of the measured environmental characteristics as explanatory variables for lake macroinvertebrate community diversity.

Once identified the most important variables, models able to define the potential richness and diversity as a function of environmental characteristics will be provided. This can allow to a priori defining the potential ecological status of lakes only on the basis of their environmental conditions.

2.2 Methods

2.2.1 Study area and sampling procedure

Nine lakes located in the plain (altitude < 800 m a.s.l.) of two Italian regions (Piedmont and Sardinia) (Fig. 2.1) were considered for the present study. Five of the lakes are natural and belongs to north-western Italy and four are reservoirs belonging to insular Italy.
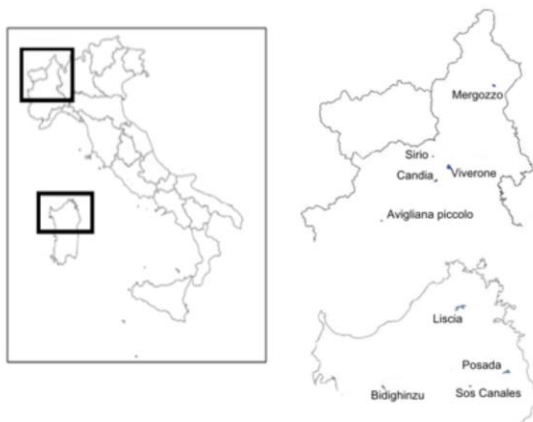
Fig. 2.1 – Study area: position of the lakes sampled in Piedmont and Sardinia, two Italian regions.

The lakes are characterised by different climatic conditions, due to their location in the Alpine and the Mediterranean Ecoregions defined by the WFD guidelines, and separated by the 44° parallel of North latitude. All the lakes are larger than 0.5 km^2 and only 2 of them have mean depth lower than 15 m. The studied lakes cover a wide trophic spectrum (O.E.C.D. 1982) from oligotrophy (L. Mergozzo) to hyper-trophy (L. Bidighinzu). In particular, all Sardinians lakes had higher trophic conditions than Piedmontese lakes, reaching only a meso-eutrophic state.

The lakes were sampled through the national standardised protocol (Boggero *et al.* 2013) considering a total area of 675 cm^2 for 3 replicates per each site per each lake zone (littoral, sublittoral and profundal) taken along transects placed in lines bounding lake-shore with the maximum depth. In each lake one or more transects were identified depending on the lake area and the habitat variability. A total of 180 replicate samples were then collected. Transparency was estimated through Secchi disk at the maximum depth of each lake. Samples were taken at lake mixing (spring) and during lake stratification (autumn), through the use of a grab on soft bottoms, sieved through a 250 µm mesh net, fixed with 5% formalin, and bottled. Grab samples for grain size analysis (60 total samples) and water Niskin samples for chemical analyses (60 total samples) were also taken, simultaneously with biological samples. Temperature was measured in the field, through an inverted thermometer positioned on the Niskin bottle. Once in the lab, chemical analyses were performed following Tartari & Mosello (1997 - http://www.idrolab.ise.cnr.it) on pH, alkalinity, conductivity, oxygen content, and nutrients, while grain size analyses was performed following Ongley (1996). Biological samples were sorted with a stereomicroscope, and specimens separated into main groups, identified and counted to the lowest practicable taxon, when possible, using taxonomic guides recognised at national and international level. For each site, the averages of the values obtained in the three samples were included in the dataset and used to calculate the number of taxa (richness) and Shannon Index (1949) (SDI) in order to represent community richness and diversity.

During summer, Lake Habitat Survey (Rowan *et al.* 2006) for characterizing the physical habitat of lakes was adopted and applied recording shoreline features in at least ten Hab-Plots,

considering shoreline characteristics and pressures, and modifications of the hydrological regime. Based on this protocol, two different summary metrics were estimated: LHMS (Lake Habitat Modification Score) related to the degree of site modification, and LHQA (Lake Habitat Quality Assessment) a measure of diversity and naturalness of physical structure of the lakes.

The Benthic Quality Index (BQIES - Rossaro *et al.* 2012) considering eutrophication as pressure was also applied to each station of all lake-dataset to provide an estimate of their water ecological status. In particular, the BQIES Index, attributing different indicators weights to the different species, assumed that a species living preferably at high diversity sites should be indicator of high environmental quality, while a species abundant in low diversity sites should indicate altered environments.

2.2.2 Data analysis

The most commonly used environmental variables in limnological studies (Tab. 2.1) were used to characterize water chemistry, lake morphology and sediment composition. For each of the 21 variables the range of variation was provided to clarify the current domain of application of the adopted methodology.

To avoid multicollinearity a stepwise selection of variables using Variance Inflation Factor (VIF) was used (Neter, Wasserman & Kutner 2004). Using the full set of explanatory variables, a VIF for each variable was calculated, the variable with the single highest value was removed, all VIF values with the new set of variables were recalculated and the variable with the next highest value was removed, and so on, until all values were below 10.

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Since the wide range of values of raw data, before quantile regression analysis all independent variables were unit-based normalized ($X'=X-X_{min}/X_{max}-X_{min}$) in order to better address their relative importance.

Tab. 2.1 – Range of variability per each environmental and chemical feature considered, and per each synthetic Index derived from Lake Habitat Survey application.

Resolution	Variable	Units	Minimum	Maximum
Sample	Sampling Depth	(m)	1.20	70.00
	Sand	(%)	51.23	99.14
	Silt	(%)	0.62	35.03
	Clay	(%)	0.00	27.48
	Temperature	(°C)	4.44	26.00
	Oxygen content	(%)	1.10	127.70
	pH		6.46	9.11
	Conductivity	(μS/cm)	52.60	561.00
	Alkalinity	(mEq/L)	13.90	398.00
	Total Phosphorus	(μg/L)	3.50	1081.00
	Total Nitrogen	(mg/L)	0.40	4.29
	Nitrate	(mg/L)	0.01	1.31
	Ammonium	(mg/L)	0.00	2.15
Lake	Elevation	(m a.s.l.)	43.00	709.00
	Surface Area	(km^2)	0.30	5.60
	Watershed Area	(km^2)	1.40	3365.78
	Maximum Depth	(m)	8.00	73.00
	Mean Depth	(m)	5.90	45.40
	Volume	(10^6 m^3)	4.30	122.00
	LHMS		14	26
	LHQA		47	62

The relationships among taxa richness and Shannon diversity, and environmental variables were explored at different quantiles (0.15, 0.20, 0.25, 0.50, 0.75, 0.80, 0.85) in order to facilitate the examination of boundaries of these relationships for upper or lower limits imposed by limiting factors.

Quantile regression fits a continuous function through the local (with respect to the independent variable) value of the quantile of a dependent variable to account for variation in the quantile with the independent variable (Koenker & Bassett 1978; Cade, Terrell & Schroeder 1999).

The statistical analyses were conducted using the *quantreg* (Koenker 2013) and *fmsb* (Nakazawa 2014) packages in the R Project software (R Core Team 2014). For each model a τ-specific version of Akaike Information Criterion, corrected for small sample size were calculated ($\mathrm{AIC}c(\tau)$).

The difference between the model $\mathrm{AIC}c(\tau)$ and the minimum $\mathrm{AIC}c(\tau)$ was used in order to choose the best-fitting model (Δ_i = $\mathrm{AIC}c(\tau)$ - min $\mathrm{AIC}c(\tau)$), considering that the model with the lowest $\mathrm{AIC}c(\tau)$ generally provides the better description of the data. Values of $\Delta_i \geq 2$ are suggested as a threshold to exclude alternative models; values of $\Delta_i < 2$ indicate substantial support for the alternative model (Burnham & Anderson 2002; Johnson & Omland 2004). For each model, we reported the Akaike weights (w_i), the relative likelihood of a model, given a data set and a set of models (Burnham & Anderson 2002).

For each univariate relationships between environmental variables and community descriptors we fitted linear, exponential, logarithmic and quadratic curves to the data and used only the best-fitting model for the comparison among variables and subsequent analyses.

We determined the best models across the studied quantiles by averaging w_i for each model from all seven quantiles model selection analyses (Allen & Vaughn 2010). As a general rule of

thumb, the confidence set of candidates include models with w_i greater than 10% of the highest one.

Using the same variables and shapes that we used in the best-fitting univariate models, we fit multivariate quantile regression models and evaluate if they improve the estimates.

Finally, we used the selected models in order to predict different ranges of the two community characteristics; the lower and upper boundaries were calculated using the 20th and 80th quantile regression model, respectively.

2.3 Results

2.3.1 Environmental variables

In the study lakes conductivity varied between 53 and 561 µS/cm, with the lowest values found in L. Mergozzo, followed by lakes Candia and Sos Canales with values > 100 µS/cm, lakes Sirio, Viverone, Liscia and Posada with values > 200 µS/cm and the highest values in lakes Avigliana piccolo and Bidighinzu (> 400 µS/cm). pH ranged between 6.5 and 9.1, with minimal variation with season mainly in lakes Candia and Sirio (1 pH unit of difference). The lowest values (<7) were found in the deepest area of lakes Mergozzo, Sirio and Sos Canales. Values higher than 8 were typical of the littoral stations of the Piedmont lakes. Alkalinity ranged between 14 and 398 mg/L, with values around 100 mg/L in lakes Viverone, Sirio and Bidighinzu, and the highest values in L. Avigliana piccolo (>300 mg/L). L. Mergozzo showed very low values similar to those of high mountain lakes.

Oxygen percent saturation was highly variable (1.1 – 128 % sat.) following sampling depth and season. In both seasons, in fact, a vertical distribution with maximum values in the littoral, exceeding in some cases 100% saturation, and lower values with increasing depth was detected. The highest values were

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found in L. Sirio in the littoral and the minimum in lakes Bidighinzu, Viverone, Sos Canales, and Liscia in the profundal reaching values close to anoxia (< 5%).

TP showed a similar increase with depth in lakes Candia, Sirio and Viverone with values around 15 µg/L in the littoral and > 140 µg/L in the profundal stations, while in L. Mergozzo occurred lower concentrations (4 µg/L), remaining constant with depth. Lake Bidighinzu displayed the highest values both in the sublittoral and in the profundal (319 and 417 µg/L, respectively). After mixing, the sole L. Mergozzo maintained TP values constant and similar to the vernal findings, whereas in lakes Bidighinzu and Liscia there was a net increase in TP, with values double, or even triple, compared to those found in spring (1,081 and 174 µg/L, respectively).

Total Nitrogen represents the sum of the concentrations of ammonium (NH_4), nitrate (NO_3) and organic nitrogen. This parameter presented a constant trend from the surface to the maximum depth in lakes Avigliana piccolo, Candia and Mergozzo, with higher values in lakes Candia and Avigliana piccolo (2.5 e 2.1 mg/L, respectively). Lakes Viverone, Sirio and the sardinian reservoirs on the contrary, showed an increase with depth. Lake Bidighinzu had the highest TN content (>3 mg/L in the profundal). After mixing all the lakes showed an increase of TN with depth, with higher concentrations in the sardinian reservoirs. Nitrate represented in the majority of cases the most abundant parameter. This statement was true only in spring for lakes Posada and Bidighinzu, in the profundal of L. Viverone, and in the littoral of L. Avigliana piccolo, but not in L. Candia where ammonium prevailed.

As regard grain-size analyses, fine sand (range 51-99%) prevailed in lake sediments, followed by silt (0.6-35%) and clay (0 − 28%). Fine sand tended to be mostly present in the

Sardinian reservoirs and in L. Avigliana piccolo, while silt was second in abundance in L. Sirio and along the littoral of L. Viverone. Clay was in any case the least abundant.

Considering the application of the Lake Habitat Survey and its synthetic Indices, L. Mergozzo exhibited the lowest LHMS values (14), immediately followed by the Sardinian reservoirs, with the exception of L. Sos Canales. The highest values (26) were assigned to lakes Candia and Viverone. The Sardinian reservoirs proved to be the least anthropically modified, in fact the only significant impact is the presence of the dam. LHQA presented a similar pattern, with higher values in natural lakes and lower in the reservoirs (with the exception of L. Liscia, presenting one of the higher values of all the lakes). Lake Viverone constituted an exception, in fact, while presenting high values of LHQA, being particularly natural, showed also high values of LHMS, because the littoral is significantly altered. Lake Mergozzo seemed to be in the best hydro-morphological conditions, since the lowest LHMS and a quite high LHQA occurred.

2.3.2 Macroinvertebrates

180 replicates samples for a total of 12,208 individuals were identified. The organisms collected belonged to 36 families and 7 classes: Arachnida, Bivalvia, Clitellata, Gastropoda, Insecta, Turbellaria and Malacostraca. The greatest number of organisms was found in L. Sos Canales (6,537 individuals), while the lowest in L. Bidighinzu (153 individuals). Lakes Mergozzo and Viverone had the larger number of taxonomic entities, respectively 49 and 43 taxa, while lakes Liscia and Bidighinzu very few (14 and 12 taxa, respectively).

In all the lakes, macroinvertebrates mainly consisted of Dipteran chironomids and oligochaetes (with values up to 80% and >90%, respectively). Even chaoborids presented high

relative abundances (from 20% to 50%), but only in 3 lakes (Avigliana piccolo, Candia and Sirio). Oligochaetes dominated the community of lakes Mergozzo (70%), Viverone (70%) and Sos Canales (about 98%), while the largest chironomids relative abundances were found in lakes Bidighinzu (60%), Posada (62%) and Liscia (78%).

The higher oligochaetes mean annual densities were found in lakes Sos Canales (20,000 ind/m^2), Mergozzo (2,400 ind/m^2), and Viverone (900 ind/m^2). Conversely, lakes Candia and Sirio showed higher chaoborids mean annual densities (900 and 550 ind/m^2, respectively), while L. Avigliana piccolo had quite similar mean annual densities for oligochaets, chironomids and chaoborids, and relatively high densities of bivalves, and gastropods.

Considering relative abundances, *Tubifex tubifex* with values of 36% and *Limnodrilus hoffmeister* with 20%, were considered abundant. There was only one common species, *Chaoborus flavicans*, with an abundance of 6%. The major part of the fauna was constituted by sparse taxonomic entities (139 taxa, including: *Potamothrix hammoniensis*, *Procladius choreus*, *Spirosperma ferox*, *Ilyodrilus templetoni* and *Tanytarsus* sp.).

SDI ranged between 0 and 2.6, with higher values (>2) in the littoral of Piedmontese lakes and in all stations in L. Posada, while the lowest values belonged to L. Sos Canales, never exceeding 1.1.

The BQIES Index presented the highest value in L. Mergozzo (0.52) and the lowest value in L. Sirio (0.22). Considering the deep lakes only, it means those with a mean depth higher than 15 m, the Index showed a good separation between lakes with high and low oxygen bottom content; therefore on the whole L. Mergozzo was chosen as a reference site since slightly altered.

2.3.3 Relationships between environmental variables

After six iteration of the VIF stepwise selection procedure all the remaining variables had a VIF value below 10. Six environmental variables were then excluded from quantile regression analysis in order to reduce collinearity.

Sand percentage was the first environmental variable to be removed followed by lake volume, mean lake depth, elevation, total nitrogen and alkalinity.

For each one of the fifteen maintained environmental variables the relationships with the two community descriptors (richness and diversity) were studied, and their performance were ranked using w_i.

2.3.4 Relationships between richness and environmental characteristics

For richness, as it is reported in Tab. 2.2 the best univariate model was the one that consider oxygen content with a quadratic relationship (w_i=0.726). The second and the third models, constructed respectively on sampling depth with a quadratic relationship (w_i=0.181) and on clay percentage also with a quadratic relationships (w_i=0.080) were not excluded from subsequent analyses. All the other models had $w_i < 0.073$ (<10% max w_i) and thus, the remaining environmental variables were excluded as a plausible explanation for richness.

Tab. 2.2 – Akaike weights (w_i) averaged from small sample Akaike information criterion (AICc(τ)) selection of univariate quantile regression models for taxa richness and SDI. Qua: Quadratic, Log: Logarithmic, Lin: Linear, Exp: Exponential.

	Richness		
Rank	Model	Shape	Averaged w_i
1	Oxygen content	Qua	0.726
2	Sampling depth	Qua	0.181
3	Clay %	Qua	0.080
4	Temperature	Qua	8.81E-03
5	Silt %	Qua	2.40E-03
6	Watershed area	Qua	3.70E-04
7	LHMS	Lin	2.22E-04
8	Null Model		1.92E-04
9	LHQA	Qua	1.58E-04
10	Nitrate	Qua	1.26E-04
11	Conductivity	Log	1.22E-04
12	Total phosphorous	Log	9.65E-05
13	Ammonium	Log	6.63E-05
14	pH	Lin	6.59E-05
15	Surface area	Qua	6.17E-05
16	Maximum depth	Qua	2.92E-05

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Tab. 2.2 (*Continued*)

Rank	Model	Shape	Averaged w_i
	SDI		
1	Oxygen content	Exp	0.461
2	Temperature	Qua	0.273
3	Sampling depth	Qua	0.263
4	Watershed area	Qua	2.41E-03
5	Clay %	Qua	6.45E-04
6	LHMS	Log	1.19E-04
7	Total phosphorous	Log	6.10E-05
8	LHQA	Log	2.94E-05
9	Surface area	Qua	2.36E-05
10	Silt %	Exp	7.95E-06
11	Ammonium	Qua	3.56E-06
12	Conductivity	Log	3.05E-06
13	Null Model		2.63E-06
14	Nitrate	Exp	1.15E-06
15	Maximum depth	Qua	6.34E-07
16	pH	Qua	3.64E-07

In order to better describe macroinvertebrate community richness, multivariate quantile regression models considering the three selected variables were tailored to the data, and results presented in Tab. 2.3.

Tab. 2.3 – Akaike weights (w_i) averaged from small sample Akaike information criterion (AICc(τ)) for the selection of multivariate quantile regression models for taxa richness and SDI. For curve code see Tab. 2.2.

Rank	Average W_i	1st Variable		2nd Variable	
		Richness			
		Variable	Shape	Variable	Shape
1	0.640	Oxygen content	Qua	Sampling depth	Qua
2	0.350	Oxygen content	Qua	Clay %	Qua
3	6.79E-03	Oxygen content	Qua		
4	1.64E-03	Sampling depth	Qua	Clay %	Qua
5	1.17E-03	Sampling depth	Qua		
6	1.04E-04	Clay %	Qua		
7	2.48E-07	Null model			
		SDI			
		Variable	Shape	Variable	Shape
1	0.661	Oxygen content	Exp	Sampling depth	Qua
2	0.296	Oxygen content	Exp	Temperature	Qua
3	0.018	Oxygen content	Exp		
4	0.014	Sampling depth	Qua	Temperature	Qua
5	9.06E-03	Sampling depth	Qua		
6	2.07E-03	Temperature	Qua		
7	2.77E-08	Null model			

The model based on sampling depth and oxygen content was (0.640/0.350) = 1.83 more likely to be the best explanation for species richness compared to the one considering oxygen content and clay percentage. Moreover the selected model was (0.640/0.007) = 91.43 more likely to be the best compared to the one considering only sampling depth and clay percentage. This model, evaluated for the 20th and 80th quantiles, enabled to describe how taxa richness values changed through the two

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environmental gradients, their visual representation and the distribution of samples across the gradients were reported in Fig. 2.2A.
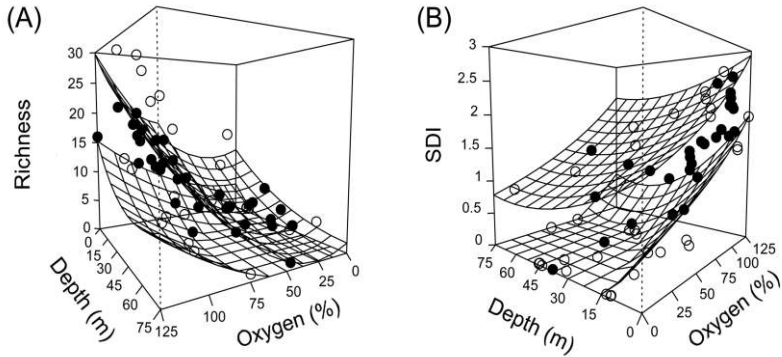
Fig. 2.2 – Graphical representation of quantile regression models: multivariate quantile regression models for taxa richness (A) and SDI (B). Surfaces represent 20th and 80th quantile regression models. Black points fall within the predicted range, white points outside.

2.3.5 Relationships between SDI and environmental characteristics

The models created for SDI provided responses similar to those created for richness, in particular, the best model was the one considering oxygen content with an exponential relationship (w_i = 0.461). The second and the third models, constructed respectively on temperature with a quadratic relationship (w_i=0.273) and on sampling depth again with a quadratic relationships (w_i=0.263) were maintained for subsequent analyses. As for richness, all the other models had $w_i < 0.046$ and thus, the remaining environmental variables were excluded as a plausible explanation for SDI.
Similarly to richness, multivariate quantile regression models considering simultaneously the selected environmental

variables were tailored to the data, and results presented in Tab. 2.3. The model containing sampling depth and oxygen content as explanatory variables for SDI, was (0.661/0.296) = 2.23 more likely to be the best compared to the one considering oxygen content and temperature and (0.661/0.018) = 36.72 times more likely to be the best compared to the one considering only oxygen content.

Tab. 2.4 – Predicted intervals (20th - 80th quantile regression models) for taxa richness and SDI as a function of both oxygen content and depth.

		Richness			
		Depth (m)			
		0	5	15	50
	30	6 - 11	4 - 10	1 - 7	0 - 1
Oxygen saturation (%)	70	8 - 15	6 - 13	3 - 11	0 - 5
	120	14 - 27	13 - 26	10 - 23	5 - 17
		SDI			
		Depth (m)			
		0	5	15	50
	30	0.86 - 1.62	0.60 - 1.51	0.15 - 1.32	0 - 0.98
Oxygen saturation (%)	70	1.24 - 2.05	0.98 - 1.94	0.53 - 1.74	0 - 1.40
	120	1.92 - 2.82	1.66 - 2.7	1.21 - 2.51	0.46 - 2.17

The use of the selected models, estimated for the 20th and 80th quantiles (Fig. 2.2B), enabled to describe how SDI values changed through the two environmental gradients. To provide a useful tool for management purposes the outcomes of the model reported in Fig. 2.2B were summarized in Tab. 2.4.

2.4 Discussion

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Eutrophication, derived from the accumulation of nutrients coming from human settlements, agricultural and zootechnical activities, and from industrial discharges, is considered one of the major cause of water deterioration (Hasler 1947) with fundamental changes at ecosystem level (Litke 1999). Since the '50s, in Italy, it became clear that an increasing number of lakes were suffering by an accelerated aging process due to human impacts, and in the last review (Premazzi *et al.* 2003) it was stated that nearly 41% of the Italian lakes are eutrophic and are undergoing significant damages to aquatic life. Since the early beginning, many limnological researches were devoted to study eutrophication (Frink 1967), its causes and effects on the biocenosis (Schelske & Stoermer 1971; Colby *et al.* 1972; Belanger 1981; Dvořák 1996), to find remedial ways to redress the obvious damages (Schindler 1974; Sharpley 2003).

The first of our results is the reduction in species richness due to oxygen depletion, which is recognised as one of the major effect of lake eutrophication in the deepest lake area (Smayda 2008). As a consequence, lakes and reservoirs deteriorate due to a growing addition of nutrients, organic matter, and silt, producing an increase of algae and macrophytes, water turbidity, with consequences on water lake volumes (Harper 1992). In other terms, water bodies considered of overriding public interest and/or benefits to the environment and the society, lose usefulness and safety as water supplies, and attractiveness as recreational activities.

Oxygen concentration was and actually is, therefore, recognised as being the main key driver of macroinvertebrate (Kagalou et al., 2006; Rakocinski, 2012) and fish community (Doudoroff & Shumway 1970; Davis 1975; McKinsey & Chapman 1998) composition under eutrophication effects,

because both require high oxygen level to thrive, to show a high diversity and, in the case of fish, to survive.

In the present study and through quantile regression analysis, despite the large number of environmental variables considered, covering the most important lake characteristics, oxygen content and sampling depth were the only parameters identified as useful in the preliminary description of lake macroinvertebrate community diversity.

Another parameter acknowledged to have profound effects on the distribution of the lacustrine macroinvertebrates fauna is depth, as we found in this paper; this was in fact recognised as another of the main key drivers since the early studies on benthic community distribution (Welch 1935). Macroinvertebrates in fact, show a rapid decline with depth, which is much more rapid when the lake is more eutrophic (Hergenrader & Lessig 1980). This distribution is due to the decrease of the structural diversity of the habitat from the littoral to the profundal zone, to the decrease in food availability, and in some cases of oxygen. As regards insects, the distribution is as well subject to the need to access to the water surface for respiratory requirements, for transformation into pupa or for the emergence to the adult stage.

Consequently, one of the critical issues faced during monitoring is represented by sampling points selection and transects position, which determines the benthic community composition along with organic matter concentration, oxygen content, temperature variation and macrophytes presence. Notwithstanding this, no agreement was found up to now, among various zonation systems as regards the precise position of boundaries (see Jørgensen and Löffler, 1990; Wetzel, 1975), helping in finding relationships with correlated factors. Through the application of quantile regression analysis this problem was effectively solved, helping water managers and

technician of the Environmental Agencies taking their decisions. The use of quantile regression compared to the conventional analyses estimating the mean density prevent the misrepresentation of the effects of the environmental variables limiting macroinvertebrate abundance and distribution.

To overcome the technical shortcomings of the Italian methodology used for the classification of the ecological status of lakes evidenced during the intercalibration exercise, Italy has improved its national assessment method (Rossaro *et al.* 2013). In this context, the development of a new methodology supporting what was done up to now for the implementation of the WFD was necessary, to enhance reliability and robustness of the proposed method.

Natural variability implicate that seasonal variations occurred in macroinvertebrate community composition (e.g. Bass and Potts, 2001; de Lima et al., 2013; Nalepa et al., 2000; Ward, 1992). Among the environmental variables tested, the values of oxygen typically decrease with increased environmental stress, while other environmental variables decrease with increased environmental stress. The positive correlation of our results with oxygen is therefore in agreement with the assumption that our index negatively responds to environmental stress (Rossaro et al. 2013).

The use of the selected multivariate model, estimated for the 20th quantile and for the optimal oxygen content enabled to provide, both for richness and SDI, a set of minimum expected values within the sampling depth gradient. These results, reported in Tab. 2.4, should be considered in the development of ecological indices referring only to anthropic pressures effects. In fact, the decrease of diversity within a community could be ascribed both to anthropogenic pressures than to natural differences in environmental characteristics. Our results could be useful in developing ecological indices which

consider the natural decrease of macroinvertebrate community diversity along a depth gradient. On the other hand, ecological indices developed not considering the depth gradient may mismatch the diversity decrease and its causes.

Furthermore, for a discrete number of combinations of sampling depth and oxygen content, values of potential richness and SDI, were provided (Tab 2.4). This result, together with site specific environmental variable monitoring, enabled water managers to earlier invest resources in lakes where the biological communities were expected to be impaired. Our results should be also useful in the identification of pristine or near-pristine lakes to be used as reference.

These findings are strictly in agreement with the guidelines of the national standard sampling protocol for lacustrine macroinvertebrates, providing for a distribution of the samples according to the generally recognized lakes zonation (Wetzel 1975; Jørgensen & Löffler 1990).

Hence the importance of a standardized sampling protocol in order to avoid the lack of comparability between sites and lakes. Moreover, this information could be particularly useful to identify, prior of any biological monitoring, which Italian lakes could or could not host a diversified macroinvertebrate community.

2.5 Conclusions

Despite the large number (21) of environmental variables considered in the present study, covering the most important lake characteristics, oxygen saturation and sampling depth were the main parameters identified as useful in the description of lake macroinvertebrate community diversity.

Even though it is difficult, at this early stage, to appreciate the real implications of this model because actually only few data are available, it is possible to indicate some of the potential

implications for the development of ecological indices based on biodiversity.

Moreover, this information could be particularly useful to identify, prior of any biological monitoring, which Italian lakes could or could not host a diversified macroinvertebrate community.

The same variables are generally recognised as being: the first, one of the major key drivers of macroinvertebrate community composition under eutrophication effects, the second, an important parameter to take into account during sampling campaign to highlight significant differences in biodiversity. From this assumption the importance of an *ad hoc* sampling campaign and of standardized sampling protocol to avoid the lack of comparability among sites.

In the near future, seasonal and annual variations should be deeply investigated and known sufficiently to ensure that quantitative values represent effectively the relation abundance/time. In the reverse case, numerical estimates of quantity will be of little or no value because of variation in density between species in relation to time and space.

The domain of application of each variable, due to a research area limited to two Italian Regions, could be considered an isolated case: future research activities considering a broader territory are needed to provide more robust and significant results on the topic.

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2.7 References

Abraham, J., Allen, P., Dunbar, J. & Dworkin, S. (1999) Sediment type distribution in reservoirs: sediment source versus morphometry. *Environmental Geology*, **38**, 101–110.

Allen, D.C. & Vaughn, C.C. (2010) Complex hydraulic and substrate variables limit freshwater mussel species richness and abundance. *Journal of the North American Benthological Society*, **29**, 383–394.

Austin, M. (2007) Species distribution models and ecological theory: A critical assessment and some possible new approaches. *Ecological Modelling*, **200**, 1–19.

Bass, D. & Potts, C. (2001) Invertebrate community composition and physicochemical conditions of Boehler Lake, Atoka County, Oklahoma. *Proceedings of the Oklahoma Academy of Science*, **81**, 21–29.

Belanger, T. (1981) Benthic oxygen demand in lake Apopka, Florida. *Water Research*, **15**, 267–274.

Boggero, A., Zaupa, S., Rossaro, B., Lencioni, V., Marziali, L., Buzzi, F., Fiorenza, A., Cason, M., Giacomazzi, F. & Pozzi, S. (2013) *Protocollo Di Campionamento E Analisi Dei Macroinvertebrati Negli Ambienti Lacustri*. MATTM-APAT, Roma.

Brinkhurst, R. (1974) *The Benthos of Lakes.* St. Martin's Press, New York.

Brodersen, K., Dall, P. & Lindegaard, C. (1998) The fauna in the upper stony littoral of Danish lakes:

macroinvertebrates as trophic indicators. *Freshwater Biology*, **39**, 577–592.

Burnham, K.P. & Anderson, D. (2002) *Model Selection and Multi-Model Inference*, 2nd ed. Springer-Verlag, New York.

Cade, B.S. & Noon, B.R. (2003) A gentle introduction to quantile regression for ecologists. *Frontiers in Ecology and the Environment*, **1**, 412–420.

Cade, B., Terrell, J. & Schroeder, R. (1999) Estimating effects of limiting factors with regression quantiles. *Ecology*, **80**, 311–323.

Campbell, R.E. & McIntosh, A.R. (2013) Do isolation and local habitat jointly limit the structure of stream invertebrate assemblages? *Freshwater Biology*, **58**, 128–141.

Colby, P.J., Spangler, G.R., Hurley, D. a. & McCombie, a. M. (1972) Effects of Eutrophication on Salmonid Communities in Oligotrophic Lakes. *Journal of the Fisheries Research Board of Canada*, **29**, 975–983.

Davis, J.C. (1975) Minimal Dissolved Oxygen Requirements of Aquatic Life with Emphasis on Canadian Species: a Review. *Journal of the Fisheries Research Board of Canada*, **32**, 2295–2332.

Doll, J.C. (2011) Predicting biological impairment from habitat assessments. *Environmental monitoring and assessment*, **182**, 259–277.

Doudoroff, P. & Shumway, D. (1970) *Dissolved Oxygen Requirements of Freshwater Fishes. FAO Fisheries Technical Paper no.86*. Rome, Italy.

Dvořák, J. (1996) An example of relationships between macrophytes, macroinvertebrates and their food resources in a shallow euthrophic lake. *Hydrobiologia*, **339**, 27–36.

Fornaroli, R., Cabrini, R., Sartori, L., Marazzi, F., Vracevic, D., Mezzanotte, V., Annala, M. & Canobbio, S. (2015) Predicting the constraint effect of environmental characteristics on macroinvertebrate density and diversity using quantile regression mixed model. *Hydrobiologia,* **742**, 153-167.

Friday, L. (1987) The diversity of macro invertebrate and macrophyte communities in ponds. *Freshwater Biology*, **18**, 87–104.

Frink, C. (1967) Nutrient budget: rational analysis of eutrophication in a Connecticut lake. *Environmental science & technology*, **1**, 425–428.

Harper, D. (1992) *Eutrophication of Fresh Waters: Principles, Problems and Restoration*. Chapman & Hall, New York.

Hasler, A. (1947) Eutrophication of lakes by domestic drainage. *Ecology*, **28**, 383–395.

Heino, J. (2000) Lentic macroinvertebrate assemblage structure along gradients in spatial heterogeneity, habitat size and water chemistry. *Hydrobiologia*, **418**, 229–242.

Henning, K., Estrup, H. & Schröder, H. (2005) Rejecting the mean: Estimating the response of fen plant species to environmental factors by non-• linear quantile regression. *Journal of Vegetation Science*, **16**, 373–382.

Hergenrader, G. & Lessig, D. (1980) Eutrophication of the Salt Valley reservoirs, 1968–73 III. The macroinvertebrate community: Its development, composition, and change in response to eutrophication. *Hydrobiologia*, **75**, 7–25.

Jeffries, M. (1991) The ecology and conservation value of forestry ponds in Scotland, United Kingdom. *Biological Conservation*, **58**, 191–211.

Johnson, J.B. & Omland, K.S. (2004) Model selection in ecology and evolution. *Trends in ecology & evolution*, **19**, 101–108.

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Jørgensen, S. & Löffler, H. (1990) *Guidelines of Lake Management. International Lake Environment Comittee, United Nations Environmental Programme*.

Kagalou, I., Economidis, G., Leonardos, I. & Papaloukas, C. (2006) Assessment of a Mediterranean shallow lentic ecosystem (Lake Pamvotis, Greece) using benthic community diversity: Response to environmental parameters. *Limnologica*, **36**, 269–278.

Koenker, R. (2013) quantreg: Quantile Regression. R package version 4.98.
http://CRAN.R-project.org/package=quantreg.

Koenker, R. & Bassett, G. (1978) Regression Quantiles. *Econometrica*, **46**, 33–50.

Lancaster, J. & Belyea, L. (2006) Defining the limits to local density: alternative views of abundance–environment relationships. *Freshwater Biology*, **51**, 783–796.

Law Decree n.152/2006. Norme in materia ambientale. G.U. 88 del 14/04/2006 - suppl. ord. n. 96.

De Lima, F., Schäfer, A. & Lanzer, R. (2013) Diversity and spatial and temporal variation of benthic macroinvertebrates with respect to the trophic state of Lake Figueira in the South of Brazil. *Acta Limnologica Brasiliensia*, **25**, 429–441.

Litke, D.W. (1999) *Review of Phosphorus Control Measures in the US and Their Effects on Water Quality. National Water Quality Assessment Program: Water-Resources Investigations Report 99-4007*.

McKinsey, D. & Chapman, L. (1998) Dissolved oxygen and fish distribution in a Florida spring. *Environmental Biology of Fishes*, **53**, 211–223.

Ministerial Decree n. 56/2009. Criteri tecnici per il monitoraggio dei corpi idrici e l'identificazione delle

condizioni di riferimento. G.U. n. 124 del 30/05/2009 - suppl. ord. n. 83.

Nakazawa, M., 2014. fmsb: Functions for medical statistics book with some demographic data. R package version 0.5.1. http://CRAN.Rproject.org/package=fmsb.

Nalepa, T., Lang, G. & Fanslow, D. (2000) Trends in benthic macroinvertebrate populations in southern Lake Michigan. *Verhandlungen des Internationalen Verein Limnologie*, **27**, 2540–2545.

Neter, J., Wasserman, W., Kutner, M. (2004). *Applied linear regression models*. 4th ed., McGraw-Hill/Irwin.

O.E.C.D. (1982) *Eutrophication of Waters: Monitoring, Assessment and Control*. OECD, Paris.

Ongley, E. (1996) Sediment measurements. *Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes* (ed UNEP/WHO), p. 15.

Premazzi, G., Dalmiglio, A., Cardoso, A. & Chiaudani, G. (2003) Lake management in Italy: the implications of the Water Framework Directive. *Lakes & Reservoirs: Research and Management*, **8**, 41–59.

R Core Team. (2013) R: A Language and Environment for Statistical Computing. *R Foundation Statistical Computing*.

Rakocinski, C.F. (2012) Evaluating macrobenthic process indicators in relation to organic enrichment and hypoxia. *Ecological Indicators*, **13**, 1–12.

Rasmussen, J. (1988) Littoral zoobenthic biomass in lakes, and its relationship to physical, chemical, and trophic factors. *Canadian Journal of Fisheries and Aquatic Sciences*, **45**, 1436–1447.

Rossaro, B., Boggero, A., Lods-Crozet, B., Free, G., Lencioni, V., Marziali, L. & Wolfram, G. (2012) A benthic quality

index for European alpine lakes. *Fauna norvegica*, **31**, 95–107.

Rossaro, B., Zaupa, S., Lencioni, V., Marziali, L. & Boggero, A. (2013) 6. Indice per la valutazione della qualità ecologica dei laghi italiani basato sulla comunità bentonica. *Indici per la valutazione della qualità ecologica dei laghi. REPORT CNR ISE, 02.13: 93-113*

Rowan, J., Carwardine, J., Duck, R., Bragg, O., Black, A., Cutler, M., Soutar, I. & Bonn, P. (2006) Development of a technique for lake habitat survey (LHS) with applications for the European Union Water Framework Directive. *Aquatic Conservation: Marine and Freshwater Ecosystem*, **16**, 637–657.

Schelske, C.L. & Stoermer, E.F. (1971) Eutrophication, silica depletion, and predicted changes in algal quality in lake michigan. *Science*, **173**, 423–424.

Schindler, D.W. (1974) Eutrophication and recovery in experimental lakes: implications for lake management. *Science*, **184**, 897–899.

Schmidt, T.S., Clements, W.H. & Cade, B.S. (2012) Estimating risks to aquatic life using quantile regression. *Freshwater Science*, **31**, 709–723.

Schooley, R. & Wiens, J. (2005) Spatial ecology of cactus bugs: area constraints and patch connectivity. *Ecology*, **86**, 1627–1639.

Sharpley, A. (2003) Development of phosphorus indices for nutrient management planning strategies in the United States. *Journal of Soil and Water Conservation*, **58**, 137–152.

Smayda, T.J. (2008) Complexity in the eutrophication–harmful algal bloom relationship, with comment on the importance of grazing. *Harmful Algae*, **8**, 140–151.

Tartari, G.A. & Mosello, R. (1997) *Metodologie Analitiche E Controlli Di Qualità Nel Laboratorio Chimico dell'Istituto Italiano Di Idrobiologia*, Consiglio Nazionale delle Ricerche.

Thienemann, A. (1925) Die Binnengewässer Mitteleuropas: Eine Limnologische Einführung. *Die Binnengewasser*, **1**, 1–25.

Timm, H. & Mols, T. (2012) Littoral macroinvertebrates in Estonian lowland lakes: the effects of habitat, season, eutrophication and land use on some metrics of biological quality. *Fundamental and Applied Limnology*, **180**, 145–156.

Ward, J. (1992) *Aquatic Insect Ecology, Part I, Biology and Habitat*. John Wiley & Sons, Ltd, New York.

Weatherhead, M. & James, M. (2001) Distribution of macroinvertebrates in relation to physical and biological variables in the littoral zone of nine New Zealand lakes. *Hydrobiologia*, **462**, 115–129.

Welch, P.S. (1935) *Limnology* (ed McGraw-Hill). New York.

Wetzel, R. (1975) *Limnology*. W.B. Saunders Company, Philadelphia.

3. Predicting the constraint effect of environmental characteristics on macroinvertebrate density and diversity using quantile regression mixed model

Abstract

Various factors, such as habitat availability, competition for space, predation, temperature, nutrient supplies, presence of waterfalls, flow variability and water quality control the abundance, distribution and productivity of stream-dwelling organisms. Each of these factors can influence the response of the density of organisms to a specific environmental gradient, inflating variability and making difficult to understand the possible causal relationship.

In our study, we used quantile regression mixed models and Akaike's information criterion as an indicator of goodness to examine two different dataset, one belonging to Italy and one belonging to Finland, and to detect the limiting action of selected environmental variables.

In the Italian dataset, we studied the relationships among five macroinvertebrate families and three physical habitat characteristics (water velocity, depth and substrate size); in the Finnish dataset the relationships between taxa richness and 16 environmental characteristics (both chemical and physical).

We found limiting relationships in both dataset and validated all of them on different datasets.

These relationships are quantitative and can be used to predict the range of macroinvertebrate densities or taxa richness as a function of environmental characteristics. They can be a tool for management purposes, providing the basis for habitat-based models and for the development of ecological indices.

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Keywords
Habitat availability, Limiting action, Quantile regression, Linear quantile mixed model, Density-Environment relationships, pH

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3.1 Introduction

Understanding of the relationships between the density of macroinvertebrates and the environment where they live is fundamental to discriminate the effects of human activities and to properly manage the riverine ecosystem. On this topic, in the past decades, impressive literature has been developed (e.g. Poff et al*.,* 1997; Hart & Finelli, 1999; Lytle & Poff, 2004).
Density–environment relations can be used in habitat-based association models to understand ecological dynamics and to predict changes in biological communities and ecosystem functions (Lancaster & Downes 2010). Different statistical and analytical methods try to relate habitat availability and some characteristics of macroinvertebrate assemblage such as density, species richness and abundance (Gore 1978; Statzner & Higler 1986). However, methods based on models testing hypotheses about central tendency (e.g. ANOVA) or central

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responses (e.g. ordinary least square regression, PCA, GAM, GLM) set aside the concurring role of other unmeasured factors (Austin 2007), such as competition for space and predation (Calizza *et al.* 2012), temperature variability (Arthur, Zischke & Ericksen 1982; Lessard & Hayes 2003), nutrient supplies (Wright 1992; Wagenhoff, Townsend & Matthaei 2012), life history trait (Statzner *et al.* 1997), retention structure types (Davies & Boulton 2009; Lacan, Resh & McBride 2010; Cabrini *et al.* 2013), presence of waterfalls or dams (Hansen & Hayes 2012; Robinson 2012; Fanny *et al.* 2013), flow variability (Townsend, Dolédec & Scarsbrook 1997) and water quality (Kail, Arle & Jähnig 2012; Canobbio *et al.* 2013; Morrissey *et al.* 2013) which control the abundance, distribution and productivity of stream-dwelling organisms.

Every homogeneous group of organisms have a range of tolerance to any given factor, and some factors are more critical than others (Gordon *et al.* 2004). Each of these factors can influence the relationships among the density of organisms and environmental gradients, inflating variability. A conceptual representation of this theoretical construct is reported in Fig. 3.1. In some cases, looking at the mean response can lead to assess biased or uninformative relationships (Lancaster & Downes 2010). Thus, it is often very difficult to disentangle the different sources of variation, of natural or anthropogenic origin, in the organism density and abundance along gradients. From the ecological point of view, testing hypotheses about the environmental gradients as limiting factors or constraints on the density of organisms could be more informative than testing them about "average" responses (Downes 2010).
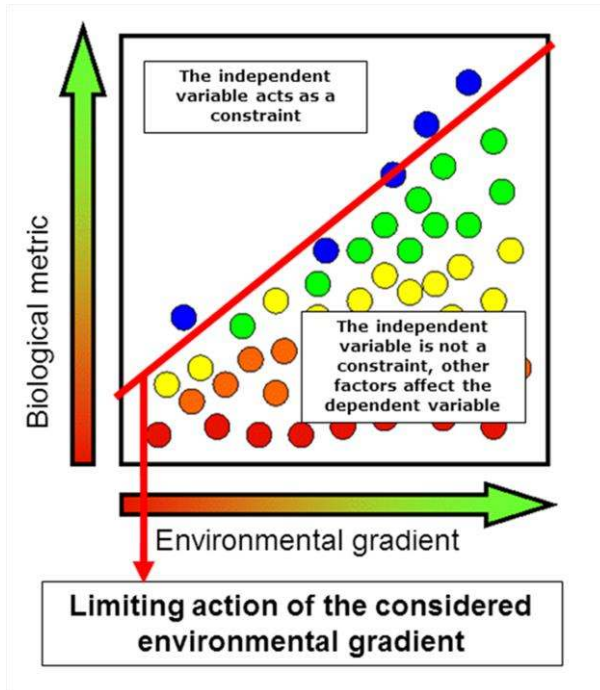
Fig. 3.1- Conceptual representation of the use of quantile regression to show the limiting action of environmental variables on biological metrics. Quantile regression analysing extreme quantiles allows to isolate the information about constraining action of a specific environmental gradient (axis x) on the distribution of the biological metric, while other variables (represented by different dot colours) are affecting it simultaneously.

Limiting factors typically result in wedge-shaped relationships with small changes in the mean value of the response variable along the gradient of the independent variable, but with large changes at the upper end of the distribution (Lancaster & Belyea 2006; Kail, Arle & Jähnig 2012). Anytime the distribution of the response in a regression model is heterogeneous, the diversified variability implies that there are multiple rates of change (slopes) embedded in the relationship.

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Moreover the distribution of density data is susceptible to stochasticity (Power *et al.* 1988).

Quantile regression allows to associate the different rates of change (slopes) to the different parts of the response distribution, being a method for estimating functional relationships among variables for all the portions of a probability distribution (Koenker & Bassett 1978; Johnson & Omland 2004; Geraci & Bottai 2007, 2014; Bolker *et al.* 2009; Grueber *et al.* 2011; Koenker 2013; Geraci 2014). This statistical tool was introduced in ecology by Cade et al. (1999) and can be used to test the role of environmental factors as constraints. Moreover, its application allows to predict not only the more probable values of the studied biological metric but also the maximum or minimum values that could be expected in environmental conditions comparable to the ones used for the model fitting (Cade & Noon 2003; Doll 2011).

The papers of Cade & Noon (2003); Henning et al. (2005); Schooley & Wiens (2005); Lancaster & Belyea (2006); Allen & Vaughn (2010); Doll (2011); Schmidt et al. (2012); Campbell & McIntosh (2013) provide a strong case for the use of quantile regression for modelling the responses of species to environmental variations.

Habitat-based models have been widely used to define a relationship between instream flow and habitat availability for various species of fish and, thus, to define the optimal or minimum flow rate (Gore, King & Hamman 1991; Maddock 1999; Ayllón *et al.* 2010). Macroinvertebrates are rarely used in habitat-based methods because of the high heterogeneity of the density response along environmental gradients. Moreover, classic suitability curves deliver information only about the *preferences* for certain habitat variables, but not about the *density* that can be reasonably expected for each value of the considered habitat variable, which would be a valuable

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information for an overall assessment and management of river ecosystems. If meaningful density-environment relationships for macroinvertebrates can be produced, they can be used, separately or along with those for fish, to have a better comprehension of the possible changes induced in the biological communities by water management strategies. The models able to predict the potential density of macroinvertebrate families only as a function of physical habitat characteristics could also be used for bioassessment purposes. In fact, predictive models such as River Invertebrate Prediction and Classification System (RIVPACS) (Wright 1995) deal with large amounts of unexplained variability and habitat-biomass relationships are assumed to partially account for this variability (Clarke, Wright & Furse 2003; Ostermiller & Hawkins 2004).

The application of the proposed methodology on a large scale dataset could allow to predict intervals for macroinvertebrate densities not only in a specific system or area (as we proposed) but also at national or international scale.

On the other hand, the finding of relationships able to predict intervals of macroinvertebrate diversity starting from widely assessed environmental variables can allow to a priori define the potential ecological status of streams only on the basis of their environmental conditions.

The aim of this work is to show the way quantile regression can be used to describe the limiting action of both physical habitat characteristics and other environmental variables, such as water quality and pH, on macroinvertebrate density and diversity.

We conducted our study in two countries: Italy and Finland.

An alpine river in northern Italy (Serio), whose conditions can be considered as near-pristine, was used to develop the model considering macroinvertebrate density. These site-specific

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models can be used within habitat-based models in order to relate instream flow and physical habitat suitability for macroinvertebrate families.

Finnish dataset of 48 headwater streams in northern Finland was used for the development of ecological indices based on site specific potential diversity.

3.2 Methods

3.2.1 Study area and sampling procedure

The Italian study area (Fig. 3.2) is the alpine valley of the Serio River in northern Italy. It was chosen because it provides a large variety of meso- and microhabitat characteristics. The river substratum varies from sand to bedrock including all the intermediate substrate classes with a heterogeneous distribution. Despite the presence of dams, this stretch of the river can be considered to be near-pristine due to absence of other anthropogenic impacts (Canobbio *et al.* 2010). The basin area of the sampling sites ranges from 90 to 268 km^2 (mean = 196 km^2), the slope from 1.1 % to 2.7%, and the elevation from 486 to 773 m a.s.l. (mean = 592 m a.s.l.).

In spring 2012, we collected quantitative data from 30 sampling points in 6 different sites (along a 15 km river sector) using a Surber net, analysing a total of 180 samples covering 0.10 m^2 each. Concurrently we also measured temperature, electric conductivity, dissolved oxygen and flow rate, to describe the environmental conditions. The sampling procedure was based on a multi-habitat scheme designed to sample invertebrate assemblages in all the available mesohabitats, i.e. steps, riffles, runs, pools and backwaters. For each sample we measured water velocity (V) and water depth (D) using a Marsh-McBirney Flo-Mate Model 2000 portable flowmeter. Water velocity was measured at 40% of the depth in order to

obtain the mean velocity of the water column in the sampling point. Near-bed velocity could be a better descriptor of the limiting action of water velocity but a more general descriptor, such as mean water velocity, may be more informative for management purpose (Mäki-Petäys *et al.* 1997) and was therefore chosen.

The substrates were classified as dominant, sub-dominant and matrix. We expressed their relative abundance in percentage according to the mineral substrate classes provided by the site description protocol in the AQEM Manual (AQEM Consortium 2002). Using these three descriptors we derived the mean substrate diameter (S) by weighting and averaging the mean particle size of each class, a method adapted from Folk (1974).

All the collected macroinvertebrates were stored in 70% ethanol and transported to laboratory where they were completely sorted. All the organisms were identified at genus level, except for Diptera order and Oligochaeta subclass that were mostly identified at family, subfamily or tribe level.

We performed quantile regression analysis only on the families that were found in at least 80% of the total samples, in order to include only well-represented populations. For the selected taxa, we proposed family-specific density models that define how the values of the physical habitat variables influence the densities of the organism.

The Finnish dataset contains 48 streams from two adjacent drainage basins (Fig. 3.2). The bedrock geology of the two basins differ, thus the 24 studied streams in Iijoki basin are circumneutral (mean pH: 6.5, range: 5.4–7.2) whereas the 24 in Oulujoki basin are naturally acidic (mean pH 5.0, range: 3.7–5.9). Drainage ditching is practiced to channel surplus water to streams and thus enhance forest growth, but it also increases the sediment load to the streams and the metal and nutrient

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concentrations of the stream waters (Åström, Aaltonen & Koivusaari 2001; Holden, Chapman & Labadz 2004). From each drainage basin, 12 drained sites [> 27% (mean: 58%) of upstream buffer of 100 m × 1000 m drained] and 12 non-drained reference sites [< 15% (mean: 6%) of upstream buffer drained] were chosen. The study sites were selected as similar as possible among them for physical and chemical characteristics. Thus we had four types of streams in the Finnish dataset: circumneutral reference, circumneutral drained, acidic reference and acidic drained.

One riffle site from each stream was sampled for macroinvertebrates in October 2010. In each site, a 2-min kick-net (net mesh size 0.3 mm) sample of 1.3 m^2 was collected and the invertebrates and associated material were preserved in 80% ethanol. The samples were taken to laboratory where invertebrates were sorted and identified mainly to species level, except for Arachnida, Diptera, Mollusca and worms which were identified to a coarser taxonomic level.

Several instream (stream slope and width, water depth, current velocity, the percentage cover of particle sizes classes) and riparian habitat (canopy cover, ratio of deciduous : coniferous trees) variables were measured in each site. Water samples were also collected from each studied site , and analyzed for Cu, Mn, Ni, Pb, Zn, electrical conductivity, pH, total phosphorus, and dissolved organic carbon (DOC). More detailed information about sampling procedures in Finnish streams are reported in Annala *et al.* (2014).

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Fig. 3.2 - Map of study regions, the triangle denotes the valley of Serio river, the circle denotes the circumneutral Finnish streams and the square denotes the naturally acidic Finnish streams.

3.2.2 Data analysis

Before carrying out quantile regression analysis all independent variables were unit-based normalized ($X'=X-X_{min}/X_{max}-X_{min}$). Since the range of values of raw data varies widely, the relative importance of the different models cannot be addressed without normalization.

In the Italian dataset we randomly selected 4 sites (sites 1, 3, 4, 6) in order to use their samples (n=120) as the training set for the implementation of the models, and we used samples

coming from the other two sites (sites 2, 5; n = 60) for the validation. Similarly, in the Finnish dataset we used 10 samples from each stream categories (n=40) for the implementation of the model and 2 (n=8) for the validation.

The relationships among the dependent and independent variables were studied at different quantiles (0.05, 0.10, 0.15, 0.20, 0.50, 0.80, 0.85, 0.90, 0.95). This choice was made in order to examine with more ease the boundaries of macroinvertebrate–habitat relationships for the upper or lower limits imposed by the limiting factors. We fitted linear, exponential, logarithmic and quadratic curves to the data.

We also used a novel linear model for quantile regression with a subject-specific random intercept that accounts for within-group correlation (Geraci & Bottai 2007). This approach is analogous to the estimate of the mean regressions with random intercepts in linear mixed models (Bolker *et al.* 2009). Fitting random slopes requires relatively large sample sizes for model convergence (Grueber *et al.* 2011), especially if we fit quantile regression models for extreme quantiles. Thus, one categorical variable for the four sites (in the Italian dataset) or for the four stream categories (in the Finnish dataset) was included in our models in order to account for their effects on intercept.

In summary, we generated 8 univariate models for each pair of the dependent and independent variables: 4 model shapes, each of them considering the random effect as present or absent. Then, we used the best-fitting univariate models (as a general rule of thumb, the models with w_i greater than 10% of the highest one) to fit multivariate quantile regression models combining the selected variables both considering the random effect as present or absent.

The statistical analyses were conducted using both the *quantreg* (Koenker 2013) and *lqmm* package (Geraci 2014; Geraci & Bottai 2014) in R Project software (R Core Team

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2014). For each model a τ-specific version of Akaike Information Criterion, corrected for small sample size (AICc(τ)), was calculated for every studied quantile.

The difference between the model AICc(τ) and the minimum AICc(τ) could be used in order to choose the best-fitting model (Δ_i = AICc(τ) - min AICc(τ)), considering that the model with the lowest AICc(τ) generally provides the best description of the data. Values of $\Delta_i \geq 2$ are suggested as a threshold to exclude alternative models; values of $\Delta_i < 2$ indicate substantial support for the alternative model (Burnham & Anderson 2002; Johnson & Omland 2004). For each model, we reported the Akaike weights (w_i), the relative likelihood of a model, given a data set and a set of models (Burnham & Anderson 2002). We determined the best models across the studied quantiles by averaging w_i for each model from all nine quantile model selection analyses (Allen & Vaughn 2010).

We used the selected models in order to predict macroinvertebrate density along the alpine valley of the Serio river and the taxa richness of Finnish streams: the lower and upper boundaries were calculated using the 10th and 90th quantile regression model, respectively. This provides an 80% prediction interval for a single new observation.

Finally we validated our models for the Italian dataset using 60 samples from the other two sampling sites. Each model could not be considered validated if more than 10% of the samples collected in each of the two validation sites had specimen densities that resulted above the upper boundary or more than 10% below the lowest one. Similarly, for the Finnish dataset we used two sites for each stream categories to validate the model, in this case the model could be considered validated if no more than one sample resulted above the upper boundary and no more than one sample resulted below the lowest boundary.

3.3 Results

3.3.1 Environmental variables and macroinvertebrate communities

In the Italian dataset the mean depth of the collected 180 samples was (mean ± st.dev) 0.36 ± 0.16 m, ranging from 0.12 to 1.00 m. Velocity ranged from 0.000 to 1.090 m/s (0.377 ± 0.264 m/s). The mean size of the substratum (obtained averaging the size of dominant, subdominant and matrix substrates) was 0.238 ± 0.132 m (range 0.015 – 0.650 m). The three considered physical variables (V, S, D) showed no mutual correlation (Pearson test: $r < 0.10$ for each couple of variables, $P > 0.10$).

Stream temperatures in the six sites were between 7.1 and 10.4 °C, which is a typical range for alpine and subalpine streams during late spring. The stream flow varied between 0.437 and 1.173 m^3/s (mean = 0.813 m^3/s) during the sampling campaign. Conductivity differed slightly among the sites (mean = 119 µS/cm, range 91 – 155 µS/cm) and the mean dissolved oxygen was 99.0 % of saturation (range 97.9 – 100.2 %), confirming that water quality could be considered good in all the studied river sectors. As the DO was always close to saturation it is unlikely, in this dataset, that it can act as a limiting factor while velocity can be important in determining the DO intake per time unit for macroinvertebrates.

These variables are related to the six sampling sites (one value for each site) and have therefore not been used for the construction of models of quantile regression at the microhabitat scale as they are not able to represent the spatial variability within sites. A possible change in the surrounding conditions at the various sites, however, was taken into account by including in the construction of the models a random effect among sites.

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The density of macroinvertebrates ranged between 3 and 1,635 specimens/0.10 m^2 (mean 214 ± 246). In the whole river sector, we found 66 macroinvertebrate taxa, from 47 different families. Among the 180 samples the mean family richness was 8 ± 3, ranging from 2 to 16.

The specimens belonging to Ephemeroptera, Plecoptera, Trichoptera (EPT) orders were the majority, 54% of the total. We found 6 families of Plecoptera (10% of the total specimens), 5 families of Ephemeroptera (37% of the total specimens), 9 families of Trichoptera (7% of the total specimens).

The most abundant macroinvertebrate families, found in at least 80% of the samples, were Leuctridae, Heptageniidae, Baetidae, Limoniidae and Chironomidae. These families comprised 76% of the 38,580 collected macroinvertebrates: 3,529 specimens of Leuctridae, a family of stoneflies (predominantly *Leuctra spp.*); 14,260 specimens of two families of mayflies, Heptageniidae (a mix predominantly of *Rhythrogena spp.* and *Ecdyonurus spp.*) and Baetidae (predominantly *Baetis spp.*); 11,516 specimens of two families of true flies, Limoniidae and Chironomidae (see Online Resource 1 for the complete description of each samples).

In the Finnish dataset 16 environmental variables were measured to describe each of the 48 sampled sites. For each variable the minimum and maximum values are provided in Tab. 3.1 to define the current domain of application of the models. In this dataset the density of macroinvertebrates ranged between 191 and 10,192 specimens/1.3 m^2 (mean 2,289 ± 1,926) and in the study area we found 112 macroinvertebrate taxa. Among the 48 samples the mean taxa richness was 26 ± 9, ranging from 9 to 46.

We collected samples from 12 streams for each of the 4 different stream categories: circumneutral reference,

circumneutral impacted, naturally acidic and naturally acidic impacted. A possible change in the surrounding conditions, not related to the measured environmental variables but linked to the differences between stream categories, was taken into account by including a random effect among stream categories in the construction of the models.

Tab. 3.1 - Range of variability per each environmental variable considered in the Finnish dataset.

Variable	Units	Mean ± St. Dv.	Range
Conductivity	mS/m	3.18 ± 1.25	1.8 - 7
pH		5.9 ± 0.8	4.2 - 7.2
TotP	µg/L	17.63 ± 12.22	7 - 79
DOC	mg/L	16.6 ± 7.18	0.8 - 40
Cu	µg/L	0.53 ± 0.41	0.1 - 1.74
Mn	µg/L	32.48 ± 30.39	0.28 - 156
Pb	µg/L	0.21 ± 0.17	0.04 - 0.96
Current velocity	m/s	0.27 ± 0.12	0.05 - 0.53
Slope	°	7.47 ± 3.07	3.2 - 18.85
Shading	%	43 ± 21	0 - 80
Depth	cm	24 ± 7	11 - 42
Width	cm	193 ± 187	36 - 890
Moss cover	%	40 ± 30	0 - 91
Substrate diversity	Simpson index	2.48 ± 0.80	1.00 – 4.19
Deciduous cover	%	58 ± 22	30 - 100
Fine sediment	%	15 ± 24	0 - 100

3.3.2 *Limiting action of environmental gradients on macroinvertebrate density and diversity*

The relationships among the density of the five macroinvertebrate families and the three measured environmental variables were analysed. The results are presented in Tab. 3.2.

Regarding Leuctridae densities, the model considering V as the independent variable and not accounting for site effect was selected as the best one (averaged w_i = 0.392). This relationship was best described by the exponential function. Variation in the density of Leuctridae increased with velocity (Fig. 3.3a). Samples collected in low velocity microhabitats had consistently low densities of Leuctridae.

V was the best descriptor also for Heptageniidae density (averaged w_i = 0.396). Differently from Leuctridae, the relationship was best described by the quadratic function and the model not considering the site effect was the best. Heptageniidae density was generally higher in high velocity habitats (Fig. 3.3b).

The relationships among the density of Baetidae specimens and the three measured physical habitat characteristics also show that the model considering V and not accounting for the site effect explained better the density distribution (averaged w_i = 0.312).

This relationship was best described by the quadratic function. This model predicts higher values of density for intermediate water velocities (Fig. 3.3c), in a similar way to what is reported in Tachet et al. (2000).

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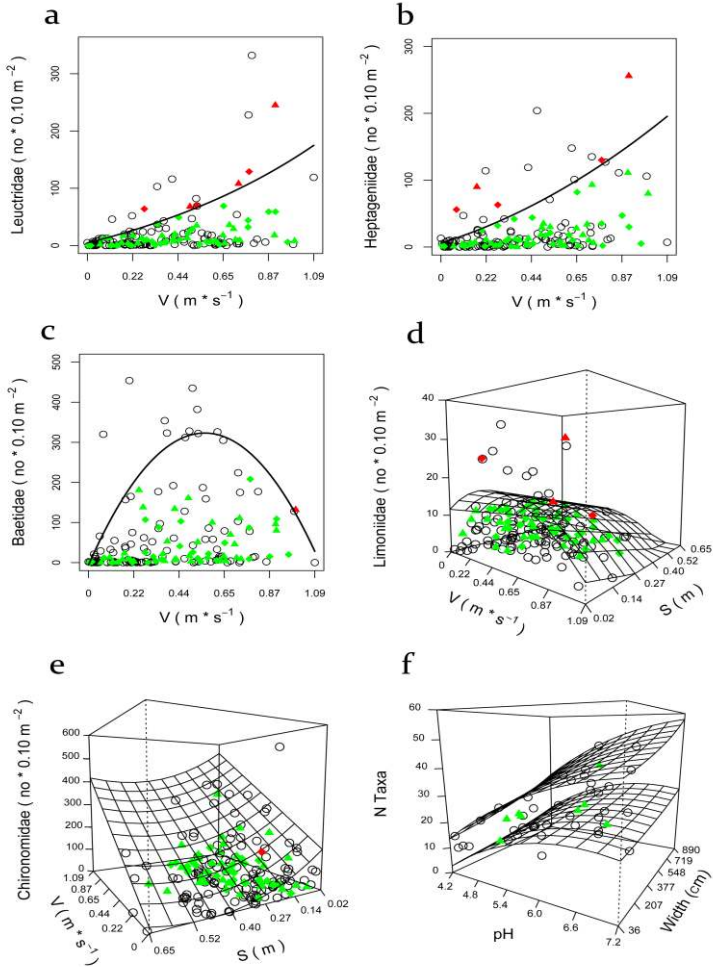
Fig. 3.3 - Selected quantile regression models and densities from both the training (empty points) and the validation datasets (solid points) of the selected macroinvertebrate families for the Italian dataset (a-e). Lines and surface represent the 90th quantile regression models. Selected quantile regression model and number of taxa from both the training (empty points) and the validation datasets (solid points) for the Finnish dataset (f). Surfaces represent the 10th and 90th quantile regression models. Green points fall within the predicted range, red points outside.

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The model considering V and S as the independent variables and not accounting for the site effect was selected as the best (averaged $w_i = 0.307$) for describing Limoniidae densities. The effect of S gradient was best described by the linear function with lower values of density for coarse substrate. On the other hand the effect of V was best described by the quadratic function which predicts higher densities for low or intermediate velocities (Fig. 3.3d).

Also the density of Chironomidae was more consistently represented by the model considering V and S simultaneously, but not accounting for the site effect (averaged $w_i = 0.575$). Chironomidae density was higher in microhabitat characterized by high velocity and fine substrate (Fig. 3.3e). The function that best described the V effect was the linear one and the effect of S was best described by a quadratic function.

All the selected models for density were not better than a constant model at the 10th quantile (for each family the constant model at the 10th quantile was: $y = 0$), thus only the upper boundaries, represented by 90th quantile, were used in order to predict the specimen densities, and in the model validation we did not consider the lower boundaries.

The relationships among the number of taxa in the Finnish dataset and the 16 environmental variables were analysed and the results are presented in Tab. 3.2.

The best fitting model was the one that considers simultaneously pH and width and does not account for the random effect between stream categories (averaged $w_i = 0.719$). The function that best described the pH effect was the quadratic one and the effect of width was best described by an exponential function. Macroinvertebrate diversity was generally higher in wider streams with high pH values. This effect was particularly evident in the 90th quantile model (Fig. 3.3f).

Tab. 3.2 - Akaike weights (w_i) averaged from small-sample Akaike information criterion (AICc) selection of quantile regression models, are also listed the variables considered with their respective shape. Only the models with 5 highest average Akaike weights (w_i) are shown.
Qua: Quadratic, Log: Logarithmic, Lin: Linear, Exp: Exponential.

		1st Variable		2nd Variable		Random
Rank	Average W_i	Variable	Shape	Variable	Shape	Effect
Leuctridae						
1	0.392	V	Exp			-
2	0.190	V	Exp	S	Lin	-
3	0.097	V	Exp	D	Lin	-
4	0.084	V	Lin			+
5	0.072	S	Lin			-
Heptageniidae						
1	0.396	V	Qua			-
2	0.251	V	Qua	D	Exp	-
3	0.143	V	Qua	S	Exp	-
4	0.091	D	Exp			-
5	0.056	S	Exp			-
Baetidae						
1	0.312	V	Qua			-
2	0.139	S	Qua			-
3	0.128	D	Qua			-
4	0.123	V	Qua	S	Qua	-
5	0.086	V	Qua	D	Qua	-

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Tab. 3.2 (*Continued*)

Chironomidae					
Rank	Average W_i	1ˢᵗ Variable		2ⁿᵈ Variable		Random Effect
		Variable	Shape	Variable	Shape	
1	0.575	V	Lin	S	Qua	-
2	0.169	V	Lin			-
3	0.148	S	Qua			-
4	0.060	D	Qua			-
5	0.023	V	Lin	D	Qua	-

Limoniidae					
Rank	Average W_i	1ˢᵗ Variable		2ⁿᵈ Variable		Random Effect
		Variable	Shape	Variable	Shape	
1	0.307	V	Qua	S	Lin	-
2	0.225	S	Lin			-
3	0.219	S	Lin	D	Log	-
4	0.144	D	Log			-
5	0.054	V	Qua			-

N taxa					
Rank	Average W_i	1ˢᵗ Variable		2ⁿᵈ Variable		Random Effect
		Variable	Shape	Variable	Shape	
1	0.719	pH	Qua	Width	Exp	-
2	0.234	pH	Qua	Deciduous %	Qua	-
3	0.023	pH	Qua	Width	Log	+
4	0.022	pH	Qua	Deciduous %	Log	+
5	0.002	Width	Exp	Deciduous %	Qua	-

3.3.3 Model Validation

For the Italian dataset the selected models were validated using the samples of the two randomly selected sites of the validation dataset. The observed densities of each sample were compared to the maximum densities given by the models, expected on the basis of the sample physical characteristics. As previously explained, we used only the upper boundary for the validation process. This makes sense from both the ecological and the limiting factor points of view as at lower quantiles the effect of other conditions, different from the considered ones, on the density of a biological population could be more relevant.

For each of the families, no more than three samples (out of 30) from each of the two validation sites had densities higher than the one predicted by the model. Thus, at least 90% of the samples for each site had densities of macroinvertebrate inside the predicted interval as it is shown in Tab. 3.3 and the models were validated. This means that all the proposed models can be used to predict the maximum density of the studied macroinvertebrate families depending on the characteristics of the physical environment at least in the upper part of Serio River. The densities obtained in the validation sites are shown in Fig. 3.3. For the Finnish dataset, the model that predict maximum and minimum values for the number of taxa on the basis of pH and width of the streams was validated using two samples from each of the four stream categories (n=8). All the samples were within the limits imposed by the model which could thus be considered as validated.

Tab. 3.3 - Percentage of samples for each validation site with macroinvertebrate densities within the acceptable boundaries.

Site	Leuctridae	Heptageniidae	Baetidae	Chironomidae	Limoniidae
2	90%	93%	97%	100%	93%
5	90%	90%	100%	97%	93%

3.4 Discussion and conclusions

The rate of change of the extreme quantile (90th percentile) models for the densities of the five selected families of macroinvertebrates was always greater than the median one. Different rates of change indicated that measured and unmeasured factors (e.g., life history, fish presence, previous conditions, floods, ecological dynamics and also stochasticity) contributed to variability in density responses. This caused low values of density in highly suitable habitats. On the other hand, the greatest effects of the considered variables were observed on quantiles above the median, showing a rate of change up to three times the median one. This is consistent with a pattern expected with ecological limiting factors. Schmidt et al. (2012) observed the same path describing the limiting action of metals on the potential macroinvertebrate densities.

In our analyses, we found that the factors limiting the densities of five macroinvertebrate families were water velocity or substratum size or both. We found that depth was never the best descriptor for the considered macroinvertebrate densities. Water velocity was the key descriptor for most specimen densities as it is shown in Tab. 3.4.

Tab. 3.4 - Effects of the studied environmental variables on the densities of the selected macroinvertebrate families (+ = significant effect; - = no significant effect) and model shapes (Qua = Quadratic; Lin = Linear; Log = Logarithmic).

Variable	Leuctridae	Heptageniidae	Baetidae	Chironomidae	Limoniidae
V	+ (Exp)	+ (Qua)	+ (Qua)	+ (Lin)	+ (Qua)
D	-	-	-	-	-
S	-	-	-	+ (Qua)	+ (Lin)
Site	-	-	-	-	-

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For Leuctridae and Heptageniidae families the selected models implies that higher velocities provide a more suitable habitat, regardless of other physical characteristics. It is known that some taxa have a major preference for high water velocities, relying on it to provide a constant replenishment of food and oxygen, to carry away waste products and to assist in the dispersal of the species (Gordon *et al.* 2004) and this is well represented by our results. Baetidae density was also limited only by water velocity but, differently, intermediate velocities represent a more suitable habitat. The maximum densities that could be expected in each new sample, as a function of water velocity, are reported in Tab. 3.5.

Tab. 3.5 - Maximum densities, as function of water velocity, for different macroinvertebrate families calculated with the best performing model. Limits were calculated using the 90th quantile regression models.

V (m/s)	Leuctridae (no/0.10 m^2)	Heptageniidae (no/0.10 m^2)	Baetidae (no/0.10 m^2)
0.000	5	6	0
0.110	15	17	110
0.220	27	30	200
0.330	39	45	266
0.440	53	62	307
0.550	69	80	323
0.650	86	100	314
0.760	105	121	280
0.870	126	144	221
0.980	149	169	137
1.090	175	196	28

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On the other hand, water depth, considered both alone and with other physical characteristics, was never involved in the model that provide the best description for the densities of the considered macroinvertebrate families (Tab. 3.4). The size of substratum played a significant role in the description of the densities for two of the studied macroinvertebrate families along with water velocity (Limoniidae and Chironomidae).

Frequently, more than one variable is needed for the description of the optimal habitats (i.a. Jowett, 1997; Maddock, 1999). The densities predicted by these models are summarized in Tab. 3.6, where different combinations of substrate size and water velocity are used to describe the characteristics of the microhabitat.

Predicted densities can vary from 0 to 18 ind/0.10 m^2 for Limoniidae and from 0 to 553 ind/0.10 m^2 for Chironomidae. The densities of Limoniidae were higher in microhabitats characterized by fine substrate and water velocities between 0.3 and 0.7 m/s, while for Chironomidae the densities were higher in microhabitats characterized by fine substrates but with fast flowing waters.

In this work we took into account the possible differences in sampling sites using the linear quantile mixed models that consider a random effect on intercept. These models were compared by AIC$c(\tau)$ to those using the same variable for fixed effects (V, S, D) but do not account for random site effect. None of the models accounting for site effect was the best. This means that the variability among sites must not be considered in the model fitting in order to generalize their conclusions to new sites. A simple explanation for this is that the samples were taken in the same stream. The six sites are all connected and probably the macroinvertebrates present in different sites belong to the same species populations.

Tab. 3.6 - Maximum densities predicted for Chironomidae and Limoniidae families with the multivariate V and S model. Limits were calculated using the 90th quantile regression models

Chironomidae (no/0.10 m^2)

						S (m)						
		0.02	0.08	0.14	0.21	0.27	0.33	0.4	0.46	0.52	0.59	0.65
	0	153	114	80	52	30	14	3	0	0	5	17
	0.11	193	154	120	92	70	54	43	38	39	45	57
	0.22	233	194	160	132	110	94	83	78	79	85	97
	0.33	273	234	200	172	150	134	123	118	118	125	137
	0.44	313	274	240	212	190	174	163	158	158	165	177
V (m/s)	**0.55**	353	314	280	252	230	214	203	198	198	205	217
	0.65	393	354	320	292	270	254	243	238	238	245	257
	0.76	433	394	360	332	310	294	283	278	278	285	297
	0.87	473	434	400	372	350	334	323	318	318	325	337
	0.98	513	474	440	412	390	373	363	358	358	365	377
	1.09	553	514	480	452	430	413	403	398	398	405	417

Limoniidae (no/0.10 m^2)

						S (m)						
		0.02	0.08	0.14	0.21	0.27	0.33	0.4	0.46	0.52	0.59	0.65
	0	12	11	10	9	8	7	6	5	4	3	2
	0.11	14	13	12	11	10	9	8	7	6	5	4
	0.22	16	15	14	13	12	11	10	9	8	7	6
	0.33	18	17	16	15	14	13	12	11	10	9	8
	0.44	18	17	16	15	14	13	12	11	10	9	8
V (m/s)	**0.55**	18	17	16	15	14	13	12	11	10	9	8
	0.65	17	16	15	14	13	12	11	10	9	8	7
	0.76	15	14	13	12	11	10	9	8	7	6	5
	0.87	12	11	10	9	8	7	6	5	4	3	2
	0.98	8	7	6	5	4	3	2	1	0	0	0
	1.09	4	3	2	1	0	0	0	0	0	0	0

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Another explanation is that the real case could involve different slopes among sites which could not be taken into account by our implementation of random effects. Including differences in slopes and in intercepts among sites in the evaluation of random effects could lead to different results but would require sample sizes larger than reasonable.

Density-environment relationships evaluating the limiting action of environmental variables can be used within habitat-based models in order to obtain a more comprehensive view of the physical habitat suitability for biological populations. Habitat-based models have been traditionally used to relate instream flow and physical habitat suitability for various life stages of fish species in order to define minimum or environmentally optimal flows. On the other hand, few studies have been carried out on habitat suitability for macroinvertebrates, in spite of their being the critical food web link to the fish community (Gore, Layzer & Mead 2001; Rosenfeld & Ptolemy 2012) and their importance as ecological indicators. The scarce use of macroinvertebrates in habitat-based models is likely due to the high heterogeneity of the density datasets. The models we developed analysing the limiting action of physical habitat descriptors on macroinvertebrate densities can solve this problem. Moreover, the traditional habitat-based models analyze the mean effect of many environmental variables and combine all these effects averaging the suitability keyed to the different variables, thus underestimating the effect of the critical one. The relationships we found can be used in a very different way: given a physical habitat described by many variables, the combined suitability is obtained by identifying the most limiting descriptors and dealing only with them. This not only makes sense from an ecological point of view, but also allows to focus on the management of the environmental variables that truly have a

limiting action on biological populations. Furthermore, unlike the suitability curves those are normally produced for fish, the relationships described in this work deliver information about the macroinvertebrate density that could be reasonably expected along the considered gradients. The optimal flows referred to available habitat for fish and to the maximum prey production can be different. This aspect has been largely ignored in routine flow assessment and should be taken into account (Rosenfeld & Ptolemy 2012).

Another field of application of the presented models is related to the prediction of reference conditions for the macroinvertebrate communities. The statistical rigour and the interpretability of ecological assessments is strongly affected by how well we predict the biological assemblages expected to occur in the absence of human-caused stress (Hawkins, Cao & Roper 2010). For example, RIVPACS purpose is to develop statistical relationships between the macroinvertebrate communities and the environmental characteristics of a large set of high quality reference sites which can be used to predict the macroinvertebrate community to be expected at any other site in the absence of anthropogenic pressure. The observed macroinvertebrate communities at new monitored sites can then be compared with their site-specific expected fauna to derive indices of ecological quality (Clarke, Wright & Furse 2003). In recent research (Sandin & K. Johnson 2004) local water quality and, above all, physical characteristics clearly explained most of the variation considering a total of 428 sampled streams that can be considered as reference. Our study highlights and quantifies the effect of the characteristics of physical habitat on the densities of five macroinvertebrate families that are frequently sampled along alpine stream and the data provided can contribute to the definition of bioassessment indices.

The model developed for the Finnish dataset on the other hand predicts minimum and especially potential community diversity as a function of environmental characteristics in different sampling sites in Finland.

The quantile regression analysis identifies pH and width as the best explanatory variables for macroinvertebrate diversity.

The effect of pH on macroinvertebrate diversity has been recognised many times in Scandinavian countries as in Heino, Muotka & Paavola (2003); Petrin *et al.* (2007); Petrin, Laudon & Malmqvist (2007); Petrin (2011); Annala *et al.* (2014). Also the effect of width is well known (Heino & Mykrä 2006; Reid, Quinn & Wright-Stow 2010), especially for headwater streams (Heino, Muotka & Paavola 2003). In this study we confirmed pH and width as the major limiting factors for macroinvertebrate diversity in headwater streams and, at the same time, we provided the range of macroinvertebrate diversity that could be expected in other sites as a function of such variables (Tab. 3.7).

This kind of evidence can help water managers in defining the priority areas of intervention selecting streams where the biological communities are expected to be impaired and identifying those which should be in pristine or near-pristine conditions. The model could also be used for developing bioassesment indices that would take into account the natural variability in macroinvertebrate diversity.

All the models developed in this study can be used only when the environmental conditions, the boundary conditions and the study area are coherent with those reported in this study. On the other hand, this kind of approach could be used in other studies and by other researchers, especially when a limiting factor point of view is coherent with the analysed problem.

Tab. 3.7 - Minimum and potential number of taxa (for Finnish streams) predicted with the multivariate model that consider simultaneously pH and stream width. Upper and lower limits were calculated using the 10th and 90th quantile regression models respectively

		Minimum Taxa richness (no/1.30 m^2)										
		Width (cm)										
		36	121	207	292	377	463	548	634	719	805	890
	4.2	3	3	4	4	5	6	7	8	9	10	11
	4.5	7	7	8	9	9	10	11	12	13	14	15
	4.8	11	11	12	12	13	14	15	16	17	18	19
	5.1	14	14	15	15	16	17	18	19	20	21	22
	5.4	16	17	17	18	19	19	20	21	22	23	25
pH	5.7	18	19	19	20	20	21	22	23	24	25	27
	6	19	20	21	21	22	23	24	24	26	27	28
	6.3	20	21	21	22	23	23	24	25	26	28	29
	6.6	21	21	22	22	23	24	25	26	27	28	29
	6.9	20	21	21	22	23	24	24	25	26	28	29
	7.2	19	20	21	21	22	23	24	25	26	27	28
		Potential Taxa richness (no/1.30 m^2)										
		Width (cm)										
		36	121	207	292	377	463	548	634	719	805	890
	4.2	14	15	16	17	18	20	21	23	25	27	30
	4.5	18	19	20	21	23	24	26	28	30	32	34
	4.8	22	23	24	26	27	28	30	32	34	36	38
	5.1	26	27	28	29	31	32	34	36	38	40	42
	5.4	30	31	32	33	34	36	37	39	41	43	46
pH	5.7	33	34	35	36	37	39	40	42	44	46	49
	6	35	36	37	38	40	41	43	45	47	49	51
	6.3	37	38	39	41	42	43	45	47	49	51	53
	6.6	39	40	41	42	44	45	47	49	51	53	55
	6.9	41	42	43	44	45	47	48	50	52	54	57
	7.2	42	43	44	45	46	48	49	51	53	55	58

We supply in the supplementary material of the published paper the complete R code and the data used for these analysis, so that other researchers using their own datasets and modifying opportunely the code could easily repeat this kind of analysis.

3.5 Acknowledgments

3.6 References:

Allen, D.C. & Vaughn, C.C. (2010) Complex hydraulic and substrate variables limit freshwater mussel species richness and abundance. *Journal of the North American Benthological Society*, **29**, 383–394.

Annala, M., Mykrä, H., Tolkkinen, M., Kauppila, T. & Muotka, T. (2014) Are biological communities in naturally unproductive streams resistant to additional anthropogenic stressors? *Ecological Applications,* **24**, 1887-1897.

AQEM Consortium. (2002) *Manual for the Application of the AQEM System.*

Arthur, J.W., Zischke, J.A. & Ericksen, G.L. (1982) Effect of elevated water temperature on macroinvertebrate communities in outdoor experimental channels. *Water Research*, **16**, 1465–1477.

_____

Åström, M., Aaltonen, E.K. & Koivusaari, J. (2001) Effect of ditching operations on stream-water chemistry in a boreal forested catchment. *The Science of the total environment*, **279**, 117–29.

Austin, M. (2007) Species distribution models and ecological theory: A critical assessment and some possible new approaches. *Ecological Modelling*, **200**, 1–19.

Ayllón, D., Almodóvar, A., Nicola, G.G. & Elvira, B. (2010) Modelling brown trout spatial requirements through physical habitat simulations. *River Research and Applications*, **26**, 1090–1102.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.-S.S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in ecology & evolution*, **24**, 127–135.

Burnham, K.P. & Anderson, D. (2002) *Model Selection and Multi-Model Inference*, 2nd ed. Springer-Verlag, New York.

Cabrini, R., Canobbio, S., Sartori, L., Fornaroli, R. & Mezzanotte, V. (2013) Leaf Packs in Impaired Streams: The Influence of Leaf Type and Environmental Gradients on Breakdown Rate and Invertebrate Assemblage Composition. *Water, Air, & Soil Pollution*, **224,** 1697.

Cade, B.S. & Noon, B.R. (2003) A gentle introduction to quantile regression for ecologists. *Frontiers in Ecology and the Environment*, **1**, 412–420.

Cade, B., Terrell, J. & Schroeder, R. (1999) Estimating effects of limiting factors with regression quantiles. *Ecology*, **80**, 311–323.

Calizza, E., Costantini, M.L., Rossi, D., Carlino, P. & Rossi, L. (2012) Effects of disturbance on an urban river food web. *Freshwater Biology*, **57**, 2613–2628.

_____

Campbell, R.E. & McIntosh, A.R. (2013) Do isolation and local habitat jointly limit the structure of stream invertebrate assemblages? *Freshwater Biology*, **58**, 128–141.

Canobbio, S., Azzellino, A., Cabrini, R. & Mezzanotte, V. (2013) A multivariate approach to assess habitat integrity in urban streams using benthic macroinvertebrate metrics. *Water Science and Technology*, **67**, 2832–2837.

Canobbio, S., Mezzanotte, V., Benvenuto, F. & Siotto, M. (2010) Determination of Serio River (Lombardy, Italy) ecosystem dynamics using macroinvertebrate functional traits. *Italian Journal of Zoology*, **77**, 227–240.

Clarke, R.T., Wright, J.F. & Furse, M.T. (2003) RIVPACS models for predicting the expected macroinvertebrate fauna and assessing the ecological quality of rivers. *Ecological Modelling*, **160**, 219–233.

Davies, J. & Boulton, A. (2009) Great house, poor food: effects of exotic leaf litter on shredder densities and caddisfly growth in 6 subtropical Australian streams. *Journal of the North American Benthological Society*, **28**, 491–503.

Doll, J.C. (2011) Predicting biological impairment from habitat assessments. *Environmental monitoring and assessment*, **182**, 259–277.

Downes, B. (2010) Back to the future: little-used tools and principles of scientific inference can help disentangle effects of multiple stressors on freshwater ecosystems. *Freshwater Biology*, **55**, 60–79.

Fanny, C., Virginie, A., Jean-François, F., Jonathan, B., Marie-Claude, R. & Simon, D. (2013) Benthic indicators of sediment quality associated with run-of-river reservoirs. *Hydrobiologia*, **703**, 149–164.

Folk, R.L. (1974) *Petrology of Sedimentary Rocks*. Hemphill Publishing, Austin, Texas.

Geraci, M. (2014) Linear quantile mixed models: The lqmm package for Laplace quantile regression. *Journal of Statistical Software*, **57**, 1–29.

Geraci, M. & Bottai, M. (2007) Quantile regression for longitudinal data using the asymmetric Laplace distribution. *Biostatistics*, **8**, 140–154.

Geraci, M. & Bottai, M. (2014) Linear quantile mixed models. *Statistics and Computing*, **24**, 461–479.

Gordon, N.D., McMahon, T.H., Finlayson, B.L., Gippel, C.J. & Nathan, R.J. (2004) *Stream Hydrology*, 2nd ed. John Wiley & Sons, Ltd.

Gore, J.A. (1978) A technique for predicting in-stream flow requirements of benthic macroinvertebrates. *Freshwater Biology*, **8**, 141–151.

Gore, J., King, J. & Hamman, K. (1991) Application of the instream flow incremental methodology to southern African rivers: Protecting endemic fish of the Olifants River. *Water SA*, **17**, 225–236.

Gore, J.A., Layzer, J.B. & Mead, J. (2001) Macroinvertebrate instream flow studies after 20 years: a role in stream management and restoration. *Regulated Rivers: Research & Management*, **17**, 527–542.

Grueber, C.E., Nakagawa, S., Laws, R.J. & Jamieson, I.G. (2011) Multimodel inference in ecology and evolution: challenges and solutions. *Journal of evolutionary biology*, **24**, 699–711.

Hansen, J. & Hayes, D. (2012) Long-Term implications of dam removal for macroinvertebrate communities in Michigan and Wisconsin Rivers, United States. *River Research and Applications*, **28**, 1540–1550.

Hart, D.D. & Finelli, C.M. (1999) Physical-Biological Coupling In Streams: The Pervasive Effects of Flow on

Benthic Organisms. *Annual Review of Ecology and Systematics*, **30**, 363–395.

Hawkins, C.P., Cao, Y. & Roper, B. (2010) Method of predicting reference condition biota affects the performance and interpretation of ecological indices. *Freshwater Biology*, **55**, 1066–1085.

Heino, J., Muotka, T. & Paavola, R. (2003) Determinants of macroinvertebrate diversity in headwater streams: regional and local influences. *Journal of Animal Ecology*, **72**, 425–434.

Heino, J. & Mykrä, H. (2006) Assessing physical surrogates for biodiversity: Do tributary and stream type classifications reflect macroinvertebrate assemblage diversity in running waters? *Biological Conservation*, **129**, 418–426.

Henning, K., Estrup, H. & Schröder, H. (2005) Rejecting the mean: Estimating the response of fen plant species to environmental factors by non-• linear quantile regression. *Journal of Vegetation Science*, **16**, 373–382.

Holden, J., Chapman, P.J. & Labadz, J.C. (2004) Artificial drainage of peatlands: hydrological and hydrochemical process and wetland restoration. *Progress in Physical Geography*, **28**, 95–123.

Johnson, J.B. & Omland, K.S. (2004) Model selection in ecology and evolution. *Trends in ecology & evolution*, **19**, 101–108.

Jowett, I.G. (1997) Instream flow methods: a comparison of approaches. *Regulated Rivers: Research & Management*, **13**, 115–127.

Kail, J., Arle, J. & Jähnig, S. (2012) Limiting factors and thresholds for macroinvertebrate assemblages in European rivers: Empirical evidence from three datasets on water

quality, catchment urbanization, and river restoration. *Ecological Indicators*, **18**, 63–72.

Koenker, R. (2013) quantreg: Quantile Regression. R package version 4.98. http://CRAN.R-project.org/package=quantreg.

Koenker, R. & Bassett, G. (1978) Regression Quantiles. *Econometrica*, **46**, 33–50.

Lacan, I., Resh, V. & McBride, J.R. (2010) Similar breakdown rates and benthic macroinvertebrate assemblages on native and Eucalyptus globulus leaf litter in Californian streams. *Freshwater Biology*, **55**, 739–752.

Lancaster, J. & Belyea, L. (2006) Defining the limits to local density: alternative views of abundance–environment relationships. *Freshwater Biology*, **51**, 783–796.

Lancaster, J. & Downes, B.J. (2010) Linking the hydraulic world of individual organisms to ecological processes: putting ecology into ecohydraulics. *River Research and Applications*, **26**, 385–403.

Lessard, J. & Hayes, D. (2003) Effects of elevated water temperature on fish and macroinvertebrate communities below small dams. *River research and applications*, **19**, 721–732.

Lytle, D. & Poff, N. (2004) Adaptation to natural flow regimes. *Trends in Ecology & Evolution*, **19**, 94–100.

Maddock, I. (1999) The importance of physical habitat assessment for evaluating river health. *Freshwater biology*, **41**, 373–391.

Mäki-Petäys, A., Muotka, T., Huusko, A., Tikkanen, P. & Kreivi, P. (1997) Seasonal changes in habitat use and preference by juvenile brown trout, Salmo trutta, in a northern boreal river. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 520–530.

_____

Morrissey, C. a., Boldt, A., Mapstone, A., Newton, J. & Ormerod, S.J. (2013) Stable isotopes as indicators of wastewater effects on the macroinvertebrates of urban rivers. *Hydrobiologia*, **700**, 231–244.

Ostermiller, J. & Hawkins, C. (2004) Effects of sampling error on bioassessments of stream ecosystems: application to RIVPACS-type models. *Journal of the North American Benthological Society*, **23**, 363–382.

Petrin, Z. (2011) Species traits predict assembly of mayfly and stonefly communuties along pH gradients. *Oecologia*, **167**, 513–524.

Petrin, Z., Laudon, H. & Malmqvist, B. (2007) Does freshwater macroinvertebrate diversity along a pH-gradient reflect adaptation to low pH? *Freshwater Biology*, **52**, 2172–2183.

Petrin, Z., McKie, B., Buffam, I., Laudon, H. & Malmqvist, B. (2007) Landscape-controlled chemistry variation affects communities and ecosystem function in headwater streams. *Canadian Journal of Fisheries and Aquatic Sciences*, **64**, 1563–1572.

Poff, N., Allan, J., Bain, M. & Karr, J. (1997) The natural flow regime. *BioScience*, **47**, 769–784.

Power, M.E., Stout, R., Cushing, C., Harper, P.P., Hauer, F.R., Matthews, W.J., Moyle, P.B., Statzner, B. & Wais De Bagden, I.R. (1988) Biotic and abiotic controls in river and stream communities. *Journal of the North American Benthological Society*, **7**, 456–479.

R Core Team. (2013) R: A Language and Environment for Statistical Computing. *R Foundation Statistical Computing*.

Reid, D.J., Quinn, J.M. & Wright-Stow, A.E. (2010) Responses of stream macroinvertebrate communities to progressive forest harvesting: Influences of harvest

intensity, stream size and riparian buffers. *Forest Ecology and Management*, **260**, 1804–1815.

Robinson, C.T. (2012) Long-term changes in community assembly, resistance, and resilience following experimental floods. *Ecological Applications*, **22**, 1949–1961.

Rosenfeld, J. & Ptolemy, R. (2012) Modelling available habitat versus available energy flux: do PHABSIM applications that neglect prey abundance underestimate optimal flows for juvenile salmonids? *Canadian Journal of Fisheries and Aquatic Sciences*, **69**, 1920–1934.

Sandin, L. & K. Johnson, R. (2004) Local, landscape and regional factors structuring benthic macroinvertebrate assemblages in Swedish streams. *Landscape Ecology*, **19**, 501–514.

Schmidt, T.S., Clements, W.H. & Cade, B.S. (2012) Estimating risks to aquatic life using quantile regression. *Freshwater Science*, **31**, 709–723.

Schooley, R. & Wiens, J. (2005) Spatial ecology of cactus bugs: area constraints and patch connectivity. *Ecology*, **86**, 1627–1639.

Statzner, B. & Higler, B. (1986) Stream hydraulics as a major determinant of benthic invertebrate zonation patterns. *Freshwater Biology*, **16**, 127–139.

Statzner, B., Hoppenhaus, K., Arens, M.-F. & Richoux, P. (1997) Reproductive traits, habitat use and templet theory: a synthesis of world-wide data on aquatic insects. *Freshwater Biology*, **38**, 109–135.

Tachet, H., Richoux, P., Bournaud, M. & Usseglio-Polatera, P. (2000) *Invertébrés D'eau Douce*. CNRS Editions, Paris.

Townsend, C.R., Dolédec, S. & Scarsbrook, M.R. (1997) Species traits in relation to temporal and spatial

heterogeneity in streams: a test of habitat templet theory. *Freshwater Biology*, **37**, 367–387.

Wagenhoff, A., Townsend, C.R. & Matthaei, C.D. (2012) Macroinvertebrate responses along broad stressor gradients of deposited fine sediment and dissolved nutrients: a stream mesocosm experiment. *Journal of Applied Ecology*, **49**, 892–902.

Wright, J.F. (1992) Spatial and temporal occurrence of invertebrates in a chalk stream, Berkshire, England. *Hydrobiologia*, **248**, 11–30.

Wright, J. (1995) Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. *Australian Journal of Ecology*, **20**, 181–197.

4. Optimal flow for brown trout: habitat – prey optimization

Abstract

Fresh water is a fundamental and limiting resource, both for the development of human society and for the maintenance of biodiversity and functionality of aquatic ecosystems. Human demand for water is constantly increasing, for both hydroelectric power production and agricultural use. At the same time the conservation of high biodiversity and ecosystem functionality depends on the availability of adequate stream flows.

The correct definition of ecosystem needs is essential in order to guide policy and management strategies to optimize the use of water. Commonly, the assessment of the optimal or minimum flow rate has been done by habitat-based models that define a relationship between instream flow and habitat availability for various species of fish.

We propose a new approach for the identification of environmental flows summing up the limiting factor approach, basic ecological relationships and the use of appropriate spatial scale for different organisms. We developed density-environment relationships for three different life stages of brown trout that show the limiting effects of hydromorphological variables at habitat scale. We used this match tense within habitat based models in order to select a range of flows that preserve most of the physical habitat for all the life stages. We also estimated the effect of varying discharge flows on macroinvertebrate biomass and used the obtained results to identify an optimal flow that maximize habitat and prey availability.

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4.1 Introduction

Fresh water is a fundamental and limiting resource, both for the development of human society and for the maintenance of biodiversity and functionality of aquatic ecosystems. Human demand for water is constantly increasing, both for hydroelectric power production and for agricultural purposes (de Fraiture & Wichelns 2010). The production of hydroelectric power is a great opportunity to reduce our dependence on fossil fuels and then the emissions of greenhouse gases. However, hydroelectric power stations necessarily modify the riverine ecosystems.
Scientists are challenged to define ecosystem needs clearly enough to guide policy and management strategies in order to optimize the use of water (Poff *et al.* 2003). In fact, the conservation of high biodiversity and ecosystem functionality depend on the release of adequate stream flows.
Habitat-based models have been widely used to define a relationship between instream flow and habitat availability for various species of fish and, thus, to define the optimal or minimum flow rate (Ayllón et al. 2010; Gore et al. 1991; Maddock 1999).

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This kind of models basically include hydraulic simulation and suitability curves of species on the basis of which it is possible to define a relationship between the flow and the availability of habitat for aquatic organisms. The conceptual basis of habitat based models is ecological. In fact, it is well known that aquatic organisms do not occupy any environment regardless of hydraulics inside the river, but they show strong preferences for certain values of the hydromorphological parameters such as water depth, current velocity, substrate size and composition (Van Liefferinge *et al.* 2005; Dolédec *et al.* 2007). When the flow rate varies, also the depth, the current velocity and often the type of substrate that is gained/lost from the river change and, consequently, the habitat availability for the reference species in the stretch of river changes.

The relationship of physical habitat to aquatic organism densities assumes that the production of benefits is limited by the availability of physical habitat. This assumption is not always true. Production may be also limited by water quality (Kail, Arle & Jähnig 2012; Morrissey *et al.* 2013), by the activities of man (Fjeldstad *et al.* 2012; Hansen & Hayes 2012), or by events and conditions occurring at a temporal and spatial scale beyond the scope of the model application (Bonada *et al.* 2008; Menge *et al.* 2011; Comte *et al.* 2013). In all cases, physical habitat is a necessary, but not sufficient condition for the production and survival of aquatic organisms. Thus, the results of habitat based models may best be viewed as indicators of population *potential* in systems where the habitat conditions described by the model are major population constraints.

In this perspective, the use of density-environment relationships that show the limiting effects of the habitat characteristics and not the average effects of the same variables seems more adequate.

Moreover, in field studies, a variety of factors such as competition for space and predation (Ayllón *et al.* 2013; Kaspersson *et al.* 2013), temperature (Lecomte & Laplanche 2012; Moore *et al.* 2012), climate change (Comte *et al.* 2013), presence of waterfalls or dams and flow variability (Fjeldstad *et al.* 2012), changes in habitat structures (Muotka & Syrjänen 2007) and stochasticity can influence the abundance and the distribution of fish. Each of these factors can influence the relationship among the density of organisms and the habitat characteristics, inflating variability.

Thus, it is often very difficult to disentangle the different sources of variation in the organism density and abundance along gradients using methods based on models testing hypotheses about central tendency (e.g. ANOVA) or central responses (e.g. ordinary least square regression, PCA, GAM, GLM) that set aside the concurring role of other unmeasured factors (Austin 2007).

From the ecological point of view, testing hypotheses about the environmental gradients as limiting factors or constraints on the density of organisms could be more informative than testing them about "average" responses (Downes 2010). Limiting factors typically result in wedge-shaped relationships with small changes in the mean value of the response variable along the gradient of the independent variable, but with large changes at the upper end of the distribution (Kail et al. 2012; Lancaster and Belyea 2006).

Quantile regression allows the association of the different rates of change (slopes) to the different parts of the response distribution, being a method for estimating functional relationships among variables for all the portions of a probability distribution (Koenker & Bassett 1978). This statistical tool was introduced in ecology by Cade et al. (1999) and can be used to test the role of environmental factors as

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constraints. Moreover, its application allows to predict not only the more probable values of the studied biological metric but also the maximum or minimum values that could be expected in environmental conditions comparable to the ones used for the model fitting (Cade & Noon 2003; Doll 2011).

Commonly, stream flows were assessed assuming that the fish abundance is directly related to habitat availability, but some critiques to this approach have already been done: i) they do not take into account the effects of flow management on macroinvertebrate populations that in many cases represent the main food source for fish (Lagarrigue *et al.* 2002; Rosenfeld & Ptolemy 2012; Sánchez-Hernández & Cobo 2012), ii) in the PHABSIM application, the suitability curves normally deliver information about where an individual fish is likely to position itself in the river at the microhabitat scale. Using this information to understand the consequences at management scale (e.g., on a stretch of river) can also be questionable (Parasiewicz & Walker 2007).

In the Alpine streams and rivers brown trout (*Salmo trutta*) was commonly considered as the target species for this kind of evaluation. The relationships among the density of brown trout and environmental variables have been deeply studied in freshwater ecosystems (Armstrong et al. 2003; Ayllón et al. 2009; Vismara and Azzellino 2001). Macroinvertebrates are rarely used in habitat-based methods because of the high heterogeneity of the density response along environmental gradients. However, density-environment relationships for macroinvertebrates were produced on the basis of the limiting factor approach (Fornaroli *et al.* 2015) and can be used, along with the one produced in this work for fish, to better understand the possible changes induced in the biological communities by water management strategies.

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In this work we propose a new concept for the identification of environmental flows summing up the limiting factor approach, basic ecological relationships and the use of appropriate spatial scale for different organisms.

4.2 Material and methods

4.2.1 Fish Sampling

In order to produce habitat suitability curves, fish sampling has been performed according to Mäki-Petäys et al. (1997) and Van Liefferinge et al. (2005). Electrofishing was conducted in 13 sites by wading along a section of the river using a generator-powered unit (Scubla ELT60 II GI), with a fixed cathode and a 2.5 m anode pole (32 cm diameter anode ring). To minimize the flight bias, which may cause the displacement of individuals from their original position, a modified point electrofishing procedure was used. The activated anode was submerged for several seconds every 0.5 – 1.0 m (measured between the anode centers of two consecutive 'dippings').

The point of the first sighting of fish was noted with a different reconnaissance symbol (colored stake) in order to know the placement of the different individuals after electrofishing.

To avoid faulty observations of habitat utilization caused by the displacement of individuals due to flight from the electric current, each study section was sampled only once with this technique.

Captured trout were measured and assigned to one of three size-classes <10, 10-15, >15 cm, roughly corresponding to fish ages of 0+, 1+, and 2+ respectively (Mäki-Petäys *et al.* 1997) before being returned unharmed to the water.

After sampling, different microhabitat variables were measured for each individual.

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We measured water velocity and water depth using a Marsh-McBirney Flo-Mate Model 2000 portable flowmeter. Water velocity was measured at 40% of the depth in order to obtain the mean velocity of the water column in the sampling point.

The substrates were classified as dominant, sub-dominant and matrix. We expressed their relative abundance in percentage according to the mineral substrate classes provided by the site description protocol in the AQEM Manual (AQEM Consortium 2002). Using these three descriptors we derived the maximum substrate size (MSS). The percent of the fine fraction was obtained summing the relative percentage of substrate classes having size below 0.06 m.

The availability of refugia was also evaluated. We defined as refugia any element that can provide protection to adult trout against adverse environmental conditions. Refugia can be vegetation (aquatic or overhanging), wood debris, undercut banks, or, more often than the other in the studied sites, discontinuities in the riverbed profile where the depth of water was higher than 0.30 m and the maximum substrate was greater than 0.5 m (boulders).

4.2.2 Characterization of sampling sites

After fish community assessment, the sampling sites were characterized measuring the above mentioned variables (depth, velocity, substrate, refugia availability) in multiple spatial referenced points (from 20 to 40 point per each site) chosen in order to represent the within site variability. Starting from this series of discrete points of measurement, a 1 m^2 cell map of the distribution for each variable was created using GRASS GIS (GRASS Development Team 2012) and an inverse distance weighting algorithm.

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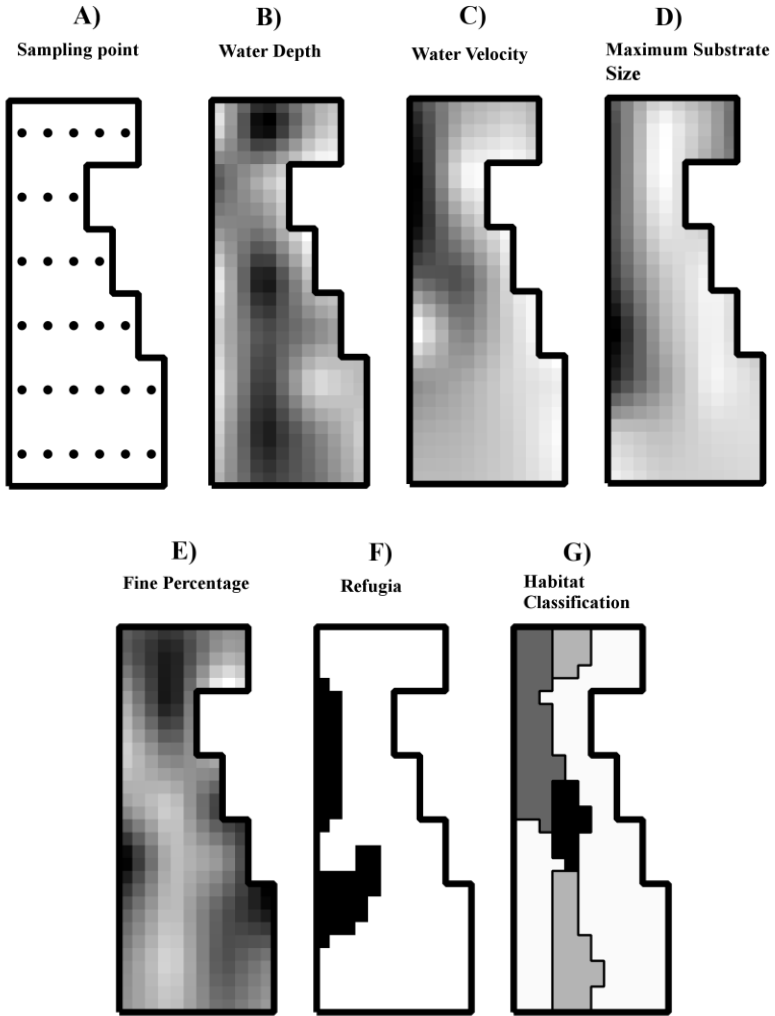
Fig. 4.1 – A) Points of measurement of environmental variables, B) Map of water depth, C) Map of water velocity, D) Map of the maximum substrate size, E) Map of the fine substrate percentage, F) Map of refugia availability, G) Reclassified habitat maps evaluated using the water velocity and water depth maps.

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Using the maps of water velocity and depth for each site a reclassified habitat map was produced following these criteria: I) if depth was lower than 0.5 meter and water velocity slower than 0.3 m/s the assigned microhabitat was shallow pool; II) if depth was higher than 0.5 meter and water velocity slower than 0.3 m/s the assigned microhabitat was deep pool; III) if depth was lower than 0.5 meter and water velocity higher than 0.3 m/s the assigned microhabitat was riffle; IV) if depth was higher than 0.5 meter and water velocity higher than 0.3 m/s the assigned microhabitat was run.

These maps were compared with the visual surveys made in the field and the identification of the different habitats was very similar between the two methods.

For each habitat in the reclassified map the mean water velocity, depth, fine percentage and maximum substrate size were evaluated averaging the values of each cell. In Fig. 4.1 are shown all the maps produced for one of the sampling sites.

Differently, the availability of refugia was calculated by dividing the number of cells classified as refugia for the number of total cells.

Each captured fish was then assigned to a specific habitat on the basis of the characteristics noted during the electrofishing and the real position in the sampling site. The abundance of each age class in each habitat was calculated by summing the number of fish captured and divided by the class area.

4.2.3 Quantitative habitat suitability models development

Before carrying out quantile regression analysis all the independent variables were unit-based normalized ($X'=X-X_{min}/X_{max}-X_{min}$). Since the range of values of raw data varies widely, normalization is needed to address the relative importance of the different models.

The relationships among the dependent and independent variables were studied at different quantiles (0.05, 0.10, 0.15, 0.20, 0.50, 0.80, 0.85, 0.90, 0.95). This choice was made in order to examine with more ease the boundaries of density–habitat relationships for the upper or lower limits imposed by the limiting factors. We fitted linear, exponential, logarithmic and quadratic curves to the data.

We also used a novel linear model for quantile regression with a subject-specific random intercept accounting for within-group correlation (Geraci & Bottai 2007). This approach is analogous to the estimate of the mean regressions with random intercepts in linear mixed models (Bolker *et al.* 2009). Thus, one categorical variable for the four habitat categories was included in our models in order to account for their effects on intercept.

In summary, we generated 8 univariate models for each pair of the dependent and independent variables: 4 model shapes, each of which considering the random effect as present or absent. Then, we used the best-fitting univariate models (as a general rule of thumb, the models with w_i greater than 10% of the highest one) to fit multivariate quantile regression models combining the selected variables considering both the presence and the absence of the random effect.

The statistical analyses were conducted using both the quantreg (Koenker 2013) and lqmm package (Geraci 2014) in R Project software (R Core Team 2014). For each model a τ-specific version of Akaike Information Criterion, corrected for small sample size ($AICc(\tau)$), was calculated for every studied quantile. The difference between the model $AICc(\tau)$ and the minimum $AICc(\tau)$ could be used in order to choose the best-fitting model ($\Delta i = AICc(\tau) - \min AICc(\tau)$), considering that the model with the lowest $AICc(\tau)$ generally provides the best description of the data. Values of $\Delta i \geq 2$ are suggested as a

threshold to exclude alternative models; values of $\Delta i < 2$ indicate substantial support for the alternative model (Burnham & Anderson 2002; Johnson & Omland 2004). For each model, we reported the Akaike weights (w_i), the relative likelihood of a model, given a data set and a set of models (Burnham & Anderson 2002). We determined the best models across the studied quantiles by averaging w_i for each model from all nine quantile model selection analyses (Allen & Vaughn 2010; Fornaroli *et al.* 2015).

We used the selected models in order to predict trout densities along the alpine valley of the Serio river and we calculated the lower and upper boundaries using the 10th and 90th quantile regression model, respectively. This provides an 80% prediction interval for a single new observation.

4.2.4 Modeled sites selection and simulation

We selected 8 sites, different from those used for the suitability evaluation, in order to model the changes induced by flow modifications on physical habitat..

We identified 2 or 3 representative cross sections in each site (for a total of 21 representative sections). The sections were placed at a distance comparable to the stream width and in order to represent properly the morphological variability, so the distance between sections was not the same in all sampling sites. We carried out several hydro-morphological surveys in order to characterize the flow. In each section we measured water velocity, depth, substrate size and refugia availability every 0.5-1 m. Water velocity and depth measurements were repeated 3 or 4 times in order to produce a calibration dataset for the PHABSIM habitat modeling. We performed these measurements in situations ranging between low and normal flows. Depending on the sites the flows varied from 0.2 up to 10 m^3/s.

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One-dimensional hydraulic models were used to compute depth and velocity in the cross-section. The STGQ (Waddle 2001) model was calibrated using the measured water surface elevations recorded during the hydraulic surveys. To calibrate the model, at least three measured water surface elevations were used. After calibration, the model simulated the water surface elevations at each cross-section for a range of flows from 0.05 to 14 m^3/s, in increments of 0.2 m^3/s for flows below 2 m^3/s, in increments of 0.5 m^3/s for flows between 2 m^3/s and 5 m^3/s and in increments of 1 m^3/s for flows over 5 m^3/s. The VELSIM velocity model with a dataset including at least 3 velocity data was calibrated using the stage-discharge method and the mean-column velocity measurements. Using this dataset, throughout PHABSIM simulation system we were able to produce maps of water velocity and depth for a wide range of discharge in each site. Then, we imported these map and those produced for maximum substrate size, fine substrate percentage and refugia availability in R software for subsequent analysis.

As we have done for the suitability evaluation, and using the same criteria, we used the maps of water velocity and depth for each site at each discharge to produce a reclassified habitat map.

For each habitat in the reclassified map the mean water velocity, depth, fine substrate percentage and the maximum substrate size were evaluated averaging the values of each cell. Since the cells areas in those maps were different from each other we used the weighted mean based on the cell area in order to evaluate the habitat characteristics. The refugia availability was calculated dividing the area of the cells classified as refugia for the total area of the considered habitat. It is important to note that a cell could be considered as a refuge for some discharge but not for others. As previously

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mentioned, often refugia are represented by streambed discontinuity but only when the water depth is over 0.3 m. That's why the distribution of refugia is not constant with varying discharge (as the fine percentage and maximum substrate size are) but varies with it.

4.2.5 Habitat availability evaluations

Once we obtained a comprehensive view of the modifications in the physical habitat, aggregated at two different spatial levels (micro- and mesohabitat), we proceeded with the evaluation of the suitability for biological populations.

We modeled the potential abundances of six macroinvertebrate families (Leuctridae, Heptageniidae, Baetidae, Limnephilidae, Chironomidae, Limoniidae) using the density models developed in Fornaroli *et al.* (2015). We evaluated the potential density (ind/m^2) in each cell of each site for each discharge and, multiplying this value for the cell area and summing the obtained results, we were able to provide a potential number of individuals in each site for each discharge.

As to brown trout population we used the density models developed in this work to evaluate the potential density of each class of trout age in each habitat per site. Then we multiplied the predicted maximum density for the habitat area and we summed the results obtained for all the habitats within a site.

The habitat classification and description were done using the same criteria used during the analyses of the field data for the development of density models.

Throughout these procedures we were able to predict the maximum number of organisms in each site at each discharge.

4.2.6 Optimal flows definition

For each site the evaluated discharges ranging between 0.050 m^3/s and the mean annual discharge of each site. The mean annual discharge in each site was evaluated using the regionalization procedure developed by Lombardy region (PTUA 2006). This procedure, starting from the measured discharge in a downstream section, estimates the mean values of the average annual flow per unit area, scaled according to the measures of average annual rainfall on the respective sub-basins.

The habitat availability – discharge relationships were evaluated by fitting a spline function for each species in each site to the output of the model and using it for making prediction for every 0.050 m^3/s in the considered range.

As to trout habitat availability we considered suitable all the discharges in which the available habitat is over the 50th percentile of the available habitat for all the life stages.

The habitat availability and so the potential abundance of the studied macroinvertebrate families were transformed in the potential dry weight of prey for trout using the equation available in Canobbio et al. (2008), Nyström and Pérez (1998), Stoffels et al. (2003) and Towers et al. (1994) and summing the results for all the six families.

The acceptable flows are the ones defined suitable for all the life stages of trout and, inside that range, the flows that maximize the macroinvertebrate (prey) availability are supposed to be better also for trout.

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4.3 Results

4.3.1 Habitat suitability models

Thirteen sites were sampled in order to develop the density-environmental models for the different life stages of brown trout. Each site was divided in different habitats as described in the methods section and a total of 73 different habitat units were characterized. The results are summarized in Tab. 4.1.

The relationships among the density of the three considered brown trout life stages and the five measured environmental variables were analyzed.

Regarding adult brown trout densities, the model considering water velocity and refugia availability as the independent variables and not accounting for habitat effect was selected as the best one (averaged w_i = 0.446). The function that best described the effects of the two independent variables was the quadratic one. Variations in the density of adult brown trout decreased with velocity and increased with refugia availability (Fig. 4.2A).

The model considering water velocity and maximum substrate size as the independent variables and not accounting for the habitat effect was selected as the best (averaged wi = 0.168) for describing juvenile brown trout densities. The effect of water velocity gradient was best described by the exponential function with lower values of density for high velocities. On the other hand the effect of maximum substrate size was best described by the quadratic function which predicts higher densities for intermediate size (Fig. 4.2B).

Also the densities of brown trout fry were more consistently represented by a multivariate model. The model considering water velocity and fine substrate percentage simultaneously, and not accounting for the habitat effect (averaged w_i = 0.335) was selected as the best performing.

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Tab. 4.1 – Mean characteristics of the habitats used for the density-environment model development and mean trout densities.

Habitat (n)	Shallow pool (31)	Deep pool (10)	Riffle (17)	Run (15)
Area (m^2)	59 ± 55	84 ± 83	117 ± 263	21 ± 22
Depth (m)	0.25 ± 0.08	0.67 ± 0.11	0.30 ± 0.08	0.65 ± 0.17
Water velocity (m/s)	0.138 ± 0.068	0.129 ± 0.049	0.442 ± 0.088	0.465 ± 0.186
MSD (m)	0.40 ± 0.25	0.55 ± 0.18	0.36 ± 0.23	0.48 ± 0.20
Fine percentage (%)	34 ± 18	33 ± 12	31 ± 18	24 ± 14
Refugia availability (%)	25 ± 34	62 ± 32	13 ± 26	35 ± 44
Trout (ind/m^2)	0.208 ± 0.184	0.219 ± 0.173	0.084 ± 0.184	0.062 ± 0.90
Adult (ind/m^2)	0.047 ± 0.073	0.156 ± 0.174	0.006 ± 0.016	0.017 ± 0.039
Juvenile (ind/m^2)	0.078 ± 0.103	0.040 ± 0.040	0.060 ± 0.098	0.014 ± 0.028
Fry (ind/m^2)	0.083 ± 0.097	0.023 ± 0.30	0.019 ± 0.042	0.032 ± 0.072

The function that best described the effects of the two independent variables was the linear one: the higher values of density are predicted for habitat with low water velocities and high percentage of fine substrate (Fig. 4.2C) as also reported by Armstrong et al. (2003).

All the selected models for density were not better than a constant model at the 10th quantile (for each life stage the constant model at the 10th quantile was: y = 0), thus only the upper boundaries, represented by 90th quantile, were used in order to predict the brown trout densities.
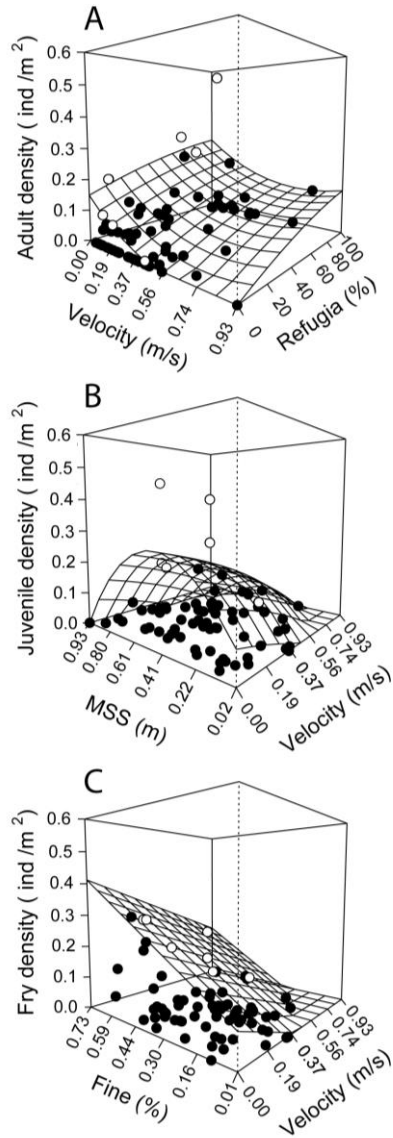
Fig. 4.2 – Multivariate quantile regression models for brown trout densities: (A) Adult, (B) Juvenile, (C) Fry. Surfaces represent 90th quantile regression models. Black points fall within the predicted range, white points outside.

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4.3.2 Optimal flows

A range of flows that maximize the habitat availability for the different life stages of brown trout were obtained for each modeled site. As it is shown in Fig. 4.3, most of the habitats for fish is already available for really low flows, while, on the contrary, the macroinvertebrate biomass increases with increasing flow.

The habitat availability, especially for young brown trout and fry, decreases rapidly for flows over 50% of the mean annual discharge in all the modeled sites, indicating that most of the physical habitat could be lost in that conditions (Fig. 4.3). Habitat availability for adult brown trout seems to be also adversely affected by high flows even if the magnitude of this trend is less clear than for fry and young life stages.

The abundance of the macroinvertebrates, and hence their dry biomass, increases monotonically with increasing discharge, in all the studied sites. These kinds of results are driven by the suitability models for the macroinvertebrates that predict higher densities in high velocity habitat for almost all the studied families.

Across the modeled sites, between the minimum and the maximum optimal flows selected for trout, the dry biomass of macroinvertebrate increases by 131 ± 98 % (Tab. 4.2).

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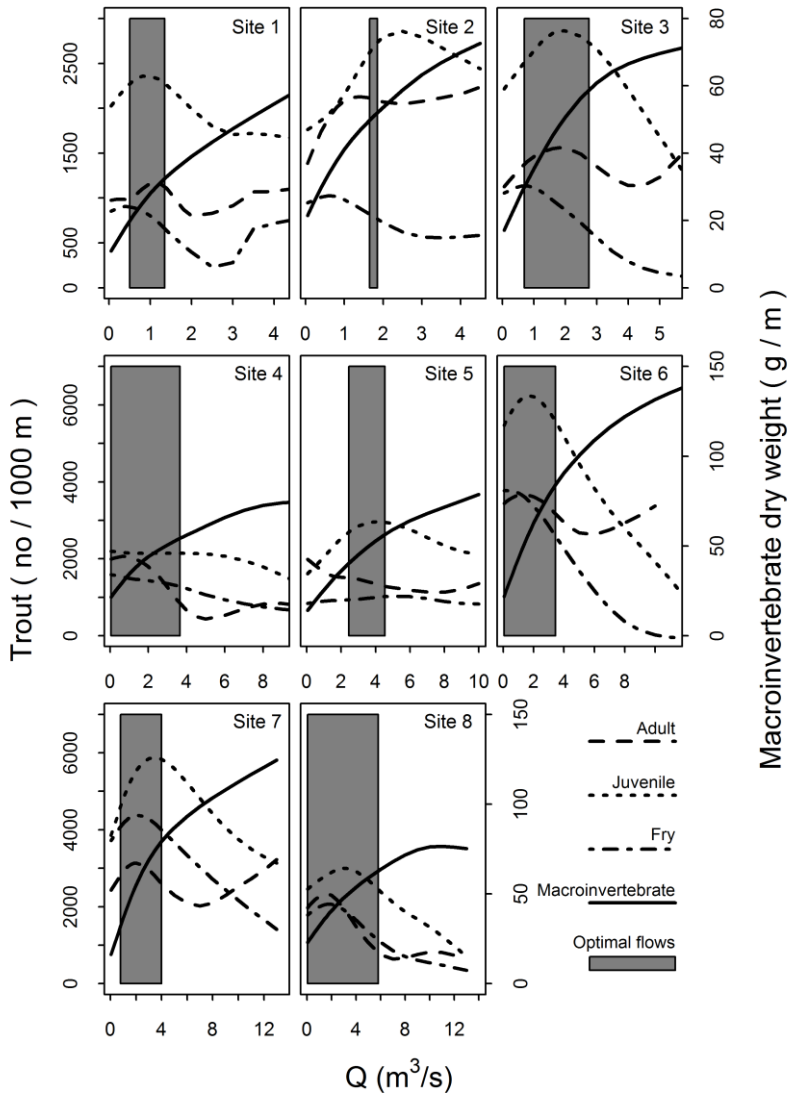
Fig. 4.3 – Trends in available habitat for adult, juvenile, fry brown trout and potential macroinvertebrate biomass in the 8 modeled sites.

These results are the key findings of this work. In fact they mean that the available energy can increase more than 2 times inside the range of flows that preserve most of the habitat for fish.

In order to provide a single value of optimal flow, we selected the ones that maximized the macroinvertebrate biomass production.

Tab. 4.2 – Optimal flows calculate for the 8 studied sites and relative biological metrics. Macroinvertebrates dry weights were compared to the worst situation inside the acceptable range of flows identified for fish.

Site	$Q_{optimal}$	Habitat availability (%)			Macroinvertebrates Biomass (% increase)
		Adult	Young	Fry	
1	1.350	96	96	73	68
2	1.850	94	96	75	5
3	2.750	91	96	57	103
4	3.650	41	98	81	167
5	4.550	65	100	99	42
6	3.450	86	89	69	310
7	4.000	78	99	91	150
8	5.800	34	82	54	200

4.4 Discussion and conclusions

Habitat models that predict flow-related changes in productive capacity are usually used for the definition of environmental flows, and thus the information they provide must be correct and useful for the water managers.

In our study we developed density-environment models for different life stages of brown trout at the habitat scale. This improves in different ways the estimate of available habitat:

- the spatial scale of the data used for developing the biological models is the same used for making predictions;

- the different behavior of the different life stages are taken into account and the optimal flows were defined on the basis of the needs of the different life stages;
- the changes in the available macroinvertebrate biomass were evaluated as the main food source for trout.

In our analyses, we found that the factors limiting the densities of trout were water velocity, substrate characteristics and refugia availability. For all the life stages, the selected models considered simultaneously two variables and implied that higher velocities provided a less suitable habitat, regardless of other physical characteristics and with different patterns. The availability of refugia, evaluated as the fractions of available habitat that were characterized by discontinuity in the riverbed profile, where the depth of the water was over 0.30 m and the maximum substrate size was greater than 0.5 m, had a significant effect only on adult trout densities. In fact, adult trout densities were greater in habitat whit a greater proportion of refugia. The density of juvenile brown trout were higher in habitats characterized by maximum substrate size between 0.4 and 0.7 m, and as for all the other life stages, slow water habitats could provide a better environment than fast flowing water habitats. On the other hand, the densities of brown trout fries increased in habitats with a great proportion of fine substrate.

In many studies (Vismara & Azzellino 2001; Strakosh *et al.* 2003; Muñoz-Mas *et al.* 2012) water depth plays an important role in the definition of habitat suitability for brown trout. In our models, water depth plays a significant role only in the description of optimal habitat for adult trout. In fact the refugia availability is strictly connected with water depth, considering that in our analyses we define as refugia only the cell with water deeper than 0.3 m. For the other life stages, the effect of water depth did not result significant, while the effect of

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substrate characteristics proved to be very important and were used for the density predictions in our models.

Habitat availability patterns were generated by the different effects of flow-dependent depth and velocity variations, but also by the substrate characteristics of the gained or lost river bed. The trout number increased rapidly till a defined flow, under which the velocity remains quite low and the gained area is, at least in part, suitable. Above such threshold, velocity turns to be unsuitable and causes the dropping of the available habitat, simply because every area increase is paid by a corresponding velocity increase, adversely affecting all of the life stages.

Besides that, macroinvertebrates show a completely different pattern. In all the study sites the total biomass of the considered macroinvertebrate families always increased with increasing discharge.

Looking at the limiting response of densities to flow - related variables seems a promising approach. The density models developed, despite not considering water depth, gave results comparable to many other studies. This is especially true when they are applied in a real context. This approach not only deals with many scattered density dataset, but involves also a different kind of interpretation of the relationships between available habitat and flows.

As physical habitat is a necessary, but not sufficient condition for production and survival of fishes, the results of habitat based models may best be viewed as indicators of population potential, in systems where the habitat conditions described by the models are the major population constraints. However, prey availability is always important for fish, and macroinvertebrates are recognized as the main food source for trout (Sánchez-Hernández & Cobo 2012). It seems clear that taking into account the effect of flows management on

macroinvertebrate population is fundamental in order to improve the assessment of environmental flows.

4.5 Acknowledgements

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4.6 References

Allen, D.C. & Vaughn, C.C. (2010) Complex hydraulic and substrate variables limit freshwater mussel species richness and abundance. *Journal of the North American Benthological Society*, **29**, 383–394.

AQEM Consortium. (2002) *Manual for the Application of the AQEM System.*

Armstrong, J.. D., Kemp, P.. S., Kennedy, G.J.. J.A., Ladle, M. & Milner, N.. J. (2003) Habitat requirements of Atlantic salmon and brown trout in rivers and streams. *Fisheries Research*, **62**, 143–170.

Austin, M. (2007) Species distribution models and ecological theory: A critical assessment and some possible new approaches. *Ecological Modelling*, **200**, 1–19.

Ayllón, D., Almodóvar, A., Nicola, G.G. & Elvira, B. (2009) Interactive effects of cover and hydraulics on brown trout habitat selection patterns. *River Research and Applications*, **25**, 1051–1065.

Ayllón, D., Almodóvar, A., Nicola, G.G. & Elvira, B. (2010) Modelling brown trout spatial requirements through physical habitat simulations. *River Research and Applications*, **26**, 1090–1102.

Ayllón, D., Nicola, G.G., Parra, I., Elvira, B. & Almodóvar, A. (2013) Intercohort density dependence drives brown trout habitat selection. *Acta Oecologica*, **46**, 1–9.

_____

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.-S.S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in ecology & evolution*, **24**, 127–135.

Bonada, N., Rieradevall, M., Dallas, H., Davis, J., Day, J., Figueroa, R., Resh, V.H. & Prat, N. (2008) Multi-scale assessment of macroinvertebrate richness and composition in Mediterranean-climate rivers. *Freshwater Biology*, **53**, 772–788.

Burnham, K.P. & Anderson, D. (2002) *Model Selection and Multi-Model Inference*, 2nd ed. Springer-Verlag, New York.

Cade, B.S. & Noon, B.R. (2003) A gentle introduction to quantile regression for ecologists. *Frontiers in Ecology and the Environment*, **1**, 412–420.

Cade, B., Terrell, J. & Schroeder, R. (1999) Estimating effects of limiting factors with regression quantiles. *Ecology*, **80**, 311–323.

Canobbio, S., Mezzanotte, V., Ballabio, D. & Pavan, M. (2008) Multi-Criteria Decision-Making Methods: A Tool for Assessing River Ecosystem Health Using Functional Macroinvertebrate Traits. *Data Handling in Science and Technology* Data Handling in Science and Technology., First Edit pp. 169–191. Elsevier.

Comte, L., Buisson, L., Daufresne, M. & Grenouillet, G. (2013) Climate-induced changes in the distribution of freshwater fish: observed and predicted trends. *Freshwater Biology*, **58**, 625–639.

Dolédec, S., Lamouroux, N., Fuchs, U. & Mérigoux, S. (2007) Modelling the hydraulic preferences of benthic macroinvertebrates in small European streams. *Freshwater Biology*, **52**, 145–164.

_____

Doll, J.C. (2011) Predicting biological impairment from habitat assessments. *Environmental monitoring and assessment*, **182**, 259–277.

Downes, B. (2010) Back to the future: little-used tools and principles of scientific inference can help disentangle effects of multiple stressors on freshwater ecosystems. *Freshwater Biology*, **55**, 60–79.

Fjeldstad, H.P., Barlaup, B.T., Stickler, M., Gabrielsen, S.E. & Alfredsen, K. (2012) Removal of weirs and the influence on physical habitat for salmonids in a Norwegian river. *River research and applications*, **28**, 753–763.

Fornaroli, R., Cabrini, R., Sartori, L., Marazzi, F., Vracevic, D., Mezzanotte, V., Annala, M. & Canobbio, S. (2015) Predicting the constraint effect of environmental characteristics on macroinvertebrate density and diversity using quantile regression mixed model. *Hydrobiologia*, **742**, 153–167.

De Fraiture, C. & Wichelns, D. (2010) Satisfying future water demands for agriculture. *Agricultural Water Management*, **97**, 502–511.

Geraci, M. (2014) Linear quantile mixed models: The lqmm package for Laplace quantile regression. *Journal of Statistical Software*, **57**, 1–29.

Geraci, M. & Bottai, M. (2007) Quantile regression for longitudinal data using the asymmetric Laplace distribution. *Biostatistics*, **8**, 140–154.

Gore, J., King, J. & Hamman, K. (1991) Application of the instream flow incremental methodology to southern African rivers: Protecting endemic fish of the Olifants River. *Water SA*, **17**, 225–236.

GRASS Development Team. (2012) Geographic Resources Analysis Support System (GRASS) Software, Version

_____

6.4.2. Open Source Geospatial Foundation. http://grass.osgeo.org.

Hansen, J. & Hayes, D. (2012) Long-Term implications of dam removal for macroinvertebrate communities in Michigan and Wisconsin Rivers, United States. *River Research and Applications*, **28**, 1540–1550.

Johnson, J.B. & Omland, K.S. (2004) Model selection in ecology and evolution. *Trends in ecology & evolution*, **19**, 101–108.

Kail, J., Arle, J. & Jähnig, S. (2012) Limiting factors and thresholds for macroinvertebrate assemblages in European rivers: Empirical evidence from three datasets on water quality, catchment urbanization, and river restoration. *Ecological Indicators*, **18**, 63–72.

Kaspersson, R., Sundström, F., Bohlin, T. & Johnsson, J.I. (2013) Modes of competition: adding and removing brown trout in the wild to understand the mechanisms of density-dependence. *PloS one*, **8**, e62517.

Koenker, R. (2013) quantreg: Quantile Regression. R package version 4.98.
http://CRAN.R-project.org/package=quantreg.

Koenker, R. & Bassett, G. (1978) Regression Quantiles. *Econometrica*, **46**, 33–50.

Lagarrigue, T. & Céréghino, R. (2002) Diel and seasonal variations in brown trout (Salmo trutta) feeding patterns and relationship with invertebrate drift under natural and hydropeaking conditions in. *Aquatic living Resources*, **15**, 129–137.

Lancaster, J. & Belyea, L. (2006) Defining the limits to local density: alternative views of abundance–environment relationships. *Freshwater Biology*, **51**, 783–796.

_____

Lecomte, J.-B. & Laplanche, C. (2012) A length-based hierarchical model of brown trout (Salmo trutta fario) growth and production. *Biometrical journal*, **54**, 108–126.

Van Liefferinge, C., Seeuws, P., Meire, P. & Verheyen, R.F. (2005) Microhabitat use and preferences of the endangered Cottus gobio in the River Voer , Belgium. *Journal of Fish Biology*, **67**, 897–909.

Maddock, I. (1999) The importance of physical habitat assessment for evaluating river health. *Freshwater biology*, **41**, 373–391.

Mäki-Petäys, A., Muotka, T., Huusko, A., Tikkanen, P. & Kreivi, P. (1997) Seasonal changes in habitat use and preference by juvenile brown trout, Salmo trutta, in a northern boreal river. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 520–530.

Menge, B. a., Gouhier, T.C., Freidenburg, T. & Lubchenco, J. (2011) Linking long-term, large-scale climatic and environmental variability to patterns of marine invertebrate recruitment: Toward explaining "unexplained" variation. *Journal of Experimental Marine Biology and Ecology*, **400**, 236–249.

Moore, a., Bendall, B., Barry, J., Waring, C., Crooks, N. & Crooks, L. (2012) River temperature and adult anadromous Atlantic salmon, Salmo salar, and brown trout, Salmo trutta. *Fisheries Management and Ecology*, **19**, 518-526.

Morrissey, C. a., Boldt, A., Mapstone, A., Newton, J. & Ormerod, S.J. (2013) Stable isotopes as indicators of wastewater effects on the macroinvertebrates of urban rivers. *Hydrobiologia*, **700**, 231–244.

Muñoz-Mas, R., Martínez-Capel, F., Schneider, M. & Mouton, A.M. (2012) Assessment of brown trout habitat suitability in the Jucar River Basin (SPAIN): comparison of data-

driven approaches with fuzzy-logic models and univariate suitability curves. *The Science of the total environment*, **440**, 123–131.

Muotka, T. & Syrjänen, J. (2007) Changes in habitat structure, benthic invertebrate diversity, trout populations and ecosystem processes in restored forest streams: a boreal perspective. *Freshwater Biology*, **52**, 724–737.

Nyström, P. & Pérez, J.R. (1998) Crayfish predation on the common pond snail (Lymnaea stagnalis): the effect of habitat complexity and snail size on foraging efficiency. *Hydrobiologia*, **368**, 201–208.

Parasiewicz, P. & Walker, J.D. (2007) Comparison of MesoHABSIM with two microhabitat models (PHABSIM and HARPHA). *River Research and Applications*, **23**, 904–923.

Poff, N., Allan, J., Palmer, M., Hart, D.D., Richter, B., Arthington, A.H., Rogers, K., Meyer, J. & Stanford, J. (2003) River flows and water wars: emerging science for environmental decision making. *Frontiers in Ecology and the Environment*, **1**, 298–306.

PTUA. (2006) Allegato 2. *Progamma di Tutela e Uso delle Acque, Deliberazione n. 2244 del 29 marzo 2006 Regione Lombardia* p. 28.

R Core Team. (2014) R: A Language and Environment for Statistical Computing. *R Foundation Statistical Computing*.

Rosenfeld, J. & Ptolemy, R. (2012) Modelling available habitat versus available energy flux: do PHABSIM applications that neglect prey abundance underestimate optimal flows for juvenile salmonids? *Canadian Journal of Fisheries and Aquatic Sciences*, **69**, 1920–1934.

Sánchez-Hernández, J. & Cobo, F. (2012) Summer differences in behavioural feeding habits and use of feeding habitat

among brown trout (Pisces) age classes in a temperate area. *Italian Journal of Zoology*, **79**, 468–478.

Stoffels, R., Karbe, S. & Paterson, R. (2003) Length-mass models for some common New Zealand littoral-benthic macroinvertebrates, with a note on within-taxon variability in parameter values among published models. *New Zealand Journal of Marine and Freshwater Research*, **37**, 449–460.

Strakosh, T., Tr, S., Rm, N. & Development, J.R.A. (2003) Development and assessment of habitat suitability criteria for adult brown trout in southern New England rivers. *Ecology of Freshwater Fish*, **12**, 265–274.

Towers, D.J., Henderson, I.M. & Veltman, C.J. (1994) Predicting dry weight of New Zealand aquatic macroinvertebrates from linear dimensions. *New Zealand Journal of Marine and Freshwater Research*, **28**, 159–166.

Vismara, R. & Azzellino, A. (2001) Habitat suitability curves for brown trout (Salmo trutta fario L.) in the River Adda, Northern Italy: comparing univariate and multivariate approaches. *Regulated rivers: research & management*, **50**, 37–50.

Waddle, T. (2001) *PHABSIM for Windows: User's Manual and Exercises. Open-File Report 01-340. US Geological Survey: Fort Collins, CO*.

5. Future climate change may affect habitat suitability in Alpine streams: a case study in Italy

Abstract

Future climate change may affect hydrological regime, and habitat suitability in Alpine stream so hampering the effectiveness of rehabilitation measures. Study of future hydrology and habitat in Alpine river requires investigation of i) future climate, ii) hydrological response of high altitude catchments, and iii) habitat preferences of riverine biota. Here the impact of prospective climate change on hydrological, and ecological status of the mountain stretch of the strongly snow fed Serio river (ca. 300 km^2), in Northern Italian Alps was investigated. A hydrological model was used to mimic the river's hydrological regime. Field experiments were carried out to build suitability curves for brown trout (*Salmo trutta*) in different stages (adult, young, spawning), and for four macro invertebrates families (Leuctridae*,* Heptageniidae*,* Limnephilidae*,* Limoniidae), considering the combined effect of flow depth, velocity, and substrate. Discharge-WUA curves were obtained for each species and stage through IFIM-PHABSIM, and habitat quality assessment was carried out at river scale via identification of characteristic river habitats, and morpho-dynamic classification by *in situ* surveys and aerial pictures. Flow driven seasonal WUA assessment at river scale was then carried out. Future (until 2100) hydrological cycle was then projected by feeding the model with temperature and precipitation outputs from two GCMs delivered from CMIP5 for the AR5 of IPCC, under three different RCPs (2.6, 4.5, 8.5), and present and prospective habitat availability was investigated. The results display potential for large flow

decrease, and shift of seasonal peaks. Variable scenarios of habitat availability were found, with potentially worse situation for all species, and stages, with differences seasonally. The study provides a *what if* analysis of potential changes of eco-hydrological status of the Serio river, somewhat representative for Italian Alpine rivers, and may aid the ongoing discussion about rivers' ecological status improvement as required by Water Framework Directive and adaptation strategies therein.

Keywords: river habitat, hydrological modeling, suitability curves, climate change projections

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5.1 Introduction

Global warming is impacting water resources distribution in temperate regions, modifying riverine ecology and hydromorphology (Mohseni *et al.*, 2003; Wilby *et al.*, 2006; Brown *et al.*, 2007; Palmer *et al.*, 2008; Buffagni *et al.*, 2009; Rijnsdorp *et al.*, 2009; Peeler *et al.*, 2011; Wenger *et al*., 2011; Loperfido, 2014). Future Changes in precipitation and temperatures as expected under transient climate change conditions will have considerable fallout upon stream flow regimes worldwide (Kang and Ramirez, 2007; Feyen and Dankers, 2009; Bocchiola *et al.*, 2011; Groppelli *et al.*, 2011; Soncini and Bocchiola, 2011; Buisson *et al.*, 2013).

Over the last 4 decades significant warming was observed within the Italian and European Alpine rivers, leading to decreased snow cover and glaciers' extent, and modifying in stream flows (Beniston *et al.*, 2003; Laternser and Schneebeli, 2003; Allamano *et al.*, 2009a, b; Maragno *et al.*, 2009; Bocchiola and Groppelli, 2010; Bocchiola and Diolaiuti, 2010; Pellicciotti *et al.*, 2010; Diolaiuti *et al.*, 2012a, b).

Recent studies (*e.g.* Bocchiola, 2014) displayed significant changes of hydrological fluxes in Alpine rivers of Italy in the last century, notably during winter, spring and summer, and future expected hydrological changes include decreased average in channel discharge, and modified incidence of extreme events, either droughts, or floods (Bavay *et al.*, 2009; Feyen and Dunkers, 2009; Confortola *et al.*, 2013).

Climate driven modification of hydrological regimes may considerably fallout upon ecological status of rivers (Arthington *et al.*, 2006; Hari *et al.*, 2006; Ficke *et al.*, 2007; Battin *et al*., 2007; Palmer *et al.*, 2008; Isaak *et al.*, 2010; Peeler and Feist, 2011, Huttunen *et al.*, 2014).

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In mountain snow fed rivers hosting salmonids, future climate change may result into lower flows in summer and autumn, increased flood flows at winter, and warmer waters in summer, eventually driving population decrease (Poff and Allan, 1995; Poff *et al.*, 2001; Battin *et al.*, 2007; Ficke *et al.*, 2007).

In Europe, hydro-morphology is a key factor controlling ecosystem behavior (Whitehead *et al.*, 2014), and alterations of rivers' morphology, including loss of connectivity with flood plains, hydraulic constructions, and loss of riparian vegetation improving fish habitat (Bocchiola *et al.*, 2006; 2008; Bocchiola, 2010) may impact river ecology. Changes in climate could also affect sediment transfer, channel morphology and inundation frequency, thereby altering ecosystems at both catchment and habitat scales (Verdonschot, 2000; Hering *et al.*, 2009).

The Water Framework Directive WFD (Directive 2000/60/EC) enacted by the European Community fostered a strong demand for cost-effective stream restoration, requiring that all European rivers reach a good ecological status by 2015 (*e.g.* Kail and Hering, 2005; Erba *et al.*, 2006). However, climate change may act against restoration, making it difficult, if not impossible, to return to the previous ecosystem status (Orr and Walsh, 2006; Wilby *et al.*, 2006), and strategies to manage freshwater ecosystems under climate change are utmost needed (Wilby *et al.*, 2010).

Habitat evaluation for fish and macro benthos is one key factor for stream ecology assessment (Lamouroux *et al.*, 1998; 1999; Hering *et al.*, 2003; Lytle and Poff, 2004; Canobbio *et al.*, 2010; 2013), and depiction under climate change (Mohseni *et al.*, 2003), as it describes the potential for colonization of riverine species under specific hydrologic conditions.

Habitat preferences with respect to hydraulic variables can be predicted based on empirically tuned equations from field

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experiments (*e.g.* Azzellino and Vismara, 2001; Lamouroux and Capra, 2002; Lamouroux and Cattanéo, 2006; Lancaster and Downes, 2010; Koljonen *et al.*, 2012a; Fornaroli *et al.*, 2015). A straightforward method to evaluate stream habitat is the estimation of weighted usable area WUA (*e.g.* Vismara *et al.*, 2001), *e.g.* by coupling hydraulic models with biological models of habitat use (Lamouroux *et al.*, 1998).

Here, the prospective effect of climate change upon habitat suitability of macroinvertebrates (four families, Leuctridae*,* Heptageniidae*,* Limnephilidae*,* Limoniidae), and salmonids (brown trout, *Salmo trutta fario*, adult, young, spawning) in the largely snow fed Alpine river Serio, in northern Italy was studied.

The study was carried out by merging i) hydraulic river modeling, ii) habitat suitability functions, iii) hydrological modeling of catchment response, and iv) climate scenarios as from downscaled GCM outputs, tightly linked but seldom exploited under a joint framework (Johnson *et al.*, 2009; Tisseuil *et al.*, 2012), to assess hydrologically driven habitat availability under future climate change, in the mountain, relatively pristine part of Serio river.

The proposed study provides a preliminary, *what if* analysis of potential changes of eco-hydrological status valid for the Serio river, somewhat representative for European Alpine rivers, under prospective climate change.

5.2 Case study: the Serio River

The Serio river drains a catchment of 1256 km^2 in Lombardy region of Italy, flowing for 124 km before joining the Adda river, a left-side tributary of the Po river. Serio river springs at 2500 m a.s.l., and it is captured by the Barbellino lake at 2129 m a.s.l., then flowing in N-S direction (Fig. 5.1a). Serio River is largely used for hydropower generation at the highest altitude, and for irrigation downstream. Channel substrate is mainly made of cobbles and boulders approximately until Valbondione, while in the mountain and piedmont floodplain (downstream Parre, Fig. 5.1b) the river is wider and has milder slope, with cobbles, gravels, and occasionally boulders and bedrocks (Canobbio *et al.*, 2010). The investigation of prospective hydrology in the Serio river is important given the large demand of water for multipurpose uses, and that the hydrological fluxes control water quality and may affect the suitability for river biota (Canobbio *et al.*, 2010). We studied the river until Parre (Fig. 5.1b), hosting valuable fishing resources, and given that downstream large water withdrawals occur**.** Köppen-Geiger climate classification places the Serio river area in the temperate/cool continental class, with considerable seasonal snow cover about 1000 m a.s.l. or so, maximum precipitation during the end of summer and autumn, and minimum precipitation in winter (ca. 1300 mm yearly). Monthly temperature ranges from +23.8° in July to -1.5° in January, and yearly average is +9.4°. Average discharge at Grabiasca is estimated into 2.7 m^{3}s^{-1}, and flow regime has two peaks, in May due to snow melt, and in November due to large precipitation. Driest periods are in August and February. Recent studies (Confortola *et al.*, 2013) display that prospective climate changing within the Italian Alps may

potentially affect Serio river stream flow regimes, so investigation of potential habitat modification is warranted.
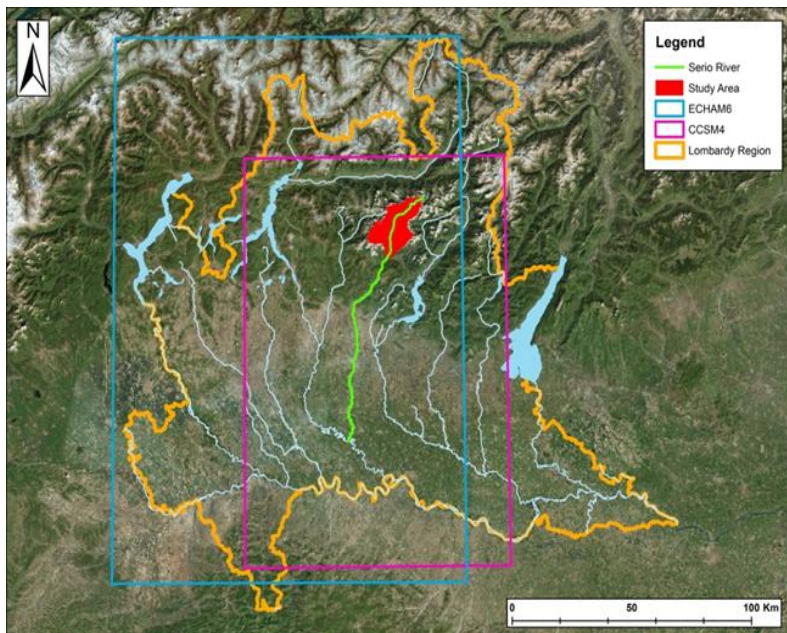
Fig. 5.1 - Case study area. a) Serio River and GCMs grids (base Google Earth^{TM}).
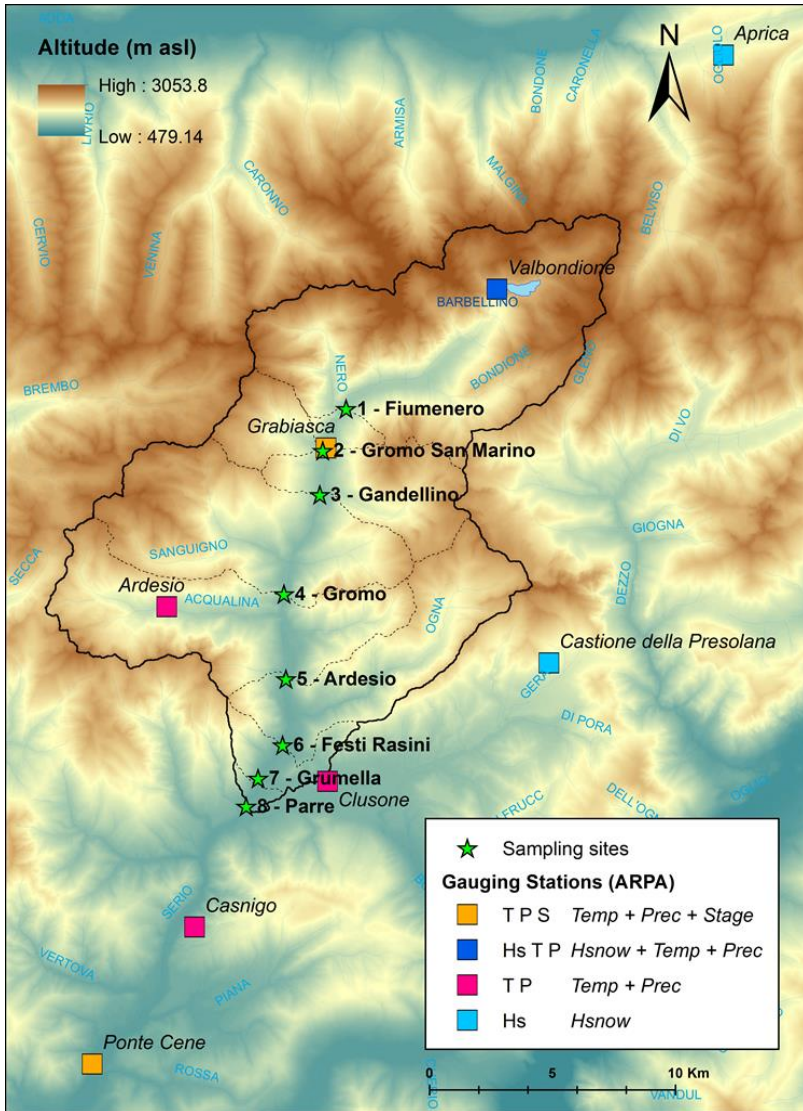
Fig. 5.1 - Case study area. b) Field data collection locations and available measuring stations.

5.3 Data and methods

5.3.1 Data base

A historical database of weather variables (hourly temperature and precipitation) was used, provided by the Regional Environmental Protection Authority (ARPA) of Lombardy Region for 2004-2012 (see Fig. 5.1). Data from different stations in the Serio catchment were available, 4 measuring temperature and precipitation, 3 measuring snow depth, and 1 measuring water level (Grabiasca). Daily discharges during 2008-2012 were estimated from water level gauged at Grabiasca station, using a stage-discharge relation provided by ARPA, that we updated during our local field surveys. ENEL hydropower company of Italy provided information concerning water abstraction nearby Grabiasca, used for back-estimation of natural in stream flows. So doing, it was possible to tune the hydrological model against supposedly natural flows. Further data used are the DTM (20 m cell size) of the Serio basin, and land use maps from CORINE Land Cover, for the estimation of soil maximum water content potential according to the SCS-CN method. In Fig. 5.1a they are reported the measured variables in the available stations.

During 2010-2012 the authors carried out spring to autumn field surveys in the Serio river. Briefly, these surveys included: i) several hydro-morphological surveys in 8 different sampling sites (Fig. 5.1b, 3 stations each), during which 24 stations were identified, classified as hydro-morphologically representative of the river habitat, ii) topographic surveys with a GPS device throughout the Alpine part of the river, to collect as many river sections as possible, iii) chemical-physical data survey, to evaluate actual water quality, iv) surveys of the biological communities (both macro and micro invertebrates) in three different sites of the river, morphologically different from each

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other, and v) survey of fish habitat use (via specimens catch using electro fishing). The sampling procedure was based on a multi-habitat scheme designed to sample invertebrate and fish assemblages in all the available meso-habitats, *i.e.* steps, riffles, runs, pools, and backwaters (Fornaroli *et al.*, 2015). For each sample water depth, and velocity were sampled, the latter at 40% of the depth using a Marsh-McBirney Flo-Mate Model 2000 portable flow meter. The substrates were classified as dominant, sub-dominant and matrix. Their relative abundance was expressed according to the mineral substrate classes provided by the site description protocol in the AQEM Manual (AQEM Consortium, 2002). Using these three descriptors the mean substrate diameter (used as Channel Index) was derived, by weighting and averaging the mean particle size of each class (Folk, 1974; Bunte and Abt, 2001).

5.3.2 Hydrological model

A hydrological model was used suitable to represent hydrological cycle of mountainous, snow fed catchments (Groppelli *et al.*, 2011b; Bocchiola *et al.*, 2011). The model is semi-distributed, based on altitude belts, and it is able to mimic snow cover and ice dynamics, evaporation losses, recharge of the groundwater reservoirs and eventually formation of channel discharge. The hydrological model worked at a daily scale, and was tuned according to the same methodology as reported in Confortola *et al.* (2013). However, due to a different control period (2008-2012), use of a new stage-discharge equation at Grabiasca validated during our field activity, and to inclusion of withdrawal upstream as reported, some parameters were slightly modified, and hydrological modeling was improved with respect to that study. Given that back-estimated natural discharges were used it is expected that the hydrological regime as simulated by the model better mirrors the natural

outflow of the river, and except for some peaks dampened by the Barbellino dam, the simulated flow fits better the observed one. Details of model calibration are not reported here for shortness, and focus is cast upon ecological status of Serio river.

5.3.3 Habitat suitability and Weighted Usable Area (WUA)

Habitat availability was assessed coupling the hydrological model with habitat suitability curves specifically developed for macroinvertebrates in a recent study (Fornaroli *et al*., 2015). In short, therein macroinvertebrates samples were collected in the sampling sites described above, and all the sampled organisms were identified at the lowest possible level, *i.e.* genus or family. Different species within a family may behave differently (*e.g.* Lancaster and Downes, 2010). However, assessment of habitat for single species may be unfeasible, so family level is mostly considered when building suitability curves (Armanini *et al.*, 2010), and for assessment of stream quality (Chessman, 2006). Therefore, preference curves were developed here at the family level. Out of 47 macroinvertebrates families, four most frequent (*i.e.* found in 80% of the surveyed samples) were isolated, and habitat suitability therein studied. To describe the potential preferences of the chosen families, quantitative relationships were obtained through quantile regression, highlighting the most limiting factor for colonization, among a set of chosen physical variables (here water depth D, water velocity V, and substrate channel index S). For our macroinvertebrates families, water velocity (in particular for Leuctridae and Heptagenidae families), substrate size (for Limnephilidae), and a combination of the two (Limoniidae) are the best descriptors of specimens' densities, while depth is a less limiting factor (Tab. 5.1). The approach by Fornaroli *et al.* (2015), was then extended to

brown trout, covering ca. 90% of surveyed fish biota, to obtain habitat suitability functions, $HS = f(D, V, S)$. Spawning habitats for brown trout were evaluated using the approach in Shirvell and Dungey (1983). The results (Tab. 5.1) indicated that adult fishes display preference for low velocity, deep water, and large channel index, whereas young trout prefer intermediate flow (especially velocity) conditions, and substrate. Spawning habitats (used during November-February) were characterized by intermediate flow (velocity, depth) conditions, and fine substrate. Here, an univariate version of the suitability curves developed by Fornaroli *et al.* (2015) was adopted (*i.e.* using each single variable independently, see *e.g.* Vismara *et al.*, 2001), to set up the PHABSIM model. A preliminary analysis indicated no noticeable changes in calculation of WUA using multivariate curves. Based upon the suitability curves, we used PHABSIM to describe the changes of Weighted Usable Area (WUA) as a function of stream flows for each of our target species and stage. The Physical Habitat Simulation System PHABSIM integrates a hydraulic model with a biological model of habitat selection based upon habitat suitability curves, to calculate the variation of WUA as a function of discharge (Ayllón *et al.*, 2012). WUA of each stream section i in each day j is estimated as a function of in stream discharge in that day Q_j (influencing flow depth D_j, and velocity V_j) by summing the partial WUA of each subsection k (*i.e.* using a 1 ca. meter step) of the n_k ones in the section, as (Eq. 1):

$$WUA_{i,j}(Q_{i,j}) = \sum_{k=1}^{n_k} WUA_{k,j}(Q_{i,j}) = \sum_{k=1}^{n_k} A_{k,j}(Q_{i,j}) \times min\left(f(V_{k,j}), f(D_{k,j}), f(S_k)\right)$$

To assess WUA along the entire river, a morphological classification was carried out, by way of a comparative analysis of field data collected during our surveys, and of aerial

pictures used to evaluate stream morphology. The upper Serio river was split into 192 morphologically distinguishable and homogeneous transects. Each transect was then classified as belonging to one of the 24 hydro-morphologic types reported above. For each of the 192 transects, a WUA-discharge relationship was built depending upon morphology, the considered species, and stage. Then, for each section and each species (stage) it was calculated the WUA corresponding to the flow rate at that section in each particular day j using Eq.(1). Eventually, WUA was calculated for each species and stage for the whole river (192 transects) for every day during the control run period CR (2008-2012). Based upon these series, duration curves of WUA (*i.e.* value of WUA against number of days of exceedance per year) were calculated for each species (and stage for trout) within each transect of the stream.

Preliminary analysis of the WUA duration curves during CR period within the 24 hydro-morphologically relevant chosen sections revealed that nearby the 20% percentile at the yearly scale (*i.e.* for values exceeded for 292 days, or not exceeded for 73 days), the curves often exhibited a kink (not shown for shortness), indicating a rapid decrease of WUA after that duration.

Tab. 5.1 - Habitat suitability preferences for flow velocity, depth, and for substrate, channel index. See Viganò (2013); Fornaroli *et al.* (2015).

Species/Life stage	Velocity	Depth	Substrate dimension
Trout/Spawning	Medium	Medium	Low
Trout/Young	Medium	Medium-Stable	Medium
Trout/Adult	Low	High	High
Leuctridae	High	-	-
Heptageniidae	High	-	Low
Limnephilidae	Stable	Stable	High
Limoniidae	Medium-high	-	Low

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This seemed to indicate a particularly critical situation, *i.e.* with large decrease of WUA, potentially indicating a situation of critically low habitat suitability. Therefore, it was used the particular value of WUA so evaluated, named henceforth $WUA_{20\%}$, specifically assessed for each species, stage, and hydro-morphologic situation, as a reference to compare changes in projected scenarios, and specifically to investigate improvement or worsening, in term of occurrence of critical situation in the future.

5.3.4 Climate projection and downscaling

In the Fifth Assessment Report (AR5) by the Intergovernmental Panel on Climate Change IPCC a new set of scenarios describing four different Representative Concentration Pathways (RCPs, 2.6, 4.5, 6.5, 8.5) was presented (IPCC, 2013). Here, temperature and precipitation data were used that were projected according to RCP2.6 (optimistic, peak in radiative forcing at 3 Wm^{-2} or 490 ppm CO_2 equivalent at 2040, and then decline to 2.6 Wm^{-2}), RCP4.5 (cautious, stabilization without overshoot pathway to 4.5 Wm^{-2}, or 650 ppm CO_2 eq., at 2070), and RCP8.5 (pessimistic, with rising radiative forcing up to 8.5 Wm^{-2}, or 1,370 ppm CO_2 eq. by 2100). Two decades were considered, 2040-2049 (hereon, 2045) and 2080-2089 (hereon, 2085) and two GCM models were used, namely ECHAM6 (Max Planck Institute for Meteorology in Hamburg, Germany) and CCSM4 (National Center for Atmospheric Research in Boulder, Colorado). Earlier versions of these models represented reasonably well the climate of Northern Italy, and especially the seasonality of rainfall, so they were used here (Groppelli *et al.*, 2011a, Soncini *et al.*, 2011; Confortola *et al.*, 2013). In Tab. 5.2 the main features of the chosen models are reported (corresponding grids in Fig. 5.1a). Precipitation was

downscaled at the spatial resolution of the Serio river, using a stochastic space random cascade, SSRC (see Groppelli *et al.*, 2011b), tuned using nine years of daily precipitation and temperature data (2004-2012) upon the Serio catchment. Temperature was shifted using a monthly *DT* approach (see Groppelli *et al.*, 2011a).

Tab. 5.2 - Main features of the adopted GCM models.

Model	Research Center	Nation	Grid size [°]	n° cells [.]	n° layers [.]
ECHAM6	Max Planck Institute of Meteorology	Germany	1.875°x1.875°	192x96	47
CCSM4	National Center for Atmospheric Research	Boulder, Colorado	1.25°x1.25°	288x144	26

5.3.5 Hydrological and habitat scenarios

Future hydrological, and habitat scenarios were estimated for two reference decades (2040-2049, and 2080-2089), by feeding the hydrological model with daily temperature and precipitation obtained from the GCM outputs, and subsequently calculating the daily WUA at stream scale (*i.e.* in the 192 homogeneous transects) from the corresponding discharges. Natural discharges (*i.e.* without including water withdrawals) were used to investigate the sole effect of climate upon flow regimes, and habitat suitability. From the so projected WUA time series, in each transect it was evaluated the frequency of occurrence (*i.e.* number of days) of the $WUA_{20\%}$ reference value. This indicates whether the number of critical days (*i.e.* those days with $WUA < WUA_{20\%}$) either increases or decreases in the future. The observed frequencies of $WUA_{20\%}$ were then averaged upon the whole catchment by weighting upon transects' length to obtain a stream wide assessment.

5.4 Results

5.4.1 Projected climate conditions

Temperature changes (after downscaling, Tab. 5.3) seem concordant for both GCMs, and in agreement with the general trend of the RCPs. In both decades yearly temperature increase is consistent between models, with monthly differences. Precipitation changes (Tab 5.4) are less consistent between models, as generally found in literature (Battin *et al.*, 2007; Groppelli *et al.*, 2011a, b; Confortola *et al.*, 2013). ECHAM6 projects a noticeable reduction in precipitation, almost halved according to RCP4.5 until 2049, with even larger decrease monthly. CCSM4 projects much smaller variations, with slight increase under RCP2.6 and RCP8.5 at 2049, and very small decrease under RCP4.5, but again with monthly differences.

Tab. 5.3 - Temperature changes with respect to CR period after downscaling (in *italic* values < 0, in **bold** values >1 and in ***italic+bold*** values >2).

Temperature [°C]							
Month		1	2	3	4	5	6
2004 - 2012	CR	0.26	1.10	5.27	9.18	13.03	16.83
Changes [°C]							
Decade	RCPs			ECHAM6			
2040 - 2049	**2.6**	0.8	*-0.6*	0.6	0.6	0.8	**1.3**
	4.5	0.6	*-0.2*	0.8	0.1	*-0.6*	**1.4**
	8.5	0.6	*-0.3*	0.2	0.2	0.0	**1.5**
2080 - 2089	**2.6**	0.8	*-0.2*	0.0	1.0	0.3	**1.8**
	4.5	**1.3**	0.4	0.9	1.0	**1.1**	***2.5***
	8.5	***2.3***	**1.2**	***2.4***	***2.4***	***2.6***	***4.5***
Decade	RCPs			CCSM4			
2040 - 2049	**2.6**	0.6	*-0.1*	*-0.5*	**1.4**	*-1.1*	*-0.1*
	4.5	0.5	*-1.2*	*-0.1*	0.1	*-1.4*	*-0.2*
	8.5	0.8	*-0.6*	0.2	0.9	*-0.2*	0.4
2080 - 2089	**2.6**	0.3	*-0.1*	*-1.2*	0.3	*-0.2*	*-0.6*
	4.5	0.8	0.5	*0.0*	*-0.1*	0.1	0.2
	8.5	**1.5**	**1.8**	**1.8**	***2.7***	***2.6***	***2.9***

Tab. 5.3 – (*Continued*)

Temperature [°C]								
Month		**7**	**8**	**9**	**10**	**11**	**12**	**Mean**
2004 - 2012	**CR**	18.23	17.63	14.50	10.46	5.13	0.58	9.35
Changes [°C]								
Decade	**RCPs**				**ECHAM6**			
2040 - 2049	**2.6**	**1.7**	0.4	0.7	0.6	0.6	**1.0**	0.7
	4.5	*2.2*	*1.1*	*1.1*	*1.5*	0.7	0.1	0.7
	8.5	**1.9**	**1.6**	**1.6**	0.9	**1.5**	**1.3**	0.9
2080 - 2089	**2.6**	**1.6**	0.4	0.7	0.9	**1.2**	**1.0**	0.8
	4.5	*3.1*	*2.2*	*2.0*	*1.1*	*1.4*	*2.5*	*1.6*
	8.5	*5.5*	*5.4*	*4.1*	*2.7*	*3.3*	*3.6*	*3.3*
Decade	**RCPs**				**CCSM4**			
2040 - 2049	**2.6**	**1.9**	**1.5**	**1.8**	**1.8**	0.8	*-0.1*	0.7
	4.5	**1.2**	0.7	**1.9**	**1.4**	**1.8**	0.5	0.4
	8.5	**1.3**	*2.3*	*2.7*	*2.1*	1.0	**1.4**	1.0
2080 - 2089	**2.6**	**1.9**	**1.1**	0.9	**1.7**	**1.1**	*-0.1*	0.4
	4.5	**1.9**	*2.1*	**1.9**	*2.1*	**1.9**	0.6	1.0
	8.5	*5.6*	*5.4*	*5.2*	*4.7*	*2.7*	*2.6*	*3.3*

Tab. 5.4 - Precipitation changes with respect to CR period after downscaling (in *italic* values >25%, in **bold** values < -25% and in *italic+bold* values < -50%).

Precipitation [mm]							
Month		**1**	**2**	**3**	**4**	**5**	**6**
2004-2012	**CR**	49	73	71	140	144	136
Changes [%]							
Decade	**RCPs**			**ECHAM6**			
2040-2049	**2.6**	-17%	0%	-9%	**-34%**	***-66%***	***-43%***
	4.5	**-44%**	**-47%**	***-60%***	-24%	***-52%***	***-59%***
	8.5	-16%	*44%*	0%	**-31%**	***-57%***	***-49%***
2080-2089	**2.6**	**-34%**	-6%	**-27%**	-18%	***-51%***	***-36%***
	4.5	***-69%***	**-33%**	**-40%**	**-37%**	***-66%***	***-54%***
	8.5	**-45%**	*10%*	**-25%**	**-36%**	***-80%***	***-45%***
Decade	**RCPs**			**CCSM4**			
2040-2049	**2.6**	*73%*	9%	-2%	**-36%**	-3%	0%
	4.5	21%	24%	23%	-22%	**-27%**	1%
	8.5	*39%*	19%	16%	-12%	-1%	-18%
2080-2089	**2.6**	*85%*	17%	24%	**-30%**	-2%	-7%
	4.5	8%	25%	*61%*	6%	**-41%**	0%
	8.5	*73%*	9%	*35%*	-5%	**-32%**	-23%

Tab. 5.4 – (*Continued*)

Precipitation [mm]								
Month		**7**	**8**	**9**	**10**	**11**	**12**	**Cum**
2004-2012	**CR**	143	154	174	110	128	130	1450
Changes [%]								
Decade	**RCPs**	ECHAM6						**Mean**
	2.6	**-31%**	24%	**-44%**	*37%*	-16%	**-45%**	-20%
2040-2049	**4.5**	**-36%**	**-38%**	**-61%**	**-72%**	**-46%**	**-57%**	**-50%**
	8.5	**-48%**	-17%	**-38%**	*-2%*	*54%*	**-27%**	-16%
	2.6	**-32%**	*44%*	**-39%**	*30%*	*31%*	15%	-10%
2080-2089	**4.5**	**-53%**	*-7%*	**-64%**	**-63%**	-14%	**-55%**	**-46%**
	8.5	**-48%**	13%	**-44%**	**-46%**	10%	**-34%**	**-31%**
Decade	**RCPs**	CCSM4						**Mean**
	2.6	*36%*	17%	**-51%**	16%	21%	9%	7%
2040-2049	**4.5**	**-32%**	7%	**-31%**	23%	5%	**-33%**	-3%
	8.5	-6%	-5%	**-46%**	*43%*	-3%	-12%	1%
	2.6	8%	19%	-17%	26%	-1%	**-40%**	7%
2080-2089	**4.5**	-12%	-16%	-25%	-5%	-19%	11%	-1%
	8.5	-19%	20%	-20%	*76%*	4%	**-31%**	7%

5.4.2 *Projected hydrological cycle*

Modified monthly stream flows of Serio river at Grabiasca for the two reference decades against CR period are reported in Fig. 5.2, and in Tab. 5.5 they are reported second order statistics of daily flow, namely mean flow E[Q] and coefficient of variation CV[Q], *i.e.* the ratio of standard deviation to mean flow. Mean flow, and CV provide a quick indication of flow magnitude and variability, which directly affects ecosystems quality (Smakhtin, 2001; Clausen and Biggs, 2002). Mean discharges are largely decreased under both models' scenarios, and all RCPs. ECHAM6 model depicts large variation of monthly flows, especially in summer for 2049 and 2089, with a net decrease of mean yearly flow rate E[Q]. Particularly striking is the projected discharge under RCP4.5, mirroring the large decrease of precipitation therein. Daily flow variability (CV[Q]) is increased under RCP2.6, while it slightly decreases under RCP4.5, and RCP8.5.

Tab. 5.5 - Serio river at Grabiasca. Projected daily stream flow statistics (mean, coefficient of variation) against those for reference period (CR).

| CR (2008-2012)	Year	Winter	Spring	Summer	Autumn
E[Q]	4.93	1.37	7.13	5.78	5.21
CV[Q]	1.09	0.72	0.61	1.14	1.05
2040-2049	**Year**	**Winter**	**Spring**	**Summer**	**Autumn**
ECHAM6			RCP2.6		
E[Q]	3.03	1.31	3.40	3.86	3.51
CV[Q]	1.16	0.94	0.68	1.03	1.34
ECHAM6			RCP4.5		
E[Q]	1.99	0.86	2.84	2.69	1.58
CV[Q]	0.98	0.82	0.73	0.55	1.51
ECHAM6			RCP8.5		
E[Q]	3.13	1.50	3.79	3.12	4.11
CV[Q]	1.08	0.83	0.59	0.71	1.33
2080-2089	**Year**	**Winter**	**Spring**	**Summer**	**Autumn**
ECHAM6			RCP2.6		
E[Q]	3.52	1.46	3.76	4.20	4.60
CV[Q]	1.18	0.73	0.84	1.06	1.23
ECHAM6			RCP4.5		
E[Q]	2.16	0.88	2.62	2.83	2.28
CV[Q]	1.09	0.84	0.49	0.73	1.61
ECHAM6			RCP8.5		
E[Q]	2.66	1.75	2.38	3.35	3.15
CV[Q]	0.97	0.64	0.82	1.03	0.89
2040-2049	**Year**	**Winter**	**Spring**	**Summer**	**Autumn**
CCSM4			RCP2.6		
E[Q]	3.99	2.15	5.20	4.64	3.93
CV[Q]	1.15	0.76	0.82	1.19	1.34
CCSM4			RCP4.5		
E[Q]	3.61	1.96	4.66	4.19	3.64
CV[Q]	1.08	0.84	0.70	1.20	1.17
CCSM4			RCP8.5		
E[Q]	3.71	2.05	5.04	3.70	4.03
CV[Q]	1.02	0.78	0.68	0.65	1.42
2080-2089	**Year**	**Winter**	**Spring**	**Summer**	**Autumn**
CCSM4			RCP2.6		
E[Q]	3.91	1.58	5.31	4.95	3.79
CV[Q]	1.37	0.78	0.99	1.32	1.56
CCSM4			RCP4.5		
E[Q]	3.63	1.93	4.82	3.97	3.79
CV[Q]	1.34	0.88	1.04	0.87	1.86
CCSM4			RCP8.5		
E[Q]	3.91	2.90	9.50	10.49	8.74
CV[Q]	1.50	1.08	0.82	1.31	1.26
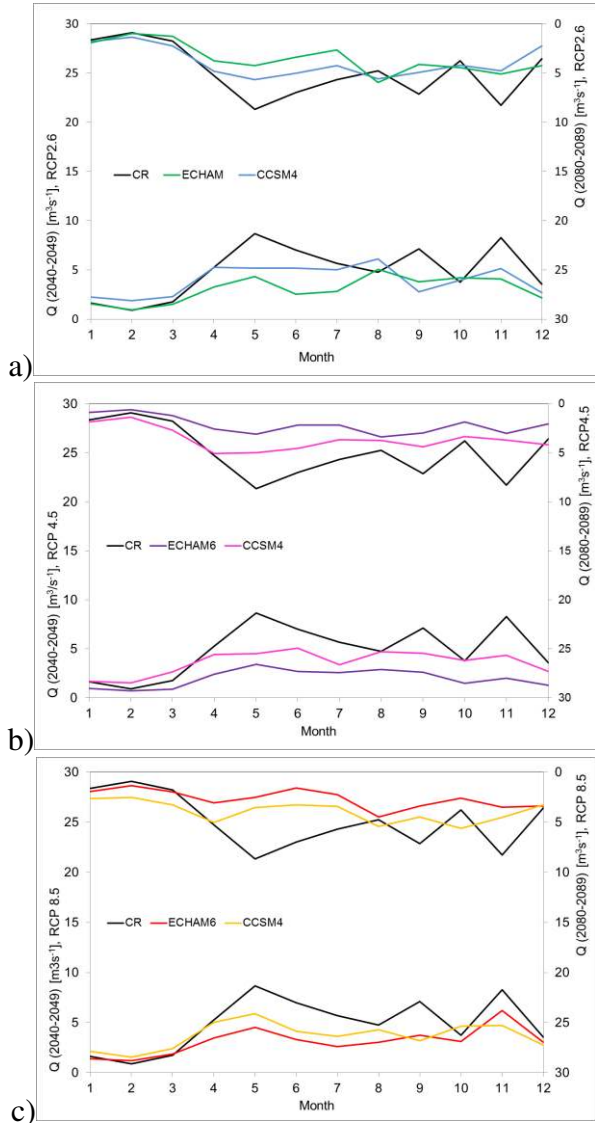
Fig. 5.2 - Serio river at Grabiasca station**.** Mean monthly discharge for ECHAM6 and CCSM4, decade 2040-2049, and 2080-2089. a) RCP2.6. b) RCP4.5. c) RCP8.5.

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CCSM4 displays similarly decreasing flows, with slightly higher values, and more consistently between the 3 RCPs. Both models, and all RCPs project a noticeable modification of the hydrological regime, passing from a largely bimodal regime now (*i.e.* with two maxima, in spring and autumn), to a more uniform one, with dampened seasonal variability. Daily flow variability increases under all RCPs, especially at 2085, thus likely leading to more variable habitat conditions.

5.4.3 Present and projected habitat suitability

The reference conditions (CR, 2008-2012) of habitat suitability averaged upon the whole catchment (*i.e.* by weighting upon transects' length) are reported in Tab. 5.6. Namely, the number of critical days n_c, and percentage in the season $n_{c\%}$ is reported, *i.e.* when WUA< WUA$_{20\%}$. Seasonal statistics are reported (*i.e.* number of critical days *vs* days in the season).

In Fig. 5.3 it is reported the spatial distribution (192 transects) of the seasonal criticalities for the four species and stages in bold in Tab. 5.6, more representative according to our analysis, namely brown trout (young and adult), Leuctridae and Heptageniidae. Both young and adults trout are largely impacted by flow velocity (*i.e.* discharge), so we found considerable variation of habitat in time. Suitability for spawning was only assessed during winter as reported, so seasonal analysis was not carried out. Concerning macroinvertebrates, Leuctridae and Heptageniidae were also most influenced by velocity, so habitat conditions are more variable in time. Limnephilidae, and Limoniidae are more weakly impacted by velocity (and Limnephilidae more largely by substrate), so habitat variation in time was smaller. From Fig. 5.3a, young trout at present undergo more critical habitat conditions during spring (AMJ, $n_{c\%}$ is 25-50% of the time, orange transects), *i.e.* when large flows at thaw provide

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increasing flow velocities (Fig. 5.3a, black line). During summer (JAS) and autumn (OND), intermediate habitat conditions are found for most of the habitat (15-25% $n_{c\%}$), in response to moderate, higher than average flows. During winter, low flow conditions provide low velocity, and few critical days in most transects (mostly, 0-5%). Adult trout (Fig. 5.3b) display a large amount of critical days during winter ($n_{c\%}$ > 50% in more than half stream), due to low flows, and low water depths, most critical to them. Spring, and especially summer conversely provide optimal conditions (critical days 0-5% mostly), due to highest flows in this period. Lower velocities in summer deliver better conditions. Autumn presents intermediate conditions (mostly $n_{c\%}$ nearby 15-25%), as given by intermediate flows, especially in October, and December. Leuctridae (Fig. 5.3c) display a similar pattern as adult trout, with critical winter ($n_{c\%}$ >50% mostly), optimal spring and summer ($n_{c\%}$ = 0-5%), and intermediate autumn conditions ($n_{c\%}$ = 15-25% mostly). Leuctridae are basically influenced by flow velocity, and the higher the more suitable. Substantially equivalent results are seen for Heptageniidae (Fig. 5.3d), also largely influenced by flow velocity, and the faster the better. From Tab. 5.6, Limnephilidae and Limoniidae undergo substantially similar patterns, with several critical days in winter ($n_{c\%}$ = 52%, and 62% on average in the stream, respectively), good conditions in spring ($n_{c\%}$ = 11%, 7%), and summer ($n_{c\%}$ = 6%, 2%), and intermediate conditions in autumn ($n_{c\%}$ = 31%, 28%).

Projected values of habitat suitability ($n_{c\%}$, yearly and seasonally) are reported in Tab. 5.7 (ECHAM6), and Tab. 5.8 (CCSM4), to be compared against those in Tab. 5.6. The presence of significant differences between the projected values of $n_{c\%}$ against the reference values during CR decade was assessed using Kolmogorov-Smirnov goodness of fit test

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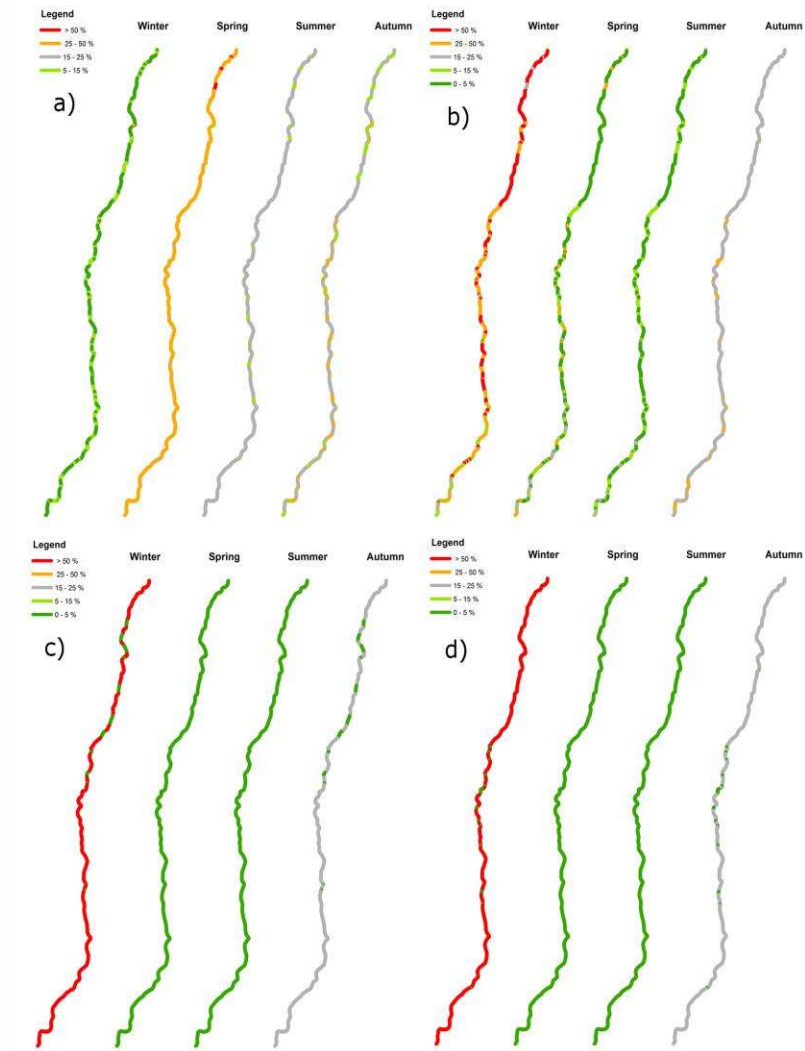($\alpha = 5\%$, Kottegoda and Rosso, 1997), applied to $WUA_{20\%}$ quantile.

Fig. 5.3 - Spatial map of the seasonal criticalities (percentage of critical days, $WUA < WUA_{20\%}$ in the season) along the Serio river, period 2008-2012 (CR). a) Young trout. b) Adult trout. c) Leuctridae. d) Heptageniidae.

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In Fig. 5.4 are displayed the mean values (*i.e.* for ECHAM6 and CCSM4, and three RCPs, mean of 6 values) of the projected seasonal average discharge in Grabiasca E[Q], and coefficient of variation CV[Q], and percentage of critical days for the control period CR, 2045, and 2085, averaged on the stream. Also the maximum and minimum (out of the 6 values) of the same variables are shown, to illustrate the range of variability of the projections.

For spawning during winter (Fig. 5.4a), critical days tend to decrease ($n_{c\%}$ from 16% in CR to 12% in 2085), also displaying low variability (according to error bars). This occurs in response to a slight increase of average discharge until the end of century, possibly providing better spawning conditions.

For young trout, analysis of the relationship between percentage of critical days $n_{c\%}$ (stream averaged, Tab. 5.6, 5.7) against stream (average) flow discharge E[Q] as calculated from the projected hydrological scenarios (Tab. 5.5) indicates increase of the former when the latter increases in all seasons, *i.e.* with seasonally high flow conditions critical for young trout. However, large spread is present, *i.e.* complex flow variability may change habitat conditions beyond such general trend. Slightly increasing discharges in winter provide increasing number of critical days $n_{c\%}$ until the end of century (Fig. 5.4a), albeit with large spread, as witnessed by large confidence bands. In spring, decreasing discharges may favor young trout instead (with decrease of $n_{c\%}$, Fig. 5.4b). In summer, similarly decreasing discharges seemingly make habitat conditions worse (with increase of $n_{c\%}$, Fig. 5.4c), especially at 2085. Notice that during summer flow variability quantified by CV[Q] is noticeably larger than during spring, both during CR, and the future decades. More largely variable flows may lead to more largely variable discharges, possibly providing more critical days. So, one may take that increased

flow variability, especially during summer may make worse habitat conditions even under average flow decrease. This issue seems even more evident in autumn (Fig. 5.4d). In that season, even with visible flow decrease, large values of CV until the end of the century may increase the number of critical days.

Concerning adult trout, critical days decrease when high flows are attained (Tab. 5.5, 5.6, 5.7), but with large spread, as a result of complex response in term of suitability (see Tab. 5.2). Adult trout adapt well to large flow depth, but not to large velocity. Higher discharges may provide larger depths, but may also lead to increased velocity. Depending upon the stream section shape, these two effects may balance, or prevail upon each other, and increasing flows may either favor, or bother adult trout. In winter as reported, flow discharge changes slightly on average, but large daily variation occurs according to different models, and scenarios (large error bars of E[Q] in Fig. 5.4a). Adults' habitat conditions tend to worsen slightly until 2045 ($n_{c\%}$ = 0.60, *vs* 0.55 under CR), and 2085 ($n_{c\%}$ = 0.63), but again large variations occur, especially at the end of the century, implying that adult trout's suitability is largely dependent upon the evolution of climate scenarios, cascading upon hydrological cycle. Similarly in spring (Fig. 5.4b), largely decreasing discharges (but again with large spread depending upon the scenario) would lead to slight decrease of habitat suitability (increasing $n_{c\%}$), especially at the end of century. In summer (Fig. 5.4c), a similar pattern is seen, with decreasing discharges, but constant habitat suitability on average (and some spread with scenarios). In autumn (Fig. 5.4d) the decrease of in stream flows would lead to a better condition, with large decrease of $n_{c\%}$. Likely, decrease of flood flows during autumn may lead to decreased flow velocity in the stream, improving habitat conditions.

a)

b)

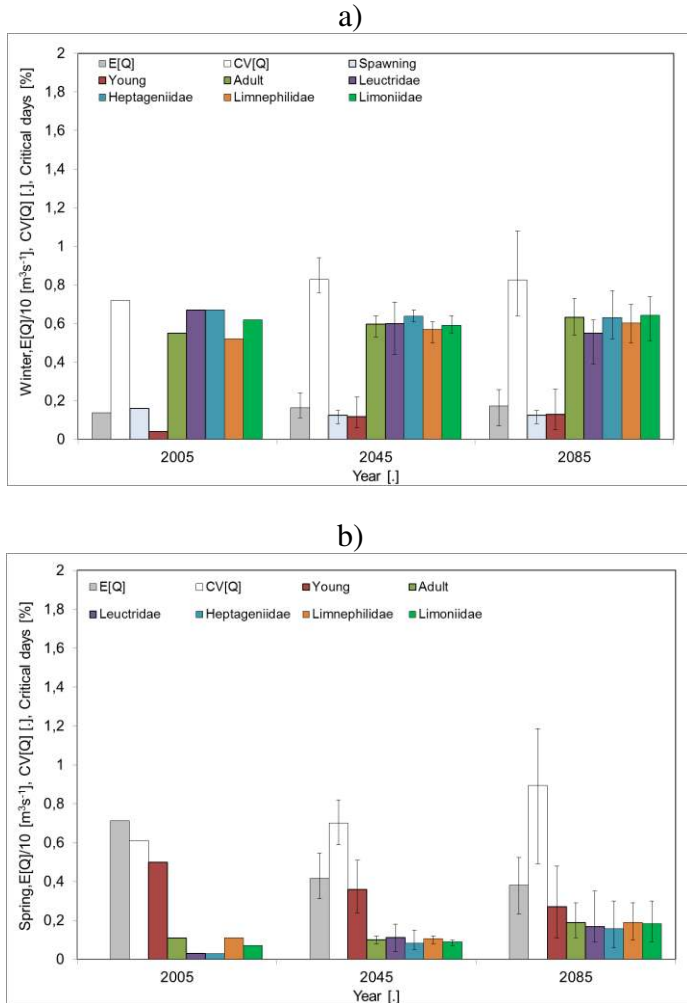Fig. 5.4 - Average of seasonal discharge E[Q] (E[Q]/10 for graphical reasons), coefficient of variation CV[Q], percentage of critical days $n_{c\%}$ for three decades (CR 2005, 2045,2085). Mean values (*i.e.* for ECHAM6 and CCSM4, and three RCPs) are taken for 2045 and 2085, and maximum and minimum values are reported as confidence bars. a) Winter. b) Spring. c) Summer. d) Autumn.

c)

d)
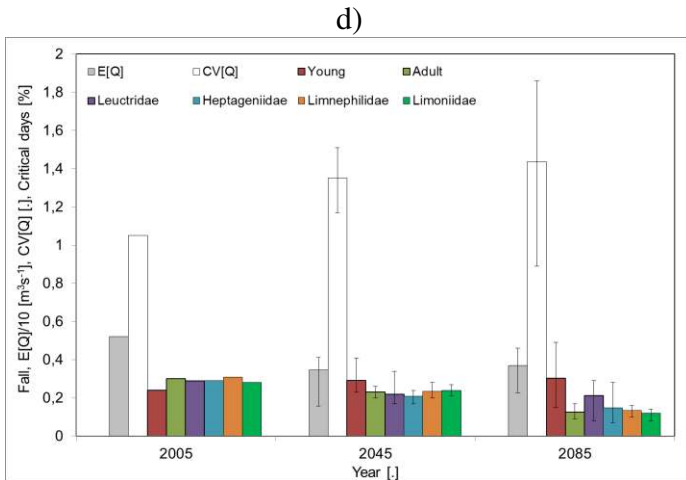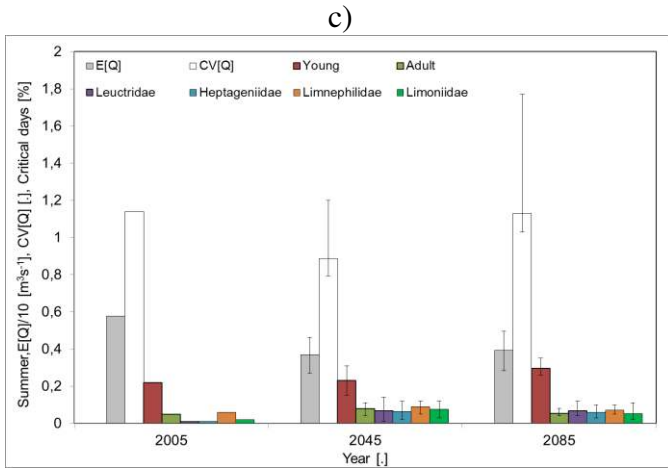
Fig. 5.4 – (*Continued*)

Tab. 5.6 - Number of critical days at yearly and seasonal scale, control run period CR 2008-2012. Species name in **bold** also reported in Fig. 5.3.

	Critical days (WUA<WUA$_{20\%}$)								
CR (2008-2012)	**Year**		**Winter**			**Spring**			
	Days	**%**	**Days**	**%**	**% Year**	**Days**	**%**	**% Year**	
Spawning	-	-	14	16%	4%	-	-	-	
Young	73	20%	3	4%	1%	36	50%	10%	
Adult	73	20%	39	55%	11%	8	11%	2%	
Leuctridae	73	20%	43	67%	12%	2	3%	0%	
Heptageniidae	73	20%	46	67%	13%	2	3%	0%	
Limnephilidae	73	20%	37	52%	10%	8	11%	2%	
Limoniidae	73	20%	44	62%	12%	5	7%	1%	

	Critical days (WUA<WUA$_{20\%}$)					
CR (2008-2012)	**Winter**			**Spring**		
	Days	**%**	**% Year**	**Days**	**%**	**% Year**
Spawning	14	16%	4%	-	-	-
Young	3	4%	1%	36	50%	10%
Adult	39	55%	11%	8	11%	2%
Leuctridae	43	67%	12%	2	3%	0%
Heptageniidae	46	67%	13%	2	3%	0%
Limnephilidae	37	52%	10%	8	11%	2%
Limoniidae	44	62%	12%	5	7%	1%

In winter (Fig. 5.4a), higher discharges on average would lead to slightly improved habitat conditions until 2045, and 2085 for Leuctridae and Heptageniidae, but to slightly worse conditions for Limoniidae (improving at half century, and worsening later), and Limnephilidae. The two first families are indeed more positively affected by changes in flow velocity (Tab. 5.1), which may explain this difference. In spring (Fig. 5.4b), all families would be negatively impacted by decrease of stream flow, given that $n_{c\%}$ always increases, and similarly so in summer (Fig. 5.4c). In autumn (Fig. 5.4d) less critical days

are seen and decreasing seasonal discharges always leaded to decreasing $n_{c\%}$.

Large flow variability during autumn (large CV[Q]) may improve habitat conditions therein.

The four macroinvertebrate families display similar results to each other. Critical days generally decrease when flow increases.

Tab. 5.7 - Percentage of critical days at yearly and seasonal scale projected with ECHAM6 (in *italic* values with changes against CR < -10%. In **bold** values with changes against CR > 10%).

Critical days (WUA<WUA$_{20\%}$)									
ECHAM6	**Year**			**Winter**			**Spring**		
RCP	2.6	4.5	8.5	2.6	4.5	8.5	2.6	4.5	8.5
2040-2049									
Spawning	-	-	-	8%	15%	14%	-	-	-
Young	*6%*	*2%*	*7%*	6%	*22%*	*11%*	*26%*	*40%*	*24%*
Adult	*16%*	19%	18%	54%	*64%*	*62%*	12%	10%	8%
Leuctridae	22%	**38%**	21%	64%	**44%**	**54%**	**15%**	**18%**	**14%**
Heptageniidae	23%	21%	23%	64%	67%	61%	**15%**	7%	5%
Limnephilidae	*16%*	18%	18%	50%	*61%*	*60%*	12%	12%	8%
Limoniidae	18%	20%	22%	55%	63%	59%	10%	10%	7%
2080-2089									
Spawning	-	-	-	*8%*	15%	10%	-	-	-
Young	*7%*	*2%*	*4%*	5%	**26%**	*11%*	*11%*	*14%*	*19%*
Adult	*13%*	17%	18%	63%	**73%**	54%	17%	11%	**29%**
Leuctridae	19%	**38%**	*29%*	62%	*51%*	*39%*	**13%**	**16%**	**35%**
Heptageniidae	20%	19%	*24%*	62%	*77%*	*52%*	**13%**	6%	**30%**
Limnephilidae	*12%*	17%	18%	*61%*	**70%**	50%	*18%*	10%	**29%**
Limoniidae	*14%*	19%	23%	68%	**74%**	*51%*	*16%*	9%	**30%**

Tab. 5.7 – (*Continued*)

Critical days (WUA<WUA$_{20\%}$)						
ECHAM6	**Summer**			**Autumn**		
RCP	2.6	4.5	8.5	2.6	4.5	8.5
2040-2049						
Spawning	-	-	-	-	-	-
Young	_31%_	15%	24%	**36%**	23%	**41%**
Adult	8%	4%	11%	26%	_23%_	20%
Leuctridae	3%	4%	**14%**	_18%_	34%	_18%_
Heptageniidae	3%	2%	**12%**	_18%_	24%	21%
Limnephilidae	10%	5%	12%	28%	_22%_	_20%_
Limoniidae	8%	3%	**12%**	27%	24%	21%
2080-2089						
Spawning	-	-	-	-	-	-
Young	**34%**	_26%_	**35%**	**49%**	34%	**35%**
Adult	4%	4%	8%	_16%_	_12%_	_9%_
Leuctridae	6%	7%	7%	_19%_	26%	20%
Heptageniidae	6%	3%	10%	_19%_	_14%_	_7%_
Limnephilidae	**29%**	5%	6%	10%	_16%_	_14%_
Limoniidae	**30%**	2%	3%	11%	_14%_	_14%_

Tab. 5.8 - Percentage of critical days at yearly and seasonal scale projected with CCSM4 (in *italic* values with changes against CR < -10%. In **bold** values with changes against CR > 10%).

Critical days (WUA<WUA20%)									
CCSM4	**Year**			**Winter**			**Spring**		
RCP	2.6	4.5	8.5	2.6	4.5	8.5	2.6	4.5	8.5
2040-2049									
Spawning	-	-	-	8%	15%	14%	-	-	-
Young	*9%*	*9%*	*9%*	<u>11%</u>	10%	10%	*<u>37%</u>*	*<u>38%</u>*	51%
Adult	<u>16%</u>	19%	18%	53%	<u>63%</u>	<u>62%</u>	12%	10%	8%
Leuctridae	<u>14%</u>	<u>12%</u>	<u>14%</u>	63%	71%	64%	<u>12%</u>	4%	4%
Heptageniidae	<u>15%</u>	21%	23%	63%	67%	61%	<u>11%</u>	7%	5%
Limnephilidae	<u>16%</u>	18%	18%	50%	<u>61%</u>	<u>60%</u>	12%	12%	8%
Limoniidae	18%	20%	22%	<u>55%</u>	64%	59%	10%	10%	7%
2080-2089									
Spawning	-	-	-	<u>8%</u>	15%	10%	-	-	-
Young	*10%*	*9%*	*8%*	7%	<u>12%</u>	**<u>17%</u>**	48%	45%	*<u>25%</u>*
Adult	<u>13%</u>	17%	18%	<u>62%</u>	**<u>73%</u>**	54%	17%	11%	**<u>29%</u>**
Leuctridae	<u>16%</u>	<u>14%</u>	<u>12%</u>	<u>58%</u>	<u>58%</u>	62%	9%	10%	**<u>18%</u>**
Heptageniidae	17%	19%	<u>24%</u>	<u>59%</u>	<u>77%</u>	*<u>52%</u>*	9%	6%	**<u>30%</u>**
Limnephilidae	<u>12%</u>	17%	18%	<u>61%</u>	**<u>70%</u>**	50%	18%	10%	**<u>29%</u>**
Limoniidae	<u>14%</u>	19%	23%	68%	**<u>74%</u>**	*<u>51%</u>*	<u>16%</u>	9%	**<u>30%</u>**

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Tab. 5.8 – (*Continued*)

Critical days (WUA<WUA20%)					
CCSM4	**Summer**			**Autumn**	
RCP	2.6	4.5	8.5	2.6	4.5	8.5
2040-2049						
Spawning	-	-	-	-	-	-
Young	27%	26%	_15%_	25%	26%	24%
Adult	9%	4%	11%	26%	_23%_	_20%_
Leuctridae	_8%_	_1%_	_11%_	_17%_	24%	_21%_
Heptageniidae	_8%_	_2%_	**_12%_**	_17%_	24%	_21%_
Limnephilidae	10%	5%	12%	28%	_22%_	_20%_
Limoniidae	8%	3%	_12%_	27%	24%	_21%_
2080-2089						
Spawning	-	-	-	-	-	-
Young	_29%_	_26%_	27%	_15%_	18%	**_31%_**
Adult	4%	4%	_8%_	_17%_	_12%_	_9%_
Leuctridae	4%	5%	**_12%_**	29%	26%	_8%_
Heptageniidae	4%	3%	_10%_	28%	_14%_	_7%_
Limnephilidae	5%	6%	10%	_16%_	_14%_	_10%_
Limoniidae	2%	3%	_11%_	_14%_	_14%_	_8%_

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5.5 Discussion and conclusions

Our results here provide some hints for discussion. The link between climate, hydrological regime, and state of hydraulic habitats in the Serio river was depicted explicitly, and subsequently the effect of potential climate change upon habitat until the end of the century could be addressed. Habitat suitability functions were explicitly derived based upon field collection of specimens, and heuristic analysis of preference against flow properties, and substrate (Fornaroli *et al.*, 2015), so being representative for the considered area. The choice of a threshold for acceptable habitat conditions, quantified via $WUA_{20\%}$ when WUA duration curves have a kink, however arbitrary, displayed that critical conditions ($WUA < WUA_{20\%}$) occur in different seasons, depending upon the considered species, and provides indication of most critical sites along the stream. Spawning tends to be favored with low discharges, so optimal conditions tend to occur during winter, which is expected. Young trout tend to prefer low velocity, and discharges, as expected in the present literature (see *e.g.* Vismara *et al.*, 2001), with spring the most critical season (Fig. 5.4a), and winter the best one.

Adult trout display a more complex response to flow patterns, *i.e.* with suitability increasing with flow depth, and decreasing with velocity, and WUA increasing slowly with discharge (*e.g.* Vismara *et al.*, 2001). Worst conditions are during winter (Fig. 5.4b), with spring and summer most favorable seasons.

Macroinvertebrates investigated here tend to display preferences for larger velocities, especially Leuctridae and Heptageniidae, again consistently with results in the present literature (*e.g.* Gordon *et al.*, 2004). Mostly WUA for our four species tends to increase with flow discharge. Most suitable seasons for habitat colonization are therefore spring and

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summer (Fig. 5.4b, c, Tab. 5.6), and less autumn, and especially winter.

These latter results provide interesting clues about Serio river ecological status, because macroinvertebrates are more indicative of local habitat conditions than fishes, which may reflect conditions over broader spatial area given their large mobility (Plafkin *et al.*, 1989; Lammert and Allan, 1999).

To investigate prospective modification of habitat conditions under climate change, we fed the hydrological model with downscaled temperature and precipitation outputs from two GCM models. Future habitat projections display variability between models, and RCPs, but on average depict consistently potential changes in future habitat.

According to our results, it is possible that future climate change provide worse habitat conditions for adult trout, and macroinvertebrates during spring, and summer. These seasons are now the most suitable for colonization from this species and stage, and decrease of habitat suitability may indeed affect their health. While some catchments in the central Alps and pre-Alps feature noticeable ice cover, possibly providing buffer for water resources during spring, and summer until down wasting, Serio river does not display any large ice cover, and future lack of snow may hardly be dampened thence. Also, young trout will be affected during their most suitable season, winter, and so possibly undergoing disturbance therein.

Few studies are available in the present literature, focusing upon the effect of potential climate change on stream habitat, and population dynamics of fish, and macroinvertebrate species, against which we can benchmark our results.

Among others, Xenopoulos *et al.* (2005) used projections from the HadCM3 model (A2, and B2 storylines) and a global hydrological model to build global scenarios of future discharge in 52 rivers worldwide (including two in Italy), and

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then used relationships between fish species abundance and discharge to build scenarios of losses of riverine fish richness. They found that in rivers with reduced discharge, up to 75% of local fish biodiversity could be extinguished by 2070. For Po and Ombrone rivers in Italy, they predicted decrease of discharge down to -25% until 2070, with decrease of biodiversity down to -7%, and -10%, respectively, only due to climate change at that time. Our results here suggest at yearly scale a loss of discharge of -23% (average between models and RCPs) until 2085, possibly consistent with their results. Habitat conditions for adult fishes here would slightly improve yearly (with decrease of $n_{c\%}$ from 20% to 16% on average at 2085, Tab. 5.7, 5.8), but with differences seasonally, being winter and spring potentially critical seasons as reported. In this sense, average flow conditions are likely not representative enough for habitat quality assessment, and daily analysis, to be summarized at least seasonally as we did here may be adopted. Battin *et al*. (2007) investigated the impacts of climate change (A2 storyline, GFDLR30, and HadCM3 models) at 2025, and 2050, on the effectiveness of habitat restoration for Chinook salmon populations in a Pacific Northwest river basin (Snohomish basin). They used a spatially explicit life-cycle model (Shiraz) to simulate the dynamics of salmon populations. They found that higher water temperatures, lower Spawning flows in autumn, and, most importantly, increased magnitude of winter peak flows are likely to decrease habitat, and increase salmon mortality, the latter results consistent with our findings here (Fig. 5.4a). Mantua *et al.* (2010) evaluated the sensitivity of Washington State's freshwater habitat of Pacific Salmon (*Oncorhynchus spp.*) to climate change. They focused upon summertime stream temperatures, seasonal low flows, and changes in peak and base flows, that they projected until 2100 using multi-

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model composites for A1B and B1 scenarios. They stated that reductions in the volume of summer/ autumn low flows might reduce the availability of spawning habitat during autumn, and increases in the intensity and frequency of winter flooding may negatively impact the egg-to-fry survival rates.

Concerning macroinvertebrates, some studies recently investigated effects of climate change, largely focusing upon stream temperatures (Durance and Ormerod, 2007; Domisch *et al.*, 2011; Li *et al.*, 2012). However, we could not find specific studies concerning future habitat conditions for macroinvertebrates under climate change scenarios, so our work here seems original in this sense.

Among others, Chessman (2009) studied the effect on macroinvertebrates of air and water temperatures increase, and of rainfall and river flows decline in the Australian state of New South Wales NSW, during 1994-2007. He found significant relationships between the *thermophily* and *rheophily* of macroinvertebrates, with families that favor colder waters and faster-flowing habitats more likely to have declined. Given that projections for future rainfall across NSW indicate a decline in (Boreal) winter precipitation, increase in summer rainfall, and more intense rain events, he concluded that invertebrate species that require fast-flowing environments such as riffles and rapids are likely to continue to suffer from scarcity of suitable habitat.

Durance and Ormerod (2009) assessed trends of macroinvertebrates in 50 southern English streams against to temperature, discharge and water quality during 1989-2007 (winter, and summer). At some sites changes in discharge explained more variation in invertebrate abundance and composition than did temperature, and the higher the discharge, the larger the richness.

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Even with some variability according to variable climate change projections, our results here tend to agree with these findings. Our target macroinvertebrates display visible preferences against flow properties, most notably for fast flows. Accordingly, future decrease of discharge during the best seasons (spring, and summer) would decrease optimal habitat availability in time.

Interestingly enough, most suitable and most critical periods for adult fishes, and macroinvertebrates coincide fair well, implying that fish and macrobenthos co-exist in similar environments. Salmonids feed largely on macroinvertebrates, and may choose their habitat in response to prey abundance (Giroux *et al.*, 2000, Koljonen *et al.*, 2012b; Rosenfeld and Ptolemy, 2012), and dynamics and fate of the two species are clearly interconnected (*e.g.* Bergonzoni *et al.*, 2014).

Accordingly, habitat suitability, and viability analysis models, including for climate change impact assessment, should take into account mutual interactions. Here, habitat suitability was modeled for each species independently, while in the future joint modeling may be tackled.

Also, besides habitat assessment we will attempt at explicitly modeling population dynamics, and potential effect under climate change.

There is evidence that decreased summer flows, and increased air temperatures may lead to increased water temperatures, with outbreaks of aquatic diseases (*e.g.* PKD, Peeler and Feist, 2011). The template built here may be used in the future as a basis to assess potential spreading of such diseases in the Serio river. Also, our method may be adopted to benchmark potential strategies for habitat improvement (*e.g.* use of wood debris, Kail and Hering, 2005; Bocchiola, 2011), against hydrological cycle, water management, and prospective climate change.

Eventually, our work here displays how future climate may affect stream flow magnitude, and future habitat conditions for brown trout, and most prominent macroinvertebrates species in the Serio river. The results here may provide hints for river management strategies for adaptation to climate change, with an eye upon hydro-morphological requirements from Water Framework Directive.

Water resources management, and ecological status of rivers in the Alps are increasingly debated topics under the observed transient climate change conditions, and policy makers need to take action rapidly. The present work may provide a benchmark for future studies and may help depiction of possible adaptation strategies, posing the methodological bases for future developments in this area.

5.6 Acknowledgements

5.7 References

AQEM Consortium (2002) *Manual for the application of the AQEM system*.

Allamano P, Claps P, Laio F. (2002) Global warming increases flood risk in mountainous areas. *Geophysical Research Letters*, **36**, L24404.

Allamano P, Claps P, Laio F. (2009) An analytical model of the effects of catchment elevation on the flood frequency distribution. *Water Resources Research,* **45**, W0140.

Armanini DG, Horrigan N, Monk WA, Peters DL, Baird DJ. (2011) Development of a benthic macroinvertebrate flow sensitivity index for Canadian rivers. *River Research and Applications*, **27**, 723-737.

Arthington AH, Bunn SR, Poff NL, Naiman RJ. (2006) The challenge of providing environmental flow rules to sustain river ecosystems. *Ecological Applications,* **16**, 1311-1318.

Ayllón, D., Almodóvar,A, Nicola, G.G ., Elvira, B. (2012) The influence of variable habitat suitability criteria on PHABSIM habitat index results. *River Research and Applications,* **28,** 1179-1188.

Azzellino A, Vismara R. (2006) Pool Quality Index: New Method to Define Minimum Flow Requirements of High-Gradient, Low-Order Streams. *Journal of Environmental Engineering*, **127**, 1003-1013.

Battin J, Wiley MW, Ruckelshaus MH, Palmer RN, Korb E, Bartz KK, Imaki H. (2007) Projected impacts of climate change on salmon habitat restoration, *PNAS*, **104**, 6720-6725.

Bavay M, Lehning M, Jonas T, Lowe H. (2009) Simulations of future snow cover and discharge in Alpine headwater catchments. *Hydrological Processes*, **23**, 95-108.

Barnett TP, Adam JC, Lettenmaier DP. (2005) Potential impacts of a warming climate on water availability in snow-dominated regions, *Nature*, **438**, 303-309.

Beniston M, Keller F, Goyette S. (2003) Snow pack in the Swiss Alps under changing climatic conditions: an empirical approach for climate impacts studies. *Theoretical and Applied Climatology*, **74**,19-31

Bergonzoni IG, Landkildehus F, Meerhoff M, Lauridsen TL, Özkan K, Davidson TA, Mazzeo N, Jeppesen E. (2014) Fish determine macroinvertebrate food webs and

_____

assemblage structure in Greenland subarctic streams. *Freshwater Biology*, **59**, 1830-1842

Bocchiola D, Diolaiuti G, Soncini A, Mihalcea C, D'Agata C, Mayer C, Lambrecht A, Rosso R. Smiraglia C. (2011) Prediction of future hydrological regimes in poorly gauged high altitude basins: the case study of the upper Indus, Pakistan. *Hydrology and Earth System Sciences*, **15**, 2059-2075.

Bocchiola D, Diolaiuti G. (2010) Evidence of climatic trends in the Adamello glacier of Italy, *Theoretical and Applied Climatology,* **100**, 351-69.

Bocchiola D, Rulli MC, Rosso R. (2006) Transport of Large Woody Debris in the presence of obstacles. *Geomorphology*, **76**, 166-178.

Bocchiola D, Rulli MC Rosso, R. (2008) A flume experiment on the formation of wood jams in rivers. *Water Resources Research,* **44**, W02408.

Bocchiola D, Groppelli B. (2010) Spatial estimation of snow water equivalent at different dates within the Adamello Park of Italy. *Cold Regions Science and Technology*, **63**, 97-109.

Bocchiola D. (2011) Modified river hydraulics and habitat suitability in presence of woody debris: a flume experiment. *Advances in Water Resources,* **34**, 1304-1319.

Brown LE, Hannah DM, Milner AM. (2007) Vulnerability of alpine stream biodiversity to shrinking glaciers and snowpacks. *Global Change Biology*, **13**, 958-966.

Buffagni A, Casalegno C, Erba S. (2009) Hydromorphology and land use at different spatial scales: expectations in a changing climate scenario for medium-sized rivers of the Western Italian Alps. *Fundamental and Applied Limnology*, **174**, 7-25.

Buisson L, Grenouillet G, Villéger S, Canal J, Laffaille P. (2013) Toward a loss of functional diversity in stream fish assemblages under climate change. *Global Change Biology*, **19**, 387-400.

Bunte K, Abt SR. (2001) Sampling Surface and Subsurface Particle-Size Distributions in Wadable Gravel- and Cobble-Bed Streams for Analyses in Sediment Transport, Hydraulics, and Streambed Monitoring. *General Technical Report RMRS-GTR-74. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station*.

Canobbio S, Azzellino A, Cabrini R, Mezzanotte V. (2013) A multivariate approach to assess habitat integrity in urban streams using benthic macroinvertebrate metrics. *Water Science and Technology*, **67**, 2832-2837.

Canobbio S, Mezzanotte V, Benvenuto F, Siotto M. (2010) Determination of Serio River (Lombardy, Italy) ecosystem dynamics using macroinvertebrate functional traits. *Italian Journal of Zoology,* **77**, 227-240.

Chessman, BC. (1995) Rapid assessment of rivers using macroinvertebrates: A procedure based on habitat-specific sampling, family level identification and a biotic index. *Australian Journal of Ecology*, **20**, 122-129.

Chessman BC. (2009) Climatic changes and 13-year trends in stream macroinvertebrate assemblages in New South Wales, Australia. *Global Change Biology*, **15**, 2791-2802.

Clausen B, Biggs BJF. (2002) Flow variables for ecological studies in temperate streams: groupings based on covariance. *Journal of Hydrology*, **237**, 184-197.

Confortola G, Soncini A, Bocchiola D. (2013) Climate change will affect hydrological regimes in the Alp. *Journal of Alpine Research*, **101**,3.

Diolaiuti G, Bocchiola D, D'agata C, Smiraglia C. (2012a) Evidence of climate change impact upon glaciers' recession within the Italian Alps: the case of Lombardy glaciers. *Theoretical and Applied Climatology*, **109**, 429-445.

Diolaiuti G, Bocchiola D, Vagliasindi M, D'Agata C, Smiraglia C. (2012b) The 1975–2005 glacier changes in Aosta Valley (Italy) and the relations with climate evolution. *Progress in Physical Geography*, **36**, 764-785.

Domisch S, Jähnig SC, Haase P. (2011) Climate-change winners and losers: stream macroinvertebrates of a submontane region in Central Europe. *Freshwater Biology,* **56**, 2009-2020.

Durance I, Ormerod, SJ. (2007) Climate change effects on upland stream macroinvertebrates over a 25-year period. *Global Change Biology*, **13**, 942-957.

Durance. I, Ormerod SJ. (2009) Trends in water quality and discharge confound long-term warming effects on river macroinvertebrates. *Freshwater Biology,* **54**, 388-405.

Erba S, Buffagni A, Holmes N, O'Hare M, Scarlett P, Stenico S. (2006) Preliminary testing of River Habitat Survey features for the aims of the WFD hydro-morphological assessment: an overview from the STAR Project. *Hydrobiologia,* **566**, 281-296.

Faggian P, Giorgi F. (2009) An analysis of global model projections over Italy, with particular attention to the Italian Greater Alpine Region (GAR). *Climatic Change*, **96**, 239-258

Feyen L, Dankers R. (2009) Impact of global warming on streamflow drought in Europe. *Journal of Geophysical Research*, **114**, D17116.

_____

Ficke AD, Myrick CA, Hansen LJ. (2007) Potential impacts of global climate change on freshwater fisheries. *Reviews in Fish Biology and Fisheries*, **17**, 581-613.

Folk R. L. (1974) *Petrology of sedimentary rocks*, Hemphill Publishing, Austin, Texas.

Fornaroli R, Cabrini R, Sartori L, Marazzi F, Vracevic D, Mezzanotte V, Canobbio S. (2015) Predicting the constraint effect of physical habitat characteristics on macroinvertebrate density using quantile regression mixed model. *Hydrobiologia*, **742**, 153-167.

Giroux F, Ovidio M, Philippart J.C, Baras E. (2000) Relationship between the drift of macroinvertebrates and the activity of brown trout in a small stream. *Journal of Fish Biology*, **56**, 1248-1257.

Gordon ND, McMahon TH, Finlayson BL, Gippel CJ, Nathan RJ. (2004) *Stream Hydrology,* John Wiley & Sons, Ltd.

Groppelli B, Soncini A, Bocchiola D, Rosso R. (2001a) Evaluation of future hydrological cycle under climate change scenarios in a mesoscale Alpine watershed of Italy. *Natural Hazards and Earth System Sciences*, **11**, 1769-85.

Groppelli B, Bocchiola D, Rosso R. (2001b) Spatial downscaling of precipitation from GCMs for climate change projections using random cascades: a case study in Italy. *Water Resources Research,* **47**, W03519.

Hari RE, Livingstone DM, Siber R, Burkhardt-Holm P, Güttinger H. (2006) Consequences of climatic change for water temperature and brown trout populations in Alpine rivers and streams. *Global Change Biology*, **12**, 10-26.

Hering, D., Buffagni, A., Moog, O., Sandin, L., Sommerhäuser, M., Stubauer, I., Feld, C., Johnson, R., Pinto, P., Skoulikidis, N., Verdonschot, P., Zahrádková, S. (2003) The development of a system to assess the

_____

    ecological   quality   of   streams   based   on
    macroinvertebrates–design of the sampling programme
    within the AQEM project. *International Review of
    Hydrobiology*, **88**, 345–361.

Hering D, Schmidt-Kloiber A, Murphy J, Dahl J, Zamora-
    Muñoz C, López Rodríguez MJ, Huber T, Graf W.
    (2009) Potential impact of climate change on aquatic
    insects: a sensitivity analysis for European caddisflies
    Trichoptera based on species traits. *Aquatic Sciences*, **71**,
    3-14.

Huttunen KL, Mykrä H, Huusko A, Mäki-Petäys A, Vehanen
    T, Muotka T. (2014) Testing for temporal coherence
    across spatial extents: the roles of climate and local
    factors   in   regulating   stream   macroinvertebrate
    community dynamics. *Ecography*, **37**, 599-608.

IPCC (2013) *The Physical Science Basis. Contribution of
    Working Group I to the Fifth Assessment Report of the
    Intergovernmental Panel on Climate Change.* Cambridge
    University Press, Cambridge, United Kingdom and New
    York, NY, USA.

Isaak DJ, Luce CH, Rieman BE, Nagel DE, Peterson EE,
    Horan DL, Parkes S, Chandler GL. (2010) Effects of
    climate change and wildfire on stream temperatures and
    salmonid thermal habitat in a mountain river network.
    Ecological Applications, **20**, 1350-1371.

Kail J, Hering D (2005) Using large wood to restore streams in
    Central Europe: potential use and likely effects.
    Landscape Ecology, **20**, 755-772.

Kang B, Ramírez JA. (2007) Response of streamflow to
    weather variability under climate change in the Colorado
    Rockies. *Journal of Hydrologic Engineering*, **12**, 63-72.

Koljonen S, Huusko A, Mäki-Petäys A, Louhi P, Muotka T.
    (2012a) Assessing habitat suitability for juvenile Atlantic

salmon in relation to in-stream restoration and discharge variability. *Restoration Ecology*, **21**, 344-352.

Koljonen S, Huusko A, Maki-Petäys A, Heikki Mykrä C, Muotkaa T. (2012b) Body mass and growth of overwintering brown trout in relation to stream habitat complexity. *River Research and Applications*, **28**, 62-70.

Kottegoda, N.T., Rosso, R. (1997) *Probability, statistics, and reliability for civil and environmental engineering*. McGraw-Hill.

Johnson, A.C., Acreman, M.C., Dunbar, M.J., Feist, S.W., Giacomello, A.M., Gozlan, R.E., Hinsley, S. a, Ibbotson, A.T., Jarvie, H.P., Jones, J.I., Longshaw, M., Maberly, S.C., Marsh, T.J., Neal, C., Newman, J.R., Nunn, M. a, Pickup, R.W., Reynard, N.S., Sullivan, C. a, Sumpter, J.P., Williams, R.J. (2009) The British river of the future: how climate change and human activity might affect two contrasting river ecosystems in England. *Science of the Total Environment*, **407**, 4787–4798.

Lammert M, Allan JD. (1999) Assessing Biotic Integrity of Streams: Effects of Scale in Measuring the Influence of Land Use/Cover and Habitat Structure on Fish and Macroinvertebrates. *Environmental Management*, **23**, 257-270.

Lamouroux N, Capra H, Pouilly M. (1998) Predicting Habitat suitability for lotic fish: linking statistical hydraulic models with multivariate habitat use models. *Regulated Rivers Research and Management*, **14**, 1-11.

Lamouroux N, Capra H (2002) Simple predictions of instream habitat model outputs for target fish populations. *Freshwater Biology*, **47**, 1543-1556.

Lamouroux N, Cattanéo F (2006) Fish assemblages and stream hydraulics: consistent relations across spatial scales and regions. *River Research and Applications,* **22**, 727-737.

_____

Lamouroux N, Capra H, Pouilly M, Souchon Y. (1999) Fish habitat preferences in large streams of southern France. *Freshwater Biology*, **42**, 673-687

Lancaster J, Downes BJ. (2010) Linking the hydraulic world of individual organisms to ecological processes: putting ecology into ecohydraulics. *River Research and Applications,* **26**, 385-403.

Laternser M, Schneebeli M. (2003) Long-term snow climate trends of the Swiss Alps (1931-99). *International Journal of Climatology*, **23**, 733-750.

Li F, Cai Q, Jiang W, Qu X. (2012) The response of benthic macroinvertebrate communities to climate change: evidence from subtropical mountain streams in Central China. *International Review of Hydrobiology*, **97**, 200-214.

Loperfido JV. (2013) Surface Water Quality in Streams and Rivers: Scaling and Climate Change. *Reference Module in Earth Systems and Environmental Sciences*, **4**, 87-105.

Lytle D, Poff N. (2013) Adaptation to natural flow regimes. *Trends in Ecology and Evolution*, **19**, 94-100.

Mantua N, Tohver I, Hamlet A. (2010) Climate change impacts on streamflow extremes and summertime stream temperature and their possible consequences for freshwater salmon habitat in Washington State. *Climatic Change*, **102**, 187-223.

Maragno D, Diolaiuti G, D'Agata C, Mihalcea C, Bocchiola D, Bianchi Janetti E, Riccardi A, Smiraglia C. (2009) New evidence from Italy (Adamello Group, Lombardy) for analysing the ongoing decline of Alpine glaciers. *Geografia Fisica e Dinammica Quaternaria*, **32**, 47-56.

Mohseni O, Stefan HG, Eaton JG. (2003) Global warming and potential changes in fish habitat in U.S. streams. *Climatic Change*, **59**, 389-409.

_____

Palmer MA, Reidy Liermann CA, Nilsson C, Flörke M, Alcamo J, Lake PS, Bond N (2008) Climate change and the world's river basins: anticipating management options. *Frontiers in Ecology and the Environment*, **6**, 81-89.

Peeler EJ, Feist SW. (2011) Human intervention in freshwater ecosystems drives disease emergence. *Freshwater Biology*, **56**, 705-716.

Pellicciotti F, Bauder A, Parola M. (2010) Effect of glaciers on stream flow trends in the Swiss Alps. *Water Resources Research*, **46**, W10522.

Plafkin JL., Barbour MT, Porter KD., Gross SK, Hughes RM. (1989) Rapid bioassessment protocols for use in stream and rivers: benthic macroinvertebrates and fish. *EPA/444/4-89-011,* US Environmental Protection Agency Washington, DC.

Poff NL, Allan JD. (1995) Functional-organization of stream fish assemblages in relation to hydrological variability. *Ecology*, **76**, 606-627.

Poff NL., Angermeier PL, Cooper SD., Lake PS, Fausch KD, Winemiller KO, Mertes LAK, Oswood MW, Reynolds J, Rahel FJ. (2001) Fish diversity in streams and rivers. In: Chapin F.S.I., Sala O.E., Huber-Sannwald E., *Global biodiversity in a changing environment: scenarios for the 21st century.* Springer, New York, 2001: 351-368.

Rijnsdorp AD, Peck MA, Engelhard GH, Möllmann C, Pinnegar JK. (2009) Resolving the effect of climate change on fish populations. *ICES Journal of Marine Science*, **66**, 1570-1583.

Rosenfeld J, Ptolemy, R. (2012) Modelling available habitat versus available energy flux: do PHABSIM applications that neglect prey abundance underestimate optimal flows

for juvenile salmonids? *Canadian Journal of Fisheries and Aquatic Sciences*, **69**, 1920-1934.

Shirvell CS, Dungey RG (1983) Microhabitats chosen by brown trout for feeding and spawning in rivers. *Transactions of the American Fisheries Society*, **112**, 355-367.

Smakhtin VU. (2001) Low flow hydrology: a review. *Journal of Hydrology*, **240**, 147-186.

Soncini A., Bocchiola D. (2011) Assessment of future snowfall regimes within the Italian Alps using general circulation models. *Cold Regions Science and Technology*, **68**, 113-23.

Tisseuil C, Vrac M, Grenouillet G, Wade AJ, Gevrey M, Oberdorff T, Grodwohl GB, Lek S. (2012) Strengthening the link between climate, hydrological and species distribution modeling to assess the impacts of climate change on freshwater biodiversity. *Science of the total environment*, **424**, 193-201.

Verdonschot PFM. (2000) Integrated ecological assessment methods as a basis for sustainable catchment management. *Hydrobiologia*, **422/423**, 389-412.

Viganò GLML. (2013) Hydrological regime and environmental suitability of Serio river (BG) under potential climate changes [*Regime idrologico e di idoneità ambientale in relazione a potenziali cambiamenti climatici*], MS Thesis. In Italian.

Vismara R, Azzellino A, Bosi R, Crosa G, Gentili G. (2001) Habitat suitability curves for brown trout (Salmo Trutta Fario L.) in the river Adda, Northern Italy: comparing univariate and multivariate approaches. *Regulated Rivers Research and Management*, **17**, 37-50.

Wenger, S.J., Isaak, D.J., Luce, C.H., Neville, H.M., Fausch, K.D., Dunham, J.B., Dauwalter, D.C., Young, M.K.,

_____

Elsner, M.M., Rieman, B.E., Hamlet, A.F., Williams, J.E. (2011) Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 14175–14180.

Whitehead PG, Wilby RL, Battarbee RW, Kernan M, Wade AJ. (2014) A review of the potential impacts of climate change on surface water quality. *Hydrological Sciences Journal*, **54**, 101-123.

Wilby RL, Orra HG, Hedger M, Forrow D, Blackmore M. (2006) Risks posed by climate change to the delivery of Water Framework Directive objectives in the UK. *Environment International,* **32**, 1043 -1055.

Wilby, R.L., Orr, H., Watts, G., Battarbee, R.W., Berry, P.M., Chadd, R., Dugdale, S.J., Dunbar, M.J., Elliott, J. a, Extence, C., Hannah, D.M., Holmes, N., Johnson, a C., Knights, B., Milner, N.J., Ormerod, S.J., Solomon, D., Timlett, R., Whitehead, P.J., Wood, P.J. (2010) Evidence needed to manage freshwater ecosystems in a changing climate: turning adaptation principles into practice. *Science of the total environment*, **408**, 4150–4164.

Xenopoulos MA, Lodge DM, Alcamo J, Marker M, Schulze K, Van Vuuren D. (2005) Scenarios of freshwater fish extinctions from climate change and water withdrawal. *Global Change Biology*, **11**, 1557-1564.

6. General conclusions

6.1 Evaluation of ecological limiting factors

Understanding the relationships among biological communities' attributes and the environment characteristics is fundamental for correctly managing freshwater ecosystems. In real cases many factors, both natural and anthropogenic, can control the abundance and the diversity of freshwater biological communities. Thus, it is often very difficult to disentangle the different sources of variation. From the ecological point of view, testing hypotheses about the environmental gradients as limiting factors or constraints could be more informative than testing them about "average" responses.

Chapter 2 and 3 show the way quantile regression can be used to describe the limiting action of environmental variables on macroinvertebrate density and diversity in different environment.

In particular, in Chapter 2, we used quantile regression to evaluate limiting responses of macroinvertebrate community in lakes environment. We selected sampling depth and oxygen percent saturation as the two variables that best explain the number of taxa present and the diversity of a site.

In this case, models able to predict the potential of community diversity and richness on the basis of environmental characteristics were provided. Results can help water managers in deciding to initially invest resources in those lakes where the biological communities can be expected to be impaired due to the limiting action of measured variables, and to identify which lakes could be recovered to pristine or near-pristine conditions.

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In Chapter 3 the limiting action of environmental variables on streams macroinvertebrate density and diversity was evaluated using both Italian and Finnish data. The results highlight and quantify the effect of the characteristics of physical habitat on the densities of five macroinvertebrate families that are frequently sampled along alpine streams. The provided results can contribute to the definition of bioassessment indices and be used within habitat based models. On the other hand, the model developed for the Finnish dataset predicts minimum and especially potential community diversity as a function of environmental characteristics in different sampling sites in Finland.

6.2 Habitat based models predictions

Habitat models that predict flow-related changes in productive capacity are normally used for the definition of environmental flows and thus it is important that the information they provide is correct and useful for the water managers.
Other applications of such models are connected to the evaluation of different kind of future scenario (e.g. climate, land use).

In Chapter 4 density-environment models for different life stages for brown trout at habitat scale were developed. The factors that limited the densities of trout were water velocity, substrate characteristics and refugia availability. For all the life stages the selected models consider simultaneously two variables and imply that higher velocities generate a less suitable habitat, regardless of other physical characteristics. It is important to note that the spatial scale of the data used for developing the biological models is the same used for the predictions. The different behaviors of the different life stages were taken into account and the optimal flows were defined on

the basis of the needs of the different life stages. Also the changes in the available macroinvertebrate biomass were evaluated as the main energy source for trout.

This study highlights that, as physical habitat is a necessary, but not sufficient, condition for the production and survival of fishes, habitat based model results may best be viewed as indicators of population potential in systems where the habitat conditions described by the model are major population constraints. However prey availability is always important for fish production and macroinvertebrate were recognized many times as the main food source for trout (Sánchez-Hernández & Cobo 2012). Therefore taking into account the effect of flow management on macroinvertebrate population is fundamental in order to improve the assessment of environmental flows.

In Chapter 5 the impact of potential climate change on the hydrological and ecological status of the alpine stretch of the Serio river was investigated. The results display the potential for a large flow decrease, and a shift of seasonal flow peaks. Variable scenarios of habitat availability were investigated, with a potentially worse situation for all species and life stages, with seasonal differences. The study provides a *what if* analysis of potential changes of the eco-hydrological status of Serio river, somehow representative for Italian Alpine rivers, and shows how the limiting factor approach can be proficiently used in the prediction of scenarios.

6.3 References

Sánchez-Hernández, J. & Cobo, F. (2012) Summer differences in behavioural feeding habits and use of feeding habitat among brown trout (Pisces) age classes in a temperate area. *Italian Journal of Zoology*, **79**, 468–478.

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