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Aquatic biomonitoring: Lessons from the past, challenges for the future

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R. Bolpagni, M. Bresciani, S. Fenoglio

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New Editor-in-Chief announcement

July 24, 2017

In recent years, it became more and more clear that, given the current volume and variety of submissions and the time commitment necessary to face it, the editorship of the *Journal of Limnology* would benefit from an enlargement and a rejuvenation.

Making a step in this direction, I am pleased to announce that Dr. Diego Fontaneto, from ISE CNR (www.cnr.it/people/diego.fontaneto), has been appointed to the position of Editor-in-Chief of the *Journal of Limnology*, joining me in this task.

Diego has been a supportive and engaged member of the Editorial Board since he joined as an Associate Editor in 2012.

As well as a leading figure in his field of expertise (speciation, macroecology and patterns of diversity) he is a wide experienced Editor of many journals in the field of water science.

I have no doubt that he will make a positive impact on the journal's future and continue the advancement of the *Journal of Limnology*.

Best wishes,

Roberto Bertoni
Roberto Bertoni

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kg m^{-2} s^{-1}	kg × m^{-2} × sec^{-1}
μg L^{-1}	ppb *or* μg/l

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Istituto per lo Studio degli Ecosistemi
Verbania Pallanza, Italy

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INTRODUCTION TO THE SUPPLEMENT ON AQUATIC BIOMONITORING

The history of bioindication and biomonitoring started in 1902 when Kolkwitz and Marsson published their work "Grundsätze für die biologische Beurtheilung des Wassers, nach seiner Flora und Fauna". A few years later, in 1909, the same authors show that it is possible to measure the quality of aquatic systems using the *Saprobien method*, still used in some countries.

In the beginning, biomonitoring analyses have been carried out on rivers because, as said by Kenneth Cummins (1992), they have always been used as a conveyor belt to transport waste to the sea. It is indeed not a coincidence that almost all most densely populated cities in history have been built along rivers. The anthropic impact on lake ecosystems and communities has only been studied from 1925-26 when August Thienemann first applied the concept of lacustrine trophy.

Today a greater political, social and scientific awareness about bioindication and biomonitoring is ratified by UE Water Framework Directive (2000) and is widespread in Europe. It is then the right time to review the state of art of the topic, identifying pros and cons of the methods available, as illustrated in the present volume.

I would like to spend few words to celebrate the memory of the late Giuseppe Morabito, whom I had the pleasure to know since his PhD, and who recently passed away tragically.

His great passion and dedication to research in aquatic ecosystems were striking to everyone knowing him, he always showed great reliability, professionalism and always was a person of few words.

Therefore, in total agreement with the editors of this Supplement of the Journal of Limnology, we would like to dedicate the present volume to the dear memory of Giuseppe Morabito, who is one of the authors of the following contributions.

Elisa Anna Fano
Department of Life Sciences and Biotechnology
University of Ferrara

Elisa Anna Fano

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PREFACE

ORIGINAL ARTICLES

J. Limnol., 2017; 76(s1): 1-4
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PREFACE

Aquatic biomonitoring: Lessons from the past, challenges for the future

Rossano BOLPAGNI,[1*] Mariano BRESCIANI,[2] Stefano FENOGLIO[3]

[1]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma; [2]Optical Sensing Group, Institute for Electromagnetic Sensing of the Environment, National Research Council of Italy, via Bassini 15, 20133 Milan; [3]Department of Science and Technological Innovation, University of Piemonte Orientale, Viale Teresa Michel 25, I-15121 Alessandria, Italy
*Corresponding author: rossano.bolpagni@unipr.it

ABSTRACT

This special issue stems from an increasing awareness on the key contribution made by biometrics and biological indices in the quality classification of aquatic ecosystems. This theme has been the subject of passionate debate during the 13th European Ecological Federation (EEF) and 25th Italian Society of Ecology's (S.It.E.) joined congresses held in Rome in September 2015. In this frame, on the margins of the special *symposium* named "*Biomonitoring: Lessons from the past, challenges for the future*", it was launched the idea of a special issue of the Journal of Limnology on the "aquatic" contributions presented at the conference. The present volume mainly reports these studies, enriched by few invited papers. Among the other things, the main message is the need of a better integration between sector knowledges and legislative instruments. This is even truer given the on-going climate change, and the necessity to record rapid changes in ecosystems and to elaborate effective/adaptive responses to them.

Key word: Bioindication; aquatic ecosystems; macroinvertebrates; diatoms; macrophytes; fish; ostracods; remote sensing.

INTRODUCTION

In recent decades, the traditional approaches used to characterize and monitor ecosystems (*e.g.*, physical and chemical, taxonomic) have been integrated and partially replaced by species- and community-based indices. For example, at the European level, the enactment of the EU Water Framework Directive (WFD, Directive 2000/60/EC; European Union, 2000) has overcome the limits imposed by a mere physical and/or chemical investigation of waters by integrating biological communities in the monitoring programs. In particular, the use of bioindicators contributes to the ecological classification of colonized habitats – in this case of colonized water bodies – thus making it possible to evaluate, if present, the deviation from the "reference conditions". This transition has the potential, among other things, to produce multi-spatial interpretations of the relationships between organisms, biogeochemistry and the physical environment. The biological communities and/or biomarkers are able to reflect the real-time quality of the system under consideration, but also to integrate in time the perturbations exerted on ecosystems. In this context, the possibility of building an integrated and intercalibrated view of the ecosystem represents an important innovation in the field of the environmental monitoring (Poikane *et al.*, 2011).

This approach appears, thus, more robust and less subjected to errors associated with transient phenomena. However, its uncritical use and the insufficient knowledge of species and communities autoecology can lead to wrong evaluations. Along with the lack of knowledge about biology and ecology at several organization levels, methodological issues such as sampling effort and imperfect detection of species, or the difficulty to exactly define the reference conditions if not properly taken into account can severely bias the results of biomonitoring (Bouleau and Pont, 2015; Baattrup-Pedersen *et al.*, 2017). Additionally, for example, the strong capability of primary producers to modulate the physical and chemical conditions in which they live can explain in part: i) the non-linear responses of many aquatic macrophyte communities to external perturbations, or ii) the clear space- and time-dependence of the evaluations provided by some macrophyte multi-metric indices (Demars *et al.*, 2012; Bolpagni *et al.*, 2016). Furthermore, the increasing spread of exotic species on a global scale is another critical factor that can alter the responses of biological communities to the rising impairment of ecosystems. In this context, basic research should be implemented to support limits and opportunities offered by biomonitoring for proper management actions.

SPECIAL ISSUE CONTENTS PRESENTATION

These critical issues have been debated in a special symposium (*Biomonitoring: Lessons from the past, challenges for the future*) of the 13th European Ecological Federation (EEF) and 25th Italian Society of Ecology's (S.It.E.) joint conference – *Ecology at the Interface: science-based solutions for human well-being* – held in Rome (Italy), September 21st - 25th September 2015. The mission of this symposium was the knowledge exchange between international groups that work with biomonitoring, also through the implementation and the support of basic discussion. In this context, our main goal was the sharing of methodological approaches to support the development of robust indicators, providing tools for their calibration and a proper use. This special issue is the symposium outcome, based on a selection of 13 peer-reviewed papers dealing with all the main biotic components of freshwater ecosystems, with special emphasis to the responses of the aquatic biological community to the main environmental and human drivers.

Four papers address the focal issue of the running water biomonitoring based on macroinvertebrates (Bo *et al.,* 2017; Burgazzi *et al.,* 2017; Guareschi *et al.*, 2017; Merritt *et al.*, 2017). They focus on multiple themes, as well as the importance of adopting functional approaches in river monitoring programs (Merritt *et al.*, 2017), or the contribution of rare *taxa* to the classification of water bodies (Guareschi *et al.*, 2017). Bo and colleagues (2017) review the history and development of macroinvertebrate indices in use in Italy, providing suggestion to improve the current biomonitoring approach. Another key aspect addressed in the present special issue is the role of mesohabitat mosaic in driving macroinvertebrate diversity and variability in braided rivers (Burgazzi *et al.*, 2017). These systems are general poorly studied, and their intrinsic structural high complexity is often neglected in biomonitoring protocols. As a major result, the summer flow reduction as a homogenizing force leads to a general loss of the most sensitive *taxa*.

In Mediterranean rivers, the strong seasonality with drought during the hot season and extreme flows in autumn-winter greatly drives the primary production and the trophic chain (Barthés *et al.*, 2015). The on-going climate change is expected to exacerbate the weather extremes with dramatic effects on river biofilm species diversity, growing rates or photosynthetic pigments (Tornés and Ruhì, 2013). In this context, Piano *et al*. (2017) investigate by regression modelling analysis the responses of benthic chlorophyll *a* concentration – assumed as a proxy of the algal biomass – to hydrological variability, including river intermittency. Specifically, they have tested the usefulness of using an *in situ* fluorimetric probe (BenthoTorch®) to discriminate between the main algal groups (*i.e.*, diatoms, cyanobacteria, and green algae) composing autotrophic biofilm. Della Bella *et al.* (2017), instead, apply a classical approach to explore the diatom diversity across the different river macrotypes recorded in the Umbria region (Central Italy). They focus on the Intercalibration Common Metric Index (ICMi; Mancini and Sollazzo, 2009), suggesting the existence of strong differences between diatomic diversity metrics comparing different Mediterranean river types, an aspect that should be taken into account in comparative studies.

Macrophytes are a further key element in monitoring programs, however additional investigations are needed to refine their use in biomonitoring because the complex interactions between aquatic primary producers and ecological drivers (Demars *et al.*, 2012; Bolpagni and Laini, 2016; Bolpagni *et al.*, 2016). In addition, alien plants may be considered one of the most critical causes of the functionality loss of aquatic ecosystems. The available knowledge needs to be improved to better manage control and mitigation programs. For this purpose, Bertrin *et al*. (2017) investigate the distribution patterns of alien species in the Aquitaine lakes, considering the influence of hydromorphology on plants morphological plasticity. All this information is fundamental to support effective actions. Similarly, to monitor and to counteract the worldwide aquatic environments decline, Sender *et al.* (2017) propose a new multi-criteria method of evaluation and assessment of the ecological status of lakes based mainly on macrophytes. Among other things, this method allows to point out a zonal evaluation of the lacustrine environment, identifying the most critic zones in terms of ecological status. In this way, it becomes easier and immediate to identify the most effective recovery actions. Additionally, with the aim of making monitoring procedures leaner and more effective, Bolpagni *et al.* (2017) explore the potential integration between the Habitat Directive (HD, European Union, 1992) and the WFD. A better integration between these two directives turned out to be a win-win strategy to obtain reliable information on the ranges occupied by macrophytes and aquatic habitats *sensu* HD, and to examine their status of conservation (Bolpagni *et al.*, 2013; Azzella *et al.*, 2014). In the general context of the macrophyte-environment relationships, another key question is the responses of the co-occurrence patterns of species to environmental gradients. This issue is investigated by Azzella *et al.* (2017) focusing on the depth distribution patterns of macrophytes in a series of volcanic lakes in Central Italy by using a null model analysis approach. Their main efforts confirm the not random co-occurrence patterns of macrophyte' communities in deep lakes. As a rule, it is fundamental to evaluate the local effects of lake trophy or human perturbations on plant dynamics before inquiring the arrangement of species.

In the last decades, remote sensing techniques have proved to be an extraordinary effective tool for monitoring ecosystems at multiple scales, especially for the aquatic ones. Bresciani *et al*. (2017) test their usefulness in the analysis of cyanobacterial blooms in the frame of the BLASCO project (CARIPLO Rif. 2014-1249). These authors verified the highly effectiveness of remote sensing for mapping cyanobacterial blooms and highlighted their main advantages, including the generation of synoptic and dynamic views. Additionally, Villa *et al*. (2017) explore the potential of airborne and spaceborne imaging sensors for mapping aquatic vegetation based on the spectral responses of its morphological and physiological features. They focused on macrophyte morphological traits (*i.e.*, fractional cover, leaf area index and above-water biomass) to discuss on the pivotal contribution offered by remote sensing to support macrophyte monitoring and management (Villa *et al.*, 2015).

Finally, one contribution addresses the role of physical and chemical drivers, as well as the functional complexity of riparian contexts in structuring the population of one of the most threatened target animal group: inland water fish. The paper by Piccoli *et al.* (2017) is finalized to assess the contribution of a complex of *Natura 2000* sites to support fish communities, with special emphasis to two endemic and one alien *Barbus* species, implementing the current data on their local spatial distribution.

Generally, all these studies stimulate a new awareness on the pivotal contribution of the biomonitoring approaches in the recovery of ecosystems and their functions, emphasizing the need of a better integration between sector knowledges and legislative instruments. This is a fundamental objective in a fast changing world, in order to improve our capability to record rapid changes in ecosystems, and then be ready to elaborate effective/adaptive responses to them.

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REFERENCES

Azzella MM, Bolpagni R, Oggioni A, 2014. A preliminary evaluation of lake morphometric traits influence on the maximum colonization depth of aquatic plants. J. Limnol. 73:1-7.

Azzella MM, Bresciani M, Nizzoli D, Bolpagni R, 2017. Aquatic vegetation in deep lakes: macrophyte co-occurrence patterns and environmental determinants. J. Limnol. 76(Suppl.1):97-108. [Epub 25 Jul 2017].

Baattrup-Pedersen A, Göthe E, Riis T, Andersen DK, Larsen SE, 2017. A new paradigm for biomonitoring: an example building on the Danish Stream Plant Index. Meth. Ecol. Evol. 8:297-307.

Barthès A, Ten-Hage L, Lamy A, Rols J-L, Leflaive J, 2015. Resilience of aggregated microbial communities subjected to drought - Small-scale studies. Microbial Ecol. 70:9-20.

Bertrin V, Boutry S, Jan G, Ducasse G, Grigoletto F, Ribaudo C, 2017. Effects of wind-induced sediment resuspension on distribution and morphological traits of aquatic weeds in shallow lakes. J. Limnol. 76(Suppl.1):84-96. [Epub 20 Jun 2017].

Bo T, Doretto A, Laini A, Bona F, Fenoglio S, 2017. Biomonitoring with macroinvertebrate communities in Italy: what happened to our past and what's the future? J. Limnol. 76(Suppl.1):21-28. [Epub 17 Oct 2016].

Bolpagni R, Laini A, 2016. Microhabitat patterns of soft-bodied benthic algae in a lowland river largely fed by groundwater. Fottea 16:244-254.

Bolpagni R, Bettoni E, Bonomi F, Bresciani M, Caraffini K, Costaraoss S, Giacomazzi F, Monauni C, Montanari P, Mosconi MC, Oggioni A, Pellegrini G, Zampieri C, 2013. Charophytes of Garda lake (Northern Italy): A preliminary assessment of diversity and distribution. J. Limnol. 72:388-393.

Bolpagni R, Racchetti E, Laini A, 2016. Fragmentation and groundwater supply as major drivers of algal and plant diversity and relative cover dynamics along a highly modified lowland river. Sci. Total. Environ. 568:875-884.

Bolpagni R, Azzella MM, Agostinelli C, Beghi A, Bettoni E, Brusa G, De Molli C, Formenti R, Galimberti F, Cerabolini BEL, 2017. Integrating the Water Framework Directive into the Habitats Directive: analysis of distribution patterns of lacustrine EU habitats in lakes of Lombardy (northern Italy). J. Limnol. 76(Suppl.1):75-83. [Epub 14 Feb 2017].

Bouleau G, Pont D, 2015. Did you say reference conditions? Ecological and socio-economic perspectives on the European Water Framework Directive. Environ. Sci. Pol. 47:32-41.

Bresciani M, Giardino C, Lauceri R, Matta E, Cazzaniga I, Pinardi M, Lami A, Austoni M, Viaggiu E, Congestri R, Morabito G, 2017. Earth observation for monitoring and mapping of cyanobacteria blooms. Case studies on five Italian lakes. J. Limnol. 76(Suppl.1):127-139. [Epub 19 Aug 2016].

Burgazzi G, Laini A, Racchetti E, Viaroli P, 2017. Mesohabitat mosaic in lowland braided rivers: Short-term variability of macroinvertebrate metacommunities. J. Limnol. 76(Suppl.1): 29-38. [Epub 14 Apr 2017].

Della Bella V, Padula R, Charavgis F, Cingolani A, Colangelo P, 2017. Mediterranean river biomonitoring in Central Italy: diatom biodiversity and characterization of communities. J. Limnol. 76(Suppl.1):49-59. [Epub 27 Oct 2016].

Demars BOL, Potts JM, Trémolières M, Thiébaut G, Gougelin N, Nordmann V, 2012. River macrophyte indices: Not the Holy Grail! Freshwater Biol. 57:1745-1759.

European Union, 1992. Council Directive 92/43/ECC of 21 May 1992 on the conservation of natural habitats and wild fauna and flora. OJ L 206, 22.07.1992, p. 7-50.

European Union, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L 327, 22/12/2000, p. 1-73.

Guareschi S, Laini A, Sánchez-Montoya MM, 2017. How do low-abundance taxa affect river biomonitoring? Exploring the response of different macroinvertebrate-based indices. J. Limnol. 76(Suppl.1):9-20. [Epub 7 Jun 2016].

Mancini L, Sollazzo C, 2009. Metodo per la valutazione dello stato ecologico delle acque correnti: comunità diatomiche. Rapporti ISTISAN 09/19. Istituto Superiore di Sanità, Roma: 32 pp.

Merritt RW, Fenoglio S, Cummins KW, 2017. Promoting a functional macroinvertebrate approach in the biomonitoring of Italian lotic systems. J. Limnol. 76(Suppl.1):5-8. [Epub 7 Jun 2016].

Piano E, Falasco E, Bona F, 2017. Mediterranean rivers: Consequences of water scarcity on benthic algal chlorophyll a content. J. Limnol. 76(Suppl.1):39-48. [Epub 7 Jun 2016].

Piccoli F, Burgazzi G, Laini A, Ferrari C, Voccia A, Bolpagni R, Nonnis Marzano F, 2017. Barbel species arrangement in a regional Natura 2000 network (Emilia Romagna, Northern Italy): an altitudinal perspective. J. Limnol. 76(Suppl.1): 140-147. [Epub 28 Jul 2017].

Poikane S, Van Den Berg M, Hellsten S, De Hoyos C, Ortiz-Casas J, Pall K, Portielje R, Phillips G, Solheim AL, Tierney D, Wolfram G, Van De Bund W, 2011. Lake ecological assessment systems and intercalibration for the European Water Framework Directive: Aims, achievements and further challenges. Procedia Environ. Sci. 9:153-168.

Sender J, Maślanko W, Różańska-Boczula M, Cianfaglione K, 2017. A new multi-criteria method for the ecological assessment of lakes: A case study from the Transboundary Biosphere Reserve 'West Polesie' (Poland). J. Limnol. 76 (Suppl.1):60-74. [Epub 14 Mar 2017].

Tornés E, Ruhì A, 2013. Flow intermittency decreases nestedness and specialization of diatom communities in Mediterranean rivers. Freshwater Biol. 58:2555-2566.

Villa P, Bresciani M, Bolpagni R, Pinardi M, Giardino C, 2015. A rule-based approach for mapping macrophyte communities using multi-temporal aquatic vegetation indices. Remote Sens. Environ. 171:218-233.

Villa P, Pinardi M, Tóth VR, Hunter PD, Bolpagni R, Bresciani M, 2017. Remote sensing of macrophyte morphological traits: Implications for the management of shallow lakes. J. Limnol. 76(Suppl.1):109-126. [Epub 7 Mar 2017].

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Promoting a functional macroinvertebrate approach in the biomonitoring of Italian lotic systems

Richard W. MERRITT,[1] Stefano FENOGLIO,[2*] Kenneth W. CUMMINS[3]

[1]Department of Entomology, Michigan State University, 288 Farm Ln #243, East Lansing, MI 48824 USA; [2]Department of Science and Technological Innovation, University of Piemonte Orientale, Via T. Michel 11, 15121 Alessandria, Italy; [3]P.O. Box 1181, Cooke City, MT 59020 USA
*Corresponding author: stefano.fenoglio@uniupo.it

ABSTRACT

Over fifty years of research on freshwater macroinvertebrates has been driven largely by the state of the taxonomy of these organisms. Significant advances have been and continue to be made in developing ever more refined keys to macroinvertebrate groups. When advances in macroinvertebrate ecological research are restricted by the level of detail in identifications, then analysis by function is a viable alternative. The focus on function, namely adaptations of macroinvertebrates to habitats and the utilization of food resources, has facilitated ecological evaluation of freshwater ecosystems. This classification is based not on what insects eat, but how they obtain their food. These categories are called “functional feeding groups”, as the name implies, denoting their functional role when describing how and where they feed. This is the basis for the functional feeding group (FFG) method that was initially developed in the early 1960s. Taxonomy is applied only to the level of detail that allows assignment to one of five functional feeding group categories: detrital shredders, scrapers, filtering collectors, gatherers, and predators. The aim of this short communication, originating from the presentation of R.W. Merritt at the Biomonitoring Symposium in Rome, 2015, is to promote the use of a functional approach in biomonitoring, especially in Italian and European lotic systems. Here, we present two case studies and we discuss the advantages of the method, especially considering the great availability of quantitative data on macroinvertebrates after the implementation of the WFD 2000/60. We are confident that the increase of functional assessment of ecosystem attributes could have important and direct repercussions in the understanding and management of running waters.

Key word: Functional Feeding Groups; ecological traits; biological traits; living stream.

INTRODUCTION

In the last 30-40 years, enormous advances have been made in the knowledge of lotic systems (Davies and Walker, 2013). Stream ecology is at present a solid discipline, with a good theoretical basis and a wide number of promising lines of research. In particular, a conceptual seed, which was a constitutive element of the River Continuum Concept (Vannote *et al.,* 1980) and previous works (Cummins, 1973, 1974), blossomed, becoming a stimulating area of investigation, with numerous applied aspects. This idea is related to the fact that, for a better understanding of lotic systems, it is important to consider not only structural elements, such as community composition and abundance, but also functional traits. In general, the importance of a functional approach has grown exponentially throughout ecological studies (Mouillot *et al.*, 2013), so that the term ‘functional diversity’ is at present widely used to indicate a component of biodiversity that generally concerns the range of things that organisms do in communities and ecosystems. In this context, feeding strategies are important and typical traits reflecting the adaptation of species to environmental conditions (Merritt and Cummins, 1996).

In lotic food webs, much of the energetic support originates from non-living sources of terrestrial organic matter origin, so that heterotrophic pathways are of greatest importance, and detritus rather than living plant material is the basis of most invertebrate food chains (*e.g.*, Cummins and Klug, 1979; Cummins *et al*., 1989). Aquatic invertebrates generally have great genetic feeding plasticity and, at least in their early instars, almost all aquatic insects can be considered omnivores (Clifford and Hamilton, 1987; Merritt *et al*., 2008). For this reason, the Functional Feeding Group approach (Cummins, 1974; Merritt and Cummins, 1996), based not on what aquatic organisms eat, but how they obtain their food, has increased the understanding of trophic dynamics in streams and rivers by simplifying the benthic community into trophic guilds. Functional Feeding Groups (FFG), based on morphological and behavioral mechanisms associated with food acquisition, are basically five: shredders, chewing or mining coarse particulate organic matter, primarily dead leaves and associated microbiota; scrapers or grazers, that scrape periphyton and generally biofilm; collectors-gatherers, that collect deposited organic fine sediments; collectors-filterers, that collect fine particulate organic matter from the water column; and predators. Appreciable differences can occur among these categories, depending on possible variations in food availability or ontogenetic shifts in diet (Malmqvist *et al.*, 1991, Fenoglio *et al.*, 2010), but the relative importance of these functional feeding groups within benthic communities can be considered to obtain useful ecological information.

The use of functional traits to investigate different ecosystem attributes has already been introduced (Merritt *et al*., 1996), and for example adopted to give practical recommendations about river oxbow and river-riparian marsh restoration in Florida (Merritt *et al*., 1999, 2002). The functional group analysis can be used as surrogates for ecosystem attribute studies (Cummins *et al*., 2005): for example, using the relationship among FFG it is possible to provide useful information about ecosystem stability, energy flow and trophic webs. The aim of this short communication, developed within the *Biomonitoring Symposium* held in Rome in September 2015 in the frame of the European Ecological Federation (EEF), is to encourage the use of the functional approach in European and especially Italian lotic environments. As an example of the potential applications of this approach, we are presenting two case studies, carried out in Alpine environments and characterized by hydrological and morphological alterations.

Case studies

Hydrological alterations – droughts

This first case study is focused on the effect of droughts in previously known perennial rivers. In particular, we re-analyzed here, through a functional approach, data from a study observed in Italian pre-Alpine environments. Droughts constitute a growing problem in Southern Europe, with dramatic ecological consequences and underestimated economic and social repercussions (Filipe *et al.*, 2013). Over-exploitation of water resources, land use transformations and particularly global climate change are among the main causes of hydrological alteration increase (Vicente-Serrano *et al.*, 2014).

In recent years, a significant part of Northern Italy lotic systems changed from naturally ‘permanent’, with continuous presence of running water, to ‘intermittent’, with extreme water scarcity or lack in some periods, mostly during summer. The study was conducted in the upper section of the Po River, the largest Italian river and tenth largest in Europe. We selected as case study at an 11-km reach, with altered flow regimes in the lowest part, where in the last few decades drought has become a regular event. From January 2004 to September 2005, we collected samples in four stations that are close to each other (<10 km from Site 1 to 4) but with different drought lengths. In the study period, superficial flowing waters were permanent in Site 1, while disappeared for 150 days in Station 2, 240 days in Station 3, and 330 days in Station 4. Discussion and conclusions about biodiversity, taxonomic richness, and invertebrate densities are reported in Fenoglio *et al.* (2007). Here, we focus on the relative importance of invertebrate functional groups as analogs of ecosystem attributes. Functional composition of benthic assemblages varied dramatically among stations, with an evident increase of collectors-gatherers and a marked reduction of shredders and scrapers in the most impacted sites. First, according to Merritt *et al.* (1996), we investigated the importance of instream primary production, and consequently the autotrophy to heterotrophy ratio, by calculating the importance of scrapers (plus live vascular hydrophyte shredders) as a proportion of shredders plus total collectors. Results are reported in Fig. 1. Moreover, we analyzed the ecological importance of allochthonous energetic inputs, markedly CPOM, in the different stations through the importance of shredders as a proportion of total collectors. In this case, we utilized only fall/winter samples, because of the seasonality of this input in the study area. Interestingly, sites that are very close in the same river reach evidenced decreasing values with the increase of drought length (Fig. 2).

These results indicate that the progressive diminution of permanent water flow profoundly alters energy fluxes and food webs of the river biota. The metabolism of instream primary producers is highly sensitive to alterations in hydrological and thermal conditions (Uehlinger, 2006). So, while in the first site the constant water permanence permits the establishment of stable periphytic biofilms, in the other stations, we can evidence a progressive reduction of autotrophy at the ecosystem level, the entity of which is inversely proportional to drought length. Furthermore, the absence of permanent water probably inhibits the microbial breakdown of allochthonous inputs (*i.e.*, the conditioning by aquatic hyphomycetes and bacteria), that is essential to make this resource available for

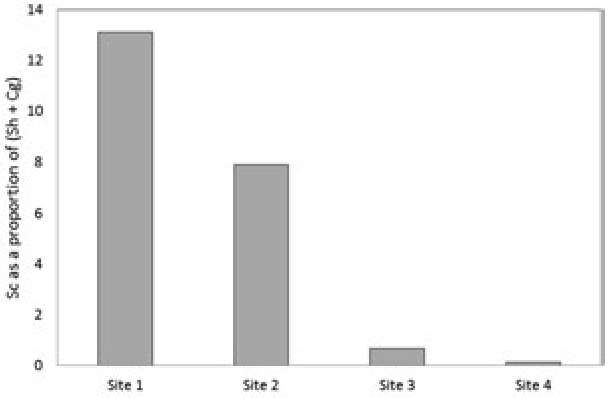

Fig. 1. Scrapers as a proportion of Shredders and Collectors in the four sites of the Po River.

macroinvertebrates and the rest of the trophic web. We can hypothesize that droughts in these naturally perennial systems cause a dramatic functional alteration, drastically reducing the importance of internal productivity and allochthonous coarse organic detritus processing. With the progressive increase of drought persistence, benthic communities became simplified and more functionally generalist, relying mainly on fine organic sediments.

Morphological alterations - siltation

In the last decades, anthropogenic pressures have increasingly altered the sediment transport-deposition cycle in many rivers. In particular, siltation, that is the deposition of unnatural amounts of fine sediments, has become a key ecological problem (Owens *et al*., 2005), especially in mountain areas that are naturally characterized by coarse substrata. Here, fine sediment accumulations can dramatically alter the environmental characteristics of streambeds, combining physical (*i.e*., clogging interstices, reducing micro- and meso-habitat heterogeneity – Bo *et al*., 2007), chemical (*i.e.*, lowering substrata permeability to oxygen and other dissolved gases, nutrients and metabolites – Pretty *et al*., 2006), and biological (*i.e.*, causing burial, constraining or preventing movement and survival of lotic organisms – Jones *et al*., 2012) effects. Siltation can cause significant changes in many aspects of stream biota, the most frequently documented being structural, such as abundance diminution and taxonomic transformation in lotic communities (Allan and Castillo, 2007). Here, we utilized the functional approach to evaluate if stream reaches impacted by anthropogenic fine sediments have similar ecosystem attributes (based on the FFG proportion). We reanalyzed data from a study conducted in two third order streams in the Cottian Alps (Piemonte, NW Italy), the Luserna and the Comba Liussa streams. These streams are very close (<7 kilometers away from each other), share the same climatic conditions, but show a great difference in fine sediment amounts, because the first drains one of the most important mining areas of Western Alps while the second is almost pristine. We performed quantitative samplings on stream macroinvertebrate communities in both streams (see details in Bona *et al*., 2015). Here we present results from the application of a functional approach. Firstly, we considered the ratio between scrapers as a proportion of shredders plus total collectors. Considering quantitative data from the two lotic systems, mean values were 4.15 (±1.45 SE, standard error) in the clogged stream and 10.12 (±3.47) in the unaltered stream. Furthermore, we calculated the ratio between total shredders and total collectors: values were 5.62 (±1.69) for the Luserna and 22.7 (±4.27) for the Comba Liussa. In addition, if no threshold levels (Merritt *et al*., 2002) have been utilized here because of the novelty of this approach in Alpine and pre-Alpine Italian lotic systems, these values underline important changes in ecosystem attributes. In particular, we can hypothesize that anthropogenic siltation severely altered energetic inputs in the Luserna stream. The elevated amounts of fine sediments in the streambed resulted in a lower instream primary production, because of the reduced survival possibilities for periphyton, and in a lower CPOM availability, due to the increased homogenization of the substrate and the consequent reduction of the coarse allochthonous detritus retention. These important functional changes were well evidenced by the different FFG ratio we reported.

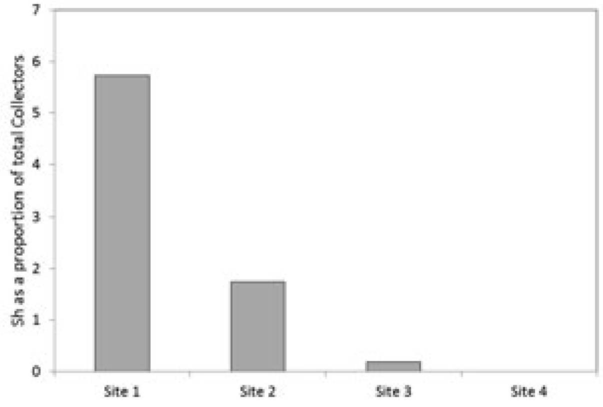

Fig. 2. Shredders as a proportion of total Collectors in the four sites of the Po River.

DISCUSSION AND CONCLUSIONS

This manuscript originates from the interesting oral communication of R. W. Merritt at the EEF 2015 Congress (Rome, September 2015) and successive conversations. The main purpose of this work is to encourage the evaluation of ecosystem attributes through the use of functional macroinvertebrate traits, in particular FFG ratios. At present, this functional approach has been rarely used in Europe and especially in Italy. This work aims to promote the use of this method, which has many advantageous applications.

Firstly, the functional group approach can be used to investigate ecosystem attributes in natural and in different kind of impacted systems. Applications can be made in the field of morpho-hydrological alterations, as shown here, or to assess ecological conditions of polluted rivers (Canobbio *et al*., 2010). This method can be applied to a wide range of river environments, across a broad geographic range. Moreover, this method provides information about ecosystem conditions that are often difficult or even quite impossible to measure directly. Finally, the diffusion of quantitative surveys of benthic macroinvertebrate communities in Italy (and in the rest of Europe) after the compliance of the Water Framework Directive 2000/60 provides the availability of a vast amount of data, which can be easily analyzed through a ‘’functional lens”, with the assignment of organisms to the different FFGs. In fact, as shown here, starting from macroinvertebrate quantitative data, appropriate functional traits can be assigned to each *taxon* and used, without problems, to evaluate ecosystem attributes.

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REFERENCES

Allan JD, Castillo MM, 2007. Stream ecology: structure and function of running waters. Springer, Dordrecht: 436 pp.

Bo T, Fenoglio S, Malacarne G, Pessino M, Sgariboldi F, 2007. Effects of clogging on stream macroinvertebrates: an experimental approach. Limnologica 37:186-192.

Bona F, Doretto A, Falasco E, La Morgia V, Piano E, Ajassa R, Fenoglio S, 2015. Increased sediment loads in Alpine streams: An integrated field study. River Res. Appl. 32:1316-1326.

Canobbio S, Mezzanotte V, Benvenuto F, Siotto M, 2010. Determination of Serio River (Lombardy, Italy) ecosystem dynamics using macroinvertebrate functional traits. Ital. J. Zool. 77:227-240.

Clifford HF, Hamilton HR, 1987. Volume of material ingested by mayfly nymphs of various sizes from some Canadian streams. J. Freshwater Ecol. 4:259-261.

Cummins KW, 1973. Trophic relations of aquatic insects. Annu. Rev. Entomol. 18:183-206.

Cummins KW, 1974. Structure and function of stream ecosystems. BioScience 24:631-641.

Cummins KW, Klug MJ, 1979. Feeding ecology of stream invertebrates. Annu. Rev. Ecol. Syst. 10:147-172.

Cummins KW, Merritt RW, Andrade PC, 2005. The use of invertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in south Brazil. Stud. Neotrop. Fauna E. 40:69-89.

Cummins KW, Wilzbach MA, Gates DM, Perry JB, Taliaferro WB, 1989. Shredders and riparian vegetation. BioScience 39:24-30.

Davies BR, Walker KF, 2013. The ecology of river systems. Springer, Dordrecht: 793 pp.

Fenoglio S, Bo T, Cucco M, Malacarne G, 2007. Response of benthic invertebrate assemblages to varying drought conditions in the Po river (NW Italy). Ital. J. Zool. 74:191-201.

Fenoglio S, Bo T, López-Rodríguez MJ, Tierno de Figueroa JM, 2010. Life cycle and nymphal feeding of Besdolus ravizzarum (Plecoptera: Perlodidae), a threatened stonefly. Insect Sci. 17:149-153.

Filipe AF, Lawrence JE, Bonada N, 2013. Vulnerability of stream biota to climate change in mediterranean climate regions: a synthesis of ecological responses and conservation challenges. Hydrobiologia 719:331-351.

Jones JI, Murphy JF, Collins AL, Sear DA, Naden PS, Armitage PD, 2012. The impact of fine sediment on macro-invertebrates. River Res. Appl. 28:1055-1071.

Malmqvist B, Sjöström P, Frick K, 1991. The diet of two species of Isoperla (Plecoptera: Perlodidae) in relation to season, site, and sympatry. Hydrobiologia 213:191-203.

Merritt RW, Cummins KW, 1996. An introduction to the aquatic insects of North America. Kendall/Hunt Publishers, Dubuque: 862 pp.

Merritt RW, Cummins KW, Berg MB, 2008. An introduction to the aquatic insects of North America. Kendall/Hunt Publishers, Dubuque: 1158 pp.

Merritt RW, Cummins KW, Berg MB, Novak JA, Higgins MJ, Wessell KJ, Lessard JL, 2002. Development and application of a macroinvertebrate functional-group approach in the bioassessment of remnant river oxbows in southwest Florida. Development 21:290-310.

Merritt RW, Higgins MJ, Cummins KW, Vandeneeden B, 1999. The Kissimmee River-riparian marsh ecosystem, Florida: Seasonal differences in invertebrate functional feeding group relationships, p. 55-79. In: D. Batzer, R.B. Rader and S.A Wissinger (eds.), Invertebrates in Freshwater Wetlands of North America. Wiley & Sons, Chichester.

Merritt RW, Wallace JR, Higgins MJ, Alexander MK, Berg MB, Morgan WT, Cummins KW, Vandeneeden B, 1996. Procedures for the functional analysis of invertebrate communities of the Kissimmee River-floodplain ecosystem. Fla. Sci. 59:216-274.

Mouillot D, Graham NA, Villéger S, Mason NW, Bellwood DR, 2013. A functional approach reveals community responses to disturbances. Trends Ecol. Evol. 28:167-177.

Owens PN, Batalla RJ, Collins AJ, Gomez B, Hicks DM, Horowitz AJ, Kondolf GM, Marden M, Page MJ, Peacock DH, Petticrew EL, Salomons W, Trustrum NA, 2005. Fine-grained sediment in river systems: environmental significance and management issues. River Res. Appl. 21:693-717.

Pretty JL, Hildrew AG, Trimmer M, 2006. Nutrient dynamics in relation to surface-subsurface hydrological exchange in a groundwater fed chalk stream. J. Hydrol. 330:84-100.

Uehlinger U, 2006. Annual cycle and inter-annual variability of gross primary production and ecosystem respiration in a flood prone river during a 15-year period. Freshwater Biol. 51:938-950.

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE, 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37:130-137.

Vicente-Serrano SM, Lopez-Moreno JI, Beguería S, Lorenzo-Lacruz J, Sanchez-Lorenzo A, García-Ruiz JM, Azorin-Molina C, Morán-Tejeda E, Revuelto J, Trigo R, Coelho F, Espejo F, 2014. Evidence of increasing drought severity caused by temperature rise in southern Europe. Environm. Res. Lett. 9:044001.

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How do low-abundance *taxa* affect river biomonitoring? Exploring the response of different macroinvertebrate-based indices

Simone GUARESCHI,[1*] Alex LAINI,[2] Maria M. SÁNCHEZ-MONTOYA[1,3]

[1]Department of Ecology and Hydrology, Regional Campus of International Importance "Campus Mare Nostrum", University of Murcia, Espinardo Campus, 30100 Murcia, Spain; [2]Department of Life Science, University of Parma, Viale G.P. Usberti 33/A, 43124 Parma, Italy; [3]Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 310, 12587 Berlin, Germany
*Corresponding author: simone.guareschi@um.es

ABSTRACT

The contribution of rare *taxa* to aquatic bioassessments remains a subject of debate, and generates contrasting positions among researchers. Very little is known about the effect of low-abundance taxa (LAT) for calculating both single and multimetric macroinvertebrate-based indices, as well as the ecological status classification. In this study, we aimed to: i) identify the aquatic macroinvertebrates that need special attention during index applications given their low abundance; ii) analyse the effect of excluding LAT on single (IBMWP and IASPT) and multimetric (STAR_ICMi) biological indices; and iii) investigate the influence of LAT on river ecological status assessments. To this end, two different river basins in SE Spain and N Italy with contrasting climatic conditions and river types were selected. Our results showed that almost all the *taxa* at the family level can act as low-abundance *taxa*. In particular, the LAT belonged mainly to Diptera, Trichoptera, Coleoptera, Gastropoda and Hemiptera. The IndVal analysis stressed Tabanidae, Cordulegasteridae and Hydroptilidae as the most characteristic low-abundance families in the Spanish data set, while Dryopidae and Athericidae were identified mostly in N Italy. Excluding LAT affected the studied index values and the resulting bioassessment classification, except for the IASPT index. Loss of the entire LAT pool reduced the ecological status for 78% of the samples for the IBMWP index. Changing took place in 41% of the samples when considering the STAR_ICM index. Relevant changes were detected even when considering loss of 50% of the LAT, especially with the IBMWP index. Similar values and patterns were obtained in each considered quality class and river type. Our results provide useful information about controversial *taxa* and stress the significance of LAT in river biomonitoring. Excluding LAT is discouraged, although different responses according to the considered index were detected. The IBMWP index always obtained lower values (coupled mainly with an underestimation of ecological classes), while STAR_ICMi and, especially the IASPT index, were less affected by excluding LAT. Paying special attention to all the protocol application stages is recommended, with emphasis placed when using the IBMWP index.

Key word: Bioassessment; rare *taxa*; ecological classes; IBMWP; IASPT; STAR_ICMi.

INTRODUCTION

River assessment is extensively based on aquatic macroinvertebrates and numerous biomonitoring indices have been developed for this purpose (Doledéc and Statzner, 2010; Birk *et al.*, 2012; Bo *et al.*, 2016). These indices are generally based on rapid biological assessment protocols (Barbour *el al.*, 1999), followed to obtain a practical and rigorous assessment of river ecological conditions. However, macroinvertebrate sampling activities may reflect just one part of the community, and documenting some *taxa* (*e.g.*, with either low abundances or small distribution ranges) is often time-consuming and cost-intensive in terms of fieldwork, laboratory work, data processing and analyses (Nijboer and Schmidt-Kloiber, 2004).

These *taxa* are generally called "rare" (Gaston 1994; Nijboer and Verdonschot, 2004) and their contribution to aquatic biomonitoring has received particular attention in multivariate analyses for bioassessment (Cao *et al.*, 2001; Marchant 2002) and predictive methods, especially in Anglo-Saxon countries (*e.g.,* the RIVPACS approach; Clarke and Murphy, 2006; Van Sickle *et al.*, 2007). Nevertheless, this debate is ongoing with researchers offering contrasting positions and findings (Cao and Williams, 1999; Marchant *et al.*, 1999; Poos and Jackson, 2012). Very little is known about the effect of rare *taxa* for calculating single or multimetric macroinvertebrate-based indices or about the effect on ecological status assessments, especially in South Europe.

Contrary to *taxa* with small distribution ranges, low-abundance *taxa* (herein referred to as LAT) might be present in numerous samples, sites and habitats, but have the general peculiarity of presenting low number of individuals during sampling activities. Although excluding this kind of *taxa* may be cost-effective (especially in the field) when applying rapid biological assessment protocols, LAT may be common contributors to the assemblage structure in undisturbed streams (Robinson *et al.*, 2000), and might even represent the largest overall richness component (Cao *et al.*, 1998).

The low abundance of a macroinvertebrate *taxon*, or even its absence, from a sample can be attributed to both structural (*i.e.*, an inhospitable environment that does not allow a species to establish) and stochastic (*i.e.*, incomplete characterisation of a macroinvertebrate community) causes (Gray, 2005). In this study, we focused specially on this second aspect as community characterisation can be strictly related with difficulties during fieldwork or laboratory procedures, such as operator efficiency (Metzeling *et al.*, 2003), sorting errors (Haase *et al*., 2010), or subsampling methods (Nichols and Norris, 2006). As a result, some methods and procedures may be biased towards large, abundant and widely distributed *taxa* (Gillies *et al.*, 2009; Haase *et al*., 2010). LAT have fewer chances of being sampled, so their distribution range can be underestimated (Nijboer and Verdonschot, 2004). The imperfect detection of these *taxa* can be a common problem that may affect numerous biological metrics; for instance, total taxon richness and EPT richness, which are two of the main metrics used in biomonitoring (Birk *et al.*, 2012).

In order to improve knowledge on this topic and to provide applied information and recommendations for environmental agencies, technicians and researchers, we aimed to: i) identify the aquatic macroinvertebrates that need special attention during biomonitoring (in different river types) given their potential low abundance; ii) analyse the effect of excluding LAT from the single (IBMWP and IASPT) and multimetric (STAR_ICMi) indices; and iii) test their influence on river ecological status assessments.

When addressing the first objective, we stressed those *taxa* that need special attention while applying protocols by discussing their ecological preferences and features. When dealing with the other two, we investigated whether the exclusion of these *taxa* could be considered crucial for determining not only the index final values, but also ecological classes, to provide useful information about efficient future sampling strategies or line guides. In this way, the comparison of different index outcomes and behaviours is possible and useful as study metrics and indices are among the most widely used tools in river bioassessments (Armitage *et al.*, 1983; Munné and Prat, 2009; Laini *et al.*, 2014; Buss *et al.*, 2015). Finally, controversial issues and possible future implications are discussed.

METHODS

Study area and sampling sites

The present study was carried out by analysing different biogeographical and climatic regions (S Spain and N Italy). We focused on the Segura Basin (SE Spain, Fig. 1a) with 30 sites located in the Murcia, Andalusia and Castilla-la Mancha regions, which were sampled mainly during three seasons in 2000-2003 for 81 sample data (Supplementary Tab. 1). The Segura Basin is an environmentally diverse basin, considered a good candidate to be utilised as a Mediterranean pilot basin (Bruno *et al.*, 2014). Agricultural impacts and dam regulation have been recognised as representing the most important pressures on aquatic ecosystems in this catchment (Kroll *et al.*, 2013; Sánchez-Montoya *et al.*, 2009; Bruno *et al.*, 2014). We considered types, reference values and class boundary values according to those proposed by Sánchez-Montoya *et al.* (2007, 2010) and developed in the Spanish study area. These biological data and types were developed as part of the GUADALMED II Project for Mediterranean Spanish rivers (Prat, 2004). To obtain a representative data set, the sites of four of the five river types in Mediterranean rivers were considered (including temporary and perennial, and different lithologies; Tab. 1).

We also studied the effect of LAT on biomonitoring in the Po Basin (N Italy, Fig. 1b). It is the widest river basin

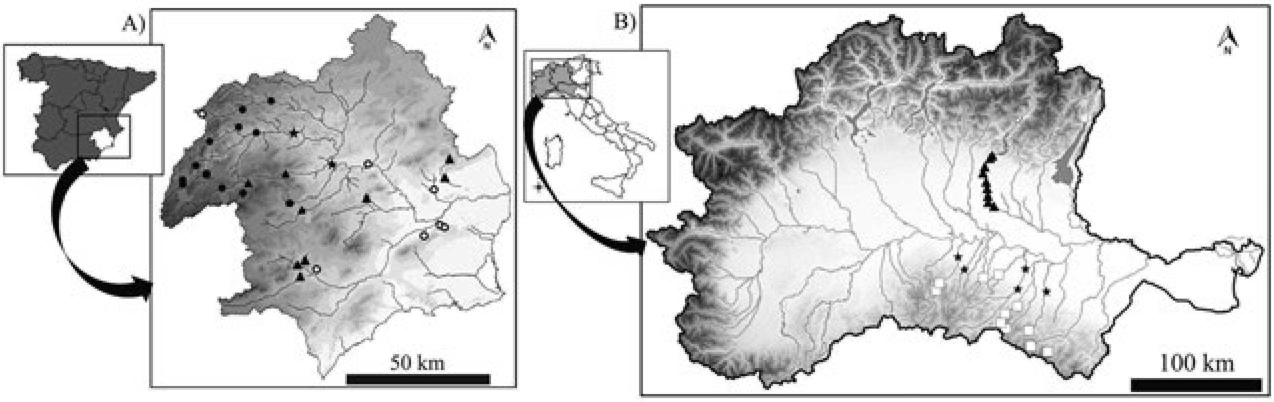

Fig. 1. Study area and location of sampling sites. A) Segura Basin (SE Spain; white circles, T1 sites; black triangles, T2 sites; black circles, T4 sites; black stars, T5 sites). B) Po Basin (N Italy, black triangles, 06GL sites; white squares, 10SS sites; black stars, 06SS sites).

in Italy. We considered 29 representative sites (Lombardia and Emilia-Romagna regions) that were sampled mainly twice (86 sample data; Supplementary Tab. 1). The macroinvertebrate data originate from the fieldwork activities performed from 2009 to 2015. Similar to the Segura Basin, agriculture and flow alterations can be considered the main pressures in the area (Laini *et al.*, 2011; Guareschi *et al.*, 2014). We considered types, reference values and class boundary values according to those published in the Italian ministerial decree DM 260/2010. The data set encompasses three river types from lowland to Apennine streams (Tab. 1, see details on Italian hydro-regions in Buffagni *et al*., 2008).

According to the Köppen-Geiger climate map of Europe (Peel *et al.*, 2007), the study area in SE Spain is classified mostly as arid (code: BSk), while Italian regions are temperate (code: Cfa).

Macroinvertebrate indices: IBMWP, IASPT and STAR_ICMi

We defined low-abundance *taxa* as all the *taxa* whose abundance was ≤3 individuals *per* sample, which corresponds to the first abundance class in the IBMWP index, and as ≤3 *per* 0.5 m^2 for the quantitative STAR_ICMi. Similar criteria have been considered in other studies (Bradley and Ormerod, 2002; Gillies *et al.*, 2009). The response of three different macroinvertebrate-based indices, IBMWP (Iberian Biological Monitoring Working Party), IASPT (IBMWP value/number of families) and STAR_ICMi (Intercalibration Multimetric Index), to LAT exclusion was tested. The first two indices were tested with the Spanish data set, while the Italian data set was used with the last one (see the details below).

The taxonomic resolution needed to calculate these indices is the family level and five 'Ecological status" levels have been established according to European legislation (WFD 2000/60/CE, European Commission 2000) for IBMWP and STAR_ICMi. IBMWP is the most widely used index in Spanish Mediterranean rivers (Alba-Tercedor *et al.*, 2002; Munné and Prat, 2009). It is currently the official index proposed in Spanish rivers (MAGRAMA, 2015) and is an adaptation of the British BMWP scoring system for the Iberian Peninsula, where each family presents a score from 0 to 10 according to their known tolerance to pollution. The IBMWP index is a single metric index (Munné and Prat, 2009) and the sample value is obtained by summing these family scores. This index is considered a simple one (Couto-Mendoza *et al.*, 2015) with a multihabitat semi-quantitative kick procedure in the field (Jáimez-Cuéllar *et al.*, 2002). IASPT represents a sensitive *taxa* index that is easily calculated for each sample as the IBMWP value divided by the number of scoring families detected. It was calculated only for the Spanish data set as this index is currently recognised as a biomonitoring tool in Spain and is commonly used in this area (*e.g.*, Sánchez-Montoya *et al.*, 2010). Unlike the other indices, STAR_ICMi is a multimetric index and is the official index used in Italy for assessing water course quality statuses according to European legislation (Buffagni *et al.*, 2006, 2008). It requires a quantitative sampling activity referee on a surface (0.5 m^2 or 1.0 m^2 depending on the Italian hydro-regions) and has also been used in Europe as an Intercalibration Common Metric Index (Buffagni *et al.*, 2006). It is composed of six metrics: ASPT (Average Score Per Taxon), logarithm of the abundances of the selected families of Ephemeroptera, Plecoptera, Trichoptera and Diptera abundances (log(sel_EPTD+1)), total number of *taxa*, number of EPT *taxa*, 1 minus the relative abundance of Gastropoda, Oligochaeta and Diptera (1-GOLD) and the Shannon index. After calculating the metrics, they were normalised with the reference community values and weighed (see Buffagni *et al.*, 2006, 2007; and the Italian ministerial decree DM 260/2010 for further details).

Tab. 1. Code and description for each river type in the study area. The details for each site are available in Supplementary Tab. 1.

Code	Description
Spanish dataset	
T1	Temporary streams
T2	Evaporite calcareous at medium altitude
T4	Calcareous headwaters at medium and high altitude
T5	Large watercourse
Italian dataset	
06GL	Floodplain watercourse originated from lakes
06SS	Floodplain streams
10SS	Northern Apennine streams

Statistical analyses

First of all, the percentage of LAT and the contribution of each taxonomic group (mainly Order) to the total number of LAT were calculated. To refine the analysis and to deal with the first goal, the Indicator Value analysis (IndVal) was carried out to select specific rare families per river type (Dufrêne and Legendre, 1997; De Cáceres *et al.*, 2010). Such affinity was calculated according to the frequency of each *taxon* in the previously identified groups by taking into account only the sub-communities that composed of LAT. The significance of the Indicator Value (IV) was tested by a Monte Carlo test (999 runs), and the alpha level was set at 0.05.

In order to test the effect of excluding LAT on river biomonitoring indices, they were removed from each sample after considering 100% and 50% of LAT (see details below). Then each index was recalculated and all the samples were reclassified in the corresponding ecological classes. The first case corresponded to excluding the entire pool of LAT (*e.g.*, relevant problems in the sorting or picking phase, operators have little experience). We also tested a more conservative exclusion threshold, which corresponded to a 50% loss of LAT. To do this, the excluded LAT were obtained by considering 100 randomisations of LAT constant loss one by one, and by stopping at the value that equalled a 50% loss of the total LAT pool. At this point, we once again recalculated each index and all the samples were reclassified in the corresponding ecological classes.

The differences between the index values obtained before and after totally excluding LAT were statistically tested by a non-parametric Mann-Whitney test. The results were reported for both the Spanish and the Italian data sets, and even after splitting each data set according to its ecological classes and river type. The behaviour of each index was also studied graphically by focusing on the trend obtained after performing the LAT loss simulations.

To determine whether possible changes in ecological status were equally distributed among the ecological quality classes, we classified the sample data into three classes: High, Good and Less than Good (codes: H, G, < G). The boundary between Good and Moderate is crucial according to European legislation (WFD 2000/60/CE) because it sets the targets for restoration plans in measuring programmes of water bodies which fail the environmental objectives of achieving a good ecological status. All the statistical analyses were performed with the statistical computing R software (R Development Core Team, 2013) with packages “Vegan”, “ade4” and “indicspecies”.

RESULTS

Low-abundance *taxa* characterisation

Ninety-five macroinvertebrate *taxa* (92 families, plus Hydrachnidia, Ostracoda, Oligochaeta) were found in the Spanish basin (mean±SD = 30±11; SD, standard deviation), with a mean value of 11 *taxa* (±5) as LAT (details in Tab. 2). Ninety-one of the 95 *taxa* were identified as rare at least once. The *taxa* with low abundances belonged mainly to Diptera (20%), Trichoptera and Coleoptera (both 14%) in this area (Fig. 2).

The IndVal analysis stressed just one indicator family in river types T1 and T2 as Baetidae and Tabanidae, respectively. Larger groups of indicator families were obtained for types T4 and T5. Cordulegasteridae and Gyrinidae showed the highest IV for T4, with Hydroptil-

Tab. 2. Mean number (±SD) of the low-abundance *taxa* (LAT) for each river type in each data set. The total number of *taxa* (TT) and the total number of LAT are also displayed.

	T1	T2	T4	T5	Total	06GL	10SS	06SS	Total
LAT	8±5	10±3	15±3	10±5	91	8±3	10±4	8±3	90
TT	73	73	83	55	95	68	66	42	92

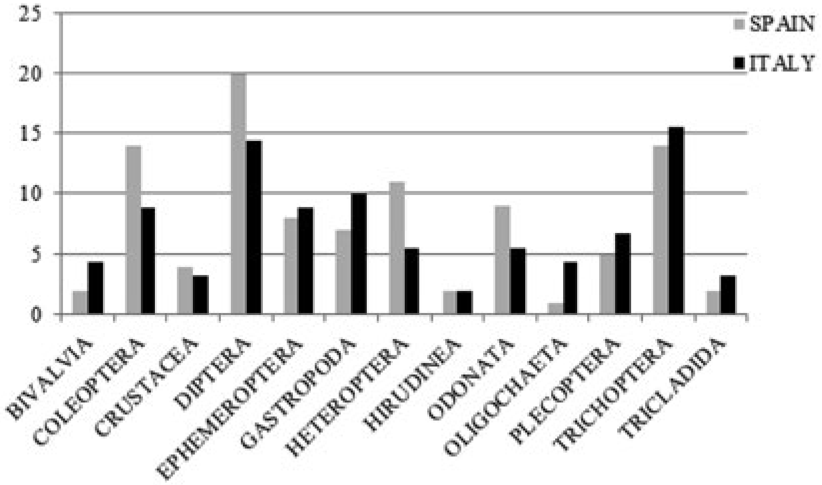

Fig. 2. Distribution of LAT (in %) among the main taxonomic groups (mainly Order). Spanish results are labelled in grey and Italian ones in the darker tone.

idae, Ceratopogonidae and Philopotamidae in the T5 ecosystems (Tab. 3).

Regarding the Italian dataset, 92 *taxa* (91 families, plus Hydrachnidia) were detected (21±5 *per* sample) with a mean value of 9±3 LAT *per* sample. Ninety *taxa* were identified as rare at least once (Tab. 2), where the highest percentage of low-abundance *taxa* belonged to Trichoptera (16%), followed by Diptera, Gastropoda and Coleoptera (Fig. 2).

The IndVal analysis showed only two families as being characteristic LAT in the 06GL river type (Corbiculidae and Aphelocheiridae), whereas a larger number of *taxa* were found in the other types (Tab. 4). Dryopidae, Leuctridae and Oligoneuriidae showed the highest IV for

Tab. 3. The IndVal results for the Spanish data set of LAT (Segura Basin) at the family level.

Order	Family	T	IV	P value
Ephemeroptera	Baetidae	T1	0.19	0.050*
Diptera	Tabanidae	T2	0.37	0.005**
Odonata	Cordulegasteridae	T4	0.32	0.005**
Coleoptera	Gyrinidae	T4	0.31	0.020*
Megaloptera	Sialidae	T4	0.29	0.020*
Diptera	Psychodidae	T4	0.26	0.025*
Trichoptera	Polycentropodidae	T4	0.25	0.040*
Odonata	Aeshnidae	T4	0.24	0.035*
Ephemeroptera	Leptophlebiidae	T4	0.24	0.010**
Plecoptera	Perlodidae	T4	0.21	0.040*
Trichoptera	Hydroptilidae	T5	0.42	0.005**
Diptera	Ceratopogonidae	T5	0.34	0.020*
Trichoptera	Philopotamidae	T5	0.32	0.020*
Odonata	Calopterygidae	T5	0.31	0.005**
Gastropoda	Planorbidae	T5	0.31	0.005**
Coleoptera	Dytiscidae	T5	0.29	0.010**
Gastropoda	Ancylidae	T5	0.22	0.050*

*T, river type; IV, indicator value; **P<0.01; *P<0.05.*

Tab. 4. The IndVal results for the Italian basin of LAT (Po Basin) at the family level.

Order	Family	T	IV	P value
Bivalvia	Corbiculidae	06GL	0.26	0.035*
Hemiptera	Aphelocheiridae	06GL	0.24	0.050*
Coleoptera	Dryopidae	6SS	0.58	0.005
Plecoptera	Leuctridae	6SS	0.30	0.020*
Ephemeroptera	Oligoneuriidae	6SS	0.29	0.005**
Ephemeroptera	Ephemerellidae	6SS	0.27	0.040*
Trichoptera	Polycentropodidae	6SS	0.25	0.040*
Amphipoda	Gammaridae	6SS	0.20	0.050*
Diptera	Athericidae	10SS	0.40	0.005**
Diptera	Empididae	10SS	0.31	0.035*
Coleoptera	Dytiscidae	10SS	0.27	0.005**
Plecoptera	Perlidae	10SS	0.23	0.040*
Trichoptera	Limnephilidae	10SS	0.23	0.010**
Trichoptera	Sericostomatidae	10SS	0.17	0.040*
Coleoptera	Scirtidae	10SS	0.13	0.025*
Hemiptera	Gerridae	10SS	0.13	0.045*
Trichoptera	Glossosomatidae	10SS	0.11	0.040*

*T, river type; IV, indicator value; **P<0.01; *P<0.05.*

floodplain rivers (06SS), while Athericidae and Empididae were identified in Apennine streams (10SS, Tab. 4).

Effect of excluding low-abundance *taxa* on single metric indices: IBWMP and IASPT

Excluding the entire pool of LAT (100%) resulted in significant differences compared with the IBMWP results (W=3321; P<0.0001). The original IBMWP mean value was 148; on the contrary when the index was recalculated, we obtained a mean value that equalled 93. The ecological quality classification of 29 of the 30 sampling sites changed at least once, and the ecological status of 78% of the samples changed to a minor class (see Tab. 5). Moreover, 82% of the values first classified as High shifted to a minor status (Good). Similar results were obtained (74-75%) when the starting point was Good or Less than Good. Substantial changes were noted for each river type in the analysis (from 33% to 100%), which was especially true for the T2 streams that exhibited huge changes in quality assessment when the starting points were High or Less than Good classes.

The 50% randomised LAT loss also led to relevant changes in the IBMWP values, but the percentage of class changes lowered compared to the total LAT loss (Tab. 5). The ecological status of about half the overall samples (48%) changed (24 of the 30 sites changed at least once). Once again, percentages were similar among different classes (43-53%). When focusing on each river type separately, the patterns were basically the same (compared with the total LAT loss), with changes falling between 33% and 100% of cases depending on ecological class. When LAT were not considered, all the river types presented similar patterns, and their IBMWP values clearly and constantly dropped (Fig. 3).

Unlike the IBMWP results, the IASPT recalculations (when all the LAT were excluded) gave values that did not statistically differ from the original ones (W=1382, P=0.1906). Graphically, the IASPT behaviour of LAT loss appeared constant and displayed a horizontal trend, with the only exception being river type T1, where more diversified responses were obtained (Fig. 4).

Effect of excluding low-abundance *taxa* on a multimetric index: STAR_ICMi

The exclusion of all the LAT (100%) led to significant differences when we compared the STAR_ICMi results (W=3781; P<0.0001). The original STAR_ICMi mean value was 0.74, which became 0.62 when recalculated. The ecological quality classification of 19 of the 29 sampling sites underwent at least one class change. The ecological status of 41% of all the samples changed to a minor class. Similar values and patterns were obtained through different ecological classes (36-41%), except when the starting point was High class (100% change, but a limited number of data were available; Tab. 6).

The 50% randomised loss of LAT brought about changes in the STAR_ICMi values (Tab. 6). The ecological status of 33% of the overall samples changed (17 of 29 sites underwent at least one change). Once again, the percentages were similar among the different classes (23-36%), expect when the original starting point was the High class. When focusing on each river type separately, and compared to the total LAT loss, the percentage of class changes lowered, except for river type 06SS (Tab. 6). When we graphically analysed the behaviour of STAR_ICMi, the response patterns were not as clear as they were for the other indices. A decreasing tendency seemed the commonest behaviour in the three studied river types when LAT were excluded (Fig. 5).

DISCUSSION

Low-abundance *taxa*

Despite the climatic and environmental differences in the two study areas, LAT belonged mainly to the same or-

Tab. 5. Number of changes of ecological quality classes for the 100% or 50% LAT loss. The results are displayed after considering the overall number of samples (Total), divided by river type (T1, T2, T4, T5) and ecological class (the classification represents the starting point class before excluding LAT) in the Spanish data set. Number of sampling data *per* river type is also displayed.

	Spain (Segura Basin)									
	Total	**Total**	**T1 (n=16)**		**T2 (n=25)**		**T4 (n=34)**		**T5 (n=6)**	
	100%	**50%**	**100%**	**50%**	**100%**	**50%**	**100%**	**50%**	**100%**	**50%**
Changes	78%	48%	12 (75%)	9 (56%)	21 (84%)	13 (52%)	27 (79%)	16 (47%)	3 (50%)	1 (17%)
No changes	22%	52%	4 (25%)	7 (44%)	4 (16%)	12 (48%)	7 (21%)	18 (53%)	3 (50%)	5 (83%)
Classes										
High	82%	53%	2 (67%)	2 (67%)	5 (100%)	5 (100%)	22 (81%)	12 (44%)	2 (67%)	1 (33%)
Good	74%	43%	7 (88%)	5 (63%)	13 (76%)	6 (35%)	5 (71%)	4 (57%)	1 (33%)	0
<Good	75%	50%	3 (60%)	2 (40%)	3 (100%)	2 (67%)	-	-	-	-

ders in both data sets (*e.g.*, Diptera, Trichoptera and Coleoptera) and were basically those stressed by Nijboer and Schmidt-Kloiber (2004) for Dutch streams. When focusing on the Spanish data set, and according to the criterion of IV>25 as a key value to judge an indicator *taxon* as being adequate (Bonada *et al.*, 2008, following Dufrêne and Legendre, 1997), Baetidae was not a good indicator for the T1 streams. On the contrary in the T2 streams, Ta-

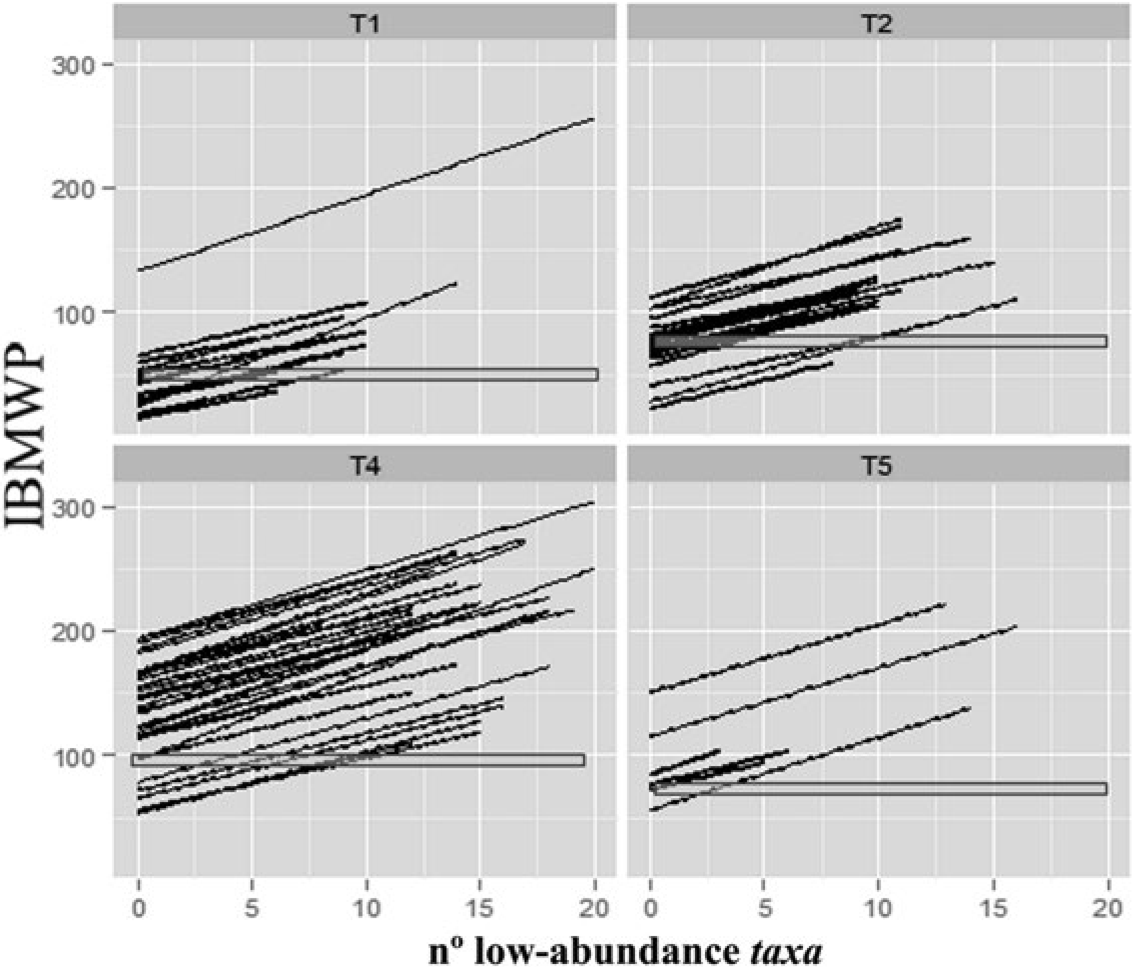

Fig. 3. IBMWP index behaviour per river type (T1, T2, T4, T5) after randomisations of constant LAT loss. The boundary value between the Good and Moderate conditions is displayed. The index values are displayed on the y-axis and the number of considered LAT is found on the x-axis.

Tab. 6. Number of changes of ecological quality classes for the 100% or 50% LAT loss. The results are displayed after considering the overall number of samples (Total), divided by river type (06GL, 10SS, 06SS) and ecological class (the classification represents the starting point class before excluding LAT) in the Italian data set. Number of sampling data *per* river type is also displayed.

	Italy (Po Basin)							
	Total	**Total**	**06GL (n=55)**		**10SS (n=24)**		**06SS (n=7)**	
	100%	**50%**	**100%**	**50%**	**100%**	**50%**	**100%**	**50%**
Changes	41%	33%	21 (38%)	17 (31%)	10 (42%)	7 (29%)	4 (57%)	4 (57%)
No changes	59%	67%	34 (62%)	38 (69%)	14 (58%)	17 (71%)	3 (43%)	3 (43%)
Classes								
High	100%	100%	-	-	-	-	3 (100%)	3(100%)
Good	41%	36%	9 (38%)	8 (33%)	8 (50%)	7 (44%)	1 (25%)	1 (25%)
<Good	36%	23%	12 (39%)	9 (29%)	2 (25%)	0 (0%)	-	-

banidae needed special attention during sample and sorting activities, as did a large group of *taxa* dominated by Cordulegasteridae and Hydroptilidae for the T4 and T5 streams, respectively. Cordulegasteridae larvae (genus *Cordulegaster*), Tabanidae and Athericidae (see below) are generally predators of other aquatic invertebrates (Tachet *et al.*, 2010). Members of these feeding groups have already been stressed as being relatively less abundant than prey species (Spencer, 2000). Hydroptilidae larvae (Trichoptera) usually prefer specific microhabitats with submerged vegetation where they anchor their cocoons which, coupled with their limited size and lack of movements (under dry and wet conditions) (Tachet *et al.,* 2010), may make them particularly difficult to detect.

None of the *taxa* stressed by the analysis was characterised by extreme IBMWP scores, and higher values were depicted by some Odonata (*e.g.*, Cordulegasteridae) or Trichoptera (*e.g.*, Philopotamidae). Most of the indicator *taxa* presented intermediate values, basically with scores of around 3, 4, or even 6 points, as confirmed by the IASPT response to LAT loss (Fig. 4). The mean *taxa* scores did not change, but stabilised with values between 4 and 6. In this situation, LAT presented a comparable IBMWP score with the commonest *taxa*. These results (Fig. 4) agree with the research of Nijboer and Schmidt-Kloiber (2004), who found that the mean scores for saprobic valences were similar for both *taxa* types (low and high abundances). If LAT had been *taxa* with an extreme IBMWP score (1 or 10), the IASPT responses to their loss would have shifted towards a marked change in their slopes.

According to the Italian results, it was difficult to suggest specific LAT for the 06GL rivers if we considered that the IndVal stressed *taxa* with IV came close to 25. On the contrary, Dryopidae (Coleoptera) and Athericidae (Diptera) were underlined as characteristic LAT in 06SS and 10SS, respectively. Special attention needs to be paid

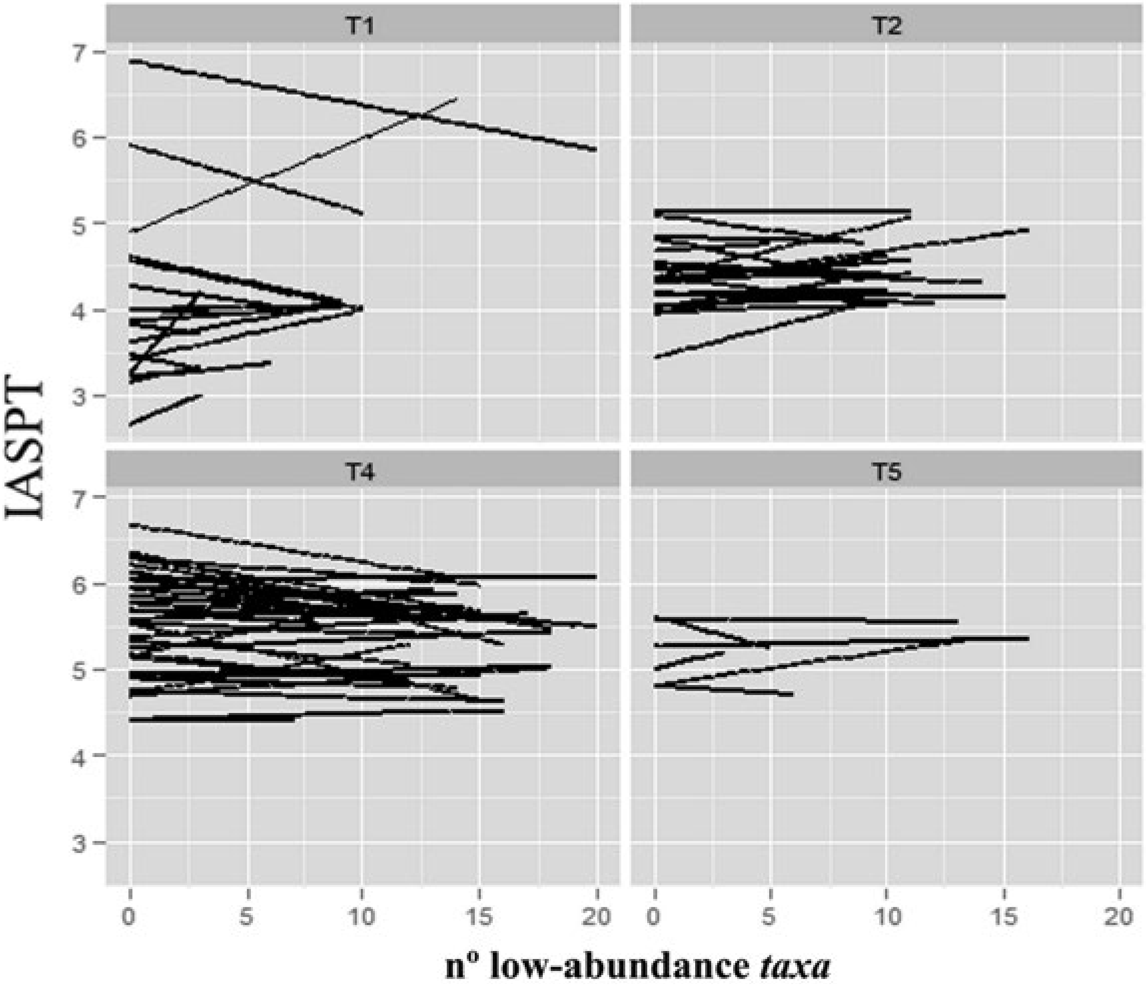

Fig. 4. IASPT index behaviour per river type (T1, T2, T4, T5) after randomisations of constant LAT loss. The index values are displayed on the y-axis and the number of considered LAT is found on the x-axis.

to these *taxa* in all the sampling phases and for all laboratory activities. Dryopidae larvae are generally semi-aquatic or riparian (Jäch and Balke, 2008) and are, therefore, rarer to detect during sampling activity as only adults are strictly aquatic. Furthermore, Dryopidae have already been stressed as infrequent in low mountainous streams in other European countries (Slovakia: Zatovičová *et al*., 2004). Regarding specific LAT features, other studies have generally found that these *taxa* are associated with standing or slowly flowing waters (Nijboer and Schmidt-Kloiber, 2004).

Our results in both data sets only partially confirmed this finding by considering that a heterogeneous group of LAT was found. This group was composed of some lotic preference taxa, but also some highly diverse taxa (Coleoptera, Diptera), and even some strictly lentic ones (Odonata, Gastropoda, and Hemiptera). However, benthic sampling methodologies like those utilised (D-net and Surber, following official legislation) could also be the reason for the low abundance found in some groups (*e.g.*, swimmers or surface skaters). Most of these *taxa* (*e.g.*, Coleoptera or Hemiptera) live on the water surface or among vegetation, and can easily escape sampling nets. Nevertheless, the LAT identified herein cannot be considered rare or endemic in terms of small range areas, especially given taxonomic resolution (family level).

Performance of indices and implication for bioassessment

Excluding LAT resulted in underestimating ecological status (values lower than reality) for STAR_ICM and IBMWP indices. This effect became considerably stronger after applying the IBMWP index, with relevant

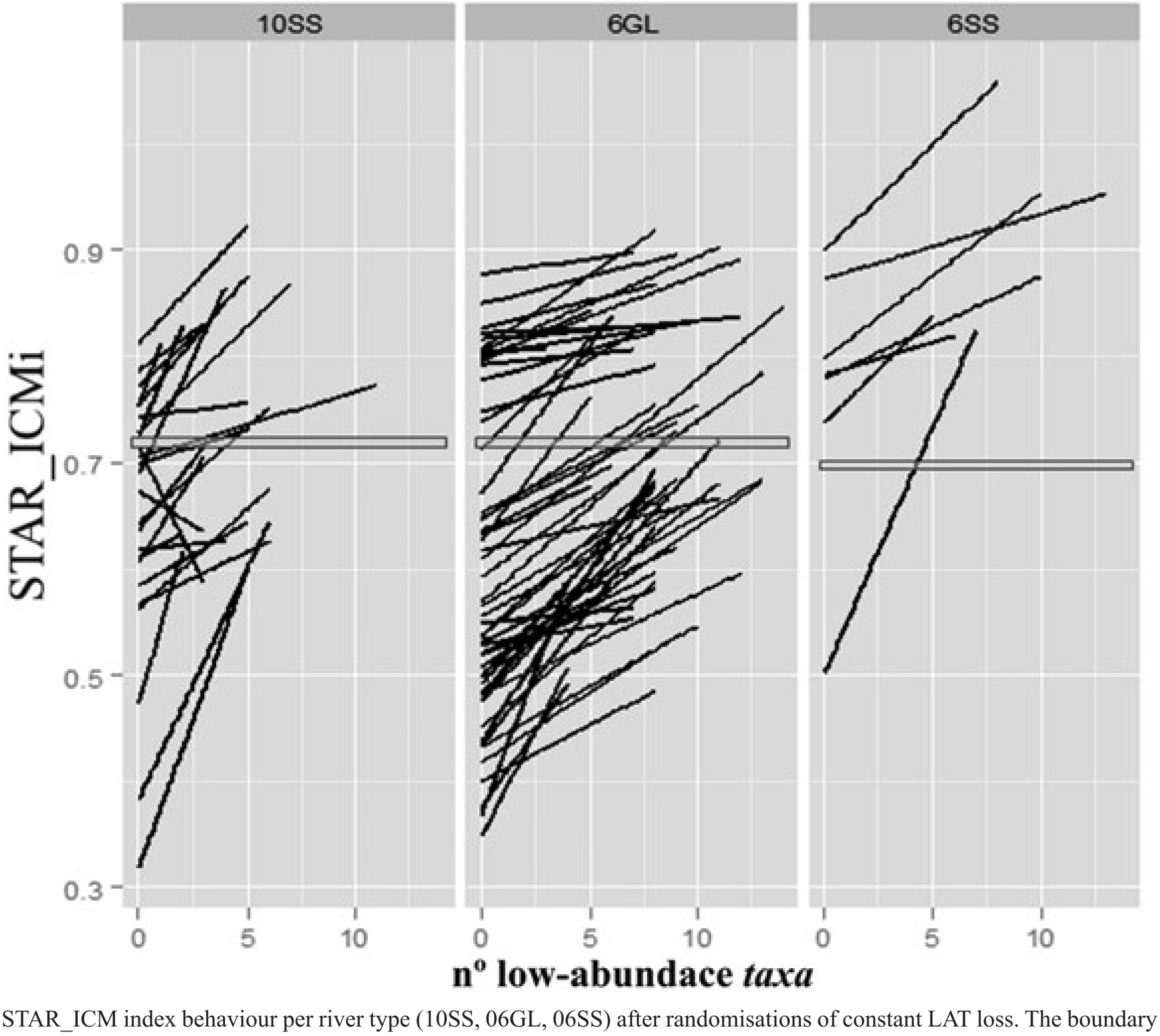

Fig. 5. STAR_ICM index behaviour per river type (10SS, 06GL, 06SS) after randomisations of constant LAT loss. The boundary value between the Good and Moderate conditions is displayed. The index values are displayed on the y-axis and the number of considered LAT is found on the x-axis.

changes in the ecological evaluations. This is strictly related with the index formula, which exclusively represents a sum of family scores and is strongly affected by a reduction in the total recognised taxa.

Different outcomes were detected when focusing on the IASPT index. This index did not appear sensitive when LAT were excluded from three of the four Mediterranean river types, and displayed a contrasting response in temporary rivers (T1). Applying biomonitoring procedures to temporary aquatic ecosystems is currently one on the main bioassessment challenges and requires specific tools (Nikolaidis *et al.,* 2013; Datry *et al.*, 2014; Prat *et al.,* 2014). The general observed lack of sensitivity of this index can be considered an interesting attribute (*i.e.*, no specific knowledge or experience required), but this approach may be questionable, or even dangerous (Metzeling *et al.*, 2003; Haase *et al.*, 2006). We should also consider that the IASPT index has been reported to be less sensitive to stressors than the IBMWP index in Mediterranean rivers (Sánchez-Montoya *et al.*, 2010).

The Italian index STAR_ICM gave an intermediate response compared to the other indices. Following LAT loss, the results were generally underestimated, but more slightly than for the IBMWP index. Once again, this is probably related with the index formula. As it was a multimetric index, and not just a mathematic sum, it could be less affected by the reduction in the total taxa considered herein. Richness metrics formed part of the index (*e.g.*, total of families, total EPT *taxa*), but the metrics with the heaviest weight in the definition of the index was the Average Score *Per Taxon* (ASPT) value. Knowing the specific behaviour of the Spanish ASPT (investigated herein) could help explain the attenuated STAR tendencies.

Unlike our results, Nijboer and Schmidt-Kloiber (2004) reported that excluding LAT overestimated the ecological quality class in Dutch lowland streams with the AQEM software (Hering *et al.*, 2004). These contrasting findings, which are especially strong between IBMWP and AQEM, may be due to the different geographic contexts and river types considered (*e.g.*, Northern *vs* Southern Europe), but could also be due to specific differences between protocols (*e.g.*, taxonomic resolution, sampling area). Despite the AQEM method and STAR_ICMi presenting similar procedures (Hering *et al.*, 2006), contrasting results were obtained as LAT exclusion did not generally lead to overestimates in the Italian data set. Nevertheless, in both cases (underestimation and overestimation), these mismatches could have serious consequences for environmental agencies and water managers during environmental management (*e.g.*, restoration where it is not necessary), which can provide equivocal pictures of river ecosystem health.

CONCLUSIONS

Our study is one of the first attempts to test the effects of excluding LAT in several widely used European indices. Previous studies have tested macroinvertebrates at the genus or species level (Cao *et al.*, 1998; Resh *et al.*, 2005), and have mainly examined the effects of LAT on richness metrics. Our results demonstrated that even at the family level and in two different study areas, LAT represent a large part of overall richness. Their exclusion strongly lowered the number of *taxa* in the samples regardless river typology, with effects on bioassessment depending on the index employed. In this context, the implementation of quality control mechanisms into macroinvertebrate assessment procedures seems recommendable.

Finally, by considering the increasing impacts and challenges of alien species on aquatic ecosystems (Havel *et al.*, 2015; Fenoglio *et al.,* 2016), alien *taxa* must be specially considered because they can be generally characterised by initial low abundances in their first invasion phases. Indeed, in our study, Corbiculidae (*e.g.*, *Corbicula fluvialis*) are reported as LAT in some Italian rivers. This fact may lead to some mismatches or variations in the final index scores, and similar problems have already been stressed in other European countries (Gabriel *et al.*, 2005). Specific reflexions and future adjustments to studied systems in both countries should be made (*e.g.*, definition of specific tolerance classes or periodically reviewing the *taxa* list by considering possible taxonomic modifications and future invaders) to improve ecological assessment tools in freshwater ecosystems.

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REFERENCES

Alba-Tercedor J, Jáimez-Cuellar P, Álvarez M, Avilés J, Bonada N, Casas J, Mellado A, Ortega M, Pardo I, Prat N, Rieradevall M, Robles S, Sáinz-Cantero CE, Sánchez-Ortega A, Suárez ML, Toro M, Vidal-Abarca MR, Vivas S, Zamora-Muñoz C, 2002. [Caracterización del estado ecológico de ríos mediterráneos ibéricos mediante el índice IBMWP (antes BMWP´)].[Article in Spanish]. Limnetica 21:175-186.

Armitage PD, Moss D, Wright JF, Furse MT, 1983. The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. Water Res. 7:333-347.

Barbour MT, Gerritsen J, Snyder BD, Stribling JB, 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Washington, D.C.

Birk S, Bonne W, Borja A, Brucet S, Courrat A, Poikane S, Solimini A, van de Bund W, Zampoukas N, Hering D, 2012. Three hundred ways to assess Europe's surface waters: an almost complete overview of biological methods to implement the Water Framework Directive. Ecol. Ind. 18:31-41.

Bo T, Doretto A, Laini A, Fenoglio S, 2017. Biomonitoring with macroinvertebrate communities in Italy: What happened to our past and what's the future? J. Limnol. 76(Suppl.1):21-28. [Epub 17 Oct 2016].

Bonada N, Prat N, Resh VH, Statzner B, 2006. Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. Annu. Rev. Entomol. 51:495-523.

Bonada N, Rieradevall M, Dallas H, Davis J, Day J, Figueroa R, Resh VN, Prat N, 2008. Multi-scale assessment of macroinvertebrate richness and composition in Mediterranean-climate rivers. Freshwater Biol. 53:772-788.

Bradley DC, Ormerod SJ, 2002. Evaluating the precision of kick-sampling in upland streams for assessments of long-term change: the effects of sampling effort, habitat and rarity. Arch. Hydrobiol. 155:199-221.

Bruno D, Belmar O, Sánchez-Fernández D, Guareschi S, Millán A, Velasco J, 2014. Responses of Mediterranean aquatic and riparian communities to human pressures at different spatial scales. Ecol. Ind. 45:456-464.

Buffagni A, Erba S, Cazzola M, Murray-Bligh J, Soszka H, Genoni P, 2006. The Star common metrics approach to the WFD intercalibration process: full application for small, lowland rivers in three European countries. Hydrobiologia 566:379-399.

Buffagni A, Erba S, Furse MT, 2007. A simple procedure to harmonize class boundaries of assessment systems at the pan-European scale. Environ. Sci. Policy 10:709-724.

Buffagni A, Erba S, Pagnotta R, 2008. [Definizione dello stato ecologico dei fiumi sulla base dei macroinvertebrati bentonici per la 2000/60/EC (WFD): il sistema di classificazione MacrOper].[Report in Italian]. Accessed on: 17 February 2015. Available from: http://www.irsa.cnr.it/Docs/Notiz/notiz2008_%28NS%29.pdf

Buss DF, Carlisle DM, Chon TS, Culp J, Harding JS, Keizer-Vlek HE, Robinson WA, Strachan S, Thirion C, Hughes RM, 2015. Stream biomonitoring using macroinvertebrates around the globe: a comparison of large-scale programs. Environ. Monit. Assess. 187:1-21.

Cao Y, Williams D, Williams NE, 1998. How important are rare species in aquatic community ecology and bioassessment? Limnol. Oceanogr. 43:1403-1409.

Cao Y, Williams D, 1999. Rare species are important in bioassessment (Reply to the comment by Marchant). Limnol. Oceanogr. 44:1841-1842.

Cao Y, Larsen DP, Thorne RS, 2001. Rare species in multivariate analysis for bioassessment: some consideration. J. N. Am. Benthol. Soc. 20:144-153.

Clarke RT, Murphy JF, 2006. Effects of locally rare taxa on the precision and sensitivity of RIVPACS bioassessment of freshwaters. Freshwater Biol. 51:1924-1940.

Couto-Mendoza MT, Vieira-Lanero R, Cobo F, 2015. More complexity does not always mean more accuracy: the case of IBMWP and METI in NW Spain. Ecohydrology 8:595-609.

Datry T, Larned ST, Tockner K, 2014. Intermittent rivers: a challenge for freshwater ecology. BioScience 64:229-235.

De Cáceres M, Legendre P, Moretti M, 2010. Improving indicator species analysis by combining groups of sites. Oikos 119:1674-1684.

Dolédec S, Statzner B, 2010. Responses of freshwater biota to human disturbances: contribution of J-NABS to developments in ecological integrity assessments. J. N. Am. Benthol. Soc. 29:286-311.

Dufrêne M, Legendre P, 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67:345-366.

European Commission, 2000. Directive 2000/60/EC of the European Parliament of the Council of 23rd October 2000 establishing a framework for community action in the field of water policy. OJ L 327, 22.12.2000, p. 1-73.

Fenoglio S, Bonada N, Guareschi S, López-Rodríguez M., Millán A, Tierno de Figueroa JM, 2016. Freshwater ecosystems and aquatic insects: A paradox in biological invasions. Biol. Lett. 12:20151075.

Gabriels W, Goethals PLM, De Pauw N, 2005. Implications of taxonomic modifications and alien species on biological water quality assessment as exemplified by the Belgian Biotic Index method. Hydrobiologia 542:137-150.

Gaston KJ, 1994. Rarity. Population and community biology. Series 13. Chapman & Hall, London: 395 pp.

Gillies CL, Hose GC, Turak E, 2009. What do qualitative rapid assessment collections of macroinvertebrates represent? A comparison with extensive quantitative sampling. Environ. Monit. Assess. 149:99-112.

Gray BR, 2005. Selecting a distributional assumption for modelling relative densities of benthic macroinvertebrates. Ecol. Model. 185:1-12.

Guareschi S, Laini A, Racchetti E, Bo T, Fenoglio S, Bartoli M, 2014. How do hydromorphological constraints and regulated flows govern macroinvertebrate communities along an entire lowland river? Ecohydrology 7:366-377.

Jáimez-Cuéllar P, Vivas S, Bonada N, Robles S, Mellado A, Álvarez M, Avilés J, Casas J, Ortega M, Pardo I, Prat N, Rieradevall M, Sáinz-Cantero CE, Sánchez-Ortega A, Suárez ML, Toro M, Vidal-Abarca MR, Zamora-Muñoz C, Alba-Tercedor J, 2002. [Protocolo Guadalmed (PRECE)].[Article in Spanish]. Limnetica 21:187-204.

Haase P, Pauls SU, Schindehütte K, Sundermann A, 2010. First audit of macroinvertebrate samples from an EU Water Framework Directive monitoring program: human error greatly lowers precision of assessment results. J. N. Am. Benthol. Soc. 29:1279-1291.

Haase P, Murray-Bligh J., Lohse S, Pauls S, Sundermann A, Gunn R, Clarke RT, 2006. Assessing the impact of errors in sorting and identifying macroinvertebrate samples. Hydrobiologia 566:505-521.

Havel JE, Kovalenko KE, Thomaz SM, Amalfitano S, Kats LB,

2015. Aquatic invasive species: challenges for the future. Hydrobiologia 750:147-170.

Hering D, Moog O, Sandin L, Verdonschot PFM, 2004. Overview and application of the AQEM assessment system. Hydrobiologia 516:1-20.

Hering D, Feld CK, Moog O, Ofenböck T, 2006. Cook book for the development of a multimetric index for biological condition of aquatic ecosystems: experiences from the European AQEM and STAR projects and related initiatives. Hydrobiologia 566:311-324.

Jäch MA, Balke M, 2008. Global diversity of water beetles (Coleoptera) in freshwater. Hydrobiologia 595:419-442.

Kroll SA, Ringler NH, De las Heras J, Goméz-Alday JJ, Moratalla A, Briggs RD, 2013. Analysis of anthropogenic pressures in the Segura Watershed (SE Spain), with a focus on inter-basin transfer. Ecohydrology 6:878-888.

Laini A, Bartoli M, Castaldi S, Viaroli P, Capri E, Trevisan M, 2011. Greenhouse gases (CO_2, CH_4 and N_2O) in lowland springs within an agricultural impacted watershed (Po River plain, northern Italy). Chem. Ecol. 27:177-187.

Laini A, Vorti A, Bolpagni R, Viaroli P, 2014. Small-scale variability of benthic macroinvertebrates distribution and its effects on biological monitoring. Ann. Limnol.-Int. J. Lim. 50:211-216.

Marchant R, Yong C, Williams D, 1999. How important are rare species in aquatic community ecology and bioassessment? A comment on the conclusions of Cao et al. Authors' reply. Limnol. Oceanogr. 44:1840-1842.

Marchant R, 2002. Do rare species have any place in multivariate analysis for bioassessment? J. N. Am. Benthol. Soc. 21:311-313.

Metzeling L, Chessman B, Hardwick R, Wong V, 2003. Rapid assessment of rivers using macroinvertebrates: The role of experience, and comparisons with quantitative methods. Hydrobiologia 510:39-52.

MAGRAMA (Ministerio de Agricultura, Alimentación y Medio Ambiente), 2015. [Criterios de seguimiento y evaluación del estado de las aguas superficiales y las normas de calidad ambiental. Boletín Oficial del Estado-BOE, 219: 80582-80677].[Report in Spanish]. Ministerio de Medio Ambiente, Madrid, Spain.

Munné A, Prat N, 2009. Use of macroinvertebrate-based multimetric indices for water quality evaluation in Spanish Mediterranean rivers: an intercalibration approach with the IBMWP index. Hydrobiologia 628:203-225.

Nichols SJ, Norris RH, 2006. River condition assessment may depend on the sub-sampling method: field live-sort versus laboratory sub-sampling of invertebrates for bioassessment. Hydrobiologia 572:195-213.

Nijboer RC, Verdonschot PF, 2004. Rare and common macroinvertebrates: definition of distribution classes and their boundaries. Arch. Hydrob. 161:45-64.

Nijboer RC, Schmidt-Kloiber A, 2004. The effect of excluding taxa with low abundances or taxa with small distribution ranges on ecological assessment. Hydrobiologia 516:347-363.

Nikolaidis NP, Demetropoulou L, Froebrich J, Jacobs C, Gallart F, Prat N, Lo Porto A, Papadoulakis V, Campana C, Skoulikidis N, Davy T, Bidoglio G, Bouraoui F, Kirkby MJ, Tournoud MG, Polesello S, González-Barberá G, Cooper D, Gomez R, Sánchez-Montoya MM, De Girolamo AM, 2013. Towards a sustainable management of Mediterranean river basins: policy recommendations on management aspects of temporary river basins. Water Policy 15:830-849.

Poos MS, Jackson DA, 2012. Addressing the removal of rare species in multivariate bioassessments: the impact of methodological choices. Ecol. Ind. 18:82-90.

Peel MC, Finlayson BL, McMahon TA, 2007. Updated world map of the Köppen-Geiger climate classification. Hydrol. Earth Syst. Sci. 4:39-473.

Prat N, 2004. El Proyecto GUADALMED. Limnetica 21:1-3.

Prat N, Gallart F, von Schiller D, Polesello S, García-Roger EM, Latron J, Rieradevall M, Llorens P, Barberá GG, Brito D, De Girolamo AM, Dieter D, Lo Porto A, Buffagni A, Erba S, Nikolaidis NP, Querner EP, Tournoud MG, Tzoraki O, Skoulikidis N, Gómez R, Sánchez-Montoya MM, Tockner K, Froebrich J, 2014. The Mirage Toolbox: An integrated assessment tool for temporary streams. River. Res. Appl. 30:1318-1334.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Resh VH, Beche LA, McElravy EP, 2005. How common are rare taxa in long-term benthic macroinvertebrate surveys? J. N. Am. Benthol. Soc. 24:976-989.

Robinson CT, Minshall GW, Royer TV, 2000. Inter-annual patterns in macroinvertebrate communities of wilderness streams in Idaho, USA. Hydrobiologia 421:187-198.

Sánchez-Montoya MM, Punti T, Suarez ML, Vidal-Abarca MR, Rieradevall M, Poquet JM, Zamora-Muñoz C, Robles S, Álvarez M, Alba-Tercedor J, Toro M, Pujante AM, Munné A, Prat N, 2007. Concordance between ecotypes and macroinvertebrate assemblages in Mediterranean streams. Freshwater Biol. 52:2240-2255.

Sánchez-Montoya MM, Vidal-Abarca M.R, Puntí T, Poquet JM, Prat N, Rieradevall M, Alba-Tercedor J, Zamora-Muñoz C, Toro M, Robles S, Álvarez M, Suárez ML, 2009. Defining criteria to select reference sites in Mediterranean streams. Hydrobiologia 619:39-54.

Sánchez-Montoya MM, Vidal-Abarca MR, Suárez ML, 2010. Comparing the sensitivity of diverse macroinvertebrate metrics to a multiple stressor gradient in Mediterranean streams and its influence on the assessment of ecological status. Ecol. Ind. 10:896-904.

Spencer M, 2000. Are predators rare? Oikos 89:115-122.

Van Sickle J, Larsen DP, Hawkins CP, 2007. Exclusion of rare taxa affects performance of the O/E index in bioassessments. J. N. Am. Benthol. Soc. 26:319-331.

Zatovičová Z, Čiampor Jr F, Kodada J, 2004. Aquatic Coleoptera (Insecta) of streams in the Nízke Beskydy Region (Slovakia): faunistics, ecology and comparison of sampling methods. Biologia 59:181-189.

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Biomonitoring with macroinvertebrate communities in Italy: What happened to our past and what is the future?

Tiziano BO,[1] Alberto DORETTO,[2] Alex LAINI,[3] Francesca BONA,[2] Stefano FENOGLIO[1*]

[1]Department of Science and Technological Innovation, University of Piemonte Orientale, Viale T. Michel 25, 15121 Alessandria; [2]Department of Life Sciences and Systems Biology, University of Turin, Via Accademia Albertina 13, 10123 Turin; [3]Life Sciences Department, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy
*Corresponding author: stefano.fenoglio@uniupo.it

ABSTRACT

This paper reviews the history and development of biological water quality assessment using macroinvertebrates in Italy. Italy was one of the first European countries to officially adopt a biomonitoring system based on benthic invertebrates, the "Indice Biotico Esteso" (IBE). After the European Water Framework Directive (WFD) 2000/60/EC, this method was replaced by the "Standardisation of River Classifications_Intercalibration Common Metrics" (STAR_ICM) index, which met the new requirements. As this method has been employed for some years, it could be useful to take a provisional stock and to provide some suggestions to ameliorate the current biomonitoring approach, also trying to minimize the break with past practices and better harmonize the history of biomonitoring in Italy. One of the most evident difference between past and current approach is related to the amount of time and effort required in the application of the two methods. STAR_ICM is a scientifically rigorous and modern method, but much more time-consuming and challenging in both field and laboratory efforts. This fact has various disturbing practical repercussions, *i.e.,* the environmental agencies have generally reduced the number of sampling stations routinely monitored during the year. The aim of our work is to propose some operational changes that would help to simplify and expedite the monitoring process. In particular, regarding fieldwork, we focus on the time and effort required for macroinvertebrate collection, while for laboratory activity we suggest a reshaping of the requested taxonomic detail. Moreover, in this way the data provided by the new approach could be compared with the long time series available from the previous application of IBE.

Key word: IBE; STAR_ICMi; WFD 2000/60; environmental quality assessment; running waters.

INTRODUCTION

A brief history of biological monitoring with macroinvertebrates

In the last decades, biological monitoring of running water systems has become increasingly important as an indispensable complement to traditional chemical-physical techniques in the evaluation of anthropic impacts (Barbour *et al.*, 1999; Birk *et al.*, 2012; Friberg, 2014). A wide range of techniques blossomed throughout the last century (Hellawell, 1986) and, whilst a variety of biological groups continued to be considered (*e.g.,* bacteria, benthic algae, fish), the use of benthic macroinvertebrates became by far the most common method (Metcalfe, 1989). Nowadays, benthic macroinvertebrates represent the most widely used group of organisms in freshwater biomonitoring, due to their different sensitivity to changes in both chemical characteristics of the water column and physical properties of habitats (Rossaro *et al.*, 2011; Szivák, and Csabai, 2012). Macroinvertebrates are a heterogeneous group of ubiquitous and abundant organisms, relatively easy to collect, identify and enumerate (Bonada *et al.*, 2006). Furthermore, the relatively long length of life cycles of many species and their constant presence in the same locality make the analysis of their community structure an effective tool to detect the occurrence of human pressures over long time periods (Allan and Castillo, 2007).

Benthic macroinvertebrates have a long history as key component of biomonitoring tools, dating back to the beginning of the 20th century (Cairns and Pratt, 1993). In fact, the idea of using macroinvertebrates as biological indicators began in Europe with the studies of Kolkwitz and Marsson (1908). Their Saprobien system relied on the fact that some organisms could be used as indicators of specific environmental conditions, and was essentially aimed at relating the organic load to the presence and distribution of benthic invertebrates in rivers. This system is still widely adopted in Central Europe, mainly in countries with German influence, while it has found minor acceptance in other areas. Its main biases are the specific and geographically restricted taxonomic approach, and limited applicability in the detection of pollution other than organic load. For these reasons other indices were developed, combining the indicator value concept with biodiversity and relative abundance of different selected groups. The Trent Biotic Index was a pioneering and seminal approach, designed to assess the water quality status of the Trent River (Woodiwiss, 1964). This method two main aspects of benthic communities: the biological richness, *i.e.* the number of collected taxa, and the presence of some key groups, characterized by different levels of tolerance to environmental alteration. The index ranges from zero (polluted condition) to ten (clean waters). In those years other methods arose, and among them specific scores were attributed to different selected taxa according to their abundance (Chandler, 1970) or tolerance. These approaches developed into true biotic score indices, among which the Biological Monitoring Working Party (BMWP; Hellawell, 1986) assumed a particular importance. In this method, taxonomic identification is easier, because performed at family level. Each family is associated to a specific score, depending on its sensitivity to environment alteration; for example, Heptageniidae are scored ten, while Chironomidae two.

Biomonitoring methods that use macroinvertebrates to assess lotic ecosystem quality are nowadays a key topic in freshwater ecology (Guareschi *et al.*, 2017) and they are employed in many countries, such as United States of America, United Kingdom, Australia, New Zealand, Canada, European Union. Furthermore, their diffusion as bioindicators is also growing in South America, East Asia, Africa and other areas (Buss *et al.*, 2015).

The Italian situation before the Water Framework Directive (2000/60/EC)

Italy was among the first European countries to adopt a biomonitoring system based on benthic macroinvertebrates. In fact, since 1975 many field studies were conducted in our country with the aim of obtaining and calibrating a reliable method. For example, in a study realized in the Parma River (Emilia Romagna), Ghetti and Bonazzi (1977) compared the reliability of several indices, while Casellato and collaborators (1980) investigated the applicability of the French Verneaux and Tuffery's Biotic Index to the Brenta River (Trentino). After some adaptations to the Italian environmental conditions and comparisons with other European methods, the "Indice Biotico Esteso" (EBI; Ghetti, 1986) later renamed IBE (Ghetti, 1997) was calibrated and adopted for running water biomonitoring in Italy. According to this procedure, benthic invertebrates were collected with kick-nets (21 mesh cm^{-1}). In wadeable environments, transects were realized kick-sampling with the net from one bank to another, and samples had to be accurately collected in all microhabitats, in order to include the entire local biodiversity. Collected macroinvertebrates were field sorted and then identified at the taxonomic level required for each group (*e.g.,* genus for Plecoptera and Ephemeroptera, family for Trichoptera and Diptera). The taxonomic list was then transformed into a numeric value, using a double entry table. This table considered the taxonomic richness in columns, varying from poor (0-1 taxa) to very rich and biodiverse environments (>35 taxa). The horizontal entry took into consideration the sensitivity of different benthic taxa: the highest row was represented by Plecoptera, whilst the lowest by Oligochaeta and Chironomidae. Combining taxonomic richness and presence of these selected groups, the final index ranged from 0 to 14. These values were finally subdivided into 5 quality classes (see Ghetti, 1997 for further details).

The Water Framework Directive (2000/60/EC) and its consequences on water biomonitoring in Europe

The Water Framework Directive (WFD) represents today the main normative reference for all EU member states in the field of water monitoring and conservation (Collins and Anthony, 2008). Published in 2000, the WFD has profoundly changed management practices placing greater emphasis on ecosystem integrity rather than on the simple detection of pollution (Hering *et al.*, 2010). In fact, this was the most noteworthy and innovative aspect, because water quality assessment shifted from a merely chemical to an ecological approach (Nõges *et al.*, 2009). In this context, the conformity of the whole community in comparison to unaffected conditions must be considered rather than the individual taxon-stressor relationship (Birk and Hering, 2006). Following this holistic approach, some innovative elements were introduced. First, several biological components, named "Biological Quality Elements" (BQEs), are simultaneously taken into consideration to provide a comprehensive evaluation of the environmental condition. For lotic environments, BQEs include phytoplankton, phytobenthos, macrophytic flora, benthic macroinvertebrates and fish (Hering *et al.*, 2003). Moreover, the WFD requires a "type-specific" approach (Hering *et al.*, 2006, Verdonschot and Nijboer, 2004), including six different categories of aquatic ecosystems: rivers, lakes, coastal waters, transitional waters, artificial and heavily modified water bodies (Borja, 2005). Within these categories, all water bodies are grouped in similar typologies according to their geo-morphological, physical and chemical features (Moog *et al.*, 2004). For each typology, reference conditions, *i.e.* "sites that show near-natural or un-impacted conditions", have to be identified so that water quality assessment is calculated as Ecological Quality Ratio (EQR) between the observed and the reference conditions (Von de Ohe *et al.*, 2007). The result is expressed in five quality classes (High, Good, Moderate, Poor and Bad) with High status meaning no differences between reference and observed conditions, while Poor and Bad classes are associated to strong differences (Birk *et al.*, 2012). Third, all member states were expected to achieve the "Good Ecological Status" for their water bodies by 2015 (Heiskanen *et al.*, 2004), encouraging the adoption of specific actions (Programmes of Measures) in each country (Logan and Furse, 2002). In the WFD context, three different types of monitoring are employed for different purposes (Buffagni and Erba, 2007). The surveillance monitoring is performed when the assessment of the overall condition of a water body is needed. When results indicate that a risk of failing to achieve the Good Ecological Status occurs, then the operative monitoring is implemented. Finally, in those occasions where further insight on the effects of specific alterations or pollution phenomena is necessary, the investigative monitoring must be adopted. Based on the type of monitoring, specific BQEs must be employed. Due to this comprehensive approach, all biological quality elements are used in the surveillance monitoring, while just one or two BQEs must be considered in operational and investigative ones.

Although the WFD introduced many innovative inputs and challenges, it was undoubtedly clear that a strong effort was necessary to make the assessment procedure adoptable, coherent and comparable across Europe (Pollard and Huxham, 1998; Reyjol *et al.*, 2014). Major problems regarded the choice of sampling methods and the setting of boundaries among quality classes, because many Member States relied on their own sampling programs. Buffagni and Furse (2006) highlighted that the WFD did not give strict indications about the sampling system because the most important objective was the harmonization of findings rather than of methods. In this context, each single State was enabled by the WFD to choose whether to improve the national method or develop a new sampling procedure. However, with the aim of ensuring an acceptable level of standardization, two crucial European projects were developed: the AQEM (Development and testing of an integrated assessment system for the ecological quality of streams and rivers throughout Europe using benthic macroinvertebrates; 2000-2002; Buffagni *et al*., 2001), and the STAR projects (Standardization of river classifications: Framework method for calibrating different biological survey results against ecological quality classifications to be developed for the Water Framework Directive; 2003-2005). The AQEM project focused exclusively on benthic macroinvertebrates, with the aim to define an operative and standard procedure for sampling and water quality assessment. The STAR project tried to solve some critical aspects related to the implementation of the Directive, especially those concerning the inter-calibration procedures (continuity with national methods, reliability of different taxa accounting for different stressors or stream types, setting procedures for the quality classes, *etc*.). Detailed information about these projects can be obtained by the reviews of Hering *et al.* (2004) and Furse *et al.* (2006), respectively; while in the next paragraph their main outcomes are briefly described with regard to biomonitoring in lotic environments.

The AQEM sampling method and the associated STAR_ICM index

The AQEM is a sampling procedure based on benthic invertebrates designed to assess the Ecological Status of running waters according to the WFD. More detailed information is available on the web site (www.aqem.de), in the associated manual (AQEM Consortium, 2002) and in an IRSA-CNR thematic publication (Buffagni and Erba, 2007). Briefly, the AQEM sampling method is a quantitative procedure that relies on a multi-habitat design, as already adopted in other contexts (*e.g.*, Rapid Bioassessment Protocol - USA; Barbour *et al*., 1999). A defined number of samples are collected from different microhabitats according to their percentage of occurrence in the examined river section. The quantitative aspect is a fundamental requirement of the WFD, while the purpose of the proportional multi-habitat approach is to provide a reliable and standardized evaluation of the structure of the sampling site. According to the official protocol, two phases are generally needed: *a priori* assessment of the monitoring (river characteristics and other aspects) and the following field activity. The *a priori* phase is fundamental to define the preliminary conditions necessary to apply the type-specific biomonitoring required by the WFD. In particular, the type of monitoring and the Hydro-Ecoregion (HER) at which the watercourse belongs are identified in this phase. According to this information, the number of samples, the total sampling area (0.5 or 1 m^2) and the mesohabitat to be sampled (riffle or pool) are defined. As previously stated, the AQEM method adopts a quantitative approach, with a set number of replicates that must be collected. This number varies according to the type of monitoring: 10 replicates are collected for the operative monitoring, while for the other types additional replicates may be added. By contrast, the selection of riffle rather than pool areas and the total sampling surface are type-specific and standardized. In the subsequent field activity, at first the percentage of occurrence of each microhabitat in the section must be recorded visually. With regard to this task, both biotic and abiotic microhabitats are considered as a continuous layer where only those with at least 10% of frequency are considered. It is important to note that the AQEM Consortium provided a list of coded microhabitats, representing a standard selection of substrata allowing an objective site description. The mineral substrata are classified according to the length of the median particle diameter (*i.e.*, gravel, megalithal, *etc*.), whereas biotic substrata are grouped according to the type of vegetation or organic matter (*i.e.*, algae, macrophytes, CPOM, *etc*.). Based on the visual estimates of microhabitat percentages, the 10 replicates are collected proportionally using the Surber net. Since most infrequent microhabitats may be ignored, additional samples may be collected as optional replicates. This is especially suggested for surveillance monitoring and reference sites.

Benthic invertebrates are identified to family level for operative monitoring and genus or Operational Units (*i.e.*, sub-genus identification, only for some selected Ephemeroptera groups) for surveillance/investigative monitoring. In addition, the abundance of individuals of each taxon is reported (although the abundance can be estimated when beyond a threshold of 10 individuals). All benthic invertebrates sampled from each microhabitat are then pooled together in order to compose a unique list of taxa. On the basis of this taxonomic list, the Ecological Status is obtained applying the STAR_ICM (Intercalibration Common Metrics) index. This is a multimetric index developed after an intense process of inter-calibration (Verdonschot and Moog, 2006; Bennett *et al*., 2011). Starting from over 50 different proposed metrics, only 6 were definitively selected to compose the index: ASPT (Average Score Per Taxon), Log10(Sel_EPTD+1) (where EPDT is the sum of selected Ephemeroptera, Plecoptera, Diptera and Trichoptera taxa), 1-GOLD (where GOLD is the sum of Gastropoda, Oligochaeta, and Diptera), total number of families, total number of EPT (Ephemeroptera, Plecoptera, and Trichoptera) families and the Shannon-Weiner diversity index (H'). The selected indices include different parameters of benthic communities: taxa sensitivity, abundance and diversity. Each metric is calculated separately and then they are combined into the overall index score, each metric with a specific weight. Finally, the values of each metrics and the final score also are normalized according to those of the reference conditions, giving the Ecological Status as an Ecological Quality Ratio (EQR) between the observed and the reference values.

Aim of the study

Aim of this study was to briefly review the history of benthic macroinvertebrates biomonitoring in Italy, with a special focus on the changes that occurred following the WFD implementation. In particular, we focused not on general, theoretical differences but on practical aspects, on the basis of our extensive experience and by applying comparatively the IBE and the STAR-ICMi to a large set of benthic macroinvertebrate samples. Since the post WFD method has been employed for a number of years (Hering *et al*., 2010), we believe this is the right time to attempt some objective assessments, which can bring insights and ideas contributing to the future progress of biomonitoring with macroinvertebrates in Italy.

Direct comparison among indices?

Ideally, macroinvertebrate biomonitoring protocols, besides being sensitive to impacts, should be reliable, efficient, cost-effective, and easy to use: the search for a satisfactory method has produced a number of comparisons, as shown by the abundant literature (Cao *et al*., 1996; Buss *et al*., 2015; Guareschi *et al*., 2017). As reported above, the IBE has been used in Italy at the national scale since 1986, but afterwards it was considered inadequate because of its inconsistence with the WFD. In particular, the most common criticisms to the application of IBE were that this method did not consider 'reference conditions' and that was not type specific, because the same scoring system and quality class boundaries were applied to all types of rivers. Moreover, the IBE seemed not to satisfy some quantitative requirements because it did not take into account taxa abundances. For these reasons, this method (as happened for many others in Europe) was replaced by newer approaches. On the other hand, some European Countries tried to maintain a connection with the past, transforming or improving their pre-WFD method (Jáimez-Cuéllar *et al*., 2002; Munné and Prat, 2009 for Spain). In Belgium, for example, sampling and identification procedures of the post-WFD Multimetric Macroinvertebrate Index Flanders were the same used in the "old" Belgian Biotic Index (Gabriels *et al*., 2010). In Italy, this did not happen and the transition was a drastic clear-cut. Comparisons between IBE and STAR_ICMi results are scarce (Mancini *et al*., 2010), but can be of some interest. Our unpublished data suggest that results of the two indices concur in general, with a modest tendency of IBE in overestimating the quality class. Nevertheless, after some years of application of the STAR_ICMi, it is possible to make some remarks, based on our research experience in different areas of the Italian peninsula, and from personal communications of ARPA (Regional Agencies for the Protection of the Environment), researchers and private consulting operators.

It is pointless to question which method performs better, because certainly the STAR_ICMi meets the WFD requirements while this cannot be said for the IBE. Moreover, the STAR_ICMi is the expression of a widely participated and complex process, based on the most modern findings and techniques.

Critical aspects in the current scenario

In our opinion, the main problem related to the hands-on, routine application of the post-WFD Italian method is that it is extremely more consuming in time and efforts than the one previously used. Considering the fact that Environmental Agencies (*e.g.*, ARPA agencies in Italy) and local governments are involved in extensive monitoring plans often carried out with scarce budgets and limited resources, the increased work required for a single sampling represents an important limiting factor. For example, as a consequence of the increased effort for each sampling point, the number of stations seasonally monitored by the ARPA in the Cuneo District (NW Italy) dropped and reduced by a quarter from 2006 to 2016 after the WFD introduction. Moreover, the current classification process is based on 6-year cycles (subdivided in two 3-year campaigns covering all significant water bodies) while the previous classification of all watercourses occurred on a 1-year basis. This reduction may allow just a partial and scarcely updated and representative "picture" of the river ecosystem health.

Considering the fact that time-related issues are of the greatest importance in planning and realizing biomonitoring campaigns, we summarize below the elements that, in our opinion, are most responsible for the increase of workload required for each sampled station.

Quantitative approach

The quantitative approach and the use of Surber nets are the most relevant innovations in field work related to AQEM/STAR_ICMi. Regarding the quantitative approach, IBE takes into account only a numerical "threshold", namely a limiting value below which the presence of a taxon is disregarded, and uses four semi-quantitative levels were considered (*i.e.*, * = below the fixed "entry'" number; I = present; L = abundant; U = dominant). The new method introduces a quantitative approach, but in the first publication (Buffagni and Erba, 2007), it is stated that beyond a threshold of 10 individuals the abundance can be estimate (page 23; Buffagni and Erba, 2007). The introduction of subsamples involves an additional decrease of quantitative accuracy (ISPRA, 2014). A further reduction in quantitative precision results from the use of subsamples, adjusted in more recent publications (APAT, 2007; ISPRA, 2014). Laini *et al.* (2014) have already expressed concerns about the new quantitative approach. In our opinion, the underestimation of abundances (and taxa richness) is a critical point. In fact, estimating necessarily implies not using real quantitative data (needed when using metrics such as Shannon Index H'). In addition, the use of the same abundance threshold value (initially set to n = 10, then increased) for all groups seems largely inadequate. It can be very misleading to count 10 individuals of some groups, and to estimate the rest of their total population, which may amount to hundreds or even thousands of individuals in one sample in some cases. Moreover, it is very different to count 10 Chironomidae or 10 Perlidae, and then estimate the rest of their population: estimation errors are obviously greater for small and cryptic organisms that for large and clearly visible ones.

Taxonomic levels

Some reconsideration on the taxonomic aspects related to the calculation of the STAR_ICMi could be useful. In the previously cited manuals (Buffagni and Erba, 2007; ISPRA, 2014), the identification at the family level is required for the operative monitoring, while the genus or Operational Units level are requested for the surveillance and investigative monitoring. However, the use of the Operational Units is limited to the Order Ephemeroptera, where this level coincides, in most cases, with the genus. *Rhithrogena*, *Caenis*, and *Baetis* represent interesting exceptions, whose identification needs a sub-genus level of detail (*e.g.*, species or groups of morphologically-similar species). Great taxonomic attention is mandatory also for other Ephemeroptera (such as *Procloeon*, *Pseudocentroptilum*) and not for other sensitive taxa such as Plecoptera and Trichoptera. In our opinion, this is an important point for future considerations. The sub-genus determination of these organisms is really time-consuming and not so easy for most of the technicians of the Environmental Agencies, so that hopefully a future update of the method could reconsider this particular aspect. Our main criticisms are the following:

i) Is it really necessary to conduct the taxonomic identification at the sub-genus level? The methods proposed for France (I_2M_2; Mondy *et al.*, 2012), Spain (Jáimez-Cuéllar *et al.*, 2002) and other European countries do not provide for such taxonomic detail, being family or genus the most detailed taxonomic resolution, and yet these methods allow achieving the monitoring goals of the WFD.

ii) What is the scientific reason to focus only on Ephemeroptera? and precisely on some selected species-groups within Ephemeroptera? Although different sensitivity to environmental alterations have been reported within these families, Baetidae and Caenidae are considered, as a whole, examples of quite tolerant mayflies in most biomonitoring systems (such as BMWP, FBI-Hilsenhoff and many others). The same occurs for *Rhithrogena* (Heptageniidae), a relatively homogeneous group of generally rhithronic, reophilous and oligotrophic organisms, considered as wholly reliable indicators of good environmental quality. We suggest to exclude the introduction of a sub-genus level identification also for Plecoptera (or Trichoptera) for the same considerations reported above. However, if a more detailed taxonomic analysis is reputed to be useful or even mandatory, why not include or consider other groups? This choice could be made considering factors such as their large-scale (geographical areas) and small-scale (mesohabitat) distribution, and their relative abundance in benthic assemblages. Chironomidae, at present grouped all at the family level, are almost ubiquitous, and include taxa with different ecological requirements and could be a good choice (Adriaenssens *et al.*, 2004). A taxonomic deepening considering the tribes or sub-family units of Chironomidae could be more useful, as it would for example allow to separate Diamesinae, inhabiting pristine waters, to the tolerant and even alpha-mesosaprobic Chironominae.

iii) Finally, the ecological information gained by using the taxonomic resolution at specific or sub-genus level in selected Ephemeroptera is less important than the information lost by grouping all Plecoptera (in the IBE considered at genus level) into families and considering this important indicator group only in the metric "number of EPT" families. For a long time, biomonitoring reports have been considered an important resource also for studies on biodiversity and biogeography studies, and the record of "Perlodidae" instead of "*Besdolus"* or Capniidae instead of "*Capnopis*" is scientifically very different.

CONCLUSIONS AND RECOMMENDATIONS

We are aware that, despite its long history, doubtless strengths, and innovative contribute, IBE is currently an outdated method. Anyway, we are also confident that the STAR_ICMi could be ameliorated.

A first, important issue that should be addressed for a better implementation of river biomonitoring is related to the direct and indirect training efforts. In fact, when the IBE was the official method in Italy, a training course was annually realized by the author of the method, Prof. Ghetti, and his collaborators for 19 years. In one week, in Trento, participants learned the use of the method with field samplings, laboratory analysis, data processing and discussions. In addition, the application of the method IBE was exhaustively explained in a single, comprehensive manual (Ghetti, 1997). The implementation of STAR_ICM perhaps lacks a similar teaching strategy, as many courses are organized independently by several institutions and associations, often without coordination and direct management of the authors. Furthermore, the information relating to STAR_ICMi application is distributed in a series of publications, while after some years of applications it would be better to concentrate them in a single, definitive manual.

Finally, we propose here two possible improvements to reduce the effort/time consumption for the data collection and processing and to better harmonize the history of biomonitoring and the comparability of the relative data in our country. Therefore, we would like to conclude this paper with some suggestions, hoping that they could be the subject of future discussion and applications.

i) The first is related to the use of Surber nets and quantitative method. Since the STAR_ICM (unlike other methods such as the French, IBGN) is not strictly quantitative, because it involves numerical estimates of organisms, samples could be collected for example using fixed-time kick-nets. Also by using these devices, the multi-habitat approach could be maintained as occurs in Denmark (Friberg *et al.*, 2005), Belgium (Gabriels *et al.*, 2010), and Spain (Munné and Prat, 2009). Interestingly, in a recent work Buss and collaborators (2015) reported that in the United States, kick-nets are used in more than 60% of the State/Federal biomonitoring protocols, whereas Surber, dredges, Hess, and other fixed-area samplers are used in ~9%.

ii) Our second suggestion is related to the taxonomic detail. Since, according to us, it is not informative to use the sub-genus level for few mayfly taxa, here we propose to re-establish family-level determination for most groups, except for Ephemeroptera and Plecoptera, which should be considered at genus level. This would allow: a) to reduce and simplify taxonomic work; b) to obtain data that are comparable with diffuse and long-term data records. We are aware that this modification may have some consequences on the reference conditions already measured, but these changes may be introduced starting from the next verification of reference conditions.

iii) Finally, in our opinion some changes could improve the reliability of the STAR_ICM. For example, there is some confusion about the source of ASPT (derived from BMWP) scores in the STAR_ICM index (Buffagni and Erba, 2007; Buffagni *et al*., 2008), and some problems could arise from the adoption of scores originating from biomonitoring in the United Kingdom (Davy-Bowker *et al.*, 2008). The adoption of BMWP scores designed for Mediterranean countries (Jáimez-Cuéllar *et al*., 2002) or the development of specific scores for Italian watercourses could be an improvement for the assessment of ecological status with the STAR_ICM index. The latter option would be possible by using information gathered with the IBE protocol during its 20 years of application. Moreover, the precision attained by the monitoring system is an essential requirement of the WFD (Clarke, 2013), and it is crucial to clearly discriminate between watercourses in good and less than good ecological status. To date little effort was made to assess the precision of the STAR_ICM (Laini *et al.,* 2014) and, more generally, to estimate the uncertainty of the biotic indexes adopted after the WFD (but see Clarke, 2013).

We are confident that the adoption of the suggested modifications, although not substantial in the architecture of the method, would have interesting repercussions. They would make it possible to shorten time, costs, and efforts required for each sampling (both in the field and in the lab), thereby increasing the number of stations that can be sampled seasonally by ARPA or local Agencies. In addition, they would re-establish a bridge with the past, allowing a better use of the long-time series of IBE data, that would share the same taxonomic detail.

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REFERENCES

Adriaenssens V, Simons F, Nguyen LT, Goddeeris B, Goethals P, De Pauw N, 2004. Potential of bio-indication of chironomid communities for assessment of running water quality in Flanders (Belgium). Belg. J. Zool. 134:31-40.

Allan JD, Castillo MM, 2007. Stream ecology: structure and function of running waters. Springer, Dordrecht: 436 pp.

APAT, 2007. [Metodi biologici per le acque. Parte I].[Book in Italian]. APAT, Rome. Available from: www.isprambiente.gov.it/it/pubblicazioni/manuali-e-linee-guida/metodi-biologici-per-le-acque-parte-i

AQEM Consortium, 2002. Manual for the application of the AQEM system. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive. Ver. 1.0: 202 pp. Available from: www.aqem.de

Barbour MT, Gerritsen J, Snyder BD, Stribling JB, 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish. EPA 841-B-99-002. U.S. Environmental Protection Agency.

Bennett C, Owen R, Birk S, Buffagni A, Erba S, Mengin N, Murray-Bligh J, Ofenbock G, Pardo I, van de Bund W, Wagner F, Wasson JG, 2011. Bringing European river quality into line: an exercise to intercalibrate macro-invertebrate classification methods. Hydrobiologia 667:31-48.

Birk S, Hering D, 2006. Direct comparison of assessment methods using benthic macroinvertebrates: a contribution to the EU Water Framework Directive intercalibration exercise. Hydrobiologia 566:401-415.

Birk S, Bonne W, Borja A, Brucet S, Courrat A, Poikane S, Solimini A, van de Bund W, Zampoukas N, Hering D, 2012. Three hundred ways to assess Europe's surface waters: an almost complete overview of biological methods to implement the Water Framework Directive. Ecol. Indic. 18:31-41.

Bonada N, Prat N, Resh VH, Statzner B, 2006. Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. Annu. Rev. Entomol. 51:495-523.

Borja A, 2005. The European Water Framework Directive: a challenge for nearshore, coastal and continental shelf research. Cont. Shelf. Res. 25:1768-1783.

Buffagni A, Erba S, 2007. [Macroinvertebrati acquatici e Direttiva 2000/60/EC (W.F.D.) – Parte A. Metodo di campionamento per i fiumi guadabili].[Article in Italian]. IRSA-CNR Notiziario dei metodi analitici 1:1-27.

Buffagni A, Erba S, Belfiore C, Hering D, Moog O, 2001. A Europe-wide system for assessing the quality of rivers using macroinvertebrates: the AQEM project*) and its importance for southern Europe (with special emphasis on Italy). J. Limnol. 60:39-48.

Buffagni A, Erba S, Pagnotta R, 2008. [Definizione dello stato ecologico dei fiumi sulla base dei macroinvertebrati bentonici per la 2000/60/EC (WFD): il sistema di classificazione MacrOper].[Article in Italian]. IRSA-CNR Notiziario dei metodi analitici 1:25-41.

Buffagni A, Furse M, 2006. Intercalibration and comparison–major results and conclusions from the STAR project. Hydrobiologia 566:357-364.

Buss DF, Carlisle DM, Chon TS, Culp J, Harding JS, Keizer-Vlek HE, Robinson WA, Strachan S, Thirion C, Hughes RM, 2015. Stream biomonitoring using macroinvertebrates around the globe: a comparison of large-scale programs. Environ. Monit. Assess. 187:1-21.

Cairns J Jr, Pratt JR, 1993. A history of biological monitoring using benthic macroinvertebrates, p. 10-27. In: M.D. Rosenberg and V.H. Resh (eds.), Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, London.

Cao Y, Bark AW, Williams WP, 1996. Measuring the responses of macroinvertebrate communities to water pollution: a comparison of multivariate approaches, biotic and diversity indices. Hydrobiologia 341:1-19.

Casellato S, Salin ML, Lorenzet T, 1980. Applicability of Verneaux and Tuffery's Biotic Index to a study of the macrobenthos in the River Brenta (Northern Italy). Boll. Zool. 47:53-61.

Chandler JR, 1970. A biological approach to water quality management. Water Pollut. Control 69:415-422.

Clarke RT, 2013. Estimating confidence of European WFD ecological status class and WISER Bioassessment Uncertainty Guidance Software (WISERBUGS). Hydrobiologia 704:39-56.

Collins AL, Anthony SG, 2008. Assessing the likelihood of catchments across England and Wales meeting 'good ecological status' due to sediment contributions from agricultural sources. Environ. Sci. Policy 11:163-170.

Davy-Bowker J, Clarke R, Corbin T, Vincent H, Pretty J, Hawczak A, Blackburn J, Murphy J, Jones I, 2008. River Invertebrate Classification Tool. WFD72C SNIFFER.

Friberg N, Baattrup-Pedersen A, Pedersen ML, Skriver J, 2005. The new Danish stream monitoring programme (NOVANA) - Preparing monitoring activities for the water framework directive era. Environm. Monit. Assess. 111:27-42.

Friberg N, 2014. Impacts and indicators of change in lotic ecosystems. WIREs Water 1:513-531.

Furse M, Hering D, Moog O, Verdonschot PFM, Johnson RK, Brabec K, Gritzalis K, Buffagni A, Pinto P, Friberg N, Murray-Bligh J, Kokes J, Alber R, Usseglio-Polatera P, Haase P, Sweeting R, Bis B, Szoszkiewicz K, Soszka H, Springe G, Sporka F, Krno IJ, 2006. The STAR project: context, objectives and approaches. Hydrobiologia 566:3-29.

Gabriels W, Lock K, De Pauw N, Goethals PL, 2010. Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). Limnologica 40:199-207.

Ghetti PF, Bonazzi G, 1977. A comparison between various criteria for the interpretation of biological data in the analysis of the quality of running waters. Water Res. 11:819-831.

Ghetti PF, 1986. [I macroinvertebrati nell'analisi di qualità dei corsi d'acqua. Manuale di applicazione Indice Biotico: E.B.I. modificato].[Book in Italian]. Provincia Autonoma di Trento, Italy.

Ghetti PF, 1997. [Manuale di applicazione. Indice Biotico Esteso (I.B.E.). I macroinvertebrati nel controllo della qualità degli ambienti di acque correnti].[Book in Italian]. Provincia Autonoma di Trento, Italy.

Guareschi S, Laini A, Sánchez-Montoya MM, 2017. How do low-abundance taxa affect river biomonitoring? Exploring the response of different macroinvertebrate-based indices. J. Limnol. 76(Suppl.1):9-20. [Epub 7 Jun 2016].

Heiskanen AS, van de Bund W, Cardoso AC, Nõges P, 2004. Towards good ecological status of surface waters in Europe - Interpretation and harmonisation of the concept. Water Sci. Technol. 49:169-177.

Hellawell JM, 1986. Biological indicators of freshwater pollution and environmental management. Elsevier.

Hering D, Buffagni A, Moog O, Sandin L, Sommerhäuser M, Stubauer I, Feld C, Johnson RK, Pinto P, Skoulikidis N, Verdonschot PFM, 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. Int. Rev. Hydrobiol. 88:345-361.

Hering D, Moog O, Sandin L, Verdonschot PFM, 2004. Overview and application of the AQEM assessment system. Hydrobiologia 516:1-20.

Hering D, Feld CK, Moog O, Ofenböck T, 2006. Cook book for the development of a Multimetric Index for biological condition of aquatic ecosystems: experiences from the European AQEM and STAR projects and related initiatives. Hydrobiologia 566:311-324.

Hering D, Borja A, Carstensen J, Carvalho L, Elliott M, Feld CK, Heiskanen AS, Johnson RK, Moe J, Pont D, Solheim AL, van de Bund W, 2010. The European Water Framework Directive at the age of 10: a critical review of the achievements with recommendations for the future. Sci. Total Environ. 408:4007-4019.

ISPRA, 2014. [Linee Guida per la valutazione della componente macrobentonica fluviale ai sensi del DM 260/2010].[Book in Italian]. ISPRA - Manuali e Linee Guida 107/2014, pp. 99. Available from: www.isprambiente.gov.it/it/pubblicazioni/manuali-e-linee-guida/linee-guida-per-la-valutazione-della-componente-macrobentonica-fluviale-ai-sensi-del-dm-260-2010

Jáimez-Cuéllar P, Vivas S, Bonada N, Robles S, Mellado A, Álvarez M, Avilés J, Casas J, Ortega M, Pardo I, Prat N, Rieradevall M, Sáinz-Cantero CE, Sánchez-Ortega A, Suárez ML, Toro M, Vidal-Abarca MR, Zamora-Muñoz C, Alba-Tercedor J, 2002. [Protocolo GUADALMED (PRECE).]. [Article in Spanish].

Kolkwitz R, Marsson M, 1908. [Ökologie der pflanzlichen Saprobien].[Article in German]. Ber. Dtsch. Bot. Ges. 26:505-519.

Laini A, Vorti A, Bolpagni R, Viaroli P, 2014. Small-scale variability of benthic macroinvertebrates distribution and its effects on biological monitoring. Ann. Limnol. Int. J. Lim. 50:211-216.

Logan P, Furse M, 2002. Preparing for the European Water Framework Directive - making the links between habitat and aquatic biota. Aquat. Conserv. 12:425-437.

Mancini D, Zanut E, Massarutto S, Piazza G, Tomasella M, Bertoli M, Pizzul E, 2010. [Valutazione biologica della qualità delle acque nel bacino del Fiume Stella (Friuli Venezia Giulia)]. [Article in Italian]. Biol. Ambient. 24:49-58.

Metcalfe JL, 1989. Biological water quality assessment of running waters based on macroinvertebrate communities: history and present status in Europe. Environ. Pollut. 60:101-139.

Mondy CP, Villeneuve B, Archaimbault V, Usseglio-Polatera P, 2012. A new macroinvertebrate-based multimetric index (I2M2) to evaluate ecological quality of French wadeable streams fulfilling the W.F.D. demands: a taxonomical and trait approach. Ecol. Indic. 18:452-467.

Moog O, Schmidt-Kloiber A, Ofenböck T, Gerritsen J, 2004. Does the ecoregion approach support the typological demands of the EU 'Water Framework Directive'? Hydrobiologia 516: 21-33.

Munné A, Prat N, 2009. Use of macroinvertebrate-based multimetric indices for water quality evaluation in Spanish Mediterranean rivers: an intercalibration approach with the IBMWP index. Hydrobiologia 628:203-225.

Nõges P, van de Bund W, Cardoso AC, Solimini AG, Heiskanen AS, 2009. Assessment of the ecological status of European surface waters: a work in progress. Hydrobiologia 633:197-211.

Pollard P, Huxham M, 1998. The European Water Framework Directive: a new era in the management of aquatic ecosystem health? Aquat. Conserv. 8:773-792.

Reyjol Y, Argillier C, Bonne W, Borja A, Buijse AD, Cardoso AC, Daufresne M, Kernan M, Ferreira MT, Poikane S, Prat N, Solheim AL, Stroffek S, Usseglio-Polatera P, Villenueve B, van de Bund W, 2014. Assessing the ecological status in the context of the European Water Framework Directive: Where do we go now? Sci. Total Environ. 497:332-344.

Rossaro B, Boggero A, Crozet BL, Free G, Lencioni V, Marziali L, 2011. A comparison of different biotic indices based on benthic macro-invertebrates in Italian lakes. J. Limnol. 70:109-122.

Szivák I, Csabai Z, 2012. Are there any differences between taxa groups having distinct ecological traits based on their responses to environmental factors? Aquat. Insects 34:173-187.

Verdonschot PFM, Nijboer RC, 2004. Testing the European stream typology of the Water Framework Directive for macroinvertebrates. Hydrobiologia 516:35-54.

Verdonschot PFM, Moog O, 2006. Tools for assessing European streams with macroinvertebrates: major results and conclusions from the STAR project. Hydrobiologia 566:299-309.

Von der Ohe PC, Prüß A, Schäfer RB, Liess M, de Deckere E, Brack W, 2007. Water quality indices across Europe - A comparison of the good ecological status of five river basins. J. Environ. Monitor. 9:970-978.

Woodiwiss FS, 1964. The biological system of stream classification used by the Trent River Board. Chem. Ind. 11:443-447.

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Mesohabitat mosaic in lowland braided rivers: Short-term variability of macroinvertebrate metacommunities

Gemma BURGAZZI,* Alex LAINI, Erica RACCHETTI, Pierluigi VIAROLI

Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Viale G.P. Usberti 33/A, 43124 Parma, Italy
*Corresponding author: gemma.burgazzi@unipr.it

ABSTRACT

Braided rivers are among the most variable and dynamic riverine systems. Changes in these environments are sudden and frequent, driven by the high hydrological variability. They host high levels of local heterogeneity, with many different habitats in close proximity establishing a mosaic of patches. This provides the conditions for high levels of biodiversity, with strong community variability in particular among the different habitats at the stream-reach level. Nevertheless, these systems are still poorly studied and their complexity is often not taken into account in biomonitoring protocols. We applied mixed effects modelling, spatial ordination techniques and beta-diversity partitioning (into nestedness and turnover components) with the aim of improving the knowledge of braided rivers, investigating: i) the organization of macroinvertebrate communities among the different habitats of a river reach, and ii) the temporal variability of this organization (both among seasons and during summer). We predicted a differentiation of macroinvertebrate communities between distinct habitats within rivers, with this differentiation increasing during the low-flow period. We carried out our study in four braided rivers and streams of the Po River basin (Northern Italy) sampling three different kinds of mesohabitats (main channel, secondary channel and pool) in eight stations during seven campaigns from June 2015 to April 2016. We found a high variability of *taxa* richness, abundance and community structure among mesohabitats, with marginal ones accounting for the greater part of macroinvertebrate diversity. Secondary channels resulted as being the habitat hosting greater *taxa* diversity, with 10 exclusive *taxa*. Surprisingly the mesohabitat communities differed greatly during the seasonal phase, whereas their dissimilarity decreased during summer. This could be explained considering the summer flow reduction as a homogenizing force, leading to a general loss of the most sensitive *taxa.* However, the summer *taxa* turnover value resulted higher than nestedness, suggesting a strong environmental control on community organization, with *taxa* well adapted to the different conditions of mesohabitats and able to manage the effects of flow reduction. Our work represents a remarkable issue for biomonitoring protocols, highlighting the importance of taking into account the whole complexity of braided rivers for a more realistic evaluation of macroinvertebrate communities.

Key word: Braided rivers; macroinvertebrate metacommunity; mesohabitat; short-term variability; macroinvertebrate distribution.

INTRODUCTION

Braided rivers (BRs) are defined as "systems composed by multiple channels, with bars and islands, often with poorly defined banks of non-cohesive sedimentary materials" (Tockner *et al.,* 2006). These systems are spread worldwide and can be found in delta areas, where rivers enter lakes and oceans, or in floodplains in presence of low slope and sandy or gravel-filled substrates (Dodds, 2002). The main feature that shapes BRs is the extreme flow variability. The alternation of sudden and frequent flow changes, spanning flash floods and dry periods generates a mosaic of patches (embracing different degrees of lotic and lentic conditions), which undergoes rapid evolution (Gray and Harding, 2009). Events like the displacement of channels or disconnection of habitats can happen within short periods, spanning from a few weeks to a few hours. Van der Nat *et al.* (2003) estimated the turnover time of the different habitats in a BR system (Tagliamento, NE Italy) reporting a high level of variation, with a total replacement of all the aquatic habitats of 82% during the period of study (2.5 years). Nevertheless, they reported that the relative proportion of the various habitats remained quite consistent. Based on these results, BRs can be conformed to the "shifting mosaic steady model" that identify systems where the habitat turnover is high but the proportions of habitats are constant (Tockner *et al*., 2006; Gray and Harding, 2011).

Based on these attributes, BRs can be considered as very suitable systems for studying metacommunity dynamics. According to the metacommunity theory (Leibold *et al*., 2004), environmental heterogeneity and *taxa* features (*e.g.,* dispersal ability and competition) determine the structure and evolution of metacommunities at different spatial scales (Siqueira *et al*., 2012; McLaughlin *et al*., 2013). In general, habitat heterogeneity has a positive effect on species richness (Poff and Ward, 1990; Garcia *et al*., 2012; Astorga *et al*., 2014), enhancing the niche availability and allowing the co-occurrence of *taxa* with different requirements. The high dynamism of BRs generates a great heterogeneity, especially at the scale of river reach, with a wide range of different habitats, spanning from lotic to lentic conditions and with a time-variable level of connection. The degree of influence of dispersal dynamics and environmental forcing is strictly related to the connectivity of habitats, besides the dispersal ability of *taxa* (Padial *et al*., 2014). In riverine systems, the level of connectivity can change widely in time and among them BRs are one of the most dynamic and complex (Ward *et al*., 2002). All these conditions are the basis for the presence of biodiversity hot-spots, with high levels of diversity variation in particular among the different habitats at the stream-reach level. In fact, several authors pointed out high levels of lateral variation in *taxa* diversity and community structure for braided systems (*e.g.*, Arscott *et al*., 2005; Gray and Harding, 2007, respectively in north-eastern Italy and New Zealand). Similar outputs were also recorded for primary producers in lowland rivers largely fed by groundwater (Bolpagni and Laini, 2016), suggesting the existence of complex metabolic gradients across habitats in hydro-systems.

The variation among habitats can be considered as a beta-diversity variation and therefore it can be ascribed to two different phenomena: nestedness and spatial turnover. Nestedness occurs when there is a non-random *taxa* loss, with the result that the poorer communities are a subset of the richer ones, while turnover is the result of *taxa* replacement (Baselga, 2010). Datry *et al*. (2016) highlighted that turnover is more related to environmental filtering, while nestedness is given by dispersal limitation. These two processes can assume differential importance in shaping local communities, in particular during low-flow periods, when connectivity among habitats is more variable.

Although these systems are widespread and considered as diversity hot-spots, for years they have been poorly studied (Gray and Harding, 2007), with a lack of knowledge, especially in how the different habitats in the river segment contribute to the total diversity and how these patterns change in time. This topic is particularly relevant considering that BRs are often located in areas heavily impacted by human activities, with all the possible consequences, like considerable water withdrawals, canalization and reduction or loss of lateral areas (Tockner *et al*., 2006; Gray and Harding 2011; Karaus *et al*., 2013). These phenomena lead to a trivialization of BRs, with the consequent reduction of habitat variability. Therefore, a good understanding of habitat heterogeneity contribution to the local diversity becomes a key point for biodiversity conservation.

The aims of this study are therefore i) to evaluate the seasonal structure and variation of benthic macroinvertebrate communities within the highly patchy environments of BRs and ii) to evaluate the short-term variability of these communities during the low-flow period. For this work, we focused on the mesohabitat sampling unit, demarcated according to the hydrodynamic characteristics in main channel, secondary channel and pool. Tickner *et al*. (2000) defined mesohabitats as "medium-scale habitats which arise through the interactions of hydrological and geomorphological forces". We hypothesize that: i) in general there is a differentiation of macroinvertebrate communities between different mesohabitats within rivers, and between considered seasons ii) during low-flow periods, with the increasing disconnection of mesohabitats there is an increase in community dissimilarity, with higher turnover in the less disconnected mesohabitats and higher nestedness in the more disconnected ones.

METHODS

Study area

The study was performed in four braided watercourses (Trebbia River, Nure Stream, Taro River and Baganza Stream) of the Po River basin (Northern Italy, Fig. 1A). A description of the studied systems is reported in Tab.1. They are fed only by wet depositions and they present two high discharge periods (in autumn and spring) and a main low water period in summer (with a secondary additional one in winter). They are included in the Cfa (humid subtropical climate) and Csa (hot-summer Mediterranean climate) climatic regions. For each watercourse we selected two sampling stations in order to take into account the within river variability. Within each station, three model mesohabitats were further selected: main channel, secondary channel, and pool (Fig. 1B). For the first part of the study (seasonal phase, T6-T7) sampling was carried out in November 2015 and in April 2016 in the whole set of systems. For the second part (summer phase, T1-T5) the set of investigated systems was reduced to two (Trebbia and Taro rivers) and the sampling was carried out in five occasions in the period of low flow, from June to September 2015. The downsizing of sampled area was operated because i) Nure and Baganza streams completely dried up during the summer season and ii) to contain the sampling and processing effort.

Physical and chemical variables

In order to check the difference between mesohabitats, for each sampling environmental data were collected with five random replicates (Fig. 1B). Flow velocity, water depth, temperature, conductivity and dissolved oxygen were recorded *in situ* by means of a current meter (FP101-FP102 Global Flow Probe) and a multi-parametric probe (HI 9828; Hanna Instruments). Water samples were collected for the determination of ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), soluble reactive phosphorous (SRP), dissolved silica (SiO_2) and total dissolved inorganic carbon (TCO_2). Detection limits were 0.01 mg L^{-1} for NH_4^+, NO_3^-, SRP and SiO_2, 0.005 mg L^{-1} for NO_2^-, and 0.02 mM for TCO_2. Precision ranged between ±3% and ±5%. Chemical analyses were performed by means of spectrophotometric techniques, according to Valderrama (1977), Golterman *et al*. (1978), and APHA (2012).

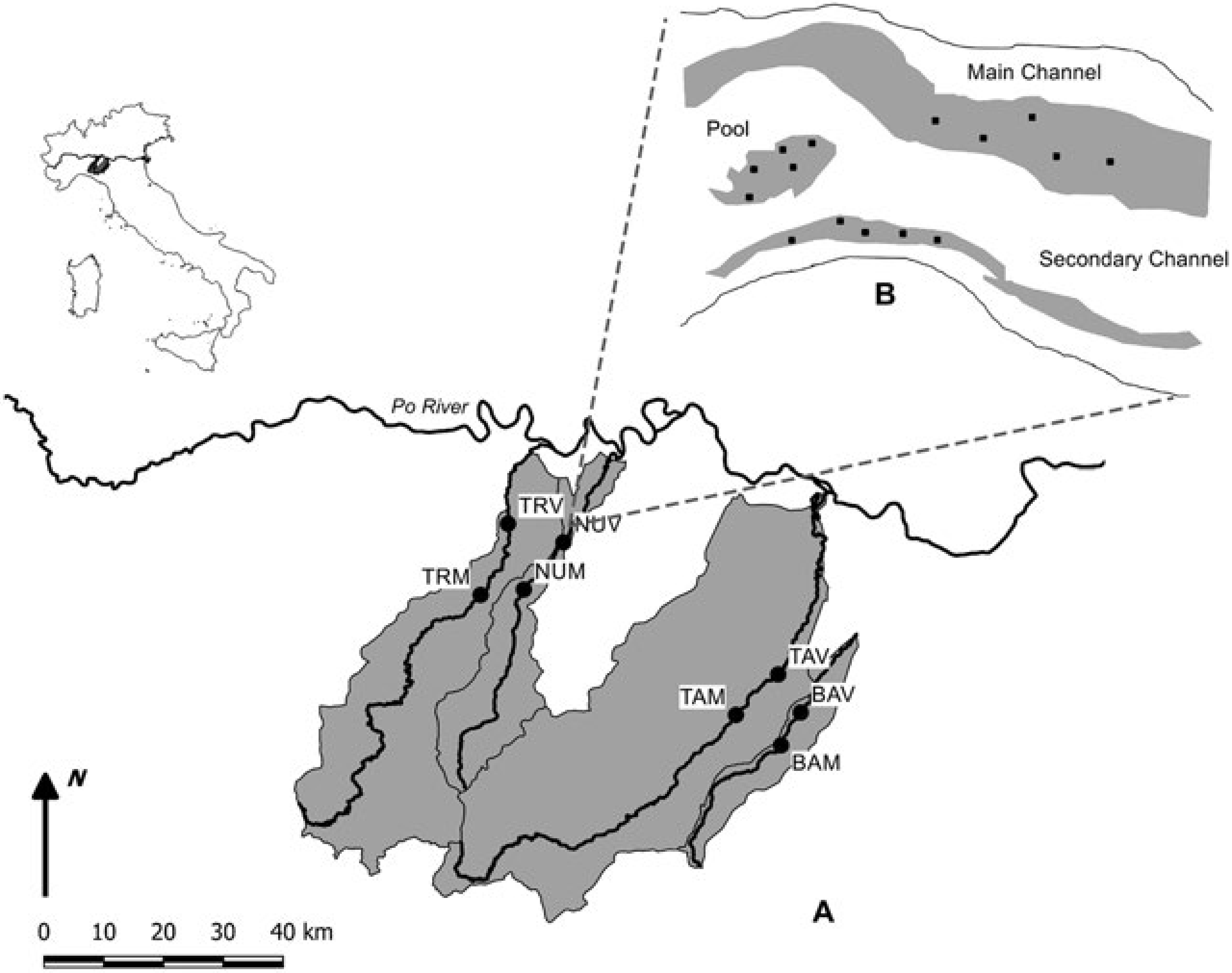

Fig. 1. Map of the sampled area (A), with the studied basins coloured in grey and the sampling stations marked with black dots, and the sampling design (B) repeated in each of the sampling stations. Black squares represent the random sampling plots.

Tab. 1. Descriptive data of the investigated rivers/streams and stations.

River/stream	Qm ($m^3 s^{-1}$)	L (km)	A (km^2)	Station	Latitude	Longitude	Altitude (m asl)	W (m)	D (km)
Trebbia	22.0	120	1083	TRM	44°51’11.05”N	9°32’11.75”E	166	298	13.9
				TRV	44°58’18.51”N	9°35’32.54”E	97	440	
Nure	15.0	77	458	NUM	44°51’52.84”N	9°37’48.73”E	212	240	9.9
				NUV	44°55’55.97”N	9°42’40.96”E	124	244	
Taro	40.5	138	2051	TAM	44°40’27.92”N	10° 4’23.03”E	151	530	10.6
				TAV	44°44’26.97”N	10°10’6.14”E	95	445	
Baganza	5.2	59	228	BAM	44°37’54.67”N	10°10’10.33”E	308	106	6.4
				BAV	44°40’54.20”N	10°12’36.90”E	213	183	

Qm, mean annual flow; L, total length; A, basin area; W, width of the riverbed; D, linear distance between stations.

Macroinvertebrates

In each mesohabitat, a ~50 m long stretch was sampled, choosing five random sampling points (Fig. 1B). Samples were collected using a surber net with frame area of 0.1 m^2 and mesh size of 500 µm. The five replicates were cumulated for each mesohabitat. Samples were filtered and preserved in 70% ethanol for laboratory sorting, where the organisms were counted and identified to family or genus level, according to Tachet *et al*. (2010).

Data analysis

The difference between mesohabitats, in terms of physical variables, was assessed by means of mixed effects modelling, considering mesohabitat and time (sampling date) as fixed effects and station and site (namely the specific sampling location) as hierarchically organized random effects. A similar approach was followed also for testing the influence of mesohabitat and time on richness and abundance. The significance was checked by means of a likelihood-ratio test. The use of these models allows us to work with correlated and non-normally distributed data (McCulloch and Neuhaus, 2005), typical of nested and hierarchical designs. The effect of covariate was tested both for seasonal and summer data. Then the distribution of *taxa* between mesohabitats at station level was checked, by estimating the mesohabitat contribution to the total number of *taxa*. We did this by computing the percentage ratio for each station between the richness of each mesohabitat and the total richness of the station.

The organization of community structure in mesohabitats was explored with a non-Metric Multidimensional Scaling (nMDS), a spatial ordination technique that represents the set of objects along a predetermined number of axes maintaining the ordering relationships among them (Borcard *et al*., 2011). As dissimilarity measure Bray-Curtis distance was used and the goodness of ordination was assessed with the stress measure.

To assess the nature of diversity variation between mesohabitats during the summer phase we performed a partition of beta-diversity, following Baselga (2010). This method produces three metrics: the total beta-diversity (the Sørensen Dissimilarity index), for all the possible pairwise comparisons, and its two additive components: nestedness, expressing the *taxa* loss between mesohabitats, and the turnover, expressing the *taxa* substitution. The values of nestedness and turnover were normalized by dividing them by the Sørensen dissimilarity value. We checked the effect of time for the Sørensen Dissimilarity index by means of linear mixed effect models and then we adjusted the p-values using the Bonferroni correction for multiple comparisons. We also applied mixed effects modelling to check the difference between beta-diversity components and their variation in time.

All analyses and graphs were performed with the statistical software R (R Core Team, 2016), with base version and with ggplot2 (Wickham, 2009), lme4 (Bates *et al*., 2015), vegan (Oksanen *et al*., 2016) and betapart (Baselga *et al*., 2013) packages.

RESULTS

Physical and chemical variables

Mean values of measured physical and chemical variables are reported in Tab. 2, according to season and mesohabitat. The distinction between mesohabitats was tested for physical variables considering the whole dataset (seasonal and summer data) and we found that they differ greatly for flow velocity ($P<0.001$) and water depth ($P<0.001$), while the others variables (temperature, conductivity and percentage of dissolved oxygen) varied significantly only in time ($P<0.001$) but not between mesohabitats.

Macroinvertebrates

A total of 74122 organisms, belonging to 94 *taxa* (75 families) was found globally. The sample with the highest *taxa* richness (34 *taxa*) was collected at the beginning of the summer period in the upstream pool of Trebbia River, while the one with the lowest (seven *taxa*) in the downstream pool of Nure Stream, during the November sampling campaign. The mean values of *taxa* richness and abundance were 18±5 and 837±743 for main channels, 22±5 and 796±546 for secondary channels and 17±6 and 426±552 for pools. The list of most abundant (A) and frequent (B) *taxa* is reported in Fig. 2. Chironomidae was both the most abundant and frequent *taxon* (abundance = 29.3%, frequency = 99.1%). Detection probabilities for the other most common *taxa* were unrelated to their abundance. Some *taxa* were found to be exclusive of one kind of mesohabitat: we found five exclusive *taxa* in the main channels (*Heptagenia, Notonecta*, Gordiidae, *Besdolus* and *Brachyptera*), six in the pools (*Pseudocentroptilum*, *Pisidium*, Dixidae, *Hydrometra*, Haplotaxidae and *Protonemura*) and 10 in the secondary channels (Hydridae, Blephariceridae, Dolichopodidae, Ephydridae, Rhagionidae, *Valvata*, *Gerris*, *Helobdella*, *Nemoura* and Lepidostomatidae).

The significance of mesohabitat and time for *taxa* richness and organism abundance was tested by means of several mixed effects models for seasonal and summer phases. Both *taxa* richness and abundance resulted related with mesohabitats, especially for seasonal data (P values 0.002 and 0.003 respectively), while for summer data these relations resulted weaker (P values 0.078 and 0.060). Time resulted significant only for the seasonal *taxa* richness ($P= 0.026$), with a variation between November and April, while no significant variation was found during summer nor for abundance. The effect of mesohabitat resulted clear also considering community composition patterns (Fig. 3 A,B): points corresponding to the three kinds of mesohabitats group into different areas of the nMDS plot, both for seasonal and summer

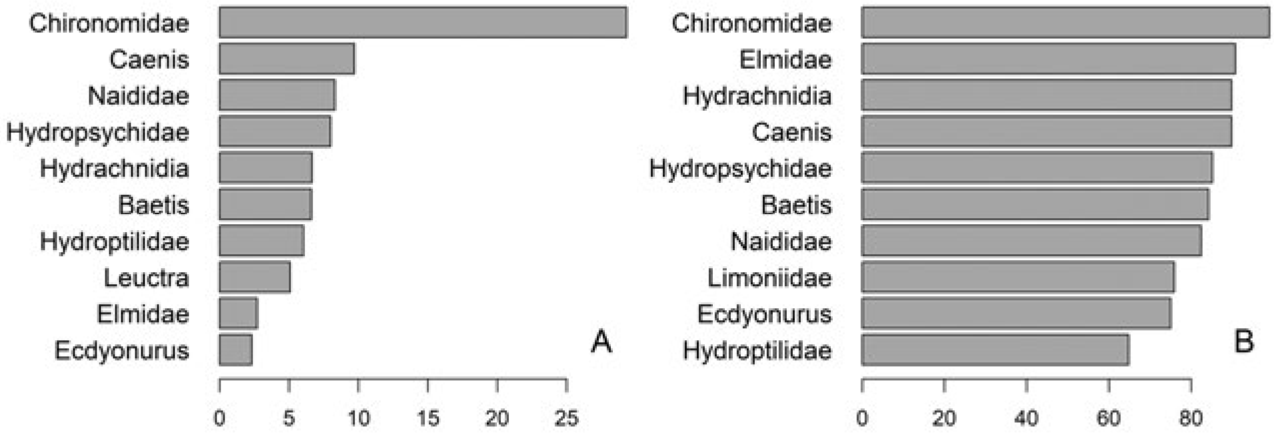

Fig. 2. Barplots of the first 10 *taxa* in terms of abundance percentage (A) and frequency percentage (B). Values are referred to the whole dataset.

Tab. 2. Physical and chemical variables for mesohabitats in each season. NH_4^+, NO_2^- and SRP values are not shown because always lower than detection values. Autumn and Spring values are for the whole set of systems, whereas Summer values are referred only to Trebbia and Taro rivers.

		Autumn		Spring		Summer	
		Mean values	SD	Mean values	SD	Mean values	SD
Flow velocity (m s^{-1})	Main	0.47	0.24	0.46	0.07	0.42	0.16
	Sec	0.31	0.36	0.14	0.07	0.22	0.19
	Pool	0.08	0.15	0.00	0.01	0.07	0.13
Water depth (cm)	Main	22.6	7.7	28.3	6.4	21.3	6.9
	Sec	13.5	10.0	10.7	8.7	14.3	8.0
	Pool	24.3	9.3	13.1	7.6	19.1	6.8
Temperature (°C)	Main	13.0	1.2	14.1	1.3	23.5	2.0
	Sec	13.8	1.5	14.9	2.2	23.7	3.8
	Pool	13.3	1.3	15.4	1.6	24.6	1.9
Conductivity (µS cm^{-1})	Main	250	29	253	24	328	45
	Sec	287	81	272	35	383	143
	Pool	268	38	274	27	375	74
Dissolved oxygen (%)	Main	104.1	5.8	103.8	12.2	107.5	8.1
	Sec	100.5	12.1	102.0	11.2	96.3	18.8
	Pool	94.0	11.3	88.3	12.6	105.1	18.4
NO_3^-	Main	0.29	0.08	0.16	0.08	0.23	0.14
	Sec	0.36	0.22	0.17	0.09	0.49	0.64
	Pool	0.31	0.07	0.21	0.06	0.44	0.67
SiO_2 (mg L^{-1})	Main	1.36	0.27	1.24	0.36	2.62	0.62
	Sec	1.37	0.32	1.32	0.38	3.00	0.80
	Pool	1.38	0.34	1.39	0.23	3.10	0.38
TCO_2 (mM)	Main	2.14	0.21	2.32	0.23	1.88	0.40
	Sec	2.34	0.58	2.20	0.29	1.92	0.50
	Pool	2.21	0.32	2.51	0.43	1.96	0.53

Main, main channels; sec, secondary channels; pool, pools.

communities. Moreover, comparing the two graphs, it can be seen that the segregation between mesohabitats resulted slightly greater during the seasonal phase (November and April) than for summer. We also found a variability of communities in time (Fig. 3C), with a clear segregation of autumn, spring and summer data in three different clusters.

The mesohabitats contribution to the *taxa* richness at station level resulted significantly different, either seasonally (P=0.002) or during the summer (P=0.045). The greater contribution was the one given by marginal mesohabitats and by the secondary channels in particular, while the importance of main channels resulted limited (Fig. 4).

Variation during the summer phase of the Sørensen Dissimilarity index and of the beta-diversity partition for the pairwise comparisons between mesohabitats are reported in Fig. 5 A,B. The dissimilarity values (Sørensen Dissimilarity index) showed similar trends in all comparisons, with a decrease in June and July (T1:T5 in Fig. 5A) and a new increase at the beginning of autumn (T6, T7). Nevertheless, this trend resulted significant only considering the comparison between main channels and pools (P=0.027). The two components of beta-diversity resulted significantly different for all the comparison (P<0.001), with higher values for *taxa* turnover. No significant temporal trends resulted from the analysis.

DISCUSSION AND CONCLUSIONS

This study highlights a strong variability of macroinvertebrate assemblages in BRs, with different mesohabitats hosting different communities. These findings are consistent with Gray and Harding (2009), Zilli and Marchese (2011), Karaus *et al*. (2013) and Starr *et al*. (2014), that reported significant levels of variation of *taxa* richness and abundance among mesohabitats inside river reaches. Arscott *et al*. (2005) found greater diversity in macroinvertebrate communities of backwaters areas of Tagliamento River, while Gray and Harding (2009) pointed out spring creeks, spring sources and ponds and Zilli and Marchese (2011) isolated lakes as mesohabitats hosting greater diversity in New Zealand rivers and in Panamá River floodplain, respectively. By contrast, in our systems secondary channels resulted as being the mesohabitat hosting greater *taxa* diversity, both for seasonal and summer data, while in the other works these mesohabitats resulted in those with low diversity compared to the other ones. This higher diversity could be explained considering that secondary channels were characterized by intermediate levels of hydrological disturb (mean discharge, depth and water velocity), were often located near the margin of riverbeds and presented a higher heterogeneity of microhabitats (cobbles, gravel, clay, algal mats and roots). These features have been shown to be critical

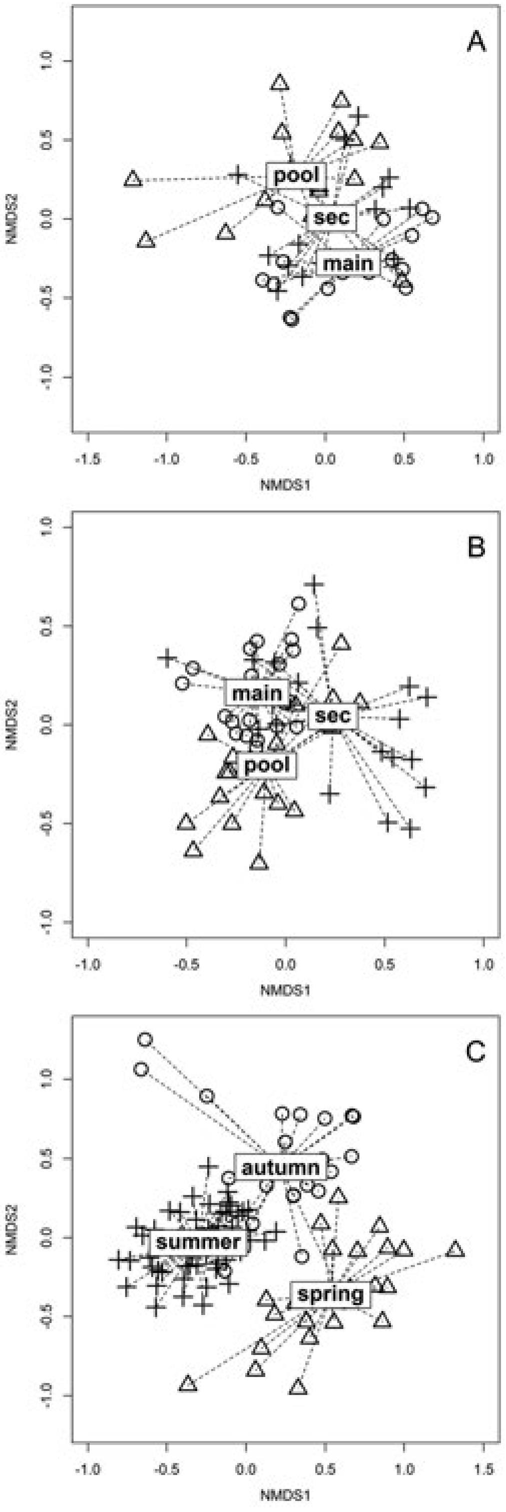

Fig. 3. nMDS ordination outputs for seasonal (A), summer (B) and all (C), data. For seasonal and summer data (A and B) the mesohabitat segregation is shown, while for the all data graph (C) the temporal (between seasons) segregation of communities is shown. Seasonal nMDS stress, 0.176; Summer nMDS stress, 0.157; all data nMDS stress, 0.175; main, main channels; sec, secondary channels; pool, pools.

in enhancing the within site richness (Downes *et al*., 2000). Additionally, they show greater shadowing levels that significantly modulate the colonization patterns of primary producers (algae, vascular macrophytes; data not shown), influencing the availability of resources for macroinvertebrates.

Several factors have been proposed as main drivers for BRs macroinvertebrate community differentiation: conductivity and percentage of sand (Zilli and Marchese, 2011), flow velocity (Arscott *et al*., 2005), nature of substrate (Beisel *et al*., 1998). In our work, we considered the mesohabitat category as a proxy of physical environment differentiation: in particular, we found that main channels, secondary channels and pools mainly differed in flow velocity and water depth. Given the high significance of mesohabitats for macroinvertebrate, we hypothesized a strong physical control of communities, with a selection of *taxa* based on their habitat needs, also suggested by the presence of unique *taxa* for different mesohabitats.

A temporal trend that arose from our results is the evident difference in the importance of mesohabitats between seasonal and summer phases, supported by both mixed effects modelling and nMDS ordination. These findings are in contrast with Starr *et al*. (2014) and Arscott *et al*. (2003), who reported an increase of compositional heterogeneity coming from the increasing isolation of sampling sites, and from flood homogenization respectively. García-Roger *et al.* (2011) instead found similar results, with a mesohabitat (riffles and pools in their study) differentiation smaller during the dry season for a decrease of mesohabitat heterogeneity. The greater differentiation of mesohabitat communities observed in the present study during seasonal samplings (November and April) could be the result of a major connectivity that allows organisms to actively choose the best living place, according to their necessities. This generates a high environmental control on the community from the moment that there are no dispersal limitations and the choice of the most suitable environmental features drives the community. On the other hand, during summer the disconnection increases, hampering the dispersion of organisms. Unlike our initial hypothesis, in this phase we observe a temporal trend of community dissimilarity reduction between mesohabitats, coupled with a prevalence of *taxa* turnover on *taxa* loss. These phenomena could be due to a general loss of the more sensitive and specialized *taxa*, which leads to the homogenization of communities. Nevertheless, the turnover remains greater than nestedness, suggesting the presence of *taxa* well adapted to the different conditions of mesohabitats and able to manage with the effects of flow reduction.

This work provides significant insights, also into the biomonitoring procedures. Hence, for BRs the Italian legislation limits the range of application of the standard biomonitoring methods to the main channel. This choice, based on our data, could lead to collect samples unrepresentative of the real communities, with a loss of 20% of families, and to obtain incorrect evaluations. In BRs the

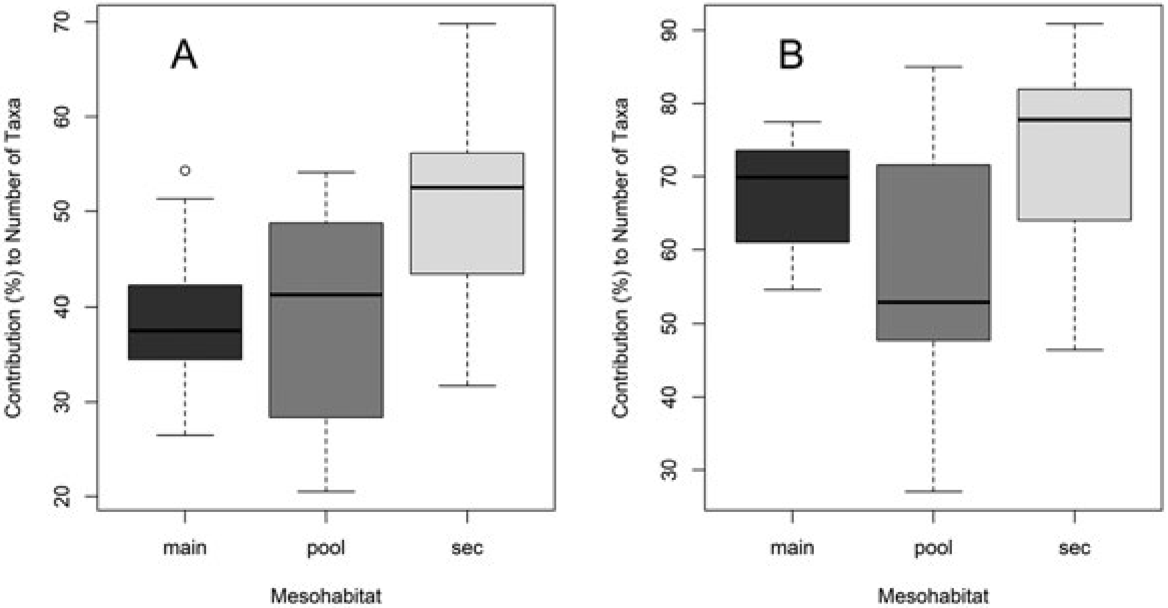

Fig. 4. Mesohabitat contribution to the total number of *taxa* for seasonal (A) and summer data (B). Main, main channels; sec, secondary channels; pool, pools.

distribution of benthic macroinvertebrates exhibits high levels of heterogeneity and therefore the ecological status cannot be evaluated considering exclusively the main channels, but it should be assessed considering the river ecosystem as a whole, including marginal water bodies (secondary channels and pools) that are common in these environments.

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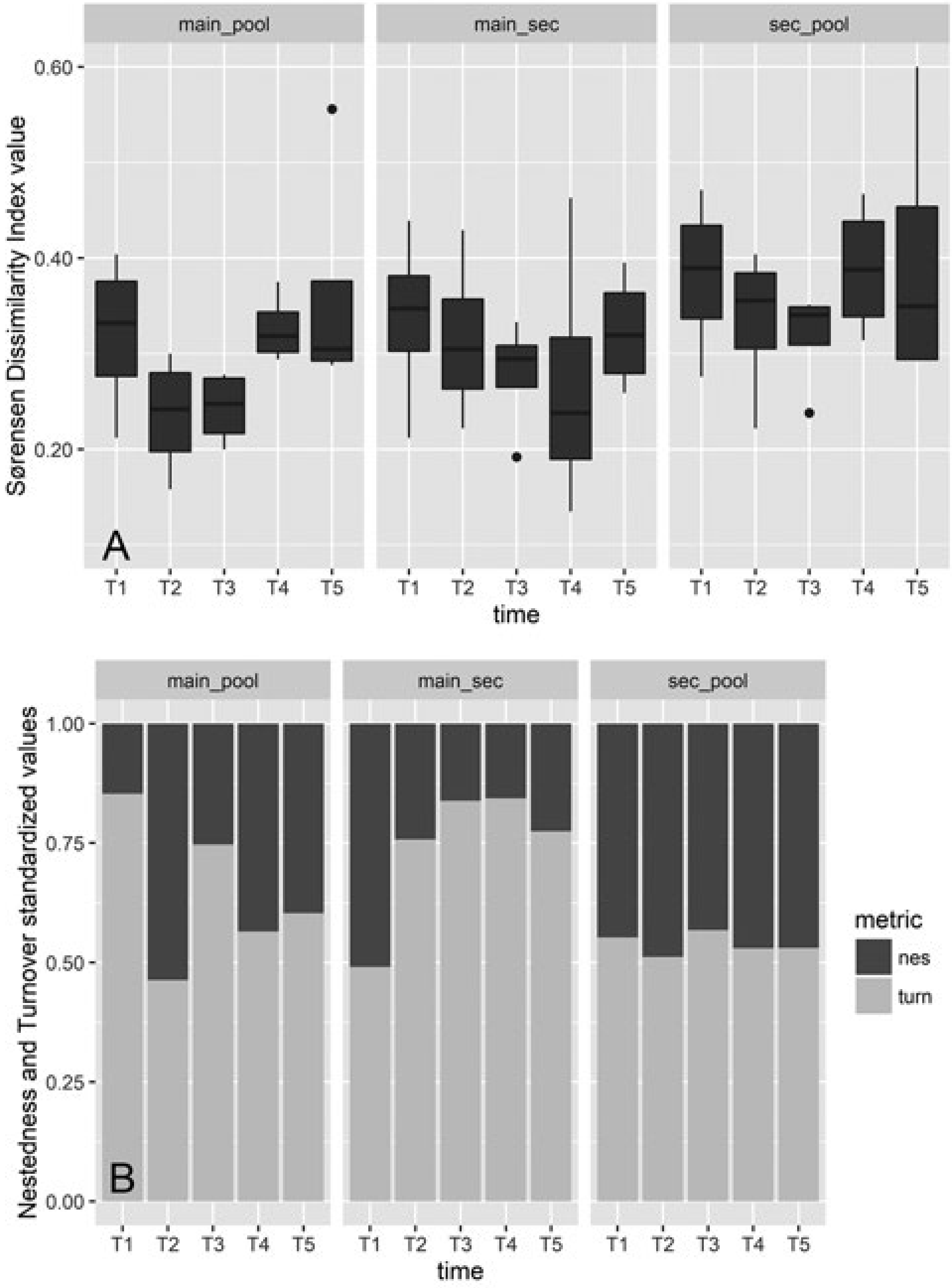

Fig. 5. Short-term variation of Sørensen dissimilarity index (A) and nestedness and turnover components (B), in the three pairwise confrontations between mesohabitats (main_pool, main channel-pool; main_sec, main channel-secondary channel: sec_pool, secondary channel-pool). Nestedness and turnover values were standardized by dividing by the Sørensen index. T1:T5, sampling campaigns.

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REFERENCES

APHA-AWWA-WPCF, 2012. Standard methods for the examination of water and wastewater. 22nd ed. Am. Publ. Health. Ass., Washington: 1360 pp.

Arscott DB, Tockner K, Ward JV, 2003. Spatio-temporal patterns of benthic invertebrates along the continuum of a braided Alpine river. Arch. Hydrobiol. 158:431-460.

Arscott DB, Tockner K, Ward JV, 2005. Lateral organization of aquatic invertebrates along the corridor of a braided floodplain river. J. N. Am. Benthol. Soc. 24:934-954.

Astorga A, Death R, Death F, Paavola R, Chakraborty M, Muotka T, 2014. Habitat heterogeneity drives the geographical distribution of beta diversity: the case of New Zealand stream invertebrates. Ecol. Evol. 13:2693-2702.

Baselga A, 2010. Partitioning the turnover and nestedness components of beta diversity. Global Ecol. Biogeogr. 19:134-143.

Baselga A, Orme D, Villeger S, De Bortoli J, Leprieur F, 2013. betapart: Partitioning beta diversity into turnover and nestedness components. R package ver. 1.3. Available from: https://CRAN.R-project.org/package=betapart

Bates D, Maechler M, Bolker B, Walker S, 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw 67:1-48.

Beisel JN, Usseglio-Polatera P, Thomas S, Moreteau JC, 1998. Stream community structure in relation to spatial variation: the influence of mesohabitat characteristics. Hydrobiologia 389:73-88.

Bolpagni R, Laini A, 2016. Microhabitat patterns of soft-bodied benthic algae in a lowland river largely fed by groundwater. Fottea 16:244-254.

Borcard D, Gillet F, Legendre P, 2011. Numerical ecology with R. Springer, New York: 306 p.

Datry T, Bonada N, Heino J, 2016. Towards understanding the organisation of metacommunities in highly dynamic ecological systems. Oikos 125:149-159.

Dodds WK, 2002. Freshwater ecology: concepts and environmental applications. Academic Press, Cambridge: 569 pp.

Downes BJ, Hindell JS, Bond NR, 2000. What's in a site? Variation in lotic macroinvertebrate density and diversity in a spatially replicated experiment. Austral. Ecol. 25:128-139.

Garcia XF, Schnauder I, Pusch MT 2012. Complex hydromorphology of meanders can support benthic invertebrate diversity in rivers. Hydrobiologia 685:49-68.

García-Roger EM, del Mar Sánchez-Montoya M, Gómez R, Suárez ML, Vidal-Abarca MR, Latron J, Rieradevall M, Prat N, 2011. Do seasonal changes in habitat features influence aquatic macroinvertebrate assemblages in perennial versus temporary Mediterranean streams? Aquat. Sci. 73:567-579.

Golterman HL, Clymo RS, Ohnstad MA, 1978. Methods for physical and chemical analysis of freshwater. 2nd ed. JNP Handbook, N. 8.

Gray DP, Harding JS, 2007. Braided river ecology: a literature review of physical habitats and aquatic invertebrate communities. Science & Technical Publ., Department of Conservation.

Gray D, Harding JS, 2009. Braided river benthic diversity at multiple spatial scales: a hierarchical analysis of β diversity in complex floodplain systems. J. N. Am. Benthol. Soc. 28:537-551.

Gray DP, Harding JS, 2011. Multi-scaled environmental determinism of benthic invertebrate communities in braided rivers: evidence for complex hierarchical controls on local communities. Fundam. Appl. Limnol./Arch. Hydrobiol. 179:3-15.

Karaus U, Larsen S, Guillong H, Tockner K, 2013. The contribution of lateral aquatic habitats to insect diversity along river corridors in the Alps. Landscape Ecol. 28:1755-1767.

Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF, Holt RD, Shurin JB, Law R, Tilman D, Loreau M, Gonzalez A, 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecol. Lett. 7:601-613.

McCulloch CE, Neuhaus JM, 2005. Generalized Linear Mixed Models. Encyclopedia of Biostatistics. 4.

McLaughlin OB, Emmerson MC, O'Gorman EJ, 2013. Chapter Four-Habitat Isolation Reduces the Temporal Stability of Island Ecosystems in the Face of Flood Disturbance. Adv. Ecol. Res. 48:225-284.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H, 2016. vegan: Community Ecology Package. R package ver. 2.3-5. Available from: https://CRAN.R-project.org/package=vegan

Padial AA, Ceschin F, Declerck SA, De Meester L, Bonecker CC, Lansac-Tôha FA, Rodrigues L, Rodrigues LC, Train S, Velho LFM, Bini LM, 2014. Dispersal ability determines the role of environmental, spatial and temporal drivers of metacommunity structure. PLoS One 9:e111227.

Poff NL, Ward JV. 1990. Physical habitat template of lotic systems: recovery in the context of historical pattern of spatiotemporal heterogeneity. Environ. Manage. 14:629-645.

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.R-project.org/

Siqueira T, Bini LM, Roque FO, Cottenie K, 2012. A metacommunity framework for enhancing the effectiveness of biological monitoring strategies. PLoS One 7:e43626.

Starr SM, Benstead JP, Sponseller RA, 2014. Spatial and temporal organization of macroinvertebrate assemblages in a lowland floodplain ecosystem. Landscape Ecol. 29:1017-1031.

Tachet H, Richoux P, Bournaud M, Usseglio-Polatera P, 2010. [Invertébrés d'eau douce: systématique, biologie, écologie].[Book in French]. CNRS Ed, Paris: 588 pp.

Tickner D, Armitage PD, Bickerton MA, Hall KA, 2000. Assessing stream quality using information on mesohabitat distribution and character. Aquat. Conserv. 10:179-196.

Tockner K, Paetzold A, Karaus U, Claret C, Zettel J, 2006. Ecology of braided rivers, p. 339359. In: G.H. Sambrook Smith, J.L. Best, C.S. Bristow and G.E. Petts (eds.), Braided rivers: Process, deposits, ecology and management. Blackwell Publishing Ltd., Oxford.

Valderrama JC, 1977. Methods used by the Hydrographic Department of National Board of Fisheries, Sweden, p. 13-40. In: Report of the Baltic Intercalibration Workshop. Annex, Interim Commission for the Protection of the Environment of the Baltic Sea.

Van Der Nat D, Tockner K, Edwards PJ, Ward JV, Gurnell AM, 2003. Habitat change in braided flood plains (Tagliamento, NE-Italy). Freshwater Biol. 48:1799-1812.

Ward JV, Tockner K, Arscott DB, Claret C, 2002. Riverine landscape diversity. Freshwater Biol. 47:517-539.

Wickham H, 2009. ggplot2: elegant graphics for data analysis. Springer, New York: 212 pp.

Zilli FL, Marchese MR, 2011. Patterns in macroinvertebrate assemblages at different spatial scales. Implications of hydrological connectivity in a large floodplain river. Hydrobiologia 663:245-257.

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Mediterranean rivers: Consequences of water scarcity on benthic algal chlorophyll a content

Elena PIANO, Elisa FALASCO, Francesca BONA*

Department of Life Sciences and Systems Biology, University of Turin, Via Accademia Albertina 13, 10123 Turin, Italy
*Corresponding author: francesca.bona@unito.it

ABSTRACT

Mediterranean rivers are subjected to strong seasonality with drought during the hot season and extreme flows in autumn-winter. In particular, drought episodes and water scarcity alter the river morphology, with repercussions on primary production and the trophic chain. In this paper, we aimed at analysing the different responses in terms of chlorophyll *a* content of the three main photosynthetic groups composing stream periphyton, namely diatoms, cyanobacteria and green algae. This work was conducted in the Ligurian Alps (NW-Italy) on five oligotrophic streams (Argentina, Impero, Merula, Quiliano, and Vallecrosia), similar in terms of physico-chemical parameters. We measured chlorophyll *a* content of diatoms, cyanobacteria and green algae by means of an in situ fluorimetric probe (BenthoTorch®). Data were collected from April to October 2014 in: i) impacted sites, where the water scarcity was exacerbated by human pressure; ii) control sites. We applied Generalized Linear Mixed Models to investigate the response of total chlorophyll *a* and its relative proportions among the three algal groups in relation to the following environmental predictors: water depth, flow velocity, canopy shading, microhabitat isolation, sampling season, dissolved oxygen, temperature, pH, nutrients, and macrophyte coverage. Results showed an opposite response of diatoms and green algae. Diatoms were favoured in the control sites and under moderate flow conditions, while the probability of green algae presence was higher in the impacted sites and during the drought season. Cyanobacteria showed a response similar to green algae, preferring warm, isolated pools typical of the drought period. Diatoms proved to be the most sensitive to drought. More specifically, we found out that percentages of diatoms below 51% with respect to total benthic chlorophyll *a* indicate high hydrological disturbance. This study provides the first evidence that the proportion of chlorophyll *a* produced by diatoms can be a suitable indicator for monitoring programs aiming at determining the effects of water scarcity on river ecosystems.

Key word: Diatoms; algal biomass; BenthoTorch®; biomonitoring; drought; GLMM.

INTRODUCTION

Hydrological disturbance affects river physicochemical and morphological features, when hydrological seasonality co-occurs with anthropogenic modifications (*e.g.,* eutrophication, habitat alteration). The intermittency in water flow, defined by McDonough *et al.* (2011) as the lack of flowing surface during some portion of the year, may cause a decline in discharge or even a total drying of the river channel. Therefore, a strong alteration of the underlying structure of freshwater foodweb (Barthès *et al.*, 2015), with consequences on water quality and morphological features, is expected (Stevenson, 1996; Boix *et al.,* 2010). Drying up of the riverbed causes fragmentation of longitudinal, lateral and vertical connectivity, while deepest pools may persist and become isolated from the main course. As a result, Mediterranean streams are characterized by marked spatial and temporal heterogeneity (Lake, 2000), which may cause severe consequences on structure and functionality of biotic communities, including autotrophic organisms. Indeed, the Mediterranean climate is characterized by seasonality and variability of rainfalls, with dry summers and rainy autumns and winters. As a consequence, Mediterranean rivers experience recurring hydrological disturbances since extreme episodes (*e.g.,* floods, droughts) are part of their cyclic temporal pattern, with droughts that develop continuously and gradually over summer, followed by sudden floods in autumn (Gasith and Resh, 1999; Sabater *et al.*, 2006).

Benthic algae and cyanobacteria represent the most important primary producers in riverine ecosystems (Vannote *et al.*, 1980). They significantly contribute to the hydrological, physical and biogeochemical processes in running waters, playing an important role in providing habitats for small invertebrates and participating in the transformation of dissolved organic matter (Barthès *et al.*, 2015). Water scarcity and drought represent major constraints for biofilm in an increasing number of aquatic ecosystems, becoming a central concern in a context of climate change (Barthès *et al.*, 2015). Several works demonstrated that the hydrological disturbance could directly or indirectly alter the biofilm species composition (Boix *et al.*, 2010; Tornés and Rhuì, 2013). Cyanobacteria are considered as better adapted to desiccation than diatoms notably thanks to the production of mucilage (Romaní *et al.*, 2012). Variation of flow also causes changes in photosynthetic pigments (*i.e.,* the production of protective carotenoids), and occurrence of cell resistance structures (*i.e.,* spores; Timoner *et al.*, 2014). High nutrient concentration and light intensity may cause an increase in biomass during stable hydrological regimes (von Schiller *et al.*, 2008). However, in Mediterranean rivers, biomass of photosynthetic organisms is highly related to seasonal variations in river discharge, which can strongly affect water temperature, light and nutrient availability (Guasch *et al.*, 1995). Moreover, according to Dallas (2013), marked spatial heterogeneity may contribute to local differentiation of river biotic communities. Concerning diatoms, Smucker and Vis (2010) observed differences in terms of species composition between different microhabitats. As a consequence, we may expect differences also in terms of algal biomass among microhabitats, especially in Mediterranean rivers, where during summer spatial heterogeneity is exacerbated. Several works examined the physiological response of photosynthetic organisms to desiccation (Caramujo *et al.*, 2008; Timoner *et al.*, 2014), or focused on the response of algal biomass to variation of light and nutrients in Mediterranean streams (Sabater *et al.*, 2000, 2011; Veerart *et al.*, 2008; Tornés and Sabater, 2010). However, in very few cases a comprehensive examination of factors affecting the algal biomass of phototrophic communities in Mediterranean streams was performed (Riseng *et al.*, 2004; Sabater *et al.*, 2008; Urrea-Clos *et al.,* 2014). Moreover, a specific analysis on the different response of the main groups composing photobiota in streams is still missing. Thus, it is presently unclear how environmental parameters affected by hydrological variability induce significant variations on primary production, especially on the relative proportion of the three main groups that constitute the autotrophic biofilm, namely diatoms, cyanobacteria and green algae.

In this study, we aim at analysing the different response to environmental parameters, in terms of benthic chlorophyll *a* concentration (chl a), assumed as a proxy of algal biomass. Our hypotheses are: i) hydrological variability influences water quality and plays a main role in determining the biomass of diatoms, cyanobacteria and green algae in Mediterranean streams during the dry season; ii) biomass of diatoms, cyanobacteria and green algae show different responses to local variations of environmental parameters so the relative proportions of the three groups can be altered. We evaluated the chl a of the three main photosynthetic groups of stream periphyton under different levels of hydrological disturbance. In particular, we applied regression models to investigate their relationship with environmental features during flow intermittency in Mediterranean streams.

METHODS

Site description

This study was conducted in five streams of Liguria (NW-Italy), belonging to the same HER (122, Ligurian Alps) in the Mediterranean region. All five study streams are comparable in terms of geology (mostly calcareous), climate and altitude, substratum size (mainly cobbles and pebbles) and water quality. We selected sites classified at least as “good” (DM 260/2010), thus guaranteeing low interference on algal biomass data. All streams are permanent in the upper part of their course, but become temporary next to the mouth in the Ligurian Sea (Fig. 1).

Sampling design

We performed eight sampling campaigns from April to October 2014: the first one during spring (04/17), with moderate flow; the other seven campaigns were performed approximately every 15-20 days from the end of June to the end of October, before the first flood event and covering the entire drought period (summer: 06/30, 07/22, 08/05, 08/28; autumn: 09/24, 10/08 and 10/28). We selected 2 sampling sections for each stream (Fig. 1), one exposed to high hydromorphological disturbance (Impacted Section, IS) and the other acting as a control (Control Section, CS). The ISs were located downstream, in urban areas, characterized by intermittent water, which dried out during the summer, with only some deep isolated pools persisting in the dry riverbed. The CSs were located upstream, in natural areas, characterized by permanent water according to historical data, where we observed just a natural flow reduction.

In each section, we identified five sampling plots (microhabitats) representing the highest possible heterogeneity in terms of flow velocity, water depth, canopy shading, macrophyte coverage and isolation from the main river course. We selected such different microhabitats in order to detect differences in the response of photosynthetic organisms to spatial heterogeneity typical of Mediterranean rivers (Tornés and Sabater, 2010). Water was always present at the sampling moment in all microhabitats, even in small amount, also during water scarcity, when the main channel of the study streams was dry.

Data collection

In each sampling section, two types of water quality parameters were measured: i) physical and chemical parameters*:* water dissolved oxygen (DO), pH, temperature and conductivity were measured with a multiparametric probe (Hydrolab mod. Quanta), while suspended sediments (TSS) were determined by gravimetry following the Italian standard methods (APAT-IRSA CNR, 2003);

ii) nutrients*:* soluble reactive phosphorous (SRP) and nitrates were determined with a LASA 100 spectrophotometer according to APAT-IRSA (2003).

In each microhabitat, we measured water depth and flow velocity with a current meter (Hydro-bios Kiel). We also visually evaluated if the microhabitat was shaded or not and if it was isolated or connected with the main course. In isolated pools, we took measures of both physical and chemical parameters and nutrients to detect possible differences with the main course. For each microhabitat we took three measures of epilithic chl a of diatoms, cyanobacteria and green algae with the BenthoTorch®, developed by BBE Moldaenke GmbH (Schwentinental, Germany). BenthoTorch® is a Pulse Amplitude Modulated (PAM) fluorimeter emitting light pulses at three different wavelengths (470, 525 and 610 nm), recording the response of cyanobacteria, diatoms and green algae at 690 nm wavelength (Kahlert and McKie, 2014). We then selected the median value of chl a concentration for each autotrophic group and we calculated their proportion with respect to the total chl a concentration.

Statistical analyses

We firstly performed data exploration in accordance with Zuur *et al.* (2009, 2010). We used Cleveland dotplots and boxplots to assess the presence of extreme values and avoid unusual observations to exert an undue influence on estimated parameters (Zuur *et al.*, 2009). We then evaluated multicollinearity among predictors using Pearson correlation test and variance inflation factors (VIFs). Variables highly correlated (R^2 correlation value >0.05 and VIF >2) were excluded to avoid confounding effects and model overfitting (Zuur *et al.*, 2009). Given the high number of zeros, we transformed the flow velocity into a categorical variable ($0\ \mathrm{m\ s^{-1}}$ = standing water; $>0\ \mathrm{m\ s^{-1}}$ = flowing water). In accordance with the results obtained from these analyses, we selected the following predictive variables: i) continuous variables*:* water depth, DO, temperature, pH, SRP, nitrates, percentage of macrophyte coverage; ii) categorical variables*:* sampling section, sampling date, flow velocity, isolation and canopy shading. We considered the sampling date as a proxy of the hydrological disturbance, since we observed a progressive and

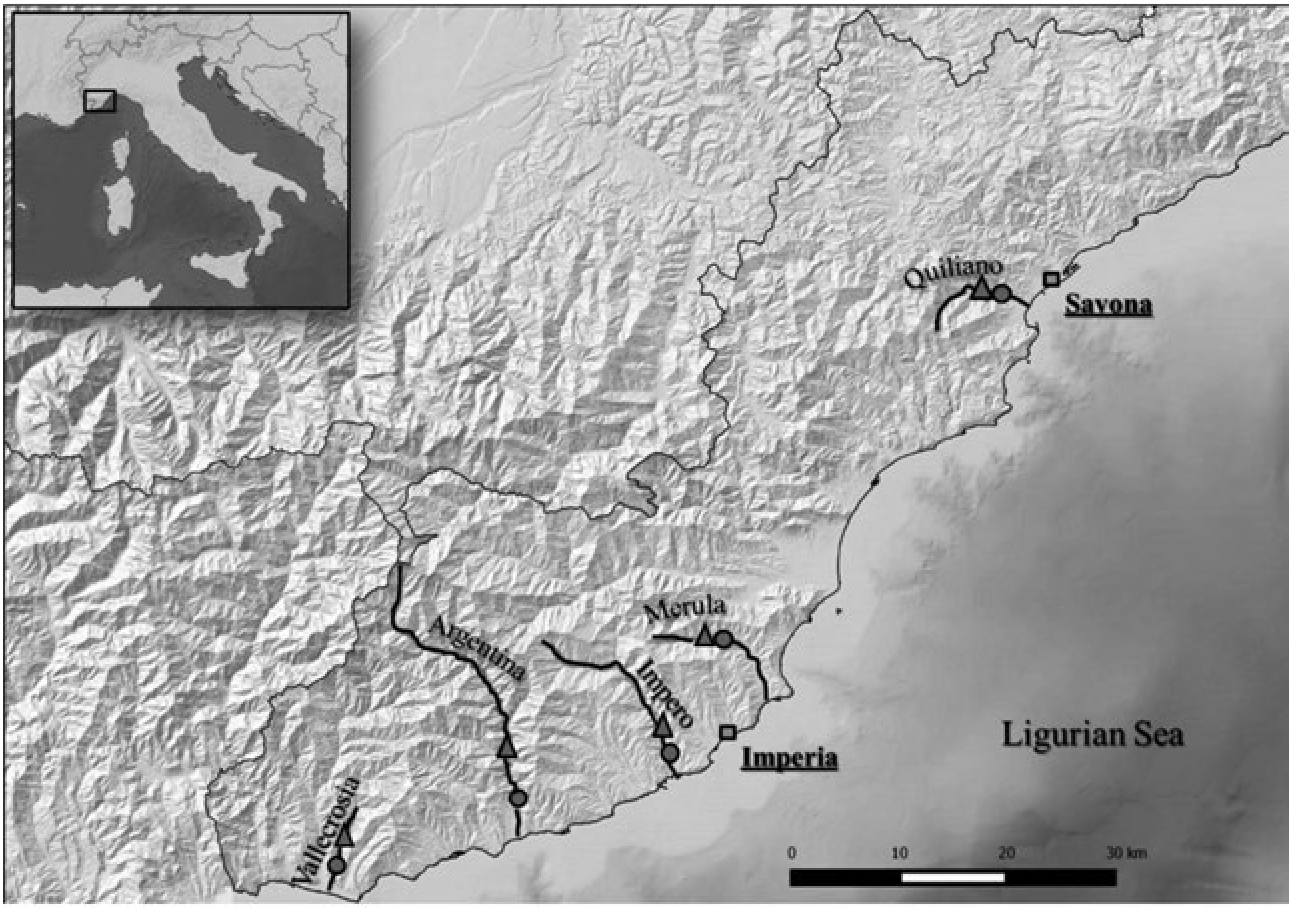

Fig. 1. Map of the five study streams and relative sampling sections. Diamonds, control sections (CSs); circles, impacted sections (ISs).

gradual reduction of water along the sampling period, together with the fragmentation in isolated pools. We tested the predictor variables and potential interactions against total chl a and relative proportions of diatoms, cyanobacteria and green algae, via Generalized Linear Mixed Models (GLMMs, in accordance with Zuur *et al.*, 2009) in R environment (R Core Team, 2014). Given the high number of zeros, green algae data were transformed into presence/absence data to obtain a more balanced dataset.

Given the spatial dependence of the data (two sections in each river), we applied the mixed procedure to include a grouping variable (river) as a random factor in order to account for the variation it introduced in our samples, rather than to test for its direct effect on the dependent variables. For the total chl a model, we assumed a gamma distribution (link function: log) which allowed us to deal with strictly positive variables, (Zuur *et al.*, 2009). For the relative proportions of the three photosynthetic groups, models were fitted with a binomial distribution (link function: log) which is able to deal with both presence/absence data (Bernoulli distribution) and proportional data (strictly binomial distribution) as recommended in Zuur *et al.*, (2009). In order to identify the best hypothesis supported by observations, we applied model selection (Johnson and Omland, 2004). We performed a backward elimination, progressively excluding variables according to AIC values (Zuur *et al.*, 2009). Variables not contributing to the fit of the model (*i.e.,* variables increasing the AIC value) were progressively dropped from the models thus avoiding overfitting (Hawkins, 2004). GLMMs were fitted via the lme4 R package (Bates *et al.*, 2014, version 1.0-6). We finally checked the correlation between the three photosynthetic groups with the Pearson correlation test.

RESULTS

Algal biomass and environmental factors

Flow velocity was higher in the CSs and presented lower values in summer and autumn during the drought season (lowest observed values 0.04 m s^{-1} in CSs and 0.00 in ISs), while no particular trend was observed for water depth (Tabs. 1 and 2). Conductivity showed an increasing trend from spring to autumn with higher values in the ISs (up to 777 µS cm^{-1}), while DO and nutrients decreased. TSS presented very low values; the only peaks (171 mg L^{-1}) were in ISs and during summer. pH was alkaline in both CSs and ISs and remained almost constant during all the sampling period. Temperature varied in accordance with the

Tab. 1. Summary of environmental parameters, in control and impacted sections. Data are expressed as mean and standard deviation of all samples.

Environmental parameters	Control sections	Impacted sections
FV (m s^{-1})	0.12 (±0.22)	0.07 (±0.16)
Water depth (cm)	28 (±18)	21 (±16)
Cond (µS cm^{-1})	401 (±159)	434 (±153)
DO (mg L^{-1})	9.3 (±1.2)	9.4 (±2.75)
Temperature (°C)	18.3 (±3.78)	19.9 (±4.15)
pH	8.53 (±0.46)	8.46 (±0.46)
TSS (mg L^{-1})	2.83 (±3.44)	8.20 (±28.34)
SRP (mg L^{-1})	0.009 (±0.013)	0.019 (±0.045)
N-NO_3 (mg L^{-1})	0.433 (±0.258)	0.675 (±0.868)
% Macrophytes	58 (±36)	65 (±34)

FV, flow velocity; Cond, conductivity; DO, dissolved oxygen; TSS, total suspended solids; SRP, soluble reactive phosphorus; N-NO_3, nitrates.

Tab. 2. Summary of environmental parameters (continuous variables), along the three seasons (spring, sampling campaign 04/17/14; summer, sampling campaigns 06/30/14, 07/22/14, 08/05/14 and 08/28/14; autumn, sampling campaigns 09/24/14, 10/08/14 and 10/28/14). Data are expressed as mean and standard deviation for each period considered.

Environmental parameters	Spring	Summer	Autumn
Flow velocity (m s^{-1})	0.16 (±0.25)	0.07 (±0.18)	0.09 (±0.18)
Water depth (cm)	22 (±17)	24 (±16)	25 (±18)
Conductivity (µS cm^{-1})	391 (±145)	393 (±163)	442 (±175)
Dissolved oxygen (mg L^{-1})	10.3 (±0.73)	8.95 (±2.1)	9.2 (±2.2)
Temperature (°C)	15.4 (±3.40)	22.3 (±2.37)	17.7 (±2.66)
pH	8.79 (±0.24)	8.68 (±0.97)	8.35 (±0.35)
TSS (mg L^{-1})	1.54 (±2.05)	7.32 (±26.65)	2.31 (±2.10)
SRP (mg L^{-1})	0.017 (±0.011)	0.010 (±0.013)	0.019 (±0.053)
N-NO_3 (mg L^{-1})	0.971 (±0.414)	0.420 (±0.205)	0.521 (±0.972)
% Macrophytes	35 (±32)	57 (±38)	69 (±31)

TSS, total suspended solids; SRP, soluble reactive phosphorus; N-NO_3, nitrates.

season with slight difference between the CSs and ISs. Total chl a showed higher values in the CSs (up to 31.4 μg cm^{-2}), with lowest values during summer (Fig. 2a). Diatoms were always the most abundant group, with higher values in CSs (up to 30.3 μg cm^{-2}), but their proportion progressively decreased in summer and autumn with respect to spring (Fig. 2b). On the contrary, cyanobacteria and green algae showed lower values but their proportions were higher in ISs and increased from spring to autumn (Fig. 2 c,d). The highest total chl a values were observed in September, which corresponded to a sharp increase in diatom primary production and a consequent reduction in proportion of green algae (Fig. 2c).

Statistical models

Results obtained from the final selected models showed clear differences between the three photosynthetic groups and total chl a in terms of environmental preferences. Total chl a was positively affected by the flow velocity and season; actually, a significant increase at the beginning of autumn was observed (Tab. 3 and Fig. 2a). According to the final selected model, diatoms proved to be favoured in environmental conditions of moderate flow periods. Indeed, their relative proportion was higher in microhabitats with flowing water and it was positively influenced by the oxygen concentration. The negative effect of drought on diatoms was revealed by the significant lower proportion in the ISs, and their decrease during the drought period (Tab. 3 and Fig. 2b). In particular, their proportion significantly decreased at the end of June and especially at the beginning of August. Cyanobacteria proved to be not so influenced by the drought, since the variables chosen as drought proxy (section and sampling date) were both excluded from the final model. However, their relative proportion was positively affected by the temperature, while the positive effect of isolation and the negative effect of depth were nearly significant, thus indicating a preference for warm, isolated shallow pools typical of the drought period. On average ISs showed a slight increase of cyanobacteria compared to CSs and a marked variability (Tab. 3 and Fig. 2c). Green algae showed an opposite trend with respect to diatoms, being

Tab. 3. For each dependent variable (total chl a; diatoms; cyanobacteria; green algae) the final selected model, estimated parameters (β-Estimate), standard errors (SE), t (or z) statistics and P values for each significant covariate are reported. For categorical variable, the reported values are referred to: section, control; date, 04/17/2014; flow velocity, class 0 (v = 0 m s^{-1}); isolation, connected.

Final selected model				
		Total chl a ~ velocity + date + (1|river)		
Variable	**β-Estimate**	**SE**	**t**	**P value**
Flow velocity (>0 m s^{-1})	0.349	0.092	3.806	0.0001
Date (24/09/2014)	0.613	0.176	3.484	0.0005
		Diatoms ~ section + velocity + oxygen + date + (1|river)		
Variable	**β-Estimate**	**SE**	**z**	**P value**
Section (impacted)	-1.675	0.385	-4.352	<0.0001
Flow velocity (>0 m s^{-1})	1.084	0.457	2.373	0.0176
Oxygen	0.174	0.080	2.179	0.0293
Date (30/06/2014)	-1.678	0.830	-2.021	0.0433
Date (05/08/2014)	-2.514	0.832	-3.022	0.0025
		Cyanobacteria ~ temperature + isolation + depth + (1|river)		
Variable	**β-Estimate**	**SE**	**z**	**P value**
Temperature	0.235	0.095	2.463	0.0138
Isolation (isolated)	1.287	0.705	1.826	0.0678
Water depth	-0.065	0.035	-1.893	0.0583
		Green algae ~ section + velocity + oxygen + macrophytes + depth + date + (1|river)		
Variable	**β-Estimate**	**SE**	**z**	**P value**
Section (impacted)	-1.223	0.353	3.463	0.0005
Flow velocity (>0 m s^{-1})	-2.173	0.543	-4.001	<0.0001
Oxygen	-0.252	0.091	-2.765	0.0057
Macrophyte	0.010	0.006	1.732	0.0833
Water depth	0.179	0.010	1.745	0.0810
Date (05/08/2014)	2.723	0.775	3.516	0.0004

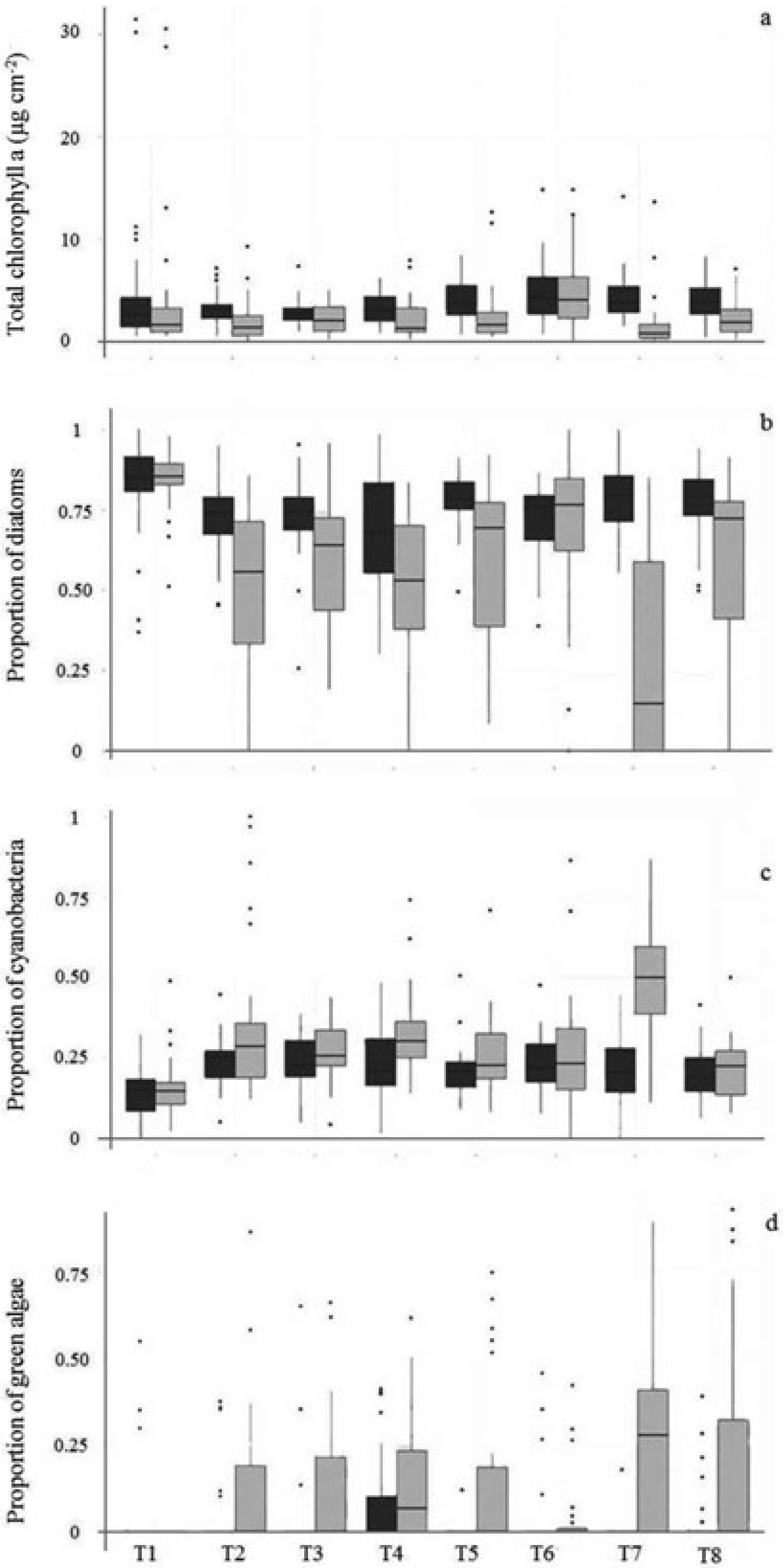

Fig. 2. Boxplots of total chlorophyll a (a) and relative proportions of diatoms (b), cyanobacteria (c) and green algae (d) in control (black) and impacted sites (grey). T1, 04/17/2014; T2, 06/30/2014; T3, 07/22/2014; T4, 08/05/2014; T5, 08/28/2014; T6, 09/24/2014; T7, 10/08/2014; T8, 10/28/2014.

favoured in environmental conditions characterising the drought season. Indeed, their presence was favoured in the ISs (Tab. 3 and Fig. 2d) and in microhabitats with standing water and low oxygen concentrations. Their probability of presence showed a positive trend during the drought period, with a significant increase at the beginning of August (Tab. 3 and Fig. 2d). A strong negative relationship between diatoms and the other two groups was revealed by the Pearson correlation test (diatoms *vs* cyanobacteria: $R = -0.59$, $t = -12.465$, $P<0.0001$; diatoms *vs* green algae: $R = -0.76$, $t = -19.844$, $P<0.0001$), while no correlation was found between cyanobacteria and green algae ($R = -0.07$, $t = -1.227$, $P=0.221$).

Proportion of diatoms was then selected as an indicator of hydrological disturbance, since it proved to be the most sensitive variable to water scarcity and it was also strongly negatively correlated with relative proportions of cyanobacteria and green algae. In order to determine a threshold to distinguish between natural drought and human-induced water scarcity we measured the median between the 75th percentile in the IS dataset and the 25th percentile in the CS dataset of diatom proportion. For having a better indication, data from the first sampling campaign were excluded in this phase. According to this procedure, the final threshold was 51% (75th percentile IS dataset = 50%; 25th percentile CS dataset = 52%).

DISCUSSION

Our data demonstrate that in Mediterranean streams the effects of hydrological variability can be quantitatively evaluated in terms of chlorophyll *a* content. More specifically, we verified the hypothesis that, among periphytic primary producers, diatoms are the most affected by hydrological disturbance, as partly suggested by previous studies (Romaní *et al.*, 2012; Barthes *et al.*, 2015), but controversial in others (Caramujo *et al.*, 2008).

The hydrological alteration was highlighted by a progressive lentification and fragmentation of the riverine habitat, with the formation of isolated pools in dry stretches. As a consequence, the flow velocity diminished from spring to autumn and from upstream to downstream (Tabs. 1 and 2). As pointed out in other studies (Gasith and Resh, 1999; Lake, 2003), the reduction of water supply and the evaporative processes caused an increase of ion concentration as demonstrated by the higher values of conductivity in the IS dataset and the increasing values from April to September. Moreover, we detected a lower oxygen concentration in isolated pools. On the contrary, nutrients did not show a pattern clearly related to the progressive hydromorphological alteration. According to literature data on Mediterreanean streams (Guasch *et al.*, 1995; Sabater *et al.*, 2006), we expected an increase of nutrient concentration with the progression of the drought; on the contrary, our summer values were lower than spring ones. The loss of lateral and longitudinal connectivity, due to the progressive drought, in parallel with reduction in precipitations, may cause a reduction in nutrient supply, as pointed out by Dahm *et al.* (2003). Moreover, the growth of riparian vegetation during the summer period may also act as a buffer zone retaining nitrates, as suggested by Sabater *et al.* (2000). Thus, during droughts nutrient input is expected to originate from groundwater and reflect the regional biogeochemistry (Clifford *et al.*, 2003).

Despite SRP and nitrate show higher values in the ISs than in Cs, due to different land uses, both CSs and ISs can be classified as oligotrophic and oligosaprobious in all five study streams according to common water quality classifications (Hofmann, 1994; Van Dam *et al.*, 1994). Accordingly, the range of total chl a corresponds to those of unenriched streams (Biggs, 1996). A further confirmation of the scarce influence of land use is given by the slightly lower values of total chl a concentration in the IS than in the CS dataset. Even if anthropogenic land uses may strongly increase algal biomass growth and alter community composition of photobiota, we observed lower values in the IS dataset, probably due to the hydrological disturbance (Taylor *et al.*, 2004; Cooper *et al.,* 2013). Our results are in accordance with Proia *et al.* (2012), who affirmed that flow variability, and in general physical disturbance, may result in a weak relationship between chl a and nutrient concentrations. However, it should be pointed out that the total chl a does not give a clear response to the reduction of water supply, since no significant differences were revealed between the CSs and ISs and no significant reduction along the sampling seasons was observed (Tab. 3). In fact, we found much clearer effects in terms of relative proportion of the three main photosynthetic groups compared to total chl a.

Diatoms largely dominated the phototrophic community over the entire study period, in accordance with Graba *et al.* (2014). However, their proportion gradually decreased during the hot season, being replaced by cyanobacteria and green algae, in accordance with Romaní *et al.* (2012). In particular, as confirmed by the Pearson correlation test, we observed an opposite trend between diatoms and green algae, similarly to what observed by Luttenton and Lowe (2006) in lentic environments. These results are also consistent with those found in artificially illuminated cave environments by Piano *et al.* (2015), who observed opposite trends for diatoms and green algae chlorophyll *a* contents. As demonstrated by the results of the statistical models, hydrological disturbance seemed to have a main role in determining the relative proportion of diatoms and green algae within the periphyton. In particular, at the beginning of August a significant decrease of the relative proportion of diatoms is combined with a significant increase in green algae probability of presence (Tab. 3). In general, diatoms were favoured in the CSs, whereas green algae probability of presence was higher in the ISs. The three photosynthetic groups showed clear different responses to hydrological changes. Microhabitat characteristics, described by flow velocity and water depth, proved to have a significant effect, confirming a microscale pattern of benthic photosynthetic microorganisms (Biggs, 1996). In particular, diatoms proved again to be the photosynthetic group most negatively influenced by drought. Their relative proportion was favoured in riffles, characterized also by high DO availability, while the presence of green algae was favoured in pools with lower DO, in accordance with Stevenson (1996). Cyanobacteria seemed not be directly influenced by the water scarcity, since their proportion did not show any differences between the two sampling sections or between the sampling dates. However, an indirect effect of low water flow could be hypothesized: cyanobacteria relative proportion significantly grows in shallow isolated pools, with high temperatures. We can assume that cyanobacteria are favoured during the hot season, as generally seen in lakes and ponds (Lake, 2003). These relationships highlighted an indirect response of cyanobacteria to the water scarcity, being favoured in environmental conditions strictly linked to this phenomenon.

Some limitations of this work should be highlighted. Indeed, our results were obtained from a limited number of streams, all belonging to the same HER and all classified at least as "good" in terms of water quality, in accordance with the WFD thresholds. There is thus a call for a validation in other streams at different water quality levels and belonging to different HERs of the Mediterranean region. In particular, a gradient of anthropic disturbance should be considered in order to disentangle the effect of nutrients on the primary production of our focus groups.

CONCLUSIONS

Comparing to phytoplankton, the assessment of benthic algal biomass has always been considered more challenging. On the one hand, it is considered as essential for tracking short and long-term changes and to assess the role of benthic algae in freshwater foodwebs (Stevenson, 1996). There have always been a series of constrains related to the costs of extensive sampling surveys needed for the high spatial and temporal variability of phytobenthic community (Kahlert and McKie, 2014). In recent years, promising methods have been developed to overcome these limitations, such as instruments for *in situ* measurements of chl a specifically conceived for benthic algae. In our study, the use of an *in situ* fluorimetric probe allowed us to discriminate between the main groups composing autotrophic biofilm and to obtain a rapid assessment of its composition in terms of primary producers. In a broader context, we suggest to use such probe as an integrative tool in supporting monitoring programs. Total chl a in itself is a good indicator of human-induced water-quality degradation and should be routinely monitored as part of an effective management program (McNair and Chow-Fraser, 2003), but as a response metric it is not stressor specific as it reflects changes in concentration of nutrients, various pollutants, physical conditions and interactions of stressors (Zalack *et al.*, 2010). In our study it did not prove to clearly distinguish between the CS and the IS datasets, thus not being a useful indicator for measuring the disturbance caused by water scarcity. On the other hand, diatoms proved to be the most sensitive group to water scarcity in terms of chl a and the group with the highest representativeness. According to our data, we can conclude that within a biofilm, a diatom proportion below the threshold of 51% could be a signal of hydrological stress caused by water scarcity in nutrient unenriched Mediterranean streams. These values could be applied in the future for the environmental impact assessment of water abstraction works. In general, the decrease of diatom proportion within a biofilm can imply negative consequences in the stream ecosystem functionality since it enhances the competitiveness of filamentous green algae and cyanobacteria that are less edible for grazers, similarly to what happens in lentic and eutrophic ecosystems (Caramujo *et al.*, 2008). Understanding the response and contribution of biofilm main components is essential to evaluate the effect of flow intermittency on stream ecosystem functioning.

The ratio between diatom chl a and total chl a can be potentially included as a metric in monitoring programs of Mediterranean streams, integrating chemical parameters and biological indices commonly adopted for classifying the ecological status, but not specifically sensitive to hydrological disturbance.

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REFERENCES

APAT- IRSA CNR, 2003. [Metodi analitici per le acque, vol I. Rapporti 29/2003].[Report in Italian]. APAT, Rome: 342 pp.

Barthès A, Ten-Hage L, Lamy A, Rols J-L, Leflaive J, 2015. Resilience of aggregated microbial communities subjected to drought - Small-scale studies. Microbial Ecol. 70:9-20.

Bates D, Maechler M, Bolker B, Walker S, 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-6 2014. http://CRAN.R-project.org/package=lme4

Biggs BJF, 1996. Patterns in benthic algae of streams, p. 31-56. In: R.J. Stevenson, M.L. Bothwell, R.L. Lowe (eds.), Algal ecology. Freshwater benthic ecosystems. Academic Press, Cambridge.

Boix D, García-Berthou E, Gascón S, Benejam L, Tornés E, Sala J, Benito J, Munné A, Solà C, Sabater S, 2010. Response of community structure to sustained drought in Mediterranean rivers. J. Hydrol. 383:135-146.

Caramujo MJ, Mendes CRB, Cartaxana P, Brotas V, Boavida MJ, 2008. Influence of drought on algal biofilms and meiofaunal assemblages of temperate reservoirs and rivers. Hydrobiologia 598:77-94.

Clifford ND, Baker MA, Moore DI, Thibault JR, 2003. Coupled biogeochemical and hydrological responses of streams and rivers to drought. Freshwater Biol. 48:1219-1231.

Cooper SD, Lake SP, Sabater S, Melack JM, Sabo JL, 2013. The effects of land use changes on streams and rivers in Mediterranean climates. Hydrobiologia 719:383-425.

Dahm CN, Baker M, Moore D, Thibault JR, 2003. Coupled biogeochemical and hydrological responses of streams and rivers to drought. Freshwater Biol. 48:1219-1231.

Dallas HF, 2013. Ecological status assessment in Mediterranean rivers: complexities and challenges in developing tools for assessing ecological status and defining reference conditions. Hydrobiologia 719:483-507.

Gasith A, Resh VH, 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annu. Rev. Ecol. Syst. 30:51-81.

Graba M, Sauvage S, Majdi N, Mialet B, Moulin F, Urrea G, Buffan-Dubau E, Tackx M, Sabater S, Sanchez-Perez M, 2014. Modelling epilithic biofilms combining hydrodynamics, invertebrate grazing and algal traits. Freshwater Biol. 59:1213-1228.

Guasch H, Martí E, Sabater S, 1995. Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. Freshwater Biol. 33:373-383.

Hofmann G, 1994. [Aufwuchs-Diatomeen in Seen und ihre Eignung als Indikatoren der Trophie], p. 1-908. In: H. Lange-Bertalot (ed), [Bibliotheca Diatomologica, Vol. 30].[Book in German]. Gebrüder Borntraeger Verlag.

Hawkins DM, 2004. The problem of overfitting. J. Chem. Inf. Comp. Sci. 44:1-12.

Kahlert M, McKie B, 2014. Comparing new and conventional methods to estimate benthic algal biomass and composition in freshwaters. Environ. Sci. Processes Impacts 16:2627-2634.

Johnson JB, Omland KS, 2004. Model selection in ecology and evolution. Trends Ecol. Evol. 19:101-108.

Lake PS, 2000. Disturbance, patchiness and diversity in streams. J. N. Am. Benthol. Soc. 19:573-592.

Lake PS, 2003. Ecological effects of perturbation by drought in flowing waters. Freshwater Biol. 48:1161-1172.

Luttenton MR, Lowe RL, 2006. Response of a lentic periphyton community to nutrient enrichment at low P:N ratios. J. Phycol. 42:1007-1015.

McDonough OT, Hosen JD, Palmer MA, 2011. Temporary streams: the hydrology, geography, and ecology of non-perennially flowing waters, p. 259-290. In: S.E. Elliot, L.E. Martin (eds), Rivers ecosystems: dynamics, management and conservation. Nova Publishers, Hauppauge.

McNair S, Chow-Fraser P, 2003. Change in biomass of benthic and planktonic algae along a disturbance gradient for 24 Great Lakes coastal wetlands. Can. J. Fish. Aquat. Sci. 60:676-689.

Piano E, Bona F, Falasco E, La Morgia V, Badino G, Isaia M, 2015. Environmental drivers of phototrophic biofilms in an Alpine show cave (SW-Italian Alps). Sci. Total. Environ. 536:1007-1018.

Proia L, Romaní AM, Sabater S, 2012. Nutrients and light effects on stream biofilms: a combined assessment with CLSM, structural and functional parameters. Hydrobiologia 695:281-291.

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: http://www.R-project.org/

Riseng CM, Wiley MJ, Stevenson RJ, 2004. Hydrologic disturbance and nutrient effects on benthic community structure in Midwestern US streams: A covariance structure analysis. J. N. Am. Benthol. Soc. 23:309-326.

Romaní AM, Amalfitano S, Artigas J, Fazi S, Sabater S, Timoner X, Ylla I, Zoppini A, 2012. Microbial biofilm structure and organic matter use in Mediterranean streams. Hydrobiologia 719:43-58.

Sabater F, Butturini A, Martí E, Muñoz I, Romaní A, Wray J, Sabater S, 2000. Effects of riparian vegetation removal on nutrient retention in a Mediterranean stream. J. N. Am. Benthol. Soc. 19:609-620.

Sabater S, Artigas J, Gaudes A, Muñoz I, Urrea-Clos G, Romaní AM, 2011. Long-term moderate nutrient inputs enhance autotrophy in a forested Mediterranean stream. Freshwater Biol. 56:1266-1280.

Sabater S, Elosegi A, Acuña V, Basaguren A, Muñoz I, Pozo J, 2008. Effect of climate on the trophic structure of temperate forested streams. A comparison of Mediterranean and Atlantic streams. Sci. Total. Environ. 390:475-484.

Sabater S, Guasch H, Muñoz I, Romaní AM, 2006. Hydrology, light and the use of organic and inorganic materials as structuring factors of biological communities in Mediterranean streams. Limnetica 25:335-348.

Smucker NJ, Vis ML, 2010. Using diatoms to assess human impacts on streams benefits from multiple-habitat sampling. Hydrobiologia 654:93-109.

Stevenson RJ, 1996. An introduction to algal ecology in freshwater benthic habitats, p. 3-30. In: R.J. Stevenson, M.L. Bothwell, R.L. Lowe (eds.), Algal ecology. Freshwater benthic ecosystems. Academic Press, Cambridge.

Taylor SL, Robert SC, Walsh CJ, Hatt BE, 2004. Catchment urbanisation and increased benthic algal biomass in streams: linking mechanisms to management. Freshwater Biol. 49:835-851.

Timoner X, Buchaca T, Acuña V, Sabater S, 2014. Photosynthetic pigment changes and adaptations in biofilms in response to flow intermittency. Aquat. Sci. 76:565-578.

Tornés E, Ruhì A, 2013. Flow intermittency decreases nestedness and specialization of diatom communities in Mediterranean rivers. Freshwater Biol. 58:2555-2566.

Tornés E, Sabater S, 2010. Variable discharge alters habitat suitability for benthic algae and cyanobacteria in a forested Mediterranean stream. Mar. Freshwater. Res. 61:441-450.

Urrea-Clos G, García-Berthou E, Sabater S, 2014. Factors explaining the patterns of benthic chlorophyll-a distribution in a large agricultural Iberian watershed (Guadiana river). Ecol. Indic. 36:463-469.

Van Dam H, Mertens A, Sinkeldam J, 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Neth. J. Aquatic. Ecol. 28:117-133.

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE, 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37:130-137.

Veerart AJ, Romaní AM, Tornés E, Sabater S, 2008. Algal response to nutrient enrichment in forested oligotrophic stream. J. Phycol. 44:564-572.

von Schiller D, Martí E, Riera JL, Ribot M, Marks JC, Sabater F, 2008. Influence of land use on stream ecosystem function in a Mediterranean catchment. Freshwater Biol. 53:2600-2612.

Zalack J, Smucker N, Vis M, 2010. Development of a diatom index of biotic integrity for acid mine drainage impacted streams. Ecol. Indic. 10:287-295.

Zuur AF, Ieno EN, Elphick SC, 2010. A protocol for data exploration to avoid common statistical problem. Methods Ecol. Evol. 1:3-14.

Zuur AF, Ieno EN, Walker NJ, Savaliev AA, Smith GM, 2009. Mixed effect models and extensions in ecology with R. Springer, Berlin: 574 pp.

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Mediterranean river biomonitoring in Central Italy: Diatom biodiversity and characterization of communities

Valentina DELLA BELLA,[1*] Rosalba PADULA,[2] Fedra CHARAVGIS,[2] Alessandra CINGOLANI,[2] Paolo COLANGELO[3]

[1]ARPA UMBRIA, Environmental Protection Agency of Umbria Region, Via C.A. Dalla Chiesa 32, 05100, Terni; [2]ARPA UMBRIA, Environmental Protection Agency of Umbria Region, Via Pievaiola 207/B-3, 06132, Perugia; [3]CNR-ISE, Institute of Ecosystems Study, Largo V. Tonolli 50, 28922 Verbania-Pallanza, Italy
*Corresponding author: v.dellabella@arpa.umbria.it

ABSTRACT

In compliance with the European and Italian regulations, the Environmental Protection Agency of Umbria Region (ARPA Umbria) defined specific river monitoring programs and networks based on river type definition, human pressures and risk analysis. The Umbria Region lies in Central Italy and it can be split into three hydro-ecoregions belonging to the Mediterranean area. Data on diatom community composition were collected in five different Mediterranean macrotypes (M1-M5) throughout the diatom-based river monitoring network that is composed by 52 sampling stations in 36 watercourses. The main aim of this study was to characterise and to analyse diatom diversity across the different regional river macrotypes. Specifically, we investigated if: i) there were differences in species diversity (species richness and Shannon Index) among macrotypes; ii) there was difference in three water quality indexes (ICMi, IPS, and TI) among sites; and iii) there was a relationship between the observed ICMi, IPS and TI value and the diatom diversity. Two-hundred diatom species and varieties were identified, and the number of species *per* sampling station ranged from a minimum of 10 to a maximum of 38 species. The most frequent and abundant species were *Amphora pediculus*, *Achnanthidium minutissimum, Navicula cryptotenella*, *Nitzschia dissipata*, and each macrotype showed some peculiar species. The ecological status evaluation based on Intercalibration Common Metric Index (ICMi) classified 69% of the water bodies in high or good class. Significant differences in diversity and ICMi value among stream macrotypes were found, with M4 (small and medium mountain) and M5 (small, lowland, temporary) typologies showing the lowest species richness, and with M5 showing the lowest Shannon Index. Conversely, M2 (small and medium lowland) and M5 showed the highest ICMi value. Lastly, significant correlations between Shannon Index and the ICMi, IPS and TI indexes were found.

Key word: Benthic algae; indicator species; Bacillariophyceae; diversity metrics; Water Framework Directive 2000/60/EC.

INTRODUCTION

Ecological status assessment based on benthic algal communities is one of the requirements of the European Water Framework Directive 2000/60/EC (WFD; European Commission, 2000), implemented in Italy with Legislative Decree 152/2006 (Italian Regulation, 2006). Diatoms are the algal group most widely used as indicators of river quality. They are the main component of phytobenthos of river water bodies and have biological and ecological characteristics that make them a good indicator for the characterization of water quality (Kelly *et al*., 1998). They have a cosmopolitan distribution and high sensitivity to water physico-chemical characteristics and environmental conditions (for a review see Prygiel *et al*., 1999).

In Italy, studies on diatom communities have been mainly focused on Alpine streams (Cantonati and Pipp, 2000; Battegazzore *et al*., 2004; Bona *et al*., 2007; Rott *et al*., 2006; Zorza and Honsell, 2008; Beltrami *et al*., 2009; Falasco *et al*., 2012) and some main watercourses in Southern Italy (Battegazzore *et al*., 2003; Finocchiaro *et al*., 2011). Existing data on benthic diatom assemblages of Mediterranean rivers of Central Italy are scattered, covering some central Apennine streams (Dell'Uomo, 1999; Scuri *et al*., 2006; Torrisi *et al*., 2008, 2010), some volcanic-siliceous streams (Della Bella *et al*., 2012), and the main river water body, the Tiber River (Cappelletti *et al*., 2005; Ciutti *et al*., 2007). A few studies on river diatom communities have been carried out on main streams of Umbria Region, Central Italy (Mancini *et al*., 2008).

In compliance with the European and national regulations, the Environmental Protection Agency of Umbria Region (ARPA Umbria) defined specific monitoring programs and networks based on river type definition, human pressures, and risk analysis (ARPA Umbria, 2008). ARPA Umbria accomplished the first biomonitoring of river diatoms between 2009 and 2012 and calculated the Intercalibration Common Metric Index (ICMi), developed in Italy for the assessment of benthic algal ecological status (Mancini and Sollazzo, 2009). The main purposes of this study were to analyse diatom diversity of regional river types and to identify the characterising diatoms of different river types in Umbria (Mediterranean river typologies defined by altitude, river basin area, and river hydrology).

This study represented a contribution to diatom-based river quality assessment following the WFD in Italy and to evaluation of differences in diversity of diatom communities in Mediterranean river types. In detail, we investigated if: i) there were differences in species diversity (species richness and Shannon Index) among river types, ii) there was difference in ICMi, IPS, and TI value among sites, and iii) there was a relationship between the observed ICMi, IPS and TI value and the diatom diversity.

METHODS

Study area

The Umbria Region is located in the Mediterranean area of Central Italy and it is included in three hydroecoregions: Tuscan Hills, Apennines Centre and Italian Volcanics (Wasson *et al*., 2006; Italian Regulation, 2008; Fig. 1). The morphology varies from lowlands in the central area to highlands in the eastern part and it is mainly characterized by a temperate climate with hot, dry summers and cool, wet winters. Almost the entire area belongs to the Tiber River basin, in the hydrographic district of Central Italy. The Tiber River begins in the northern part of the Apennine Mountains and crosses the Umbria Region from North to South, collecting waters from several tributaries and with a length of 400 km before draining into the Tyrrhenian Sea in the Lazio Region. Tributaries from the eastern part of the region show steady flows due to carbonate sources from the Apennines and a good ecological quality. On the contrary, all over the central and western area the rivers show a high flow variability and significant impacts on water quality, due to human activities.

According to national legislation (DM 131/08; Italian Regulation, 2008), 135 river waterbodies were included in the monitoring network and assigned to 19 types, which were grouped in five main Mediterranean macrotypes (M1-M5) defined in the European Intercalibration exercise (European Commission, 2008; Tab.1). Most of the regional water bodies belongs to macrotypes M5 (39%) or M1 (34%) and are characterized by small basins and low or temporary discharges, while there are few big rivers (8%) with significant flows in floodplain areas (macrotype M3). Several rivers, altered by human activities such as land drainage, dredging, flood protection, water abstraction, building of dams to create reservoirs, have been designated as "artificial" (1; AWB) or "heavily modified" (20; HMWB) water bodies (Fig. 2).

In order to define monitoring networks and programs, a risk analysis and an anthropic pressure assessment have been carried out (ARPA Umbria, 2008). Data were collected using the diatom-based river monitoring network that is composed by 52 sampling stations distributed in 36 watercourses. Data were collected between 2009 and 2012. Almost 100 diatom samples were collected and analysed. Data gathered allowed to calculate for 48 out of 52 sites the Intercalibration Common Metric Index (ICMi), developed for the assessment of benthic algal ecological status follow-

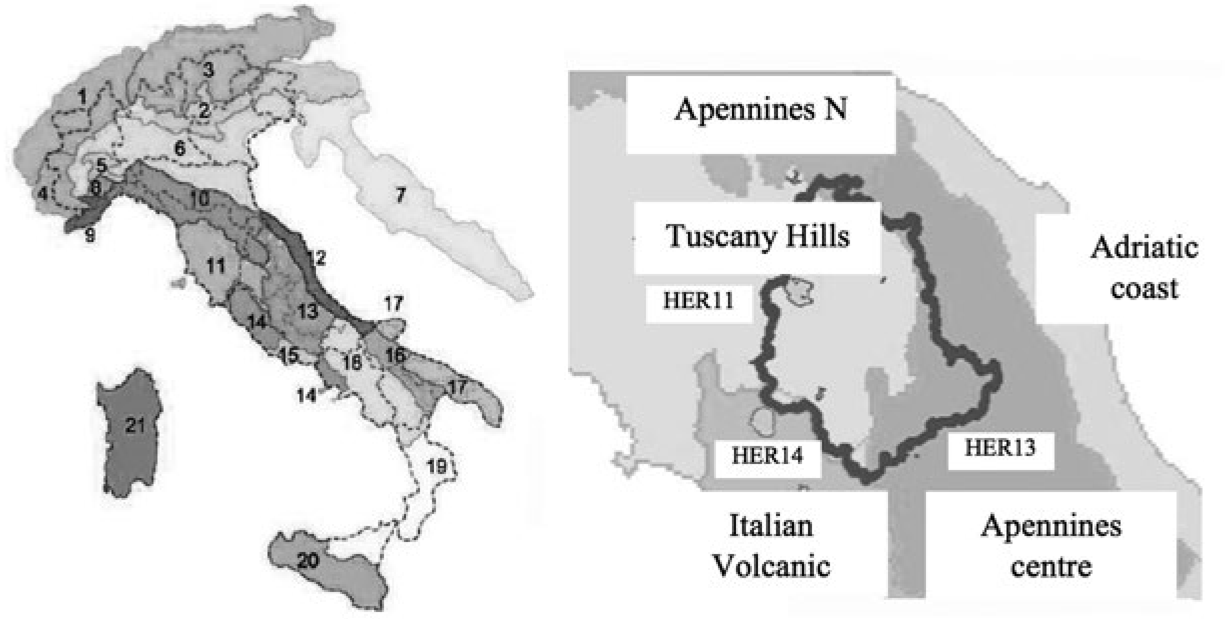

Fig. 1. Hydro-ecoregions (HER; WFD 2000/60/CEE) defined by CEMAGREF for Italy (Wasson *et al.*, 2006), then verified at the local/regional scale and identified according to Basin Authority, Regions, Regional Environmental Agency and Italian Ministry of Environment (DM 131/08; Italian Regulation, 2008). HER11, Tuscany Hills; HER13, Apennine Centre; HER14, Italian volcanic regions.

ing the formula: ICMi index = (RQE_IPS + RQE_TI)/2 (Mancini and Sollazzo, 2009). The ICMi is based on two sub-indices: the IPS index (CEMAGREF, 1982), which mainly assesses the sensitivity of species to organic pollution and the TI Index (Rott *et al*., 1999), which is based on the species sensitivity to trophic pollution. For the diatom-based ecological status assessment, the value of the two sub-indices have to be expressed as Ecological Quality Ratio (RQE_IPS and RQE_TI) with the respective reference values for each river macrotype following the DM 260/2010 (Italian Regulation, 2010). Boundaries between quality classes adopted for river macrotypes M1-M2-M3-M4 are: High/Good = 0.80, Good/Moderate = 0.61, Moderate/Poor = 0.51, Poor/Bad = 0.25; and for M5 are: High/Good = 0.88, Good/Moderate = 0.65, Moderate/Poor = 0.55, Poor/Bad = 0.26 (reported values are the lowest value of higher class). We evaluated the ecological status on the basis ICMi for 38 out of 48 sites because ten sites

Tab. 1. River Mediterranean macrotypes identified in the Region Umbria.

River macrotype	River macrotype description	Number of water bodies	Sampling sites
M1	Small mid-altitude streams (200-800 m asl)	45	20
M2	Small and medium lowland streams (<400 m asl)	23	12
M3	Large lowland rivers	11	10
M4	Small and medium mountain streams (400-1500 m asl)	2	2
M5	Small, lowland, temporary (<300 m asl)	54	8

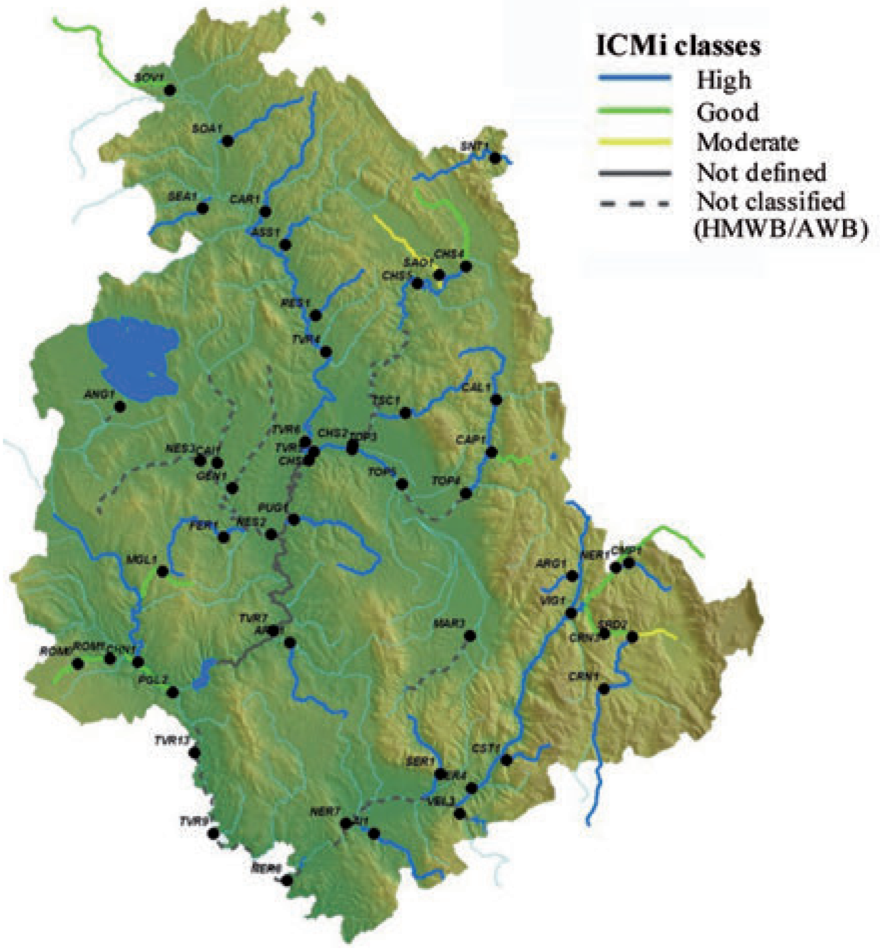

Fig. 2. Diatom-based river classification using ICMi Index.

were defined as heavily modified/artificial water bodies (HMWB/AWB).

Sampling activities, data analysis and statistics

Diatom sampling, sample treatment and laboratory work were carried out according to the European recommendations (European Committee for Standardization, 2003, 2004) and national guidelines (APAT, 2007). In order to sample epilithic forms, the upper surface of five stones was brushed with a toothbrush in each sampling site. Diatom samples were immediately placed in an ice bag and carried to the laboratory. In order to identify the diatom frustules, the diatom valves were cleaned using hydrogen peroxide to eliminate organic matter and with hydrochloric acid to dissolve calcium carbonate. Clean diatom frustules were mounted in a synthetic resin with high-refraction index (Naphrax©). Successively in each sample up to 400 valves were counted and classified at the species or the variety level using a light microscope with 1000x magnification. Morphometric measurements were made with the aid of image analysis software (CellB, Imaging Software for Life Sciences Microscopy© OLYMPUS Soft Imaging Solutions GmbH, Münster, Germany). The main references for diatom taxonomy were Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b, 2000), Krammer (2000), Lange-Bertalot (2001), and Hofmann *et al*. (2011).

In order to analyse diatom diversity and identify the characteristic species of different river macrotypes, we performed two types of statistical analyses. To define the characterizing species of river macrotypes, we applied the Indicator Species Analysis (ISA, Dufrene and Legendre, 1997). This analysis establishes indicator values for different species combining the information on relative abundances of species in a particular group of samples with the relative frequency of the species occurring in the group. Successively, in order to test if there were differences in species diversity among macrotypes and hydro-geographic regions (HER), we used a GLM procedures including the diatom species richness or the Shannon Index as the response variables (assuming a Poisson distribution for species richness and a Gaussian for Shannon) and using in turn the macrotype (5 categories) and HER (2 categories) as independent variable. The diatom species richness and Shannon Index were calculated at site level. Successively, six separate GLMs were performed in order to test the presence of a significant difference among macrotypes and HERs for the three indexes ICMi, TI and IPS. Lastly, GLM was used in order to test if there are any significant relationship between ICMi, IPS and TI and diatom diversity (the observed species richness and Shannon at the site level). These analyses were also replicated excluding the 10 sites not classified because defined as heavily modified/artificial water bodies (HMWB/AWB). Significance of all GLMs was tested against a null model (*i.e.*, no significant relationships with factors) by chi-squared test. All the statistical analyses were performed using the R statistical environment ver. 3.30 (R Core Team, 2015).

RESULTS AND DISCUSSION

A total of 200 diatom species and varieties were identified (Supplementary Tab. 1) in 96 collected samples (almost 41,000 counted frustules). The number of species *per* sample varied from 10 to 38 with a mean of 22 species. The most frequent and abundant species were *Amphora pediculus* (Kützing) Grunow, *Achnanthidium minutissimum* (Kützing) Czarnecki, *Navicula cryptotenella* Lange-Bertalot, *Nitzschia dissipata* (Kützing) Grunow ssp. *dissipata* (Tab. 2).

A. pediculus and *A. minutissimum* are cosmopolitan

Tab. 2. List of most frequent and abundant species. Only species found in more than 2/3 of sampling sites are listed.

Species	Sites	Samples	Mean relative abundance (%)	Median relative abundance (%)	Max relative abundance (%)
Amphora pediculus (Kützing) Grunow	47	91	10.52	6.00	55.67
Achnanthidium minutissimum Kützing	47	87	20.09	10.27	79.12
Navicula cryptotenella Lange-Bertalot	45	79	4.53	2.10	23.90
Nitzschia dissipata (Kützing) Grunow	45	72	2.58	0.67	25.24
Cocconeis placentula Ehrenberg	42	64	8.84	0.85	86.82
Navicula tripunctata (Müller) Bory	39	60	2.27	0.25	22.76
Gomphonema parvulum (Kützing) Kützing	37	57	0.98	0.25	10.82
Gomphonema olivaceum (Hornemann) Brébisson	40	54	1.65	0.25	19.01
Rhoicosphenia abbreviata (Ag.) Lange-Bertalot	34	52	2.40	0.23	69.51
Nitzschia palea (Kützing) W. Smith	35	47	2.14	0.00	21.13

species, very common and abundant in the Italian rivers and streams, often dominant in diatom communities and considered pioneer species (Falasco and Bona, 2013). *A. pediculus* can tolerate high concentration of nutrients in water with low organic load, while *A. minutissimum* has a wide ecological range as it can tolerate large ranges of organic and inorganic pollution (Falasco *et al*., 2013). The high abundance and frequency of these both pioneer species were probably due to the high flow variability of rivers for the most part of the region in analysis. *N. cryptotenella* is also a cosmopolitan and mobile species. Like *A. pediculus*, this species is quite sensitive to organic pollution, and it can be found in oligotrophic to eutrophic waters (Falasco *et al*., 2013). *N. dissipata*, also found in several sampling sites though with less abundance, may become a dominant species in diatom community in site with medium - high content of nutrients such as nitrate and total phosphorus (P_{tot} >46.5 µg L^{-1}; Falasco *et al*., 2013; Hofmann, 1994). Among the most common and abundant species there were also *Gomphonema olivaceum* (Hornemann) Brébisson, and *Gomphonema parvulum* (Kützing) Kützing. *G. olivaceum* is sensitive to organic pollution but can tolerate a moderate trophic load. Hence, it is generally abundant in the limestone streams with high conductivity (Falasco *et al*., 2013). *G. parvulum*, belonging to a complex of species with a large ecological value and a high tolerance to trophic and organic pollution, it is also widespread in sites affected by anthropogenic pressure due to urbanization and agriculture practices (Della Bella *et al*., 2007; Falasco *et al*., 2013).

Among the species found in more than half of the monitoring sites was *Rhoicosphenia abbreviata* (Agardh) Lange Bertalot, an epiphytic species which can be often found on aquatic macrophytes in brackish water and/or characterized by limestone substrates. It is characterized by a moderate tolerance to eutrophication (Della Bella *et al*., 2007). Both *N. dissipata* and *R. abbreviata* are characteristic species of sites with a surrounding land use of the catchment area devoted to agriculture that reflected in high values of nutrients in waters, as shown by studies carried out in other Italian regions (Bona *et al*., 2007; Della Bella *et al*., 2012). During the study, we also identified *Reimeria uniseriata* Sala Guerrero & Ferrario, a diatom species that is considered an alien species in Italy (Falasco *et al*., 2013), and *Diadesmis confervacea* Kützing var. *confervacea*, a taxon considered non-native in Europe coming from tropical or subtropical areas (Coste and Ector, 2000). Both these species were found with low abundances (<5.5%) and in only four samples belonging to M1, M2 and M3 river typologies.

The Indicator Species Analysis (ISA) suggested that in each river type some typical species occur (Tab. 3). For example, temporary rivers (M5) are characterized by *A. minutissimum*, which is a pioneer and mobile species, capable of a fast river substratum recolonization after repeated annual dry phases. Plain large rivers (M3), instead, are characterized by slow water flow, which allows the development of planktonic species that can be also found in benthic communities, like *Cyclotella meneghiniana* Kützing.

According to the GLM analyses we found significant difference among macrotypes in terms of species richness (df=4, deviance=16.271, P=0.003; Fig. 3) and Shannon Index (df=4, deviance=2.523, P=0.019; Fig. 3). When we evaluated how species richness and Shannon Index were distributed across macrotypes (Fig. 3), we found that M4 and M5 showed a significant lower species richness. M5 showed also a significant lower value of the Shannon indexes (Tab. 4). By contrast, when we focussed on HERs (Fig. 3), we found that only the species richness was significant different between HER 11 and HER 13 (df=1, deviance=5.1931, P=0.023) with the last showing a significant lower estimate of species richness values (Tab. 4).

Tab. 3. Characteristic species, defined by Indicator Species Analysis, for the five Mediterranean river macrotypes in analysis.

River macrotype ***Species***
M1 - Small mid-altitude streams
Achnanthidium pyrenaicum (Hustedt) Kobayasi
Amphora inariensis Krammer
Encyonema silesiacum (Bleisch in Rabh.) D.G. Mann
M2 - Small and medium lowland streams
Cymatopleura solea (Brébisson) W. Smith
Gomphonema parvulum (Kützing) Kützing
Navicula tripunctata (Müller) Bory
M3 - Large lowland rivers
Cyclotella meneghiniana Kützing
Navicula capitatoradiata Germain
Navicula cincta (Ehrenberg) Ralfs
Navicula cryptocephala Kützing
Navicula cryptotenella Lange-Bertalot
Nitzschia frustulum (Kützing) Grunow
M4 - Small and medium mountain streams
Denticula tenuis Kützing
Encyonema minutum (Hilse in Rabh.) D.G. Mann
Nitzschia fonticola Grunow
Nitzschia palea (Kützing) W. Smith
M5 - Small, lowland, temporary
Achnanthidium minutissimum (Kützing) Czarnecki
Encyonopsis microcephala (Grunow) Krammer
Fragilaria recapitellata Lange-Bertalot & Metzeltin
Gomphonema tergestinum Fricke

Tab. 4. Estimated richness and Shannon index coefficients. GLM results were reported for the three significant models. Estimates and their standard errors, t or z test values and associate significance for each level of the fixed factors were reported.

		Macrotypes richness estimates		
Coeff.	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	3.129	0.033	94.596	<0.001
M2	0.017	0.054	0.323	0.747
M3	0.080	0.067	1.196	0.232
M4	-0.281	0.125	-2.252	0.024
M5	-0.168	0.066	-2.556	0.011
		Macrotypes Shannon index estimates		
Coeff.	**Estimate**	**Std. Error**	**t value**	**Pr(>|z|)**
(Intercept)	2.008	0.073	27.460	<0.001
M2	0.032	0.119	0.268	0.79
M3	0.246	0.152	1.614	0.11
M4	-0.271	0.243	-1.117	0.28
M5	-0.310	0.137	-2.266	0.026
		Hydro-ecological regions richness estimates		
Coeff.	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	3.143	0.026	122.888	<0.001
HER 13	-0.110	0.049	-2.261	0.024

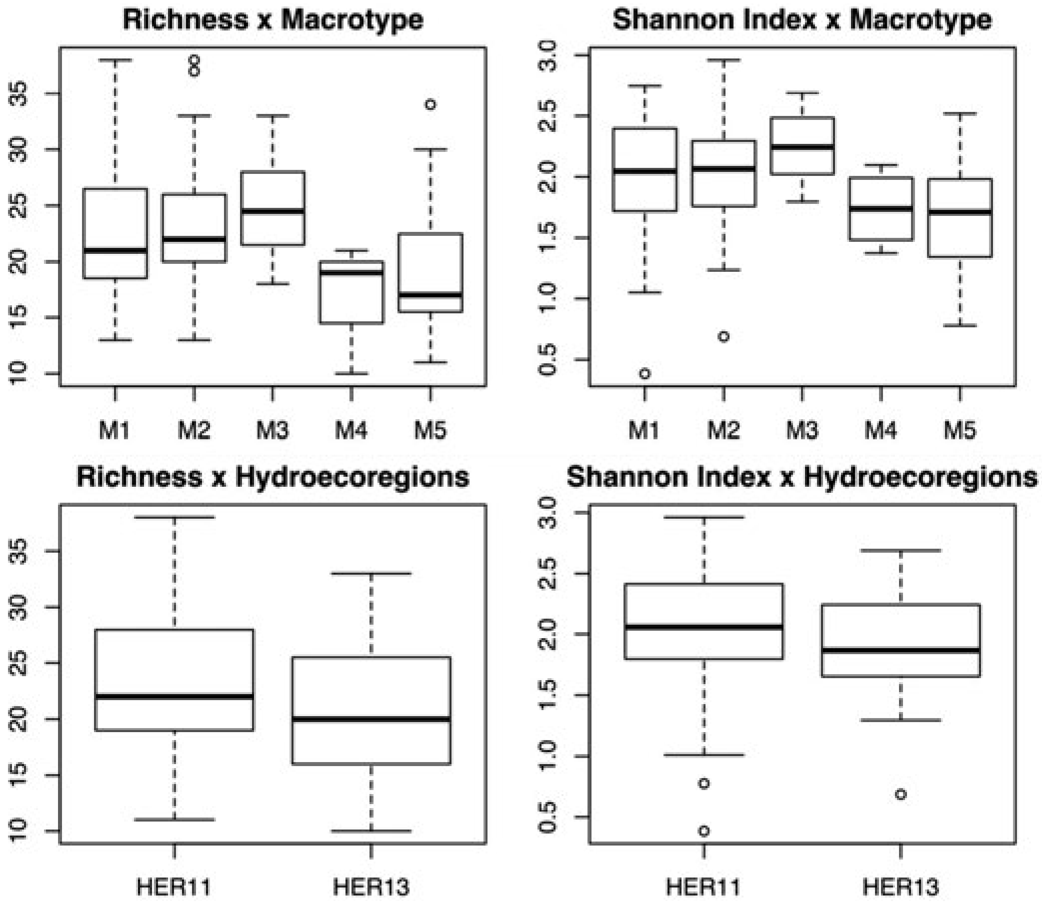

Fig. 3. Box plots of species richness and Shannon Index (on Y axis) among river macrotypes and among hydroecoregions (respectively, M and HER on X axis). The inner line is the median, the box margins are the 25th and 75th percentile, bars extend to 5th and 95th percentile.

Intercalibration Common Metric Index (ICMi) classified 69% of water bodies in high or good class. Forty-two species (about 20% of the total) were not included in the list of taxa for the calculation of ICMi Index, because these diatom species were not included in the TI Index (Rott *et al*., 1999), as their sensitivity (TW) and reliability (G) values are still unknown.

We found a significant ICMi (df=4, deviance=0.67824, P<0.001), IPS (df=4, deviance=55.867, P<0.001) and TI (df=4, deviance=3.8236, P<0.001) variation among macrotypes (Fig. 4; Tab. 5). Particularly, the highest ICMi was found in M2 and M5, IPS was found to be significant lower in M2, M3 and M4 while M3 and M5 showed the lowest TI (Tab. 5). When we investigated variation between HERs we found that only the TI was significant different (df=1, deviance=1.9075, P=0.008) with HER13 showing a significant lower value.

Finally, we did not find any significant correlation between species richness, Shannon Index and the three indexes when we took into account the total of 48 sites. However, when we limited the analysis to the 38 sites for which was possible to evaluate the ecological status on the basis of ICMi, we found that only the Shannon Index showed a significant negative correlation with the ICMi Index and IPS, while TI showed a significant positive correlation with both species richness and Shannon Index (Fig. 5).

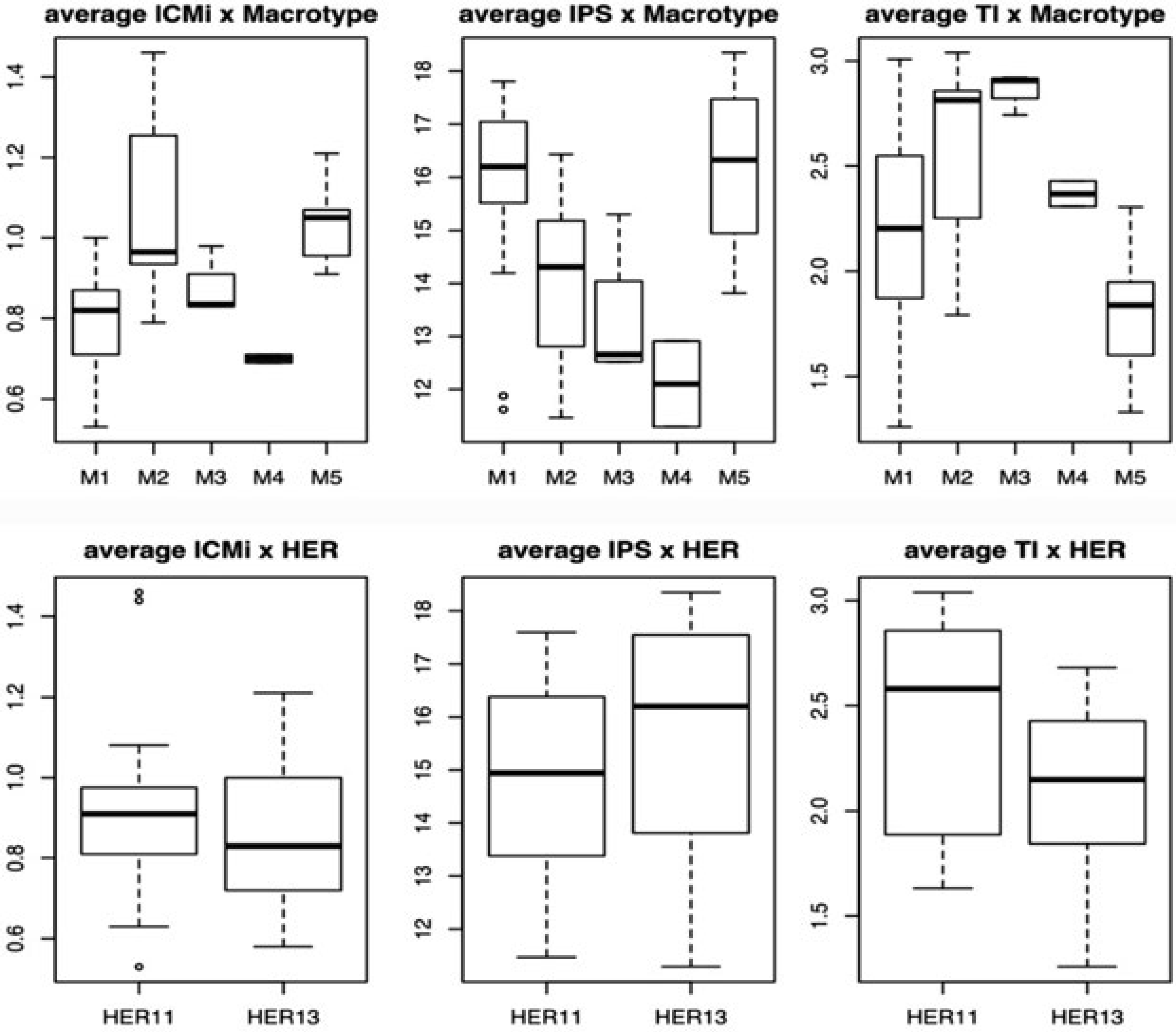

Fig. 4. Box plots of ICMi, IPS, TI values (Y axis) among macrotypes and among hydroecoregions (respectively, M and HER on X axis). The inner line is the median, the box margins are the 25th and 75th percentile, bars extend to 5th and 95th percentile.

Tab. 5. Estimated values for the three indexes among macro-types and hydro-ecological regions.

	Macrotypes ICMi estimates			
	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.777	0.037	20.885	<0.001
M2	0.288	0.066	4.382	<0.001
M3	0.089	0.085	1.049	0.302
M4	-0.082	0.115	-0.717	0.478
M5	0.253	0.069	3.671	0.001
	Macrotypes IPS estimates			
	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	15.785	0.423	37.313	<0.001
M2	-1.720	0.748	-2.300	0.028
M3	-2.499	0.969	-2.578	0.015
M4	-3.682	1.304	-2.824	0.008
M5	0.405	0.783	0.518	0.608
	Macrotypes TI estimates			
	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	2.225	0.101	22.137	<0.001
M2	0.359	0.178	2.018	0.052
M3	0.644	0.230	2.798	0.009
M4	0.142	0.310	0.460	0.649
M5	-0.429	0.186	-2.306	0.028
	Hydro-ecological regions TI estimates			
	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	2.420	0.099	24.332	<0.001
HER 13	-0.367	0.168	-2.188	0.035

CONCLUSIONS

According to this result the diatom species richness did not result as a community variable related to the ecological quality of the studied river. A similar conclusion was already reported in previous works on other Italian (Della Bella *et al*, 2012) and European catchments (Blanco *et al*., 2012), and on other aquatic ecosystems (Cohen *et al*., 1993; Chipps *et al*., 2006; Della Bella and Mancini, 2009). Diatom diversity metrics exhibited poor linear correlations with environmental factors indicating ecological status because of complex environmental influences. The relationship between diatom diversity indices and productivity has remained unclear and then some authors suggested that these indices are not suitable for evaluating ecological conditions (Blanco *et al*., 2012). Archibald (1972) found linear negative and Lavoie *et al*. (2008) found positive relationships between diversity and nutrients. Soininen (2009) indicated that other variables than nutrients determine diatom diversity. For example, Stenger-Kovács *et al*. (2014) found that stream order is a relevant typological parameter which can basically influence the diatom species number and diversity. Species richness and diversity indices, like the Shannon Index, are two important aspects of diversity, but it is not obvious that both respond in a similar way to varying intensities of disturbance (Svensson *et al*., 2012). Indices of diversity generally include both the number of species and their relative abundances, which make assessment of their responses more complex. Diversity indices based on species relative abundances (*e.g.*, Shannon Index) could show misleading responses and could be unsuitable for comparison of biological communities. A recent study on other biological community showed that the majority of the biodiversity metrics increased whereas the most abundant species declined and highlighted that increasing metrics of diversity may occur in parallel with substantial losses of individuals (Schipper *et al*., 2016).

The present study highlighted differences in diversity metrics (species richness and Shannon Index) of diatom communities in Mediterranean river types, and this finding suggested that this aspect should be taken into account when comparison studies were made among different river typologies. Although our analyses could be affected by an imbalance in the number of observations among macrotypes and further testing with additional data of different hydroecoregion are necessary, our results indicated

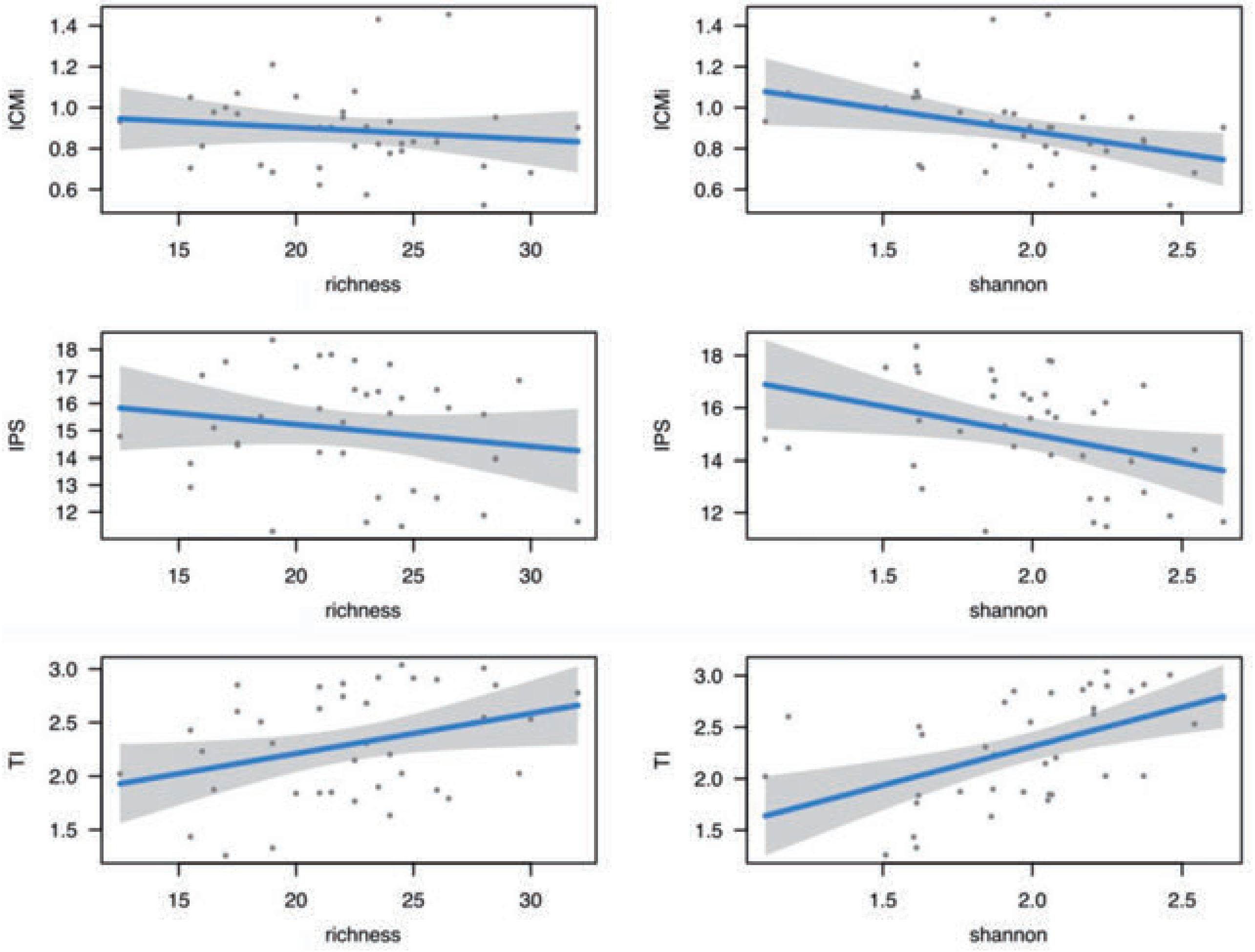

Fig. 5. Predicted values (blue continuous line) and confidence intervals (95%, light grey area) for species richness and Shannon Index of diatoms in relation to the ICMi, IPS and TI index values.

that the diatom diversity metrics could be considered complementary parameters in river biomonitoring for the ecological status assessment based on diatoms.

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REFERENCES

APAT, 2007. [Protocollo di campionamento e analisi delle diatomee bentoniche dei corsi d'acqua]. In: APAT (ed.), [Metodi Biologici per le acque dolci. Parte I].[in Italian]. Manuali e Linee Guida XX/2007. Available from: http://www.isprambiente.gov.it/it/pubblicazioni/manuali-e-linee-guida/metodi-biologici-per-le-acque-parte-i

Archibald REM, 1972. Diversity and some South African diatom association and its relation to water quality. Water Res. 6:1229-1238.

ARPA Umbria, 2008. [Definizione della rete di monitoraggio dei corpi idrici superficiali della regione Umbria ai sensi della Direttiva 2000/60/CE (DLgs 152/06 e s.m.i.)].[in Italian]. ARPA Umbria, Perugia, Italy. Available from: http://http://www.arpa.umbria.it/pagine/acque-superficiali

Battegazzore M, Gallo L, Lucadamo L, Morisi A, 2003. Quality of the main watercourses in the Pollino National Park (Appennine Mts, S Italy) on the basis of diatom benthic communities. Studi Trent. Sc. Nat. Acta Biol. 80:89-93.

Battegazzore M, Morisi A, Gallino B, Fenoglio S, 2004. Environmental quality evaluation of Alpine springs in NW Italy using benthic diatoms. Diatom Res. 19:149-165.

Beltrami ME, Ector L, Ciutti F, Cappelletti C, Hoffmann L, Rott

E, 2009. Longitudinal variations of benthic diatoms and water quality along a large river (Adige River) in Northern Italy. Verh. Internat. Verein. Theor. Angew. Limnol. 30:915-918.

Blanco S, Cehudo-Figuerias C, Tudesque L, Bécares E, Hoffmann L, Ector L, 2012. Are diatom diversity indices reliable monitoring metrics? Hydrobiologia 695:199-206.

Bona F, Falasco E, Fassina S, Griselli B, Badino G, 2007. Characterization of diatom assemblages in mid-altitude streams of NW Italy. Hydrobiologia 583:265-274.

Cantonati M, Pipp E, 2000. Longitudinal and seasonal differentiation of epilithic diatom communities in the uppermost sections of two mountain spring-fed streams. Verh. Internat. Verein. Theor. Angew. Limnol. 27:1591-1595.

Cappelletti C, Ciutti F, Crippa A, Mancini L, Beltrami ME, Pierdominici E, Dell'Uomo A, 2005. [Diatomee come indicatori della qualità biologica dei corsi d'acqua. EPI-D ed altri metodi europei a confronto: il caso di studio del fiume Tevere].[Article in Italian]. Biol. Amb. 19:103-108.

CEMAGREF, 1982. [Etude des methodes biologiques quantitative d'appreciation de la qualité des eaux].[Book in French]. Rapport Q.E. A.F. Bassin Rhone-Mediterranee-Corse, Lyon: 218 pp.

Chipps ST, Hubbard DE, Werlin KB, Haugerud NJ, Powell KA, Thompson J, 2006. Association between wetland disturbance and biological attributes in floodplain wetlands. Wetlands 26:497-508.

Cohen AS, Bills R, Cocquyt CZ, Caljon AG, 1993. The impact of sediment pollution on biodiversity in Lake Tanganyika. Conserv. Biol. 7:667-677.

Coste M, Ector L, 2000. [Diatomees invasives exotiques ou rares en France: principales observations effectuees au cours des dernieres decennies].[Article in French]. Syst. Geogr. Pl. 70:373-400.

Ciutti F, Della Bella V, Beltrami ME, Puccinelli C, Marcheggiani S, Cappelletti C, Martinelli A, Mancini L, 2007. [Le comunità diatomiche del bacino del fiume Tevere: uno studio pilota]. Proceedings 27th Congr. SITE-AIOL "Ecologia, Limnologia e Oceanografia: quale futuro per l'ambiente?" [Article in Italian]. Ancona, Italy.

Dell'Uomo A, 1999. Use of algae for monitoring rivers in Italy: current situation and perspectives, p. 17-25. In: J. Prygiel, B.A. Whitton and J. Bukowska (eds.), Use of algae for monitoring rivers III. Agence de l'Eau Artois-Picardie, Douai.

Della Bella V, Mancini L, 2009. Freshwater diatom and macroinvertebrate diversity of coastal permanent ponds along a gradient of human impact in a Mediterranean ecoregion. Hydrobiologia 634:25-41.

Della Bella V, Marcheggiani S, Puccinelli C, Mancini L, 2007. Benthic diatom communities and their relationship to water variables in wetlands of central Italy. Ann. Limnol.-Int. J. Lim. 43:89-99.

Della Bella V, Pace G, Barile M, Zedde A, Puccinelli C, Ciadamidaro S, Danieli PP, Andreani P, Aulicino FA, Belfiore C, Mancini L, 2012. Benthic diatom assemblages and their response to human stress in small-sized volcanic-siliceous streams of central Italy (Mediterranean eco-region). Hydrobiologia 695:207-222.

Dufrêne M, Legendre P, 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67:345-366.

European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L 327, 22/12/2000, p. 1-73.

European Commission, 2008. Commission decision of 30 October 2008 establishing, pursuant to Directive 2000/60/ EC of the European Parliament and of the Council, the values of the Member State monitoring system classifications as a result of the intercalibration exercise. OJ L 332, 10/12/2008, p. 20-44.

European Committee for Standardization, 2003. European Standard EN 13946. Water quality - Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers. CEN, Brussels: 14 pp.

European Committee for Standardization, 2004. European Standard EN 14407. Water quality - Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters. CEN, Brussels: 12 pp.

Falasco E, Bona F, 2013. Recent findings regarding non-native or poorly known diatom taxa in north-western Italian rivers. J. Limnol. 72:35-51.

Falasco E, Mobili L, Risso AM, Bona F, 2012. [Considerazioni sull'applicazione dell'indice diatomico ICMi (Intercalibration Common Metric index) nell'Italia nord-occidentale].[Article in Italian]. Biol. Amb. 26:1-8.

Falasco E, Piano E, Bona F, 2013. [Guida al riconoscimento e all'ecologia delle principali diatomee fluviali dell'Italia Nord Occidentale].[Article in Italian]. Biol. Amb. 27:1-277.

Finocchiaro M, Torrisi M, Ferlito A, Dell'Uomo A, Ector L, 2011. [Biodiversità algale in Sicilia: diatomee rare o notevoli del bacino idrografico del fiume Simeto].[Article in Italian]. Biogeographia 30:13-29.

Hofmann VG, 1994. [Aufwuchs Diatomeen in Seen und ihre Eignung als Indikatoren der Trophie].[Article in German]. Biblioth. Diatomol. 30: 1-241.

Hofmann VG, Werum M, Lange-Bertalot H, 2011. Diatomeen im Süßwasser - Benthos von Mitteleuropa].[Book in German]. A.R.G. Gantner Verlag, Vaduz: 908 pp.

Italian Regulation, 2006. [Norme in materia ambientale].[in Italian]. Legislative Decree No. 152, 3 April 2006. Ordinary Supplement No. 96 to the OJ No. 88, 04/04/2006.

Italian Regulation, 2008. [Regolamento recante i criteri tecnici per la caratterizzazione dei corpi idrici (tipizzazione, individuazione dei corpi idrici, analisi delle pressioni) per la modifica delle norme tecniche del decreto legislativo 3 aprile 2006, n. 152, recante norme in materia ambientale, predisposto ai sensi dell'articolo 75, comma 4, dello stesso decreto].[in Italian]. Ministry Decree No. 131/2008. Ordinary Supplement No. 189 to the Official Journal No. 187, 11/08/2008.

Italian Regulation, 2010. [Regolamento recante i criteri tecnici per la classificazione dello stato dei corpi idrici superficiali, per la modifica delle norme tecniche del decreto legislativo 3 aprile 2006, n. 152, recante norme in materia ambientale, predisposto ai sensi dell'articolo 75, comma 3, del medesimo decreto legislativo].[in Italian]. Ministry Decree No. 260/2010. Ordinary Supplement No. 31 to the Official Journal No. 30, 07/02/2011.

Kelly MG, Cazaubon A, Coring E, Dell'Uomo A, Ector L, Gold-smith B, Guasch H, Hurlimann J, Jarlman A, Kawecka B, Kwandrans J, Laugaste R, Lindstrom EA, Leitao M, Marvan P, Padisàk J, Pipp E, Prygiel J, Rott E, Sabater S, van Dam H, Vizinet J, 1998. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. J. Appl. Phycol. 10:215-224.

Krammer K, 2000. The genus Pinnularia. A.R.G. Gantner Verlag K.G., Vaduz: 703 pp.

Krammer K, Lange-Bertalot H, 1986. [Bacillariophyceae 1 Teil: Naviculaceae].[Book in German]. Gustav Fischer Verlag, Stuttgart: 876 pp.

Krammer K, Lange-Bertalot H, 1988. [Bacillariophyceae 2 Teil: Bacillariaceae, Epithemiaceae, Surirellaceae].[Book in German]. Gustav Fischer Verlag, Stuttgart: 596 pp.

Krammer K, Lange-Bertalot H, 1991a. [Bacillariophyceae 3 Teil: Centrales, Fragilariaceae, Eunotiaceae].[Book in German]. Gustav Fischer Verlag, Stuttgart: 576 pp.

Krammer K, Lange-Bertalot H, 1991b. [Bacillariophyceae 4 Teil: Achnanthaceae. Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema].[Book in German]. Gustav Fischer Verlag, Stuttgart: 437 pp.

Krammer K, Lange-Bertalot H, 2000. [Bacillariophyceae 5 Teil: English and French translation of the keys].[German and French edition]. Gustav Fischer Verlag, Stuttgart: 311 pp.

Lange-Bertalot H, 2001. Navicula sensu stricto. 10 genera separated from Navicula sensu lato. Frustulia. A.R.G. Gantner Verlag K.G., Vaduz: 526 pp.

Lavoie I, Campeau S, Darchambeau F, Cabana G, Dillon J, 2008. Are diatoms good integrators of temporal variability in stream water quality? Freshwater Biol. 53:827-841.

Mancini L, Puccinelli C, Della Bella V, Marcheggiani S, Beltrami ME, Cappelletti C, Ciutti F, 2008. Freshwater diatoms of the Tiber River basin (Central Italy). In: Proceedings of "Central European Diatom Meeting" (CEDIATOM2), Trentino Nature & Science Museum, Trento, Italy.

Mancini L, Sollazzo C, 2009. [Metodo per la valutazione dello stato ecologico delle acque correnti: comunità diatomiche].[Report in Italian]. Rapporti ISTISAN 09/19. Istituto Superiore di Sanità, Rome: 32 pp.

Prygiel J, Coste M, Bukowska J, 1999. Review of the major diatom-based techniques for the quality assessment of rivers. State of art in Europe, p. 122-127. In: J. Prygiel, B.A. Whitton and J. Bukowska (eds.), Use of algae for monitoring rivers III. Agence de l'Eau Artois-Picardie, Douai.

R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.R-project.org/

Rott E, Pfister P, van Dam H, Pipp E, Pall K, Binder N, Ortler K, 1999. [Indikationslisten fur Aufwuchsalgen. Teil 2: Trophieindikation sowie geochemische Praferenz, taxonomische und toxikologische Anmerkungen].[Report in German]. Bundesministerium fur Land und Forstwirtschaft, Wien: 248 pp.

Rott E, Cantonati M, Fureder L, Pfister P, 2006. Benthic algae in high altitude streams of the Alps - a neglected component of the aquatic biota. Hydrobiologia 562:195-216.

Schipper AM, Belmaker J, Dantas de Miranda M, Navarro LM, Böhning-Gaese K, Costello MJ, Dornelas M, Foppen R, Hortal J, Huijbregts MAJ, Martín-López B, Pettorelli N, Queiroz C, Rossberg AJ, Santini L, Schiffers K, Steinmann ZJN, Visconti P, Rondinini C, Pereira HM, 2016. Contrasting changes in the abundance and diversity of North American bird assemblages from 1971 to 2010. Glob. Change Biol. 22:3948-3959.

Scuri S, Torrisi M, Cocchioni M, Dell'Uomo A, 2006. The European Water Framework Directive 2000/60/EC in the evaluation of the ecological status of watercourses. Case study: The River Chienti (central Apennines, Italy). Acta Hydroch. Hydrob. 34:498-505.

Soininen J, 2009. Is diatom diversity driven by productivity in boreal streams? Diatom Res. 24:197-207.

Stenger-Kovács C, Tóth L, Tóth F, Hajnal É, Padisák J, 2014. Stream order-dependent diversity metrics of epilithic diatom assemblages. Hydrobiologia 721:67-75.

Svensson JR, Lindegarth M, Jonsson PR, Pavia H, 2012. Disturbance-diversity models: what do they really predict and how are they tested? P. Roy. Soc. B-Biol. Sci. 279:2163-2170.

Torrisi M, Dell'Uomo A, Ector L, 2008. [Evaluation de la qualité des rivières des Apennins (Italie) au moyen des indices diatomiques: le fleuve Foglia].[Article in French] Cryptogam. Algol. 29:45-61.

Torrisi M, Scuri S, Dell'Uomo A, Cocchioni M, 2010. Comparative monitoring by means of diatoms, macroinvertebrates and chemical parameters of an Apennine watercourse of central Italy: The river Tenna. Ecol. Indic. 10:910-913.

Wasson JW, Garcia Bautista A, Chandesris A, Pella H, Armanini D, Buffagni A, 2006. [Approccio delle Idro-Ecoregioni Europee e tipologia fluviale in Francia per la Direttiva Quadro sulle Acque (2000/60/EC)].[Report in Italian]. IRSA-CNR Notiziario dei Metodi Analitici 1:39-64.

Zorza R, Honsell G, 2008. [Studio sulle diatomee bentoniche del fiume Natisone (Italia nord-orientale)].[Article in Italian]. Gortania 30:73-88.

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A new multi-criteria method for the ecological assessment of lakes: A case study from the Transboundary Biosphere Reserve 'West Polesie' (Poland)

Joanna SENDER,[1] Weronika MAŚLANKO,[1] Monika RÓŻAŃSKA-BOCZULA,[2] Kevin CIANFAGLIONE[3*]

[1]Department of Landscape Ecology and Nature Conservation, University of Life Sciences in Lublin, 37 B. Dobrzański Street, 20-262 Lublin, Poland; [2]Department of Applied Mathematics and Computer Science, University of Life Sciences in Lublin, 28 Głęboka Street, 20-950 Lublin, Poland; [3]EA 2219 Géoarchitecture, UFR Sciences & Techniques, Université de Bretagne Occidentale, 6 Avenue Le Gorgeu, CS 93837, 29238 Brest Cedex, France
*Corresponding author: kevin.cianfaglione@univ-brest.fr

ABSTRACT

A new multi-criteria method of evaluation and assessment of the ecological status of lakes is proposed. It is based on macrophytes analysis integrated with geomorphological, landscape and catchment sources of threats. A total of 22 lakes in the Transboundary Biosphere Reserve 'West Polesie' (Poland) were investigated along trophic (available nutrients) and human pressure gradients, testing the proposed method with ESMI and TRS indices. Therefore, the present indexation included 22 criteria (*i.e.,* catchment land use, phytolittoral area, number of plant species) concerning three different assessing zones (lakeshore, littoral and surrounding area), and provided a five-class ecological classification. The proposed index, in addition to the general ecological conditions assessment of lakes, allows to point out a zonal evaluation, identifying the most critic zones in terms of ecological status. The proposed method can be universally adapted for any type of lakes, regardless of their geographical characteristics. It can be applied to system monitoring, and to support lakes biodiversity, functionality, conservation, restoration, water protection and uses, as well as water, territory and landscape management actions.

Key word: Multi-metric Index; macrophytes; lake functionality; ABC method; waters and land management; new proposal.

INTRODUCTION

The worldwide progressive human pressure on water bodies is among the key causes of aquatic environments decline (Melzer, 1999; Palmer and Roy, 2001; Baattrup-Pedersen *et al*., 2006; Brucet *et al*., 2013). The main causes are burial, erosion, pollution, water characteristics variation (*i.e.*, flow regimes, functionality, physical and chemical changes); together with water bodies, land forms, dynamics artificialization; and biodiversity regression (*i.e*., Suominen, 1968; Bolpagni *et al*., 2013; Sossey-Alaoui and Rosillon, 2013; Cianfaglione, 2014; Szoszkiewicz *et al*., 2014; Baláži *et al*., 2014; Bolpagni and Piotti, 2015, 2016).

To monitor and try to counteract this trend, the development of fast multi-criteria methods, indicating the main causes of the degradation processes, may represent a pivotal choice for introduce effective planning and remedial actions (Villa *et al.*, 2013). Focusing on macrophytes and ecological features, a new multi-criteria method for the ecological status assessment of lakes is presented in order to improve standard methods developed in the frame of the Water Framework Directive (WFD) from European Parliament and Council European Union (2000).

Macrophytes are used successfully in good long time monitoring of ecologic variations, because they are characterized by low mobility (if compared with phytoplankton, fishes or invertebrates), and also in a relative short time monitoring because they are closely linked to biodiversity and the environmental conditions (Botineau and Ghestem, 1995; Van der Molen *et al*., 2004; Kolada, 2008; Cianfaglione, 2011; Khadija *et al*., 2015; Bolpagni *et al*., 2016a, 2016b). They are widely used for the aquatic ecosystems assessment, integrating the environmental changes in their frequency, communities structure, phenological rhythm, development (functional traits *s.l.*), vegetation series and dynamical tendencies (Carvalho *et al.,* 2006; Ali *et al*., 2007; Feldmann and Nöges, 2007; Sender, 2016; Cianfaglione and Bioret, 2017). For that, macrophytes indices are generally related to the composition, frequency, abundance and presence of specific *taxa* (Spence, 1967; Suominen, 1968; Newbold and Holmes, 1987; Murphy *et al*., 1990; Whitton and Kelly, 1995). Despite this, the reliability of the macrophyte-based indices can be limited to (more or less) restricted geographic areas (De Lange and Van Zon, 1983; Holmes *et al*., 1988; Carbiener *et al*., 1990; Haury and Peltre, 1993; Stelzer *et al*., 2005; Haury *et al*., 2006; Penning *et al*., 2008).

Until the 90's, the macrophytes were still not so commonly used in the assessment of water quality, but the WFD boosted their role as bioindicators in Europe (*i.e.*, Schneider and Meltzer, 2003; Schaumburg *et al*., 2004; Meilinger *et al*., 2005; Stelzer *et al*., 2005; Kolada, 2008; Pall and Moser, 2009; Sender, 2012a; Bertrin *et al.,* 2012).

Since 2007, "macrophyte methods" for the diagnosis and monitoring of aquatic ecosystems were recognized as one of the official monitoring methods by the Polish state (Rozporządzenie Ministra Środowiska, 2011), and adopted for monitoring water bodies in Poland (Ciecierska *et al.,* 2010).

METHODS

Study area

In 2012, between Poland, Ukraine and Belarus, the 'West Polesie' Transboundary Biosphere Reserve was created. Polish part of the Biosphere Reserve covers the whole mesoregion of the Łęczna-Włodawa Lake District (Wilgat, 1954), also called as Łęczna-Włodawa Plain (Kondracki, 2002). The region is rich in several dozens of lakes, located between the middle course of the Wieprz and Bug rivers; 61 of them are larger than 1 ha, and only 16 lakes are over 50 ha (Chmielewski, 2009). The majority of them are small and shallow lakes, and their formation is associated with the global warming occurred in late Pleistocene and Holocene, after the dissolution/degradation of permafrost and consequent development of thermokarst (Wojtanowicz, 1994).

These lakes are represented by four trophic types (meso-, eu-, hyper-, and dystrophic), undergoing various forms of human pressure. From the end of the 70's of the XX century, most of these lakes were subjected to huge negative changes due to the hydrological regime changes of the area. Also for their originalities, there is no data concerning the current ecological status of these lakes, except only for a small group of them, monitored by the Regional Inspectorate for Environment Protection (Sender, 2012a). Some of them are subjected to very rapid burial processes, eutrophication, banalization of the aquatic communities and species loss. It is estimated that over the last 50 years, from among 68 lakes, five disappeared, and two reduced their surface below 1 ha (Chmielewski, 2009).

The study area corresponds to 22 lakes from the Łęczna-Włodawa Lake District (Fig. 1). These lakes are original in terms of morphology and human pressure diversity, making difficult to assess them by other methods already developed for other Polish lakes; requiring to treat them case by case, individually, as specific sites, showing how it is necessary to implement indexing procedures, to carry out more fine policies and management actions.

Sampling procedures

Field research was carried out from 2012 to 2014. According to Jensen (1977) and Sender (2009, 2012b), it was conducted through horizontal transects, from the shoreline to the central part of the lakes, following the maximum range of the macrophytes occurrence as possible. Surface of macrophyte communities (coverage), submerged macrophytes share, and species frequency were analysed according to Szmeja (2006). The vegetation was mapped relying on orthophoto-maps (where one pixel representing 0.5 m in the field) provided by the Polish Head Office of Geodesy and Cartography-Geoportal Web-Site (www.geoportal.gov.pl) ver. 2013, integrating the field observations. This was useful to define vegetation series, interpreting the spatial distribution and surfaces of studied communities according to De Bolòs (1963), Géhu (1991), Biondi (1996), and Rivas-Martinez (1994). This helped us to better define syndynamics (*i.e*., succession, transgression, regression, fluctuation, regeneration, degeneration) and to relatively adjust the human pressure related values during the assessment (according to Faliński, 1999; Falińska, 2003; Pedrotti, 2013). Surfaces, distributions and phytolittoral were determined by ArcGIS 10.2 software. In order to define plant communities, plant specimens were collected, examined, and identified following Mirek *et al.* (2002), whereas the syntaxonomic nomenclature follows Matuszkiewicz (2008).

Ecological assessment procedure

During 70's, macrophytes was rarely used to carry out lake assessments in Poland; Tomaszewicz and Kłosowski (1985) used them in sigma-associations by syn-phytosociological relevés for assessing lakes of the Sejneńskie Lake District.

Macrophyte bio-indicators index (MFI) followed during 80's, also based on the structural-spatial vegetation systems (Rejewski, 1981); not only determining the cover degree, but also the dynamical tendencies by two divergent trends, representing renaturalization *vs* human impact, indicated as succession (+) and synanthropisation (-). The MFI method was implemented and adapted to the requirements of the WFD, becoming the "Ecological Status Macrophyte Index" (ESMI), accepted to monitor the stagnant lakes in Poland (Ciecierska *et al*., 2010); however, it is not considerable to be successfully applied to all Polish lake types. In Poland, there are over nine thousands of lakes, and most of them are from glacial origin (Soszka and Cydzik, 2003) as is the case of our study area. ESMI is also considerably not so sensitive to the human pressures that can accelerate the eutrophication, and it should not be applied in lakes where the natural forms can negatively affect the expression of the vegetation (*i.e.*, deepness, slope steepness) limiting the macrophyte communities' expansion (Ciecierska *et al*., 2006; Ciecierska, 2008; Ciecierska and Kolada, 2014). As example, the ESMI is not successfully applicable to the Polesie region lakes (Ciecierska and Kolada, 2014), because their particular morphology and small water surface: as pointed out by Ciecierska *et al*. (2006). In most the Polesie region lakes, the phytolittoral is naturally dominated by emergent communities (Sender, 2009);

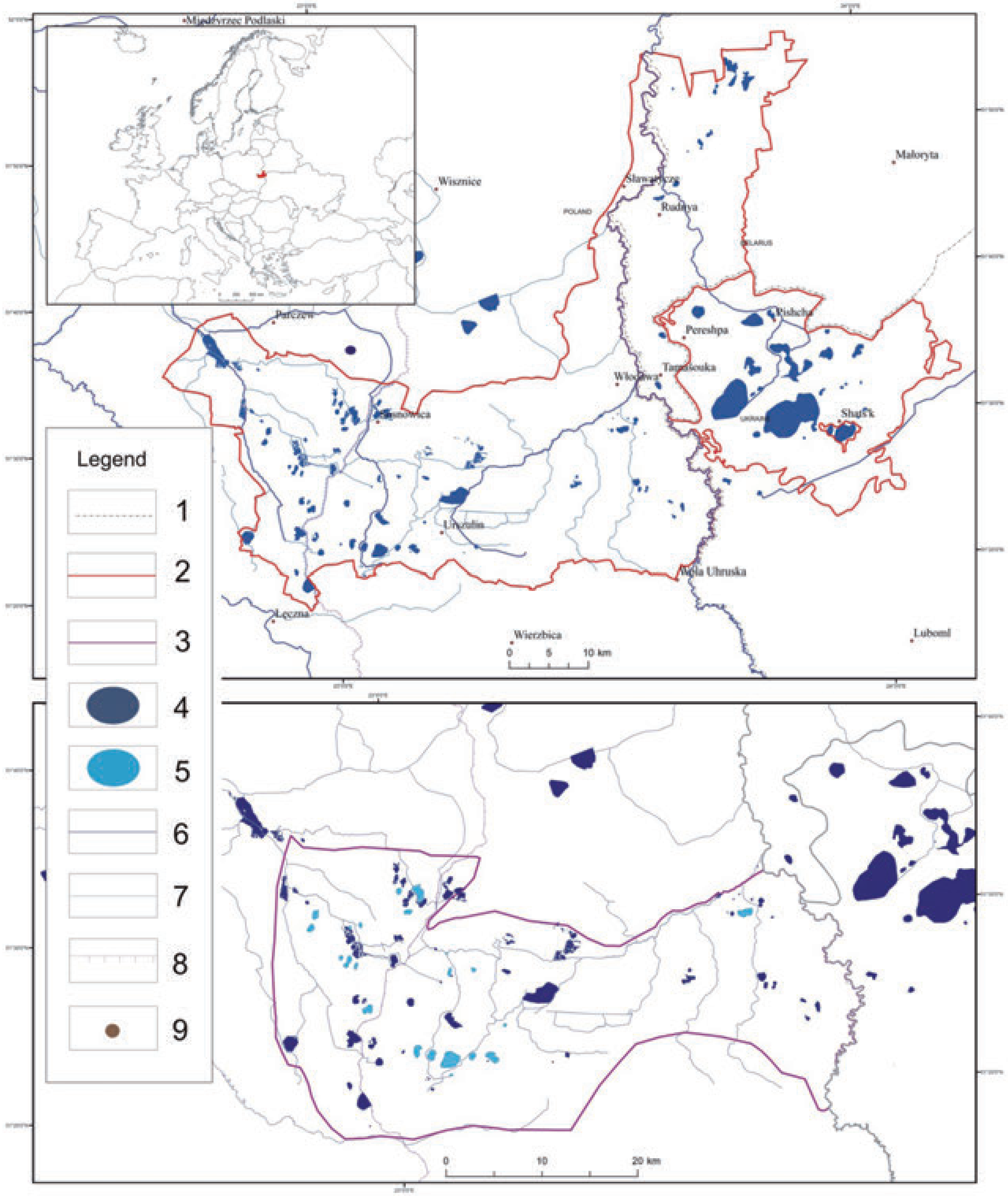

Fig. 1. Investigated area. 1, Polish national border; 2, 'West Polesie' International Transboundary Biosphere Reserve; 3, Łęczna-Włodawa Lake District; 4, lakes; 5, investigated lakes; 6, main rivers; 7, other rivers; 8, Wieprz-Krzna channel; 9, main settlements.

the analysis of these lakes, based on others existing indexes showed that the specificity of these lakes requires more elastic methods for their ecological status evaluation, according to Ciecierska *et al*. (2006).

Following past experiences and these observations, the main goal of our research was to elaborate a new fast and easy to use index, useful to be more generically applied in lakes, assessing their ecological status, paying attention to try to limit the sampling technique and diminish the inter-surveyor variability related problems (Kolada *et al.*, 2014). The proposed index is based on macrophyte communities (*i.e.*, non-ligneous plants between *Spermatophyta*, *Pteridophyta*, *Bryophyta*, macroscopic Algae and even some Lichens), integrated with data related to ecology, syndynamics, geomorphology (shoreline, catchment, water chemistry) and land use features (*i.e.*, disturbance, management, threats). Attention was paid to the qualitative and quantitative structure of the macrophytes (Lacoul and Freedman, 2006) as bio-indicator, trying to develop a simple, fast and accurate tool to monitor lake systems. The method we proposed, in addition to the overall assessment of the ecological status of lakes, it provides also an indication of the more threatened zones; making possible to improve their ecological status, reducing threats or fostering restoration activities and landscape planning policies. The catchment analysis was considered necessary because lakes are strongly influenced from its origin, status and structure (Brucet *et al.*, 2013; Bolpagni, 2013; Alahuhta *et al*., 2014). Therefore, it is assumed that the functioning of the lakes is linked also with lake's features, as well as the lake morphology (Azzella *et al.*, 2014a, 2014b). Following this, the new proposed ABC method offers to determine which factor is the most influential on the lakes functionality, as well to point out which zones are under a negative effect, and which are the main factors that can limit the macrophyte communities' occurrence; trying to understand the more influential factors that can contribute to perturb the vegetation dynamics.

The overall assessment of this proposed method (ABC) was calibrated to the analysis of three predefined zones: catchment (the more external one), shoreline (shallow littoral), and littoral zone (the inner part of water, covered by macrophytes). Three groups of criteria were distinguished: two of them concerned the assessment of lake vegetation quality (A and B), whereas the third concerned the assessment of the catchment status (C). For each zone, to understand the human pressure, is necessary to evaluate pollution, modification of land forms and water flows, soil use, and related effect on water/vegetation. To define vegetation changes and dynamics, as possible is also necessary to understand the potential vegetation; and how we are far from it. Roughly is enough, but more it is possible to be precise, more accurate will be the result.

Proposed for small reservoirs evaluation, Juszczak and Arczyńska-Chudy (2003), and Skwierawski (2005) were used as basis model, modifying or replacing few points, concerning the natural variability of biotic and abiotic conditions of lakes, taking as case study the Polesie region.

In the assessment of the shoreline zone (A), we considered the human pressure, and how shoreline communities are far from the potential vegetation. We decided to not consider the peat-bog species presence in the catchment area, because frequently lakes surroundings can be characterized by several other communities with huge ecological difference (*i.e*., grasslands, fen, cultivations and forests), as it is in the study area. This homogenization better allows comparisons between different types of biotopes. In other hand, in order to eventually underline a remarkable species or a special communities' presence to be monitored, it can be useful to mark them in additional notes. However, we added the number of emergent plant communities because some authors consider them as indicator of changes than individual species (Ciecierska *et al.*, 2006). We paid particular attention also on submerged macrophytes, considered important in ecological assessment of lakes, according to Sondergaard *et al*. (2010). In evaluation of littoral zone (B) we took in consideration the number of submerged macrophyte communities (pleustonic plants included); share of submerged macrophytes in the lake surface and the total phytolittoral surface. Skwierawski's method focus especially on pleustonic macrophytes, because this group often appears in small reservoirs; but in bigger lakes it occurs more randomly and usually among the helophytes (Wołek and Kościółek, 2012).

As follows, we choose to do not considered few features, when they are not always significant, easy or so fast to know: grouping, replacing, deleting them or adding new points following our goal to develop an efficient, fast, simple and generically applicable evaluation criterion. We did not consider the "water level stability" as single factor, but we considered it between the threats, according to Keto *et al.* (2006) and Zohary and Ostrovsky (2011) indicating that drastic water level fluctuations (fluctuations out from the normal cyclical ranges) are disadvantageous for aquatic species. Also, waters coming not from the natural water cycle ("alien waters inputs") was considered as threat for similar reasons and for their relative pollution risks. This also allows to relativize and discriminate among the normal natural fluctuations (*i.e.*, *per* season, *per* year), to the fluctuation produced by man activities influence: giving more space into the assessment. We replaced also watercolor and turbidity with Secchi disk visibility, giving us an easy and "fast to take" information about light condition and possibilities of submerged macrophytes occurrence. Maximal and minimal values of studied feature (Tab. 1) were defined according to Skwierawski (2005). Three intermediate groups were distinguished and progressively numbered, allowing the determination of community conditions.

For the evaluation of shores (A), seven criteria were taken into account: i) shoreline development; ii) share of helophytic communities belonging to *Phragmition* or *Magnocaricion* alliances, as emergent macrophytes in shoreline; iii) number of helophytic communities; iv) number of species presence; v) average width of helophytes; vi) woodlots and shrubs in the shore zone; and vii) share of helophytes in phytolittoral.

Each feature of evaluation was pointed in a scale from zero to five points (see Tab. 1 about more details). About emergent vegetation, we found more correct to treat this community in *sensu stricto*; for that, we considered floodable meadows as "out" of shoreline (or marginal), because occurring too far from the shoreline, because of their transitional characteristic (strong amphibious seasonal nature): considering them in catchment analysis, also if

Tab. 1. Assessment criteria of: shoreline zone in lakes (A); lakes littoral zone (B); and lake catchment (C).

Zone	Feature\ punctuation	5	4	3	2	1	0
	Shoreline development	Heterogeneous	Diversified slopes		Homogeneous slopes		Converted / anthropic shore (embanking)
			With abundance of less steep slopes	With abundance of steep slopes	Less steep slopes	Very steep slopes	
A	Share of the belt rushes (%) in shoreline	75-100	50-74	25-50	10-25	<10	Lack or vestigial
	Number of emergent macrophytes communities	>10	9-8	7-6	5-4	3-2	>1
	Number of species	>20 with rare or protected species	>20	16-20	15-10	9-6	<5 or with ruderal species
	Average width of rushes (m)	>26	25-21	20-16	15-11	10-6	<5
	Woodlots and shrubs in the shore zone (%)	100	99-80	89-60	59-30	Single trees	Lack
	Share of emergent macrophytes in phytolittoral (%)	<39	40-49	50-59	60-69	70-84	>85
B	Secchi disk visibility (m)	>4.6	4.5-2.5	2.4-1.5	1.4-1.0	0.9-0.5	<0.5
	Conductivity ($\mu s \cdot cm^{-1}$)	<150	151-250	251-350	351-450	451-550	>500
	Laker surface (ha)	>300	299-150	149-70	69-30	<29	-
	Max depth in lake (m)	>19	19-15	14-10	9-5	4-2	<2
	Colonization depth of macrophyte occurrence (m)	>5 or to the bottom	4.5-4	3.9-3	2.9-2	1.9-1	<1
	Share of submerged macrophytes in the lake surface (%)	>75	74-61	60-46	45-31	30-15	<14
	Phytolittoral surface (%)	50-60	61-70	71-75; 49-40	39-30; 76-85	29-24; 81-80	>90; <20
	Number of submerged macrophyte communities	>10	9-8	7-6	5-4	3-2	>1
	Number of species	≥7 with rare or protected	6	5	4	3	≤2
C	Catchment area (ha)	>100	101-200	201-300	301-400	401-500	<500
	Catchment usage	F, P, G >75%	F, P, G >50%	G, A-50-75%, B >25%	A >75%, B 26-50%	B >50%	B >50% lack of sewage
	Average slope (‰)	<2	2-3	3-5	5-10	10-20	>20
	Type of flow	Lack of inflows, outflows permanent	Lack of inflows, outflows periodic	Lack of inflows and outflows	Periodic inflows and outflows	Constant flow (channel), periodic inflow	Only inflow
	Threats	Lack of threats/ effective form of protection	Very low (or occasional/ potential) risk	Low risk	Moderate risk	High risk	Very high risk
	Ratio of shore length (m)/capacity (m^3)	>0.5	0.6-1.5	1.6-2.5	2.6-3.5	3.6-4.5	<4.6

F, forest; P, peat-bog; G, grassland; A, agricultural land; B, buildings.

some helophyte and hygrophyte species can occur. To give adequate values during catchment analysis it is crucial to understand how far we are from the potential vegetation; considering the catchment area status: human influence, plant communities' dynamics (+ or -), and risks. Floodable meadows communities can have an important species diversity and an important productive or cultural importance, but being blocked in an artificial dynamic (anthropogenic fluctuation) they are less in equilibrium, being less quality indicator of trends (+ and -). In that way, more we are distant from the potential vegetation, worst is the catchment value influence. In other hand, when worthy, any secondary plant formations need an additional but apart assessment, specifically calibrated to point out the biodiversity and cultural (heritage) of the landscape: focusing on its functionality, aesthetics, stability, risks, homogeneity/heterogeneity degree, and man uses.

For the assessment of littoral zones (B), nine criteria were taken into account: i) turbidity, as Secchi disk visibility; ii) water conductivity; iii) lake surface; iv) max depth of lake; v) max depth of macrophyte occurrence in lake; vi) share of submerged macrophytes in the lake surface; vii) share of submerged macrophytes in the phytolittoral surface; viii) number of submerged macrophyte communities (with pleustonic and floating leaves plants); and ix) number (amount) of submerged macrophyte species.

The third group of factors (C) consisted of lake surrounding's characteristics and their potential effects on the aquatic ecosystem, including six criteria: i) the catchment area status; ii) the catchment usages; iii) the catchment average slope; iv) type of water flows; v) sources of threats (*i.e.*, industry, agriculture, recreation, anthropogenic transformation of shoreline, pollution); and vi) shore length capacity ratio. In general, all these descriptors allow the assessment of lakes degradation threats, and let us to distinguish five classes of lakes, starting from well-preserved and favorable habitat conditions, to degraded (strongly modified) lakes (Tab. 2), which require corrective/restoration actions (Skwierawski, 2005; Juszczak and Arczyńska-Chudy, 2003. Average point values were normalized to indexation in accordance to the guidelines of the WFD scale, from 0.0 to 1.0. This make possible to distinguish five classes of ecological status of investigated lakes: ≥0.90 (high); 0.89-0.86 (good); 0.85-0.70 (moderate); 0.69-0.64 (poor); and ≤0.63 (bad), according to Ciecierska *et al.* (2006). At this point, we considered only 14 among 22 lakes, because only they could be successfully compared using others indices. In order to verify the analysis undertaken for selected lakes: the Polish index (ESMI) and the English Trophic Ranking Scores (TRS) were specified (Palmer *et al.,* 1992; Ciecierska, 2008).

An analysis of lake similarity was made regarding three ecological zones A, B, C (in Statistica Software, ver. 10.0). For this purpose, a hierarchical method based on the technique of agglomeration was used. About statistical analysis, concerning the point distance, we adopted the Euclidean distance, whereas the Ward's method was used to estimate the distance between the clusters (Stanisz, 2007).

Tab. 2. Scoring and classification of investigated lakes.

Zone	Points	Rank	Interpretation
A+B	65-55	I	Lakes very well preserved, with favorable habitat conditions, with domination of submerged macrophytes
	54-45	II	Lakes well preserved with macrophytes in balance
	44-35	III	Lakes with slight disturbances, in moderate status, with a slightly dominant group of emergent macrophytes
	34-25	IV	Lakes in poor status, with a significant level of transformation, with a clearly dominant group of emergent macr phytes; often lack submerged
	<24	V	Lakes with poor ecological status, degraded or capped by living biomass, often with a lack of macrophytes
C	35-30	I	Any danger of degradation
	29-24	II	Moderate risk of degradation (1)
	23-18	III	Endangered lakes (2-3)
	17-11	IV	Significantly endangered lakes (4)
	<10	V	Very strong endangered lakes
A+B+C	100-85	I	Lakes in very good condition: natural valuable
	84-65	II	Lakes with high natural values, in good condition, but with some dangers
	64-45	III	Lakes with moderate natural values, slightly transformed
	44-25	IV	Lakes of slight natural values, very transformed
	<24	V	Lakes strongly transformed/endangered; requiring restoration or corrective actions

A, lakes; B, lakes littoral zone; C, lake catchment.

RESULTS AND DISCUSSION

Based on a comprehensive valuation (*i.e.,* considering all the zones in analysis A, B, and C), the majority of the investigated lakes fell in the III quality class (intermediate). This category included lakes with moderate natural values and risk of degradation (corresponding to the 59.1% of the 22 lakes *alias* 13 lakes). The lowest values were found in two lakes (Uścimowskie and Zienkowskie) that exhibited a poor ecological status (IV class); despite they showed significantly differences in terms of water surface and quality. However, they both had the catchment area devoted mainly to agricultural purposes, and they both served as wastewater receivers. In other hand, by our assessment, we not found lakes in the worst class (V class, namely degraded), frequently characterized by scarce and banal plants presence; often without aquatic plants; requiring attentions or urgent interventions (Fig. 2).

Getting a look to the quality assessment of the surrounding areas (C), we observed a clear predominance of lakes in IV class (5). There were also lakes included in the V class, corresponding mainly to lakes embedded in a strongly human impacted landscape. Based on the analysis of the littoral zone (B), the largest lake group was the one with moderate natural values and slightly perturbed (III class) (Fig. 3).

Comparing the ecological assessment at zonal (A, B and C) scale, especially for lakes felling into III class, we identified the zones characterized by the highest rates of perturbation. In general, the shoreline zone (A) reached the highest value in all lakes except those that fell in II class, whereas littoral zone (B) was highly evaluated in III and IV class categories of lakes. In the lakes where the catchment was dominated by agricultural land and buildings, the catchment zone (C) reached the lowest values (Fig. 3). Białe Włodawskie, Bialskie, Piaseczno, Rotcze, Uściwierz, and Moszne lakes were evaluated as lakes with a high natural value and a slight risk of degradation, namely with a high ecological value (II class), although of their significant seasonal anthropogenic pressure (*i.e.*, touristic/recreational purposes) (Fig. 3). Due to their rather high depth and large surface, these ecosystems seemed to be enough resistant to this touristic type of pressure.

Lakes in the II class are characterized by the presence of several *Chara* species, and by the dominance of submerged macrophytic beds. An exception was represented by the Lake Moszne, that is shallow, polymictic, and subject to a full preservation by the highest level of Polish law protection.

The analysis of the lake similarity based on the analyzed parameters allowed us to identify three clusters with very similar conditions. The first cluster contained the most impacted and degraded lakes (Zienkowskie, Uścimowskie, Gumienko, Sumin, Gumienek, Białe Sosnowickie) (S1). The second group included lakes in III class, typically eutrophic lakes subjected to various forms of pressure, with well-developed submerged macrophyte beds (Płotycze Urszulińskie, Głębokie Uścimowskie, Ściegienne, Miejskie, Bikcze, Firlej, Krasne, Rotcze, Moszne, and Czarne Sosnowickie lakes) (S2). The third group contained the best-preserved lakes (II class), with the presence of several *Chara* species (Maśluchowskie, Uściwierz, Kleszczów, Piaseczno, Białe Włodawskie, Bialskie lakes) (S3) (Fig. 4).

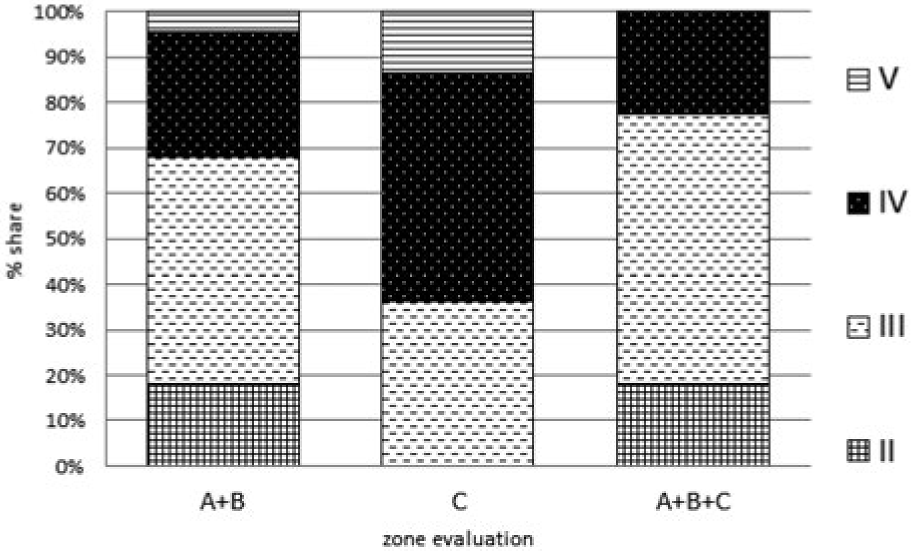

Fig. 2. Percentage share of lakes with different classes of evaluation (II-IV class of lakes).

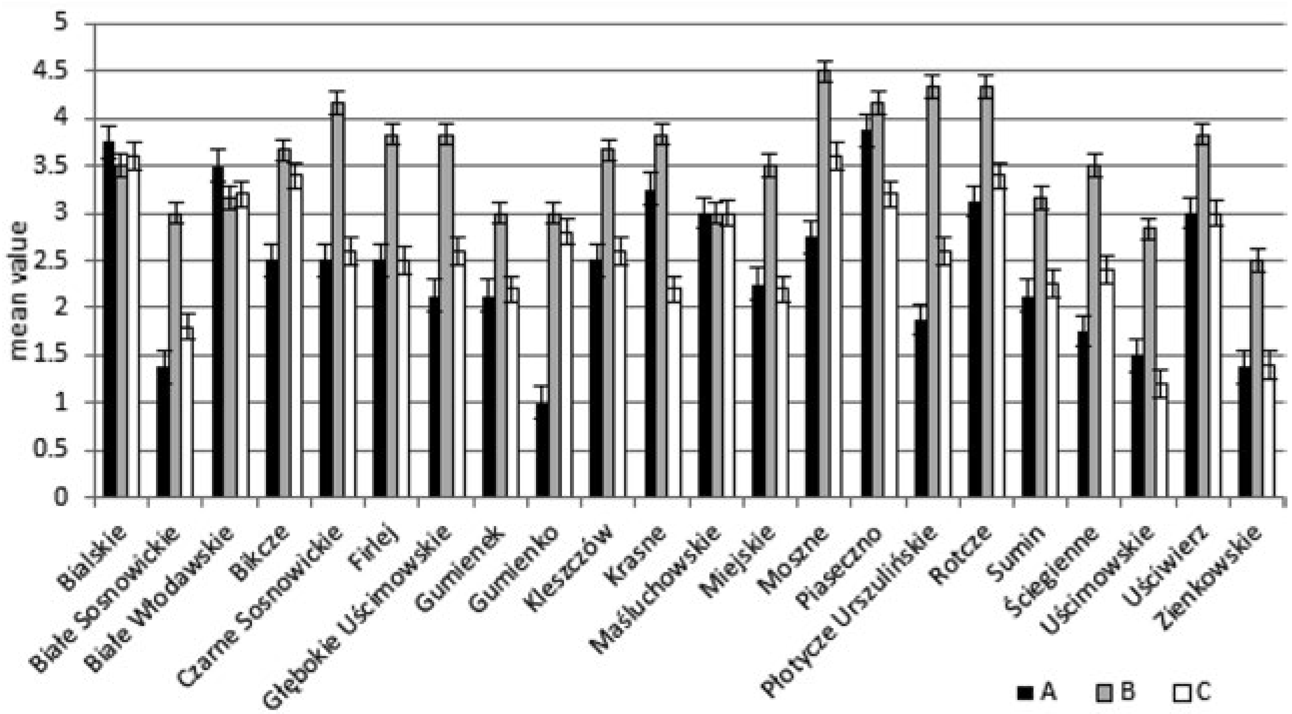

Fig. 3. Average value of evaluation for each zone (A, of shore; B, littoral; C, catchment).

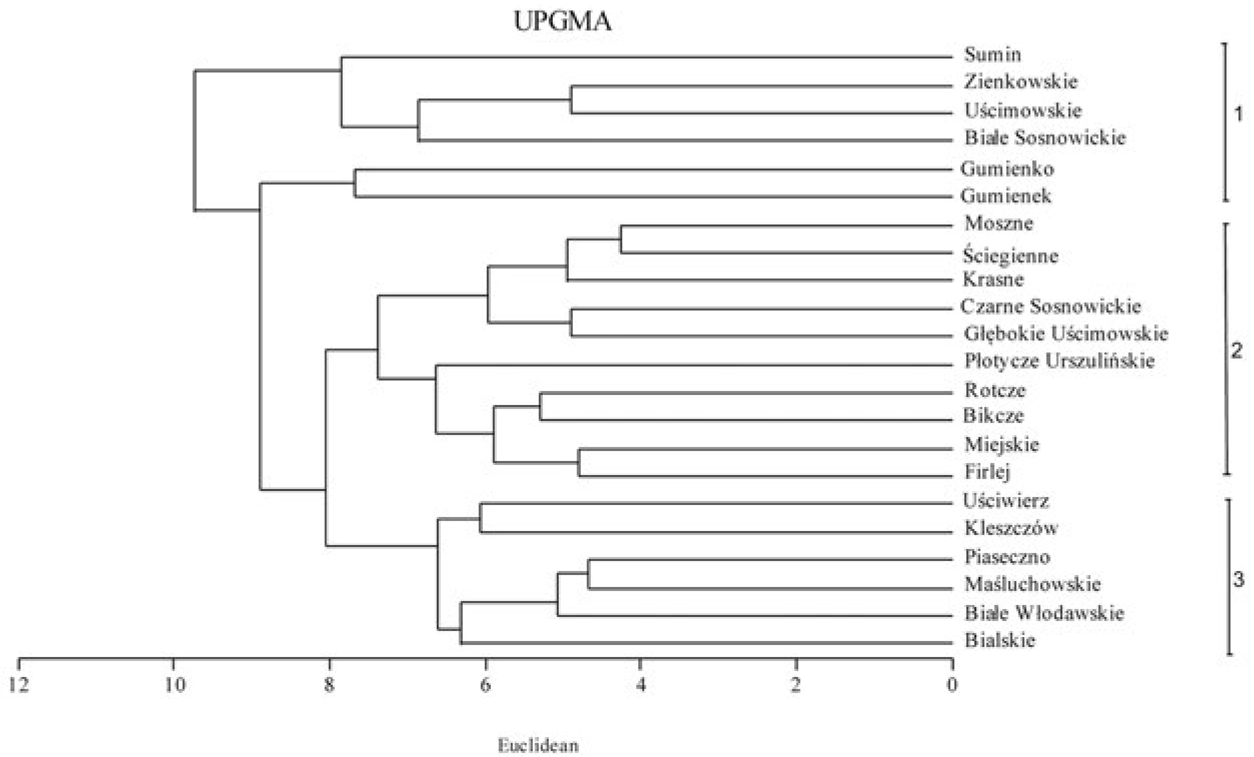

Fig. 4. Mean of similarity coefficient of examined lakes: 1 (=S1), 2 (=S2), 3 (=S3) groups of lakes.

A test of variance analysis (Tab. 3) clearly indicated that all the investigated zones are extremely important and significant in the overall valuation and assessment of the ecological status of the lakes, determining the membership into a specific group (category) ($P<0.05$). The statistical analysis of any zone (A, B, and C) of lakes (Fig. 4) showed that lakes belonging to the degraded lakes group (S1) had the lowest values in all analyzed zones; constituting a group of lakes that has strongly differing parameters from the average status. After analyzing lakes falling in the others two groups, it can be concluded that lakes in the second group (S2, eutrophic) were much better from the viewpoint of the littoral and surrounding zones (B and C), while worse from the shore conditions (A). This trend substantially changed for the lakes in the third group (S3), with the highest overall assessments and the best ecological status, for each zone.

The three identified lake classes showed a clear difference in vegetation types, in terms of macrophyte communities' distribution. Some of floating leaves plant communities (*i.e.*, *Nupharo-Nymphaeetum albae* Tomasz. 1977, *Potametum natantis* Soó 1923, *Hydrocharitetum morsus-ranae* Langendonck 1935, *Nymphaeetum candidae* Miljan 1958), and pleustophytes [*i.e.*, *Lemno minoris-Salvinietum natantis* (Slavnić 1956) Korneck 1959, *Lemnetum trisulcae* (Kelhofer 1915) Knapp et Stoffers 1962, *Spirodeletum polyrhizae* (Kelhofer 1915) Koch 1954 em. R.Tx. et A. Schwabe 1974 in R.Tx.1974] occurred in lakes with the lowest ecological value. The clear domination of helophytic communities [among them *Scirpetum lacustris* (Allorge 1922) Chouard 1924, *Typhetum angustifoliae* (Allorge 1922) Soó 1927, *Phragmitetum australis* (Gams 1927) Schmale 1939, *Typhetum latifoliae* Soó 1927, *Thelypteridi-Phragmitetum* Kuiper 1957, *Caricetum acutiformis* Sauer 1937, *Phalaridetum arundinaceae* (Koch 1926 n.n.) Lib. 1931, *Caricetum ripariae* Soó 1928 and others similar but less represented communities] occurred in lakes with good and moderate ecological status. The most balanced participation of all macrophyte groups occurred in lakes with the highest value of evaluation (II class) (Fig. 5). Kendall's correlation between ecological status and the number of the different groups of macrophytes in each lake was medium but significant ($\tau=0.52$, $P<0.05$).

ABC method evaluation *versus* other macrophyte indexes

We used and compared the ABC method with two different, widely used, macrophyte-based indexes elaborated for assessing the ecological status of lakes: The Polish "ESMI", and the English "TRS". The ecological status assessment obtained using the ESMI index and the present method (ABC) showed a slight similarity (38%), whereas the lowest similarity was between ABC index and TRS index (only 15%). The Pearson's correlation coefficient (comparing ABC and ESMI) was $r=0.62$, $P<0.05$. Lakes fell into I and II classes were mesotrophic or slightly eutrophic, in any case they can be considered in a good functional status. The greater differences were found among lakes felling into III class, namely eutrophic lakes. Comparing ESMI and ABC method, few lakes showed different results. Values were lower for seven lakes, five were the same and two higher (Tab. 4). These differences probably are due to the ESMI method, that is based on littoral zone data, and submerged macrophytes are its main element of evaluation. In our system, we also propose more factors to take under control, than ESMI is more focused to underline the human pressure as eutrophication process, however, lakes are subject also to others pressures.

Smallest differences in lake's ecological values were observed between the ABC and the English TRS index, but following this method, the largest group belongs from eutrophic lakes. In our opinion, this view can be too generic, because between eutrophic lakes it is possible to find different stages of eutrophication, combined not always with human pressure. In this way, our method try to combine a wide range of parameters (between vegetation, ecological features and uses) that can influence macrophytes community. The ABC method could be applied to all types of lakes, regardless the lake size/shape, the macrophyte occurrence, trophy or lake origin. Analyzing the different zones (A, B and C) it is possible to know which one is in poor condition, differing to others methods based only on macrophytes and less focused on ecological and dynamical features assessment.

The results allow to define lakes ecological status and which zones of them may require remedial action. For this reason, the ABC index can be applied to support biodi-

Tab. 3. Variance analysis test of A, B and C zones.

Svar	SSb	df	SSw	df	F	P value
A	15.89	2	5.11	19	29.53	<0.001
B	15.78	2	5.22	19	28.69	0.000002
C	13.68	2	7.32	19	17.76	0.000045

Svar, Source of variation; SS, sum of square; SSb, SS between; df, degrees of freedom; SSw, SS within; F, F test; A, lakes; B, lakes littoral zone; C, lake catchment.

versity, functionality, uses, conservation, restoration, water, territory and landscape management actions.

Practical remarks on the use of phytosociological approach in lakes analysis

In our proposal, it is possible to use any known type of plant/community “nomenclature approach”, and any method to assess the vegetation cover. We opted for the phytosociological approach to discriminate the recorded vegetation under a standardized and hierarchized framework. In this way, it was possible to place any communities in a *syntaxon* by a known procedure. Using this type of communities’ decoupage/nomenclature lets us to be more fine discriminating between similar communities; being universal, because if necessary, the holotypes are published and relevés can be converted into others

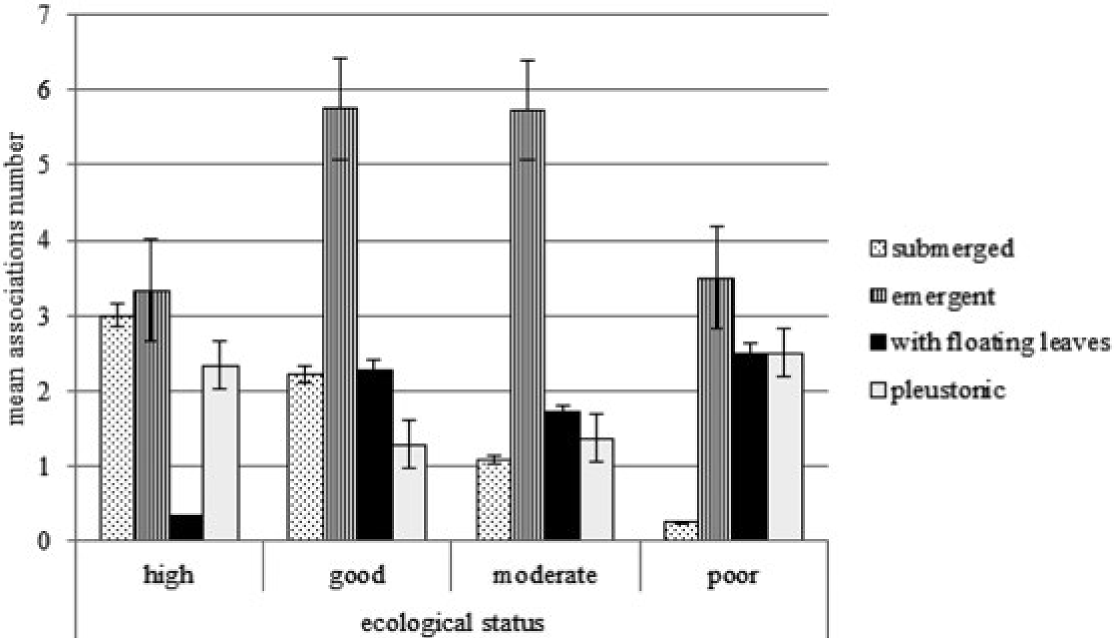

Fig. 5. Macrophyte groups/plant associations’ ratio, per different ecological lake status type.

Tab. 4. Ecological status assessment of selected lakes by different compared methods.

Evaluation / Lake	ABC	Status	ESMI	Status	TRS	Status
Białe Włodawskie	0.91	Very good	0.74	Very good	7.1	Mesotrophic
Firlej	0.83	Moderate	0.514	Good	8.2	Eutrophic
Głębokie Uścimowskie	0.83	Moderate	0.34	Good	8.1	Eutrophic
Kleszczów	0.82	Moderate	0.99	Very good	7.3	Mesotrophic
Krasne	0.87	Good	0.5	Good	8.0	Eutrophic
Maśluchowskie	0.86	Good	0.95	Very good	8.0	Eutrophic
Miejskie	0.77	Moderate	0.67	Good	8.2	Eutrophic
Moszne	0.90	Very good	0.88	Very good	8.1	Eutrophic
Piaseczno	0.95	Very good	0.8	Very good	8.0	Eutrophic
Płotycze Urszulińskie	0.81	Moderate	0.455	Good	8.3	Eutrophic
Rotcze	0.90	Very good	0.394	Good	7.9	Mesotrophic
Sumin	0.76	Moderate	0.104	Poor	8.4	Eutrophic
Uściwierz	0.90	Very good	0.83	Very good	8.2	Eutrophic
Zienkowskie	0.68	Poor	0.207	Moderate	8.6	Hypertrophic

nomenclatural approaches. In addition, when necessary (*i.e*., communities with new floristic combination, or not well expressed) it is possible to know the relevés accuracy level by the hierarchic *syntaxon* used (*i.e*., group, variant, association, sub-association, alliance, *etc*.). According to some authors, similar communities, or communities dominated by the same species, are not necessary similar in ecological, floristic or geobotanical traits, reflecting a different ecological value (Whittaker, 1962; Westhoff and Van der Maarel, 1973; Whittaker, 1975; Pott, 1995; Willner *et al*., 2009; Lötter *et al*., 2013; Pérez-Harguindeguy *et al*., 2013; Pillar *et al*., 2013). Consequently, these communities can differ in type of response to global changes (*i.e.*, climate, land uses, water, pollution, artificialization, burial), needing different management strategies.

In the context of the debated conceptual problems about biological communities' definition and their limits, we believe that the integration of ecology with nomenclature and empirical approaches based on plant sociology may be an interesting fast way to study, classifying, and cataloguing plant communities. These approaches need to be more explored as useful tool in the solution of biogeography and ecology problems (Poore, 1955; Jennings *et al*., 2003; Blasi and Frondoni, 2011; Van der Maarel, 2012; Cianfaglione and Pedrotti, 2016), and relative applications as proposed in certain ways from others authors (*i.e.*, Faliński, 1993; Gillet and Gallandat, 1996; Mucina, 1997; Pedrotti, 1999; Biondi, 2011; Decocq, 2016).

Ecological remarks

Bearing in mind that the use of the macrophyte index ESMI for most lakes of Polesie is not possible, an attempt was made to develop alternative method for assessing a macrophytes based on ecological status of lakes. In this way, our index allows to determine the lakes ecological status, and in other hand it can be helpful applied to choose methods and scope of protection/restoration policies, allowing to determine which zone require more remedial action. Our observations underlined that even the highest environmental protection standards cannot guarantee from the "risks" if a human pressure is (potentially) present. Certainly, they help to limit (and prevent) the risk, but they cannot ensure the preservation of habitats or the natural dynamics constancy, according to Kopeć *et al.* (2011). For example, until the late 90's Lake Moszne was frequently inhabited by stonewort mats [*i.e.*, *Charetum aculeolatae* (Corillion 1957) Dąmbska 1966, and *Charetum fragilis* Corillion 1949], recently replaced by a dense and more banal watermilfoil stands (*Myriophylletum spicati* Soó 1927), according to Sender (2008). As known, there are many factors influencing resilience and resistance to the lakes degradation. Observations in study area confirmed this, and personal observations (made by the authors mainly in Poland, but also in France and in Italy) underlined how the resilience of lakes appear greater, when the surface and depth are greater, according to Azzella *et al.* (2014a) and Sender *et al*. (2014). Smaller and shallower lakes are easier to fill and pollute, with a relative overgrowth of *Phragmitetalia australis* communities and other graminoids communities, so-called "roseliere" (*sensu* Géhu, 2006) formations.

In evaluation of water ecosystem ecological status, the catchment plays a highly important role (Sender *et al.,* 2014; Szoszkiewicz *et al.,* 2014), also as buffer zone (Alahuhta *et al.,* 2014). Both, the presence of intensive/extensive agriculture and housing in catchment areas, as well as the lack of a buffer zone in surveyed lakes, usually decreased their quality: adversely affecting plants occurrence (*i.e.*, Uścimowskie, and Zienowskie lakes). Definitely, the highest value lakes were in the deepest lakes, with a large share of bogs, grasslands and forests in their catchments: that resulted in the dominance of submerged macrophytes in phytolittoral, and a significant share of *Charophyta* (Piaseczno, Bialskie, and Białe Włodawskie lakes).

A dominance of emergent macrophytes, the disappearance of submerged vegetation in lakes or a depletion of the species composition may suggest that the deteriorating light conditions (turbidity) is related to burial, or mass development of phytoplankton (Sondergaard *et al.,* 2010). The spread of "roseliere" means a progressive process of burial, with graminoids communities' overgrowth, transforming it into a sort of sedge, fen or "flat peat-bog"; and burial rates can increase to very high levels following human pressure. Urbanization, deforestation or logging, modification of landforms or water flow, industrial or agricultural development, pollution and eutrophication can easily amplify that process (Arbuckle and Downing, 2001; Heathcote and Downing*,* 2012; Sender, 2012c; Sawtschuk and Bioret, 2012). Land use can also amplify the burial process because lakes receive increased erosional loads (Bennett *et al.,* 2001), and the volume of sediment deposited *per* unit time varies in a function of lake and watershed size: smaller impoundments had greater deposition and accumulation rates per unit area (Downing *et al*., 2008). Also in forested areas, the patterns of increasing sediment and nutrient delivery are remarkable, directly after deforestation, logging or land clearance (Rask *et al.,* 1998; Dearing and Jones, 2003; Sender, 2016). Less is known about temporal trends related to other land cover types (such as grasslands), which may substantially differ in response to land clearance, according to Jobbágy and Jackson (2000) hypothesis, also if more in general consequences can be presumably similar.

CONCLUSIONS

The studied lakes in the Łęczna-Włodawa Lake District shows a different ecological status, concerning conservation and functionality. Macrophytes and functional conditions of the surroundings were a basis for their classification. The ABC method for lakes ecological status assessment, allowed us to distinguish them into five classes. The largest group was the one with a good or moderate ecological status. The zones with the lowest values, often demanding remedial actions, were pointed out. In the overall assessment of the lakes ecological status, all the zones (A, B and C) demonstrated to be extremely important, affecting the functionality of the aquatic ecosystems.

The results of this newly index as a system of lakes status evaluation was comparable to the Ecological Status Macrophyte Index (ESMI), especially for lakes with a high natural value; however, its application is wider, and allows a more accurate representation of the actual ecological status, risks and functionality, by zones.

REFERENCES

Alahuhta J, Kanninen A, Hellsten S, Vuori K, Kuoppala M, Hämäläinen H, 2014. Variable response of functional macrophyte groups to lake characteristics, land use, and space: implications for bioassessment. Hydrobiologia 737:201-214.

Ali MM, Mageedb AA, Heikal M, 2007. Importance of aquatic macrophyte for invertebrate diversity in large subtropical reservoir. Limnologica 37:155-169.

Arbuckle KE, Downing JA, 2001. The influence of watershed land use on lake N:P in a predominantly agricultural landscape. Limnol. Oceanogr. 46:970-975.

Azzella MM, Bolpagni R, Oggioni A, 2014a. A preliminary evaluation of lake morphometric traits influence on the maximum colonization depth of aquatic plants. J. Limnol. 73:400-406.

Azzella MM, Rosati L, Iberite M, Bolpagni R, Blasi C, 2014b. Changes in aquatic plants in the Italian volcanic-lake system detected using current data and historical records. Aquat. Bot. 112:41-47.

Baattrup-Pedersen A, Szoszkiewicz K, Nijboer R, O'Hare M, Ferreira T, 2006. Macrophyte communities in unimpacted European streams: variability in assemblage patterns, abundance and diversity. Hydrobiologia 566:179-196.

Baláži P, Hrivnák R, Ot'ahelová H, 2014. The relationship between macrophyte assemblages and selected environmental variables in reservoirs of Slovakia examined for the purpose of ecological assessment. Pol. J. Ecol. 62:541-558.

Bennett EM, Carpenter SR, Caraco NF, 2001. Human impact on erodable phosphorus and eutrophication: A global perspective. BioScience 51:227-234.

Bertrin V, Boutry S, Dutartre A, 2012. Ecological quality assessment of lakes based on aquatic macrophyte assemblages in the context of the Water Framework Directive (WFD): The French Macrophyte Index for lakes (IBML). Journees Internationales de Limnologie et d'Oceanographie, Clermont-Ferrand, France: 18 pp.

Biondi E, 1996. [L'analisi fitosociologica nello studio integrato del paesaggio, p. 13-22].[In Italian]. In: J. Loidi (ed.), Proceedings 1st Symp. on "Avances en Fitosociología", Universidad del Pais Vasco.

Biondi E, 2011. Phytosociology today: methodological and conceptual evolution. Plant Bios. 145:19-29.

Blasi C, Frondoni R, 2011. Modern perspectives for plant sociology: the case of ecological land classification and the ecoregions of Italy. Plant Biol. 145:30-37.

Bolpagni R, 2013. Multimetric indices based on vegetation data for assessing ecological and hydromorphological quality of a man-regulated lake. Ann. Bot. (Roma) 3:87-95.

Bolpagni R, Piotti A, 2015. Hydro-hygrophilous vegetation diversity and distribution patterns in riverine wetlands in an agricultural landscape: a case study from the Oglio River (Po plain, Northern Italy). Phytocenologia 45:69-83.

Bolpagni R, Piotti A, 2016. The importance of being natural in a human-altered riverscape: Role of wetland type in supporting habitat heterogeneity and vegetation functional diversity. Aquat. Conserv. 26:1168-1183.

Bolpagni R, Laini A, Azzella MM, 2016a. Short-term dynamics of submerged aquatic vegetation diversity and abundance in deep lakes. Appl. Veg. Sci. 19:711-723.

Bolpagni R, Racchetti E, Laini A, 2016b. Fragmentation and groundwater supply as major drivers of algal and plant diversity and relative cover dynamics along a highly modified lowland river. Sci. Tot. Environ. 568:875-884.

Bolpagni R, Bettoni E, Bonomi F, Bresciani M, Caraffini K, Costaraoss S, Giacomazzi F, Monauni C, Montanari P, Mosconi MC, Oggioni A, Pellegrini G, Zampieri C, 2013. Charophytes of Garda Lake (Northern Italy): A preliminary assessment of diversity and distribution. J. Limnol. 72: 388-393.

Botineau M, Ghestem A, 1995. Caractérisation des communautés de macrophytes aquatiques (plantes vasculaires, bryophytes, lichen) en Limousin. Leurs relations avec la qualité de l'eau. Acta Bot. Gall. 142:585-594.

Brucet S, Poikane S, Lyche-Solheim A, Birk S, 2013. Biological assessment of European lakes: ecological rationale and human impacts. Freshwater Biol. 58:1106-1115.

Carbiener R, Trémolières M, Mercier JL, Orcheit A, 1990. Aquatic macrophyte communities as bioindicators of eutrophication in calcareous oligosaprobe stream waters (Upper Rhine plain, Alsace). Vegetatio 86:71-88.

Carvalho L, Lepisto L, Rissanen J, Pietilainen O P, Rekolainen S, Torok L, Lyche Solheim A, Saloranta T, Ptacnik R, Tartari G, Cardoso AC, Premazzi G, Gunn I, Penning E, Hanganu J, Hellsten S, Orhan I, Navodaru I, 2006. Nutrients and eutrophication in lakes, p. 3-32. In: A. Solimini, A.C. Cardoso and A.S. Heiskanen (eds.), Indicators and methods for the ecological status assessment under the Water Framework Directive: Linkages between chemical and biological quality of surface waters. Official Publications of the European Communities, Luxembourg.

Chmielewski TJ, 2009. [Ekologia krajobrazów hydrogenicznych Rezerwatu Biosfery Polesie Zachodnie].[Book in Polish]. Wydawnictwo Uniwersytet Przyrodniczy w Lublinie: 344 pp.

Cianfaglione K, 2011. The *Ricciocarpetum natantis* association in the Springs of Capo Pescara, Abruzzo (Italy). In A. Bensetiti, F. Bioret, and F. Pedrotti (eds.), Centenarie de la Phytosociologie. Braun-Blanquetia 46:186-190.

Cianfaglione K, 2014. The variations of water level and influence of artificial regulations in marshy woodland of *Alnus glutinosa* in the Peligna Valley (Abruzzo, central Italy): 4

years of investigation. Contribuţii Botanice 49:121-127.

Cianfaglione K, Pedrotti F, 2016. The vegetation of the Pie' Vettore Debris (Sibillini Mountains, Central Italy), p. 363-374. In: E.O. Box (ed.), Vegetation structure and function at multiple spatial, temporal and conceptual scales. Springer, Dordrecht.

Cianfaglione K, Bioret F (2017). Autoecological and synecological resilience of *Angelica heterocarpa* M.J. Lloyd, observed in the Loire Estuary (France). In: A. Greller, K. Fujiwara and F. Pedrotti (eds.), Geographical changes in vegetation and plant functional types. Geobotany studies. Springer, Dordrecht (In press).

Ciecierska H, 2008. [Makrofity jako wskaźniki stanu ekologicznego jezior. Rozprawy i Monografie].[Book in Polish]. Wydawnictwo Uniwersytetu Warmińsko-Mazurskiego w Olsztynie: 139 pp.

Ciecierska H, Kolada A, 2014. ESMI a macrophyte index for assessing the ecological status of lakes. Environ. Monit. Assess. 186:5501-5517.

Ciecierska H, Kolada A, Soszka H, Gołub M, 2006. [Opracowanie podstaw metodycznych dla monitoringu biologicznego wód powierzchniowych w zakresie makrofitów i pilotowe ich zastosowanie dla części wód reprezentujących wybrane kategorie i typy. Etap II Opracowanie metodyki badań terenowych makrofitów na potrzeby rutynowego monitoringu wód oraz metoda oceny i klasyfikacji stanu ekologicznego wód na podstawie makrofitów, Tom 2, Jeziora].[Book in Polish]. Narodowego Funduszu Ochrony Środowiska i Gospodarki Wodnej. IOŚ-UWM, Warszawa-Olsztyn.

Ciecierska H, Kolada A, Soszka H, Gołub M, 2010. A method for macrophyte-based assessment of the ecological status of lakes, developed and implemented for the purpose of environmental protection in Poland. BALWOIS, Ohrid.

De Bolòs O, 1963. [Botánica y Geografía].[Article in Spanish]. Memorias Real Academia Ciencias y Artes Barcelona 34:443-480.

De Lange L, Van Zon JCJ, 1983. A system for the evaluation of aquatic biotypes based on the composition of macrophyte vegetation. Biol. Conserv. 25:273-284.

Dearing JA, Jones RT, 2003. Coupling temporal and spatial dimensions of global sediment flux through lake and marine sediment records. Global. Planet. Change 39:147-168.

Decocq G, 2016. Moving from patterns to processes: A challenge for the phytosociology of the twenty-first century?, p. 407-424. In: E.O. Box (ed.), Vegetation structure and function at multiple spatial, temporal and conceptual scales. Springer, Dordrecht.

Downing JA, Cole JJ, Middelburg JJ, Striegl RG, Duarte CM, Kortelainen P, Prairie YT, Laube KA, 2008. Sediment organic carbon burial in agriculturally eutrophic impoundments over the last century. Global. Biogeochem. Cy. 22:GB1018.

European Union, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L 327, 22/12/2000, p. 1-73.

Faliński JB, 1993. Applied geobotany and "ecologization" of geobotanical map. Fragm. Florist. Geobot. 2:501-512.

Faliński JB, 1999. Geobotanical cartography: subject, source basis, transformation and application fundamentals of maps. Phytocoenosis Suppl. Cart. Geobot. 11:43-65.

Falińska K, 2003. Alternative pathways of succession: species turnover patterns in meadows abandoned for 30 years. Phytocoenosis Archiv. Geobot. 9:1-104.

Feldmann T, Nöges P, 2007. Factors controlling macrophyte distribution in large shallow Lake Vortsjarv. Aquat. Bot. 87:15-21.

Géhu JM, 1991. [L'analyse phytosociologique et géosymphytosociologique de l'espace. Théorie et méthodologie].[Article in French]. Colloq. Phytosoc 17:11-46.

Géhu JM, 2006. [Dictionnaire de sociologie et synécologie végétales].[Book in French]. J. Cramer Ed., Berlin-Stuttgart, Germany: 899 pp.

Gillet F, Gallandat, JD, 1996. Integrated synusial phytosociology: some notes on a new, multiscalar approach to vegetation analysis. J. Veg. Sci. 7:13-18.

Haury J, Peltre MC, 1993. [Intérêts et limites des «indices macrophytes» pour qualifier la mésologie et la physicochimie des cours d'eau: exemples armoricains, picards et lorrains].[Article in French]. Ann. Limnol. 29:239-253.

Haury J, Peltre MC, Trémoliéres M, Barbe J, Thiébaut G, Bernez I, Daniel H, Chatenet P, Haan-Archipof G, Muller S, Dutartre A, Laplace-Treyture C, Cazaubon A, Lambert-Servien E, 2006. A new method to assess water trophy and organic pollution - the Macrophyte Biological Index for Rivers (IBMR): its application to different types of river and pollution. Hydrobiologia 570:153-158.

Heathcote AJ, Downing JA, 2012. Impacts of eutrophication on carbon burial in freshwater lakes in an intensively agricultural landscape. Ecosystems 15:60-70.

Holmes NTH, Boon P J, Rowell TA, 1988. Revised classification for British rivers based on their plant communities. Aquat. Conserv. 8:555-578.

Jennings M, Loucks O, Glenn-Lewin D, Peet R, Faber-Langendoen D, Grossman D, Damman A, Barbour M, Pfister R, Walker M, Talbot S, Walker J, Hartshorn G, Waggoner G, Abrams M, Hill A, Roberts D, Tart D, 2003. Guidelines for describing Associations and Alliances of the U.S. National vegetation classification. The Ecological Society of America. Vegetation Classification Panel, vers. 2.0; March 28.

Jensen S, 1977. An objective method for sampling the macrophytes vegetation in lakes. Vegetatio 33:107-118.

Jobbágy EG, Jackson RB, 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol. Appl. 10:423-436.

Juszczak R, Arczyńska-Chudy E, 2003. [Ekologiczna i melioracyjna waloryzacja małych zbiorników wodnych w krajobrazie rolniczym Wielkopolski].[Article in Polish]. Zeszyty Problemowe Postępów Nauk Rolniczych 1:145-160.

Keto A, Tarvainen A, Hellsten S, 2006. The effect of water level regulation on species richness and abundance of aquatic macrophytes in Finnish lakes. Verh. Int. Verein Limnol. 29:2103-2108.

Khadija SA, Francis R, Bernard T, 2015. Trend analysis in ecological status and macrophytic characterization of watercourses: Case of the Semois-Chiers Basin, Belgium Wallonia. J. Water Res. Prot. 7:988-1000.

Kolada A, 2008. [Wykorzystanie makrofitów w ocenie jakości jezior w Europie w świetle wymogów Ramowej Dyrektywy

Wodnej – przegląd zagadnienia].[Article in Polish]. Ochrona Środowiska i Zasobów Naturalnych 37:24-42.

Kolada A, Ciecierska H, Ruszczyńska J, Dynowski P, 2014. Sampling techniques and inter-surveyor variability as sources of uncertainty in Polish macrophyte based metric for lake ecological status assessment. Hydrobiologia 737:256-279.

Kondracki J, 2002. [Geografia regionalna Polski].[Book in Polish]. Wydawnictwo PWN, Warszawa: 440 pp.

Kopeć D, Halladin-Dąbrowska A, Zając I, 2011. Flora dynamics in strictly protected nature reserve. Pol. J. Environ. Stud. 20:107-113.

Lacoul P, Freedman B, 2006. Environmental influences on aquatic plants in freshwater ecosystems. Environ. Rev. 14:89-136.

Lötter MC, Mucina L, Witkowski ETF, 2013. The classification conundrum: species fidelity as leading criterion in search of a rigorous method to classify a complex forest data set. Comm. Ecol. 14:121-132.

Matuszkiewicz W, 2008. [Przewodnik do oznaczania zbiorowisk roślinnych Polski].[Book in Polish]. Wyd. Naukowe PWN, Warszawa: 536 pp.

Meilinger P, Schneider S, Melzer A, 2005. The reference index method for the Macrophyte based assessment of rivers – A contribution to the implementation of the European Water Framework Directive in Germany. Int. Rev. Hydrob. 90:322-342.

Melzer A, 1999. Aquatic macrophyres as tools for lake management. Hydrobiologia 395/396:181-190.

Mirek Z, Piękoś-Mirkowa H, Zając A, Zając M, 2002. Flowering plants and pteridophytes of Poland, a checklist. Szafer Inst. of Botany, Polish Academy of Sciences, Kraków: 442 pp.

Mucina L, 1997. Classification of vegetation: Past, present, and future. J. Veg. Sci. 8:751-760.

Murphy KJ, Rorslett B, Springuel I, 1990. Strategy analysis of submerged lake macrophytes communities: an international example. Aquat. Bot. 36:303-323.

Newbold C, Holmes NTH, 1987. Nature conservation: water quality criteria and plants as water quality monitors. Water Pollut. Control. 86:345-364.

Pall K, Moser V, 2009. Austrian Index Macrophytes (AIM-Module 1) for lakes: A Water Framework Directive compliant assessment system for lakes using aquatic macrophytes. Hydrobiologia 633:83-104.

Palmer MA, Bell SL, Butterfield I, 1992. A botanical classification of standing waters in Britain: applications for conservation and monitoring. Aquat. Conserv. 2:125-143.

Palmer MA, Roy DB, 2001. A method for estimating the extent of standing fresh waters of different trophic states in Great Britain. Aquat. Conserv. 11:199-216.

Pedrotti F, 1999. [Cartografia della vegetazione e qualità dell'ambiente].[Article in Italian]. Nat. Alp. 4:21-41.

Pedrotti F, 2013. Plant and vegetation mapping. Springer, Dordrecht: 249 pp.

Penning E, Mjelde M, Dudley B, Hellsten S, Hanganu J, Kolada A, Van Den Berg M, Mäemets H, Poikane S, Phillips G, Willby N, Ecke F, 2008. Classifying aquatic macrophytes as indicators of eutrophication in European Lakes. Aquat. Ecol. 42:237-251.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte M, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, Wright IJ, Ray P, Enrico L, Pausas JG, de Vos AC, Buchmann N, Funes G, Quétier F, Hodgson JG, Thompson K, Morgan HD, ter Steege H, van der Heijden MGA, Sack L, Blonder B, Poschlod p, Vaieretti MV, Conti G, Staver AC, Aquino S, Cornelissen JHC, 2013. New handbook for standardised measurement of plant functional traits worldwide. Aust. J. Bot. 61:167-234.

Pillar VD, Blanco CC, Müller SC, Sosinski EE, Joner F, Duarte LDS, 2013. Functional redundancy and stability in plant communities. J. Veg. Sci. 24:963-974.

Poore MED, 1955. The use of phytosociological methods in ecological investigations. J. Ecol. 43:226-244.

Pott R, 1995. [Die Pflanzengesellschaften Deutschlands].[Book in German]. Ulmer, Stuttgart.

Rask M, Nyberg K, Markkanen SL, Ojala A, 1998. Forestry in catchments: Effects on water quality, plankton, zoobenthos and fish in small lakes. Boreal Environ. Res. 3:75-86.

Rejewski M, 1981. [Roślinność jezior rejonu Laski w Borach Tucholskich].[Book in Polish]. Uniwersytet Mikołaja Kopernika, Rozprawy, Toruń.

Rivas-Martinez S, 1994. Dynamic-zonal phytosociology as landscape science. Phytocoenologia 24:23-25.

Rozporządzenie Ministra Środowiska, 2011. [R. w sprawie klasyfikacji stanu ekologicznego, potencjału ekologicznego i stanu chemicznego jednolitych części wód powierzchniowych].[Report in Polish]. Dz.U. 2011, nr 258, poz. 1549.

Sawtschuk J, Bioret F, 2012. [Analyse diachronique de la dynamique spatiale de la végétation de l'estuaire de la Loire. Photo-Interprétation].[Article in French]. Eur. J. Appl. Remote Sensing 3:15-28.

Schaumburg J, Schranz C, Hofmann G, Stelzer, Schneider S, 2004. Macrophytes and phytobenthos as indicators of ecological status in German lakes - a contribution of the implementation of the Water Framework Directive. Limnologica 34:302-314.

Schneider S, Meltzer A, 2003. The trophic index of macrophytes (TIM) - A new tool for indicating the trophic state of running waters. Int. Rev. Hydrobiol. 88:49-67.

Sender J, 2008. Long term changes of macrophytes in the Lake Moszne (Poleski National Park). Teka Komisji Ochrony i Kształtowania Środowiska Przyrodniczego Oddział Lublin PAN 5:154-163.

Sender J, 2009. [Analiza zmian sukcesyjnych zachodzących w fitocenozach wodnych i florze makrolitów badanych jezior w latach 1960-2009, p. 139-161]. In: T.J. Chmielewski (ed.), [Ekologia krajobrazów hydrogenicznych Rezerwatu Biosfery Polesie Zachodnie].[Book in Polish]. Wyd. UP Lublin.

Sender J, 2012a. Possibilities of macrophyte indicators, application for assessment of ecological status of lakes. Transylv. Rev. System. Ecol. Res. 14:115-128.

Sender J, 2012b. Quantitative investigations of vascular flora in deep and shallow eutrophic lake. Teka Komisji Ochrony i Kształtowania Środowiska Przyrodniczego Oddział Lublin PAN 9:215-223.

Sender J, 2012c. The dynamics of macrophytes in a lake in an agricultural landscape. Limnol. Rev. 2:93-100.

Sender J, 2016. The effect of riparian forest shade on the structural characteristics of macrophytes in a mid-forest lake. Appl. Ecol. Environ. Res. 14:249-261.

Sender J, Cianfaglione K, Kolejko M, 2014. Evaluation of ecological state of small water reservoirs in the Bystrzyca river valley. Teka Komisji Ochrony i Kształtowania Środowiska Przyrodniczego Oddział Lublin PAN 11:173-180.

Skwierawski A, 2005. [Ocena stanu małych zbiorników wodnych na terenach wiejskich. Cz. I. Metoda waloryzacji małych zbiorników].[Article in Polish]. Zeszyty Problemowe Postępów Nauk Rolniczych 506:391-402.

Sondergaard M, Johansson L, Lauridsen TL, Jorgensen TB, Liboriussen L, Jeppesen E, 2010. Submerged macrophytes as indicator of ecological quality of lakes. Freshwater Biol. 55:893-908.

Sossey-Alaoui K, Rosillon F, 2013. Macrophytic distribution and trophic state of some natural and impacted watercourses-Belgium Wallonia. Int. J. Water Sci. 2:1-11.

Soszka H, Cydzik D, 2003. Changes in water quality of Polish lakes in the years 1991-2000 (based on lake monitoring results). Limnol. Rev. 3:53-58.

Spence DHN, 1967. Factor controlling the distribution of freshwater macrophytes with particular reference to the lochs of Scotland. J. Ecol. 55:147-170.

Stanisz A, 2007. [Przystępny kurs statystyki z zastosowaniem].[Book in Polish].Statistica PL na przykładach z medycyny, Kraków vol.3.

Stelzer D, Schneider S, Melzer A, 2005. Macrophyte-based assessment of lakes - a contribution to the implementation of European Water Framework Directive in Germany. Int. Rev. Hydrobiol. 90:223-237.

Suominen J, 1968. Changes in the aquatic macro-flora of the polluted lake Rautrvesi. SD.W. Finland. Ann. Bot. Fenn. 5:65-81.

Szmeja J, 2006. [Przewodnik do badań roślinności wodnej].[Book in Polish]. Wyd. UG, Gdańsk: 307 pp.

Szoszkiewicz K, Ciecierska H, Kolada A, Schneider SC, Szwabińska M, Ruszczyńska J, 2014. Parameters structuring macrophyte communities in rivers and lakes - results from a case study in North-Central Poland. Knowl. Manag. Aquat. Ecosyst. 415:08.

Tomaszewicz H, Kłosowski S, 1985. [Roślinność wodna i szuwarowa jezior na Pojezierzu Sejneńskim].[Article in Polish]. Monogr. Bot. 67:69-141.

Van der Maarel E, 2012. Vegetation ecology - an overview, p. 1-51. In: E. Van der Maarel and J. Franklin (eds.), Vegetation ecology, 2nd ed. Wiley-Blackwell.

Van der Molen DT, Latour P, Stronkhorst J, Van der Wal B, 2004. [Ecologische referenties en maatlatten voor de Kaderrichtlijn Water].[Article in Dutch]. H2O 6:10-12.

Villa P, Laini A, Bresciani M, Bolpagni R. 2013. A remote sensing approach to monitor the conservation status of lacustrine *Phragmites australis* beds. Wetl. Ecol. Manag. 21:399-416.

Westhoff V, van der Maarel E, 1973. The Braun-Blanquet approach, p. 617-726. In: R.H. Whittaker (ed.), Handbook of vegetation science. Part 5, Classification and ordination of communities. Junk, The Hague.

Whittaker H, 1962. Classification of natural communities. Bot. Rev. 28:1-239.

Whittaker RH, 1975. Communities and ecosystems. MacMillan Publishing, London: 352 pp.

Whitton BA, Kelly MG, 1995. Use of algae and other plants for monitoring rivers. Aust. J. Ecol. 20:45-56.

Wilgat T, 1954. [Jeziora Łęczyńsko-Włodawskie].[Article in Polish]. Annales UMCS, sec. B 8:37-122.

Willner W, Tichy L, Chytry M, 2009. Effects of different fidelity measures and contexts on the determination of diagnostic species. J. Veg. Sci. 20:130-137.

Wojtanowicz J, 1994. [O termokrasowej genezie Jezior Łęczyńsko-Włodawskich].[Article in Polish]. Annales UMCS sec. B 49:1-18.

Wołek J, Kościółek A, 2012. [Występowanie, struktura i ekologia zbiorowisk pleustonowych (klasa *Lemnetea minoris*) w województwie małopolskim (Polska)].[Article in Polish]. Fragm. Florist. Geobot. Polon. 19:99-115.

Zohary T, Ostrovsky I, 2011. Ecological impacts of excessive water level fluctuations in stratified freshwater lakes Tamar. Inland Waters 1:47-59.

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Integrating the Water Framework Directive into the Habitats Directive: Analysis of distribution patterns of lacustrine EU habitats in lakes of Lombardy (northern Italy)

Rossano BOLPAGNI,[1*] Mattia M. AZZELLA,[2] Chiara AGOSTINELLI,[3] Andrea BEGHI,[3] Eugenia BETTONI,[3] Guido BRUSA,[4] Cristina DE MOLLI,[4] Riccardo FORMENTI,[3] Filippo GALIMBERTI,[3] Bruno E.L. CERABOLINI[4]

[1]Department of Chemical Science, Life and Environmental Sustainability, University of Parma, Viale G.P. Usberti 33/A, 43124 Parma, Italy; [2]Via G. Bellucci 94, 00156 Rome, Italy; [3]Lombardy Regional Environmental Protection Agency, Via Rosellini 17, 20124 Milan, Italy; [4]Department of Theoretical and Applied Sciences, University of Insubria, Via J.H. Dunant 3, 21100 Varese, Italy
*Corresponding author: rossano.bolpagni@unipr.it

ABSTRACT

The existence of strong potential synergies between the Water Framework Directive (WFD) and the Habitats Directive (HD) is widely acknowledged. Indeed, ensuring favourable conservation conditions for aquatic habitats and species of conservation concern is closely related to the achievement of a good ecological status in water bodies. However, since these two sets of European laws are generally applied without any coordination, an inefficient use of resources may adversely affect their goals. The main negative outcome is an increase in the cost of monitoring programs for collecting data in nature (*i.e.*, physical and chemical parameters, species and habitats, plant communities). The use of macrophytes as a bioindicator, as imposed by the WFD, may instead help to integrate data on aquatic EU habitats and enhance knowledge of such habitats outside the Natura 2000 network. The aim of present study was to evaluate the usefulness of data collected in WFD monitoring surveys as a means of inferring the occurrence and the distribution of lacustrine aquatic habitats in countries belonging to the European Union (EU). The main aim of the analysis was to identify the depth gradient distribution of diagnostic macrophyte *taxa* in two EU habitats (3140, *i.e.*, *Chara*-dominated benthic communities, and 3150, *i.e.*, natural eutrophic lakes) using data collected in lakes in Lombardy (northern Italy), some of which are included in the Natura 2000 network (10 out 16). While recognizing the limitations of the data collected within the two frameworks, the results confirmed the marked usefulness of WFD data as a means of enhancing the knowledge available on lacustrine aquatic habitats in the EU. WFD data can actively help to improve the basic information on aquatic habitats, thereby more effectively supporting regional strategies for biodiversity conservation as well as recovery programs.

Key word: WFD; HD; monitoring programs; aquatic vegetation; vascular macrophytes; charophytes; nutrient drivers; macrophyte depth distribution.

INTRODUCTION

The Water Framework Directive (WFD, European Union, 2000) and the Habitats Directive (HD, European Union, 1992) are strongly interrelated (Janauer *et al.*, 2015). The synergy between these two directives represents an intriguing goal aimed at pursuing an effective conservation of aquatic ecosystems. It being universally accepted that the achievement of favourable conservation conditions for aquatic habitats or species of conservation concern is closely related to the possibility of guaranteeing a good ecological status of colonized water bodies (Janauer *et al.*, 2015). However, each of these directives is currently implemented on its own, thereby potentially hampering the achievement of their respective goals (Beunen *et al.*, 2009). One major effect of the lack of coordination between the directives is an increase in the cost of monitoring programs for collecting data in nature (*i.e.*, physical and chemical parameters, species and habitats, plant communities). Indeed, the synergy and potential conflicts between these directives have mainly been investigated in terms of the attainment of their respective goals rather than of the possible reciprocal operational support (Ecke *et al.*, 2010; Collins *et al.*, 2012; Janauer *et al.*, 2015).

WFD monitoring activities include both biotic elements and physical and chemical features (European Union, 2000), providing the opportunity to integrate the structural/compositional investigation of biotic communities with their environmental drivers (*e.g.*, hydro-morphological, physical and chemical determinants). Indeed, the depth distribution of lacustrine macrophytes is affected by several ecological factors: light (Canfield *et al.*, 1985; Chambers and Kalff, 1985), phosphorus (Søndergaard *et al.*, 2013), temperature and depth of the thermocline (Genkai-Kato and Carpenter, 2005), and pressure (Dale, 1986). Although light seems to be the most representative ecological factor, phosphorus availability is also important on account of the role it plays in the regulation of macrophyte distribution in a lake as well as of their occurrence at the regional scale (Duarte and Kalff, 1990). Furthermore, the maximum depth of macrophyte colonization (Zc_{max}) is strongly dependent on the size of the lake, which is in turn directly regulates the depth of the thermocline (Genkai-Kato and Carpenter, 2005). Where water transparency is high, the Zc_{max} is greater in large lakes than in medium-sized and small lakes (Azzella *et al.*, 2014a, 2014b). These factors may be useful for monitoring the conservation status of aquatic European Union (EU) habitats if we bear in mind that that the HD reporting activities are – *inter alia* – based on an evaluation of the area and range occupied by habitats (Evans and Arvela, 2011).

The level of information available for aquatic vegetation is generally far lower than that available for terrestrial vegetation. A greater integration between these two directives may represent a win-win strategy to fill the gaps in knowledge on aquatic vegetation and to assess the effectiveness of WFD legislation in improving the quality of water bodies. This is particularly interesting if we consider that aquatic ecosystems are among the ecosystems threatened most on a worldwide scale and that, besides the inherent difficulties associated with sampling, the cost of providing reliable data is high (*e.g.*, boat availability, poor weather conditions, wind) (Azzella *et al.*, 2013a).

A potential change in this scenario may lie in the WFD. It provides for the use of several biotic communities as a means of assessing the quality status of water bodies, including macrophytes, fish and macroinvertebrates, which are actually the same target elements as those found in the HD (Janauer *et al.*, 2015). By focusing on lacustrine macrophytes, we may be able to integrate the HD database with routine monitoring information yielded by the WFD. Indeed, almost all the aquatic vegetation in lakes, whether it is dominated by vascular plants or charophytes, can be referred to the conservation habitats defined by the HD (European Union, 1992; Bolpagni, 2013a).

The main aim of the present study was to verify the occurrence of the EU habitat codes 3140 and 3150 by using WFD data, both within and outside the Natura 2000 network in Lombardy. By considering the depth distribution of their diagnostic *taxa*, we also investigated the predictive role of water transparency (expressed as SD) in modelling the depth distribution of lacustrine EU habitats.

METHODS

Study area

The study was conducted on data collected from 16 different lakes in the Lombardy Region (northern Italy), 10 of which are included in the Natura 2000 network (Tab. 1; Fig. 1). The lakes covered a wide range of ecological conditions (Tab. 2), both as regards their physical (*e.g.*, depth) and trophic (*e.g.*, water transparency investigated by means of Secchi disk, SD) characteristics. Lake depth ranged from 3 m (Lake Ganna) to 370 m (Lake Maggiore), whereas the SD ranged from ~0.80-1.00 m (lakes of Mantova) to 8.42 m (Lake Monate).

Macrophyte EU target habitats

The target habitats of the present study are the EU habitats 3140 (hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp.) and 3150 (natural eutrophic

Tab. 1. Interactions between lakes and Natura 2000 network; the official name of the site, its national code and type, as well as the percentage of the lacustrine area included in the site is reported for each lake included in a Natura 2000 site. The overall assessment of EU habitat 3150 is also included (updated in February 2016).

Lake	Natura 2000 site name	National code	Type	LA	3150
Comabbio	Lago di Comabbio	IT2010008	SAC	100%	B
Ganna	Lago di Ganna	IT2010001	SAC	100%	
Iseo	Torbiere d'Iseo	IT2070020	SAC/SPA	<1%	B
Maggiore	Canneti del Lago Maggiore	IT2010502	SPA	<1%	B
	Palude Bruschera	IT2010015	SAC	<1%	B
Mantova Superior	Ansa e Valli del Mincio	IT20B0017	SAC	40%	C
	Valli del Mincio	IT20B0009	SPA	100%	C
Mantova Middle	Valli del Mincio	IT20B0009	SPA	100%	C
Mantova Inferior	Valli del Mincio	IT20B0009	SPA	100%	C
Mezzola	Lago di Mezzola e Pian di Spagna	IT2040042	SAC	100%	A
	Lago di Mezzola e Pian di Spagna	IT2040022	SPA	100%	A
Montorfano	Lago di Montorfano	IT2020004	SAC	100%	
Varese	Alnete del Lago di Varese	IT2010022	SAC	<1%	B
	Lago di Varese	IT2010501	SPA	100%	B

LA, lacustrine area; SAC, Special Area of Conservation; SPA, Special Protection Areas; A, excellent value; B, good value; C, significant value.

lakes with *Magnopotamion* or *Hydrocharition*-type vegetation). The former includes oligo- to meso-trophic waters with well-developed benthic vegetation dominated by stoneworts (charophytes); the latter includes the free-floating and rhizophytic plant communities of eutrophic lakes (Biondi *et al*., 2009; Azzella *et al*., 2013b; Bolpagni, 2013a).

Generally, *Chara*-dominated vegetation has a low species richness and is strictly controlled by water nutrient content (especially by phosphorous availability) (Blindow,

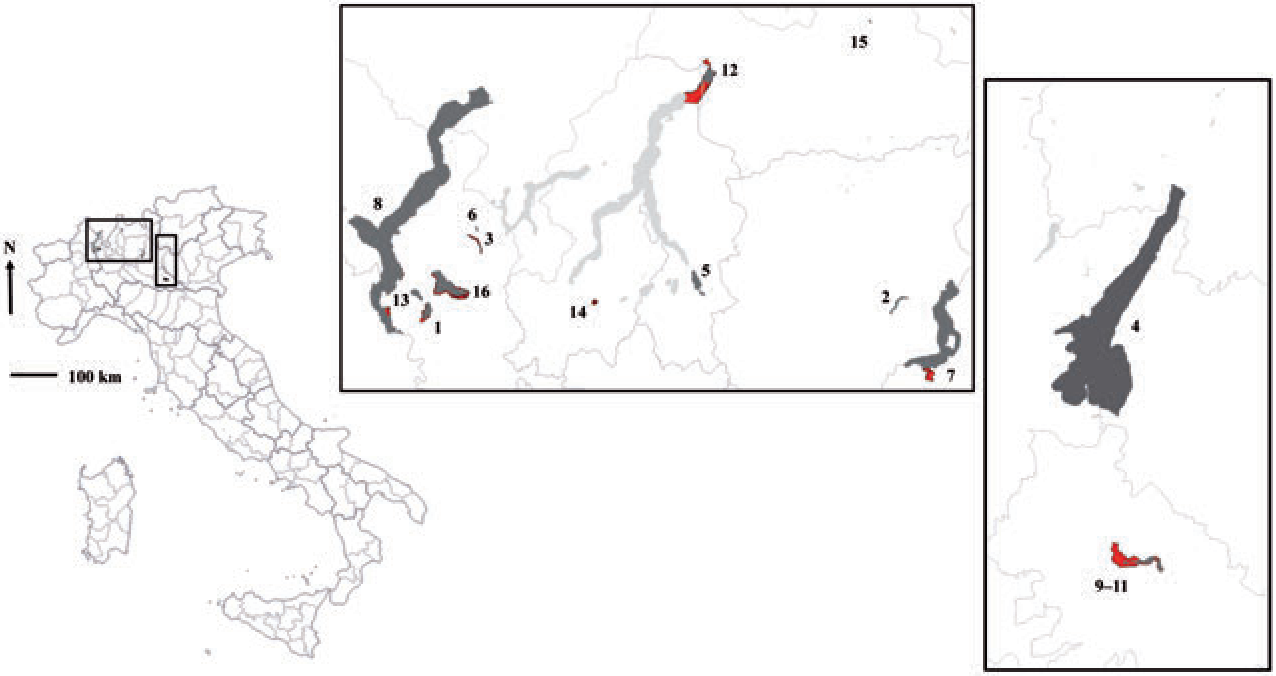

Fig. 1. Study area; the spatial distribution of the lakes analyzed (in dark grey), and the Natura 2000 sites (in red) are reported; see Tab. 1 and Tab. 2 for the Natura 2000 site codes and the lake names, respectively.

Tab. 2. Lakes features and sampling effort for each investigated lake.

	Lake	Alt (m asl)	A (km^2)	D (m)	V (m^3 10^6)	SD (m)	TP (μg L^{-1})	IY	#TR	#SP
1	Comabbio	243	3.6	8	16.6	3.23	5.3	2008	23	78
2	Endine	334	2.1	9	11.9	2.42	19.0	2008	67	157
3	Ganna	390	0.1	3	0.1	2.50	2.5	2008	5	15
4	Garda	65	368.0	350	49031.0	8.20	17.7	2011	82	760
5	Garlate	205	4.6	34	70.0	5.62	23.0	2011	20	164
6	Ghirla	415	3.2	14	nd	4.93	2.5	2008	18	74
7	Iseo	185	61.0	251	7600.0	4.67	61.5	2008	109	743
8	Maggiore	193	213.0	370	37500.0	6.42	4.4	2012	198	1056
9	Mantova Middle	15	3.7	12	nd	0.85	50.0	2010	28	65
10	Mantova Inferior	15	1.5	9	nd	0.82	37.5	2010	20	51
11	Mantova Superior	18	1.1	15	nd	0.99	55.0	2010	45	119
12	Mezzola	200	5.9	69	149.0	2.72	11.3	2011	30	189
13	Monate	266	2.5	34	45.0	8.42	2.5	2008	40	172
14	Montorfano	397	0.5	7	1.9	2.83	20.5	2012	12	48
15	Palù	1921	0.2	50	nd	6.43	5.0	2011	10	52
16	Varese	238	14.8	26	160.0	3.38	39.3	2008	44	130

Alt, altitude; A, area; D, depth; V, volume; SD, Secchi disk; TP, total phosphorous; IY, investigation year; #TR, total number of transects; #SP, sampling plots; nd, not detected.

1992). Since the *Chara*-dominated communities in lakes in northern Italy have seldom been investigated, few data are available on their composition and conservation status (Bolpagni *et al*., 2013). By contrast, more detailed investigations have been conducted on the volcanic lakes of central Italy (Azzella *et al*., 2013a, b, 2014; Bolpagni *et al.,* 2016). The free-floating and rhizophytic plant communities are found both in eutrophic lakes and ponds. Almost all aquatic plant communities can be referred to this habitat type (*e.g.*, *Lemnetea* and *Potametea* vegetation classes) (Biondi *et al*., 2009), including free-floating [*e.g.*, *Lemna* ssp. or *Salvinia natans* (L.) All. dominated stands] and rooted vegetation consisting of both submerged (*e.g.*, *Vallisneria natans* L., *Potamogeton lucens* L. dominated stands) and emergent [*e.g.*, *Nuphar lutea* (L.) Sm., *Nymphaea alba* L., *Potamogeton natans* L. dominated stands] plant communities (Bolpagni and Piotti, 2015, 2016).

No record of the presence of EU habitat 3140 is present in the standard data forms of the Natura 2000 sites, whereas the presence of EU habitat 3150 is recorded in 8 out of 10 lakes (lakes Comabbio, Iseo, Maggiore, Mantova Superior, Middle and Inferior, Mezzola, and Varese) (Tab. 1). Furthermore, no standardized information on any EU habitats is available for the lakes outside the Natura 2000 sites (lakes Endine, Garda, Garlate, Ghirla, Monate, and Palù). Thus, the data currently available indicate that EU habitat 3140 is not present, whereas EU habitat 3150 covers ~298 ha.

Study design and data analysis

We analyzed data collected in WFD monitoring surveys conducted between 2009 and 2012. Data included SD and TP values (Tab. 2) and the depth of macrophyte distribution. Macrophyte data was collected by applying the Italian national protocol, as described by Oggioni *et al*. (2011). Accordingly, the lakeshores were divided into homogeneous sections by inspecting helophytic and macrophyte vegetation. Data on macrophytes were recorded from a depth of 0.5 m to the maximum depth of colonization at 1-meter depth intervals along transects in a randomly selected section (Bolpagni, 2013a,b). The cover-abundance – expressed as a percentage – was estimated for all the *taxa* identified.

Three different morpho-functional groups of *taxa* were identified (Tab. 3) on the basis of the diagnostic

Tab. 3. Total representativeness of diagnostic *taxa* (total sampling plots colonized) of the EU habitat codes 3150 (natural eutrophic lakes; 1: free-floating and floating-leaved rhizophyte-dominated stands, and 2: submerged plant-dominated stands) and 3140 (3: charophyte-dominated benthic communities).

Habitat code		Species	Total
3150	1	*Nuphar lutea* (L.) Sm.	97
3150	1	*Nymphaea alba* L.	312
3150	1	*Nymphoides peltata* (S.G. Gmel.) Kuntze	40
3150	1	*Trapa natans* L.	241
3150	2	*Ceratophyllum demersum* L.	1651
3150	2	*Myriophyllum spicatum* L.	1683
3150	2	*Najas marina* L.	1849
3150	2	*Najas minor* All.	73
3150	2	*Potamogeton lucens* L.	31
3150	2	*Potamogeton pectinatus* L.	297
3150	2	*Potamogeton perfoliatus* L.	916
3150	2	*Potamogeton pusillus* L.	274
3150	2	*Potamogeton trichoides* Cham. & Schltdl.	20
3150	2	*Vallisneria spiralis* L.	2809
3150	2	*Zanichellia palustris* L.	582
3140	3	*Chara globularis* Thuiller	1439
3140	3	*Chara intermedia* A.Braun	36
3140	3	*Chara tomentosa* Linnaeus	25
3140	3	*Nitella flexilis* (Linnaeus) C.Agardh	61
3140	3	*Nitella gracilis* (J.E.Smith) C.Agardh	27
3140	3	*Nitella hyalina* (De Candolle) C.Agardh	36
3140	3	*Nitellopsis obtusa* (N.A.Desvaux) J.Groves	84

power of each species detected in terms of life-growth form and the phytosociological literature. The free-floating and floating-leaved rhizophytes (group 1) and submerged rhizophytes (2) were referred to EU habitat 3150; charophytes (3) were instead referred to EU habitat 3140. *Taxa* with at least 20 records (=22) were considered in the analysis (Supplementary Tab. 1). Overall, 751 transects were analyzed, which resulted in a total of 3,873 plots being investigated and 14,130 *taxon* records being collected. We considered a habitat present when at least one stonewort or vascular diagnostic species was present with an estimated cover-abundance higher than 35%.

Simple regression analysis was used to evaluate the relationships between the depth distribution of the EU habitats being analyzed and SD as a proxy of the underwater light conditions. The area and depth descriptors were excluded from the analyses owing to their high collinearity with SD. All the analyses were performed in the R environment (R Development Core Team, 2016), considering the depth distribution of the EU habitats' diagnostic *taxa*, considering the three morpho-functional groups of species separately: i) free-floating and floating-leaved plants, ii) submerged plants, and iii) charophytes.

RESULTS

Macrophyte EU habitat distribution patterns

Based on the diagnostic species spatial arrangement, we confirmed the presence of EU habitat 3150 in all the lakes investigated; by contrast, EU habitat 3140 was present in only 7 of the 16 lakes (43.8%) (Fig. 2).

The emergent plant communities of EU habitat 3150 grew to a maximum depth of 4.5 m (recorded at Lake Monate), with a mean colonization depth of 1.3±0.8 m (± SD) (Fig. 2), whilst the submerged EU 3150 plant communities grew down to 10.5 m (recorded at Lake Garda), with a mean colonization depth of 2.9±1.9 m (Fig. 2). The emergent EU 3150 communities were dominated by *N. lutea*, *N. alba*, *Nymphoides peltata* (S.G. Gmel.) Kuntze, and *Trapa natans* L., the submerged communities by *Ceratophyllum demersum* L., *Myriophyllum spicatum* L., *Najas marina* L., *Potamogeton lucens* L., *P. pectinatus* L., *P. perfoliatus* L., and *Vallisneria spiralis* L.

EU habitat 3140 exhibited a greater variability in terms of colonized depths than the vascular plant communities, with a maximum depth of 16.5 m at Lake Garda, and a mean colonization depth of 3.8±2.6 m (Fig. 2). The prevalent (dominant) species were *Chara globularis* Thuiller, *C. intermedia* A.Braun, *C. tomentosa* Linnaeus, *Nitella flexilis* (Linnaeus) C.Agardh, and *Nitellopsis obtusa* (N.A.Desvaux) J.Groves.

Depth distribution of lacustrine EU habitats *vs* SD

The linear regression analysis revealed that the mean depth range colonized by submerged vegetation correlated with SD (Fig. 3). Both submerged EU 3150 and *Chara*-dominated communities positively correlated with SD (R^2 adj=0.501, P<0.01, n=16, and R^2 adj=0.737, P<0.01, n=7, respectively); by contrast, no correlation was detected between emergent plant communities (EU 3150) and SD (R^2 adj=0.138, P=0.864, n=9).

DISCUSSION

The present data confirm that information collected during WFD monitoring programs are able to make an important contribution to our knowledge of aquatic EU habitats in lacustrine environments. These results may, in addition, be used to evaluate the effectiveness of local water conservation policies. For example, the fact that EU habitat 3140 had not previously been found in the Natura 2000 sites considered in this study may be due to a progressive improvement in the chemical and physical conditions of lakes. As is widely known, *Chara*-dominated communities are considered to be among the aquatic habitats that are most sensitive to external pressures, especially in terms of nutrient loading (*e.g.*, phosphorous availability or algal blooms) (Blindow, 1992; Auderset Joye *et al.*, 2002). We cannot, however, exclude inaccuracies in the assessment of the structure and composition of the submerged habitats during the past routine HD surveys given the intrinsic difficulties encountered when monitoring water bodies (Azzella *et al.*, 2013b), as well as the rarity or temporal dynamicity of these communities (Bolpagni *et al.*, 2016). Nevertheless, these uncertainties further support the pivotal contribution of the use of the standardized approaches adopted within the framework of the WFD to integrate current knowledge on the presence, areal distribution and representativeness of aquatic EU habitats.

Aquatic EU habitats in the lakes of Lombardy

On the basis of Lombardy Regional Environmental Protection Agency surveys, the results of our study highlight the local predominance of vascular submerged communities in the depth range of 1 to 4 m, which are dominated by *V. spiralis*, *N. marina* and *M. spicatum*. Indeed, vascular plant communities were limited to the upper water depths, whereas communities that live at the maximum growing depths in lakes are composed of non-vascular species (Hutchinson, 1975). Accordingly, we substantiated the existence of a fairly clear differentiation between the two habitats we analyzed (EU 3140 and 3150), thereby highlighting the pivotal role played by the ability of vascular and charophyte species to capture light in shaping the depth distribution of aquatic vegetation. Indeed, EU habitat 3140 was found to mainly occur in deep lakes, not all of which are included in the local Natura 2000 network. This suggests that the regional policy regarding this habitat needs to be reassessed. This finding is in agreement with a preliminary evaluation of the representativeness of *Chara*-dominated stands in Lake Garda, which may account for approximately 20% of the overall area occupied by this habitat at national scale (1000-1200 ha out of a total of ~5000 ha) (Bolpagni *et al.*, 2013).

In the lakes we investigated, EU habitats 3140 and

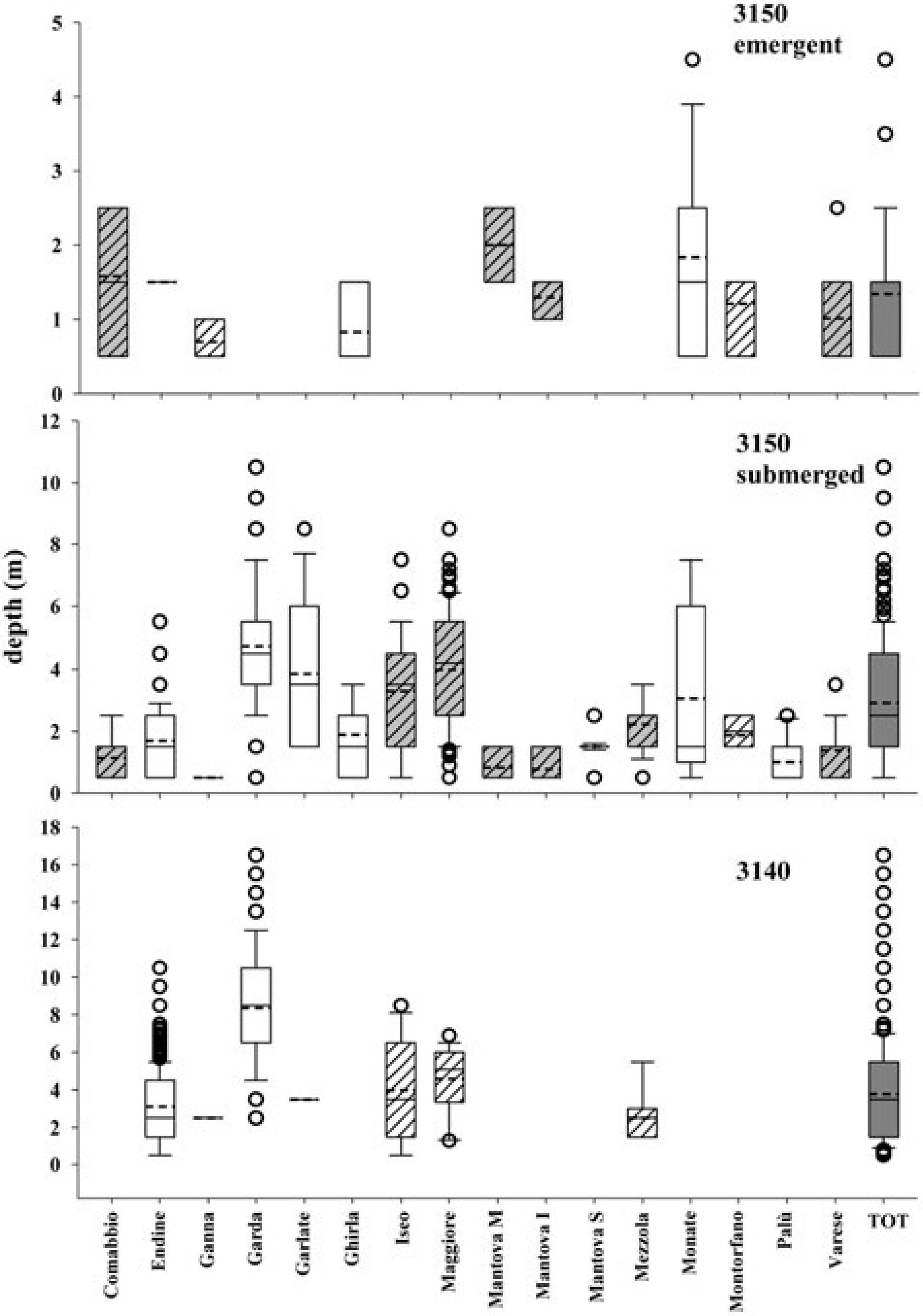

Fig. 2. Box plot graph of the depth distribution of the diagnostic species of the EU habitats 3150 (considering both the emergent and submerged plant communities) and 3140. The box indicates 25th-75th percentile; the solid and dotted lines indicate median and mean values, respectively. The lakes with previous habitat records are highlighted in grey, the coarse pattern indicates the lakes included in the Natura 2000 network. Mantova M, Mantova Middle; Mantova I, Mantova Inferior; Mantova S, Mantova Superior; TOT, all data together.

3150 displayed a comparable range of colonized depths that was partially overlapped (0.5-4.5 m *vs* 1.5-5.6 m; Fig. 3), which reflects the wide range of SD conditions considered. Hence, the variability in underwater light conditions affects both the presence/absence and the extent of the depth range colonized by diagnostic species, which change significantly in different lakes. Although the dataset we used was limited in size, our findings corroborate the strong interdependence between aquatic habitat depth ranges and the underwater light environment (Canfield *et al.*, 1985; Chambers and Kalff, 1985). This is substantiated by the differences that emerged between the linear regression models generated, with the increased steepness of the estimated slope revealing a greater sensitivity of charophytes to a progressive worsening in water conditions (Fig. 3). Even a small reduction in SD may be assumed to be associated with a non-negligible reduction in the area occupied by *Chara*-dominated stands. By contrast, vascular-dominated stands appear to be more "resilient", with a lesser dependency on SD variations. One explanation for the findings yielded by the lakes we investigated may be the replacement of sensitive aquatic vascular plants (mainly species belonging to the genus *Potamogeton*) by more tolerant species (*e.g.*, *M. spicatum*, *V. spiralis*) or alien *taxa*, such as *Lagarosiphon major* (Ridl.) Moss and *Elodea nuttallii* (Planch.) H. St. John (Bolpagni *et al.*, 2015; Soana and Bartoli, 2013, 2014).

Practical remarks on the WFD and HD integration

Although the standardized WFD methods used for macrophyte characterization cannot be considered exhaustive to describe vegetation, mainly because they do not provide a full and detailed floristic account of plant communities (Oggioni *et al.*, 2011), it is worth bearing in mind that their primary objective is the ecological classification of water bodies. This type of information can be used to define the spatial distribution and depth rearrangement of the diagnostic species of many EU habitats of conservation concern. Furthermore, if compared with the methods commonly used to characterize aquatic vegetation (*i.e.*, phytosociology), the standardized WFD methods ensure the acquisition of ancillary data on the physical and chemical conditions of the colonized environments. These data are important as a means of assessing the quality of habitats and colonized sites as well as of evaluating the future prospects of habitats (Collins *et al.*, 2012; Louette *et al.*, 2015 and references therein). This is a focal point that supports the integration of current programs aimed at protecting lacustrine aquatic EU habitats not only in Lombardy, but also across Europe as a whole. Bearing all this in mind, we wish to stress the need to implement strategies for the conservation of aquatic habitats by integrating trophic trends of lakes in terms of nutrient availability and water transparency as well as of the relative size of water bodies (Azzella *et al.*, 2014b).

By focusing on the distribution patterns of aquatic habitats of conservation concern, this paper attempts, to our knowledge for the first time, to actively combine the WFD directive with the HD. The standard WFD data on macrophytes appear to complement the needs and the requirements of HD, especially for defining the area (range) occupied by a specific lacustrine aquatic EU habitat. The use of data collected by the WFD will also enable us to make robust comparisons between biogeographic regions and states, thereby strengthening evaluations of current trends

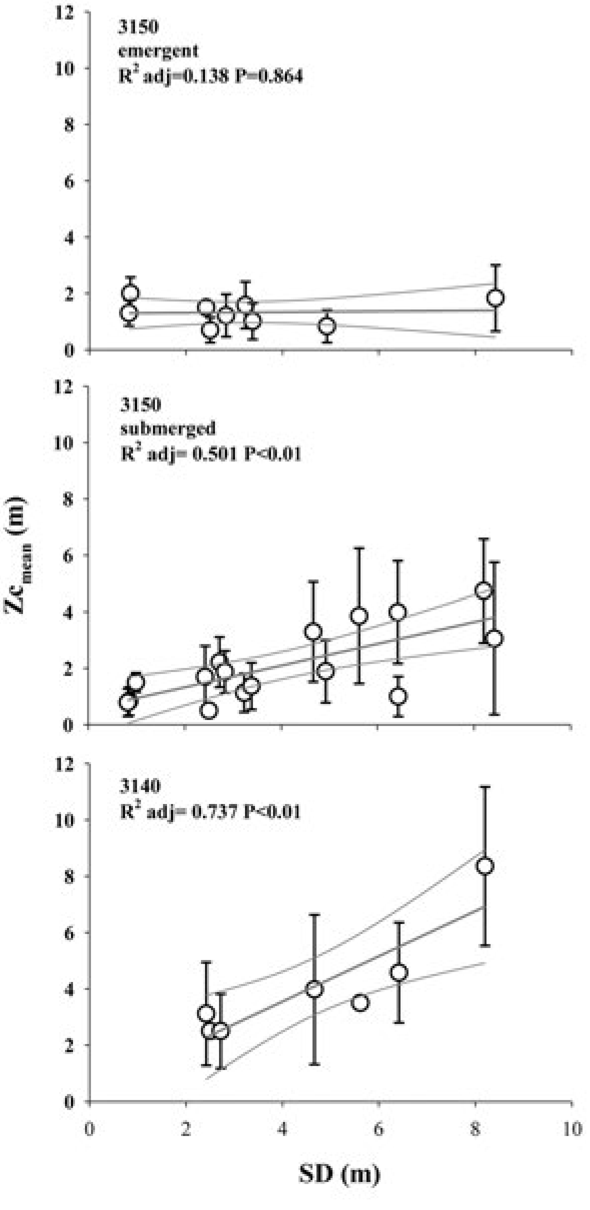

Fig. 3. Linear regression between water transparency, expressed as Secchi disk (SD, in m), and the mean depth of the habitat distribution (Zc_{mean} ± standard deviation, in m).

in aquatic vegetation on a large spatial scale. Furthermore, the possibility of gathering reliable and valid information on the physical and chemical conditions of water and sediments may make a fundamental contribution to defining favourable conservation conditions of habitats as well as to planning the distribution of areas of aquatic vegetation in response to climate change or direct human impacts (*i.e.*, pollution, water abstraction). This is especially important for aquatic environments, which are among the most vulnerable ecosystems in the world (Verhoeven *et al*., 2006).

Within this context, a better integration between HD, WFD and remote sensing techniques may represent a key strategy to obtain reliable information on the ranges occupied and to examine current dynamic trends in aquatic vegetation, as has previously been shown by many authors (Villa *et al.*, 2013, 2014, 2015; Giardino *et al.*, 2015). Indeed, remote sensing is an effective tool for recognizing and mapping vegetation thanks to the advantages of the synoptic view (in time and space) over the field survey (Vis *et al*., 2003; Silva *et al.*, 2008). Furthermore, the vegetation indices yielded by airborne and satellite images can effectively be used to implement standard monitoring approaches, thereby providing new opportunities in the assessment of vegetation status, growth or biophysical features. This may be considered a further step toward maximizing monitoring efforts and assessment outcomes.

CONCLUSIONS

This study highlights, for the first time, the usefulness of approaches implemented and adopted by the WFD to assess macrophyte communities in lakes as a means of also evaluating the presence of EU habitats and the size of the areas they occupy. Furthermore, the data collected within the framework of the WFD monitoring networks are comparable because they are obtained by means of similar sampling procedures, calibrated at the ecoregion scale by specific Geographical Intercalibration Groups. In addition, the use of these data may minimize the limitations usually associated with local flora and vegetation surveys, which are often restricted to a single, or a few, Natura 2000 sites. WFD data effectively address the urgent need to extend the information available on the presence and state of conservation of EU habitats beyond the Natura 2000 network.

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REFERENCES

Auderset Joye D, Castella E, Lachavanne JB, 2002. Occurrence of Characeae in Switzerland over the last two centuries (1800-2000). Aquat. Bot. 72:369-385.

Azzella MM, Bolpagni R, Oggioni A, 2014a. A preliminary evaluation of lake morphometric traits influence on the maximum colonization depth of aquatic plants. J. Limnol. 73:400-406.

Azzella MM, Iberite M, Fascetti S, Rosati L, 2013a. Loss detection of aquatic habitats in Italian volcanic lakes using historical data. Plant Biosyst. 147:521-524.

Azzella MM, Ricotta C, Blasi C, 2013b. Aquatic macrophyte diversity assessment: Validation of a new sampling method for circular-shaped lakes. Limnologica 43:492-499.

Azzella MM, Rosati L, Iberite M, Bolpagni R, Blasi C, 2014b. Changes in aquatic plants in the Italian volcanic-lake system detected using current data and historical records. Aquat. Bot. 112:41-47.

Beunen R, vand der Knaap WGM, Biesbroek GR, 2009. Implementation and integration of EU environmental directives. Exp. Netherlands Environm. Poll. Governan. 19:57-69.

Biondi E, Blasi C, Burrascano S, Casavecchia S, Copiz R, Del Vico E, Galdenzi D, Gigante D, Lasen C, Spampinato G, Venzanzoni R, Zivkovic L, 2009. [Manuale italiano di interpretazione degli habitat della Direttiva 92/43/CEE].[Book in Italian]. Accessed on: 10 December, 2016. Available from: http://www.vnr.unipg.it/habitat

Blindow H, 1992. Decline of charophytes during eutrophication: comparison with angiosperms. Freshwater Biol. 28:9-14.

Bolpagni R, 2013a. Macrophyte richness and aquatic vegetation complexity of the Lake Idro (Northern Italy). Ann. Bot. (Roma) 3:77-85.

Bolpagni R, 2013b. Multimetric indices based on vegetation data for assessing ecological and hydromorphological quality of a man-regulated lake. Ann. Bot. (Roma) 3:87-95.

Bolpagni R, Piotti A, 2015. Hydro-hygrophilous vegetation diversity and distribution patterns in riverine wetlands in an agricultural landscape: a case study from the Oglio River (Po plain, Northern Italy). Phytocoenologia 45:69-84.

Bolpagni R, Piotti A, 2016. The importance of being natural in a human-altered riverscape: Role of wetland type in supporting habitat heterogeneity and vegetation functional diversity. Aquat. Conserv. 26:1168-1183.

Bolpagni R, Bettoni E, Bonomi F, Bresciani M, Caraffini K, Costaraoss S, Giacomazzi F, Monauni C, Montanari P, Mosconi MC, Oggioni A, Pellegrini G, Zampieri C, 2013. Charophytes of the Lake Garda (Northern Italy): a preliminary assessment of diversity and distribution. J. Limnol. 72:388-393.

Bolpagni R, Laini A, Soana E, Tomaselli M, Nascimbene J, 2015. Growth performance of *Vallisneria spiralis* under oligotrophic conditions supports its potential invasiveness in mid-elevation freshwaters. Weed Res. 55:185-194.

Bolpagni R, Laini A, Azzella MM, 2016. Short-term dynamics of submerged aquatic vegetation diversity and abundance in deep lakes. Appl. Veg. Sci. 19:711-723.

Canfield D, Langeland K, Linda S, Haller W, 1985. Relations between water transparency and maximum depth of macrophyte colonization in lakes. J. Aquat. Plant. Manage. 23:25-28.

Chambers PA, Kalff J, 1985. Depth distribution and biomass of submersed aquatic macrophyte communities in relation to Secchi depth. Can. J. Fish. Aquat. Sci. 42:701-709.

Collins A, Dieudonne-Guy O, Hoare D, Voulvoulis N, 2012. Implementing the Water Framework Directive: a transition from established monitoring networks in England and Wales. Environ. Sci. Pol. 17:49-61.

Ecke F, Hellsten S, Mjelde M, Kuoppala M, Schlacke S, 2010. Potential conflicts between environmental legislation and conservation exemplified by aquatic macrophytes. Hydrobiologia 656:107-115.

Evans D, Arvela M, 2011. Assessment and reporting under Article 17 of the Habitats Directive - Explanatory Notes & Guidelines for the period 2007-2012. ETC-BD.

Dale H, 1986. Temperature and light: the determining factors in maximum depth distribution of aquatic macrophytes in Ontario, Canada. Hydrobiologia 133:73-77.

Duarte CM, Kalff J, 1990. Patterns in the submerged macrophyte biomass of lakes and the importance of the scale of analysis in the interpretation. Can. J. Fish. Aquat. Sci. 47:357-363.

European Union, 1992. Council Directive 92/43/ECC of 21 May 1992 on the conservation of natural habitats and wild fauna and flora. OJ L 206, 22/07/1992, pp. 7-50.

European Union, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L 327, 22/12/2000, pp. 1-73.

Genkai-Kato M, Carpenter SR, 2005. Eutrophication due to phosphorus recycling in relation to lake morphometry, temperature, and macrophytes. Ecology 86:210-219.

Giardino C, Bresciani M, Valentini E, Gasperini L, Bolpagni R, Brando VE, 2015. Airborne hyperspectral data to assess suspended particulate matter and aquatic vegetation in a shallow and turbid lake. Remote Sens. Environ. 157:48-57.

Janauer GA, Albrecht J, Stratmann L, 2015. Synergies and Conflicts between Water Framework Directive and Natura 2000: Legal Requirements, Technical Guidance and Experiences from Practice, p. 9-29. In: S. Ignar, M. Grygoruk (eds.), Wetlands and Water Framework Directive. GeoPlanet: Earth and Planetary Sciences.

Hutchinson G, 1975. A treatise on limnology: limnological botany. 3. Wiley & Sons, New York: 660 pp.

Louette G, Adriaens D, Paelinckx D, Hoffmann M, 2015. Implementing the Habitats Directive: How science can support decision making. J. Nature Conserv. 23:27-34.

Oggioni A, Buzzi F, Bolpagni R, 2011. [Indici macrofitici per la valutazione della qualità ecologica dei laghi: MacroIMMI e MTIspecies, p. 52-80]. In: A. Marchetto, A. Lugliè, B.M. Padedda, M.A. Mariani, N. Sechi, N. Salmaso, G. Morabito, F. Buzzi, M. Simona, L. Garibaldi, A. Oggioni, R. Bolpagni, B. Rossaro, A. Boggero, V. Lencioni, L. Marziali, P. Volta, M. Ciampittiello (eds.), [Indici per la valutazione della qualità ecologica dei laghi], Report CNR-ISE 03-11. [Book in Italian]. CNR Publication.

R Development Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: http://www.R-project.org

Silva TSF, Costa MPF, Melack JM, Novo EMLM, 2008. Remote sensing of aquatic vegetation: Theory and applications. Environ. Monit. Assess. 140:131-145.

Soana E, Bartoli M, 2013. Seasonal variation of radial oxygen loss in *Vallisneria spiralis* L.: an adaptation to sediment redox? Aquat. Bot. 104:228-232.

Soana E, Bartoli M, 2014. Seasonal regulation of nitrification in a rooted macrophyte (*Vallisneria spiralis* L.) meadow under eutrophic conditions. Aquat. Ecol. 48:11-21.

Søndergaard M, Phillips G, Hellsten S, Kolada A, Ecke F, Mäemets H, Mjelde M, Azzella MM, Oggioni A, 2013. Maximum growing depth of submerged macrophytes in European lakes. Hydrobiologia 704:165-177.

Verhoeven JTA, Arheimer B, Yin C, Hefting MM, 2006. Regional and global concerns over wetlands and water quality. Trends Ecol. Evol. 21:96-103.

Villa P, Laini A, Bresciani M, Bolpagni R. 2013. A remote sensing approach to monitor the conservation status of lacustrine *Phragmites australis* beds. Wetl. Ecol. Manage. 21:399-416.

Villa P, Bresciani M, Braga F, Bolpagni R, 2014. Comparative assessment of Broadband Vegetation Indices over aquatic vegetation. IEEE J. Sel. Top. Appl. 7:3117-3127.

Villa P, Bresciani M, Bolpagni R, Pinardi M, Giardino C, 2015. A rule-based approach for mapping macrophyte communities using multi-temporal aquatic vegetation indices. Remote Sens. Environ. 171:218-233.

Vis C, Hudon C, Carignan R, 2003. An evaluation of approaches used to determine the distribution and biomass of emergent and submerged aquatic macrophytes over large spatial scales. Aquat. Bot. 77:187-201.

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Effects of wind-induced sediment resuspension on distribution and morphological traits of aquatic weeds in shallow lakes

Vincent BERTRIN,* Sébastien BOUTRY, Gwilherm JAN, Greta DUCASSE, Florent GRIGOLETTO, Cristina RIBAUDO

Irstea, UR EABX - 50 avenue de Verdun, 33612 Cestas, France
*Corresponding author: vincent.bertrin@irstea.fr

ABSTRACT

The spread of invasive aquatic plants (*i.e.*, aquatic weeds) requires a broader knowledge of the factors determining their settlement at the lake scale, in order to improve management practices and biomonitoring. Among hydrodynamic pressures, wave action might influence submerged vegetation distribution in shallow lakes and potentially engender modifications of plant morphological traits. We here report a field survey conducted between 2014 and 2015 in French Atlantic Lakes to assess the spatial distribution and biomass production of two rooted aquatic weeds, *Egeria densa* Planch. and *Lagarosiphon major* (Ridl.) Moss, in relation to wind-induced sediment resuspension, water depth and sedimentary features. Moreover, we explored the relation between plant morphological traits and hydrodynamic disturbance under *in situ* conditions. At the lake scale, *E. densa* and *L. major* formed extensive monospecific stands, and occurred in the same areas only at low biomass. Both monospecific and mixed stands preferentially developed in shallow-sheltered or deep-exposed areas. Plant morphological traits showed different patterns in function of sediment resuspension according to the species and the lake. The influence of resuspension was however not systematic, with many cases where morphological traits were not affected at all. Maximum stem length appeared to be the most correlated trait towards sediment resuspension. Moreover, we found a significant correlation between the biomass and the sedimentary organic matter, indicating an interaction between the organic substrate preference of the plants and the effect of the canopy on fine particles sedimentation. On the whole, we highlighted the link between plant distribution, phenotypic plasticity and sediment resuspension, with calm-water zones favouring the settlement of dense vegetated stands. Our study could thus contribute to improve prediction models for identifying suitable areas for potential colonization by aquatic weeds. Further research is needed to better understand the role played by hydraulic forces in structuring the habitats in shallow lakes.

Key word: Exotic invasive hydrophytes; sedimentary organic matter; wind exposure; biomonitoring; colonization depth; alien plant management.

INTRODUCTION

Wave exposure is one of the abiotic factors influencing aquatic plant communities in freshwater ecosystems (Bornette and Puijalon, 2010 and references therein). Waves strongly affect the distribution of submerged macrophytes in shallow lakes, potentially driving to morphological damages on plant architectural features (Keddy, 1982; Strand *et al.*, 1996; Riis and Hawes, 2003; Schutten *et al.*, 2004). Water movement impacts on rooted aquatic plants depend on the magnitude of the hydraulic forces, the resistance capacity of the plants (*i.e.*, root anchorage and stem breaking strength) and the sediment type (Schutten *et al.*, 2005). For instance, mechanical stress directly shapes morphological features and biomass allocation. The plasticity in plant biomass (*i.e.*, the root to shoot ratio, R:S), can be considered as an adaptation to physical disturbance (Chambers *et al.*, 1991; Barrat-Segretain, 2001). Permanent exposure to water movement is able to disturb or alter plants development (Doyle, 2001), shoot elongation (Ellawala *et al.*, 2011), branch length and branch number (Strand and Weisner, 2001), seedling and germination (Foote, 1988) and shoot density (Chambers *et al.*, 1991). As a consequence, aquatic plants demonstrate capacities to morphologically adapt when they are confronted to hydrodynamic forces (Puijalon *et al.*, 2008).

Waves may also exert indirect impacts on aquatic plants through sediment resuspension. Indeed, turbidity, associated to light attenuation, and depth gradient are structuring factors for rooted macrophytes distribution at intermediate depth (Lehmann *et al.*, 1997). Submerged macrophytes distribution is then potentially limited by wave energy, with some species colonizing deeper parts of the lakes in order to avoid water mixing and sediment suspension caused by the orbital velocity of waves reaching the shoreline (Chambers, 1987). Waves are able to influence epiphytic growth, sediment grain size and water quality by water mixing, that selecting aquatic species according to their resistance against breakage (Koch, 2001; Bornette and Puijalon, 2010). On the other hand, the presence of dense vegetated mats can increase the sedimentation of fine particles and favor organic matter build-up (Madsen *et al.*, 2001).

Hydrocharitaceae family gathers aquatic rooted macrophytes, usually submerged, among which many species are considered as aquatic weeds. This is particularly the case for the two species *Egeria densa* Planch. and *Lagarosiphon major* (Ridl.) Moss, which occur in European freshwater ecosystems in both lentic and lotic waters (Dutartre *et al.*, 1999; Celesti-Grapow *et al.*, 2010; Hussner, 2012; Brundu, 2015). In natural ecosystems, they form large and dense stands causing important biogeochemical and management problems when climatic, hydromorphologic and trophic characteristics are advantageous for their development (Bini *et al.*, 1999; Bini and Thomaz, 2005; Yarrow *et al.*, 2009; Ribaudo *et al.*, 2014). Light attenuation and fetch are among the most important variables determining the occurrence of the genus *Egeria* (Bini and Thomaz, 2005). Moreover, the onset of dense vegetated stands and the subsequent deposition of fine particles may significantly contribute to water clarity and improve light penetration (Madsen *et al.*, 2001; Siffedine *et al.*, 2011).

Several studies on the response of submerged plants to hydrodynamic forces have been carried out in mesocosms, whereas *in situ* conditions embed interactions with light, temperature and depth (Doyle, 2001; Sand-Jensen, 2003; Ellawala *et al.*, 2011; Riis *et al.*, 2012; Redektop *et al.*, 2016). The investigation of wave exposure on aquatic weeds through field surveys is thus a key element for improving biomonitoring approaches in shallow lakes. Indeed, the integration of water depth, wind velocity and fetch in prediction models might help in detecting preferential habitats and physical thresholds for the spread of invasive macrophytes. In this study, we report the results of a survey conducted between 2014 and 2015 to assess the plant coverage and the biomass production of two rooted aquatic weeds, *E. densa* and *L. major*, in relation to sediment resuspension probability and sedimentary features. The objectives of the research were i) to obtain a quantitative assessment of aquatic weeds distribution and biomass in French Atlantic Lakes; ii) to evidence the dependence of plant distribution on sediment resuspension and water depth; and iii) to highlight the relation between morphological traits and physical disturbance within *in situ* conditions.

METHODS

Study area

French Atlantic Lakes (Carcans-Hourtin, Lacanau, Cazaux-Sanguinet and Parentis-Biscarrosse) are large shallow lakes located in the south-western coast of France, less than 5 km from the ocean (Fig. 1). These lakes were originated from the barrage of coastal rivers by littoral dunes (Tastet *et al.*, 2008). The eastern bottom is characterized by a very gentle slope while the western bottom presents a steep slope at the dunes' foot. These four lakes are classified from oligo- to mesotrophic and constitute the southern distribution limit for isoetid lawns in Europe (Cellamare *et al.*, 2012; Bertrin *et al.*, 2013; Ribaudo *et al.*, 2017). The main uses of these lakes are recreational activities such as boating, bathing, hunting and angling during a limited part of the year. Here, *L. major* appeared in Cazaux-Sanguinet in 1960, started to be signaled as a nuisance in late '70s and spread all over the four lakes in the following decades, showing a slow dynamic of invasion between 1985 and 2005 (Dutartre and Capdevielle, 1982; Bertrin *et al.*, 2013). *E. densa* was not reported in the vegetation surveys of these lakes until 2006, and only two of them were concerned (Bertrin *et al.*, 2013). Thanks to mild water temperatures (>16°C from April to October), the vegetative period of the two aquatic weeds is here extended during the year.

Dense stands mapping and traits measurement

In order to assess the dense stands boundaries of the two hydrophytes, vegetation surveys were carried out during the standing crop period (May-September) by echo-sounding, during 2014 in Carcans-Hourtin (HOU) and Lacanau (LAC) lakes and during 2015 in Cazaux-Sanguinet (CAZ) and Parentis-Biscarrosse (PAR) lakes. The scan sonar surveys were carried out on a boat equipped with a GPS coupled to a Humminbird 1197C. Transects were irregularly spaced along the lake's surface, according to the local bathymetry, and were concentrated along the shallowest areas of the lakes (<5 m), typically perpendicularly to the shoreline. Profiles were intensified when plant density was high; additionally, GPS points were marked in correspondence with the boundaries of each dense stand. Due to the presence of restricted areas for military purpose, in CAZ and PAR lakes some areas were not prospected by boat, but by feet. Concomitantly, plant collection was systematically carried out with a rake (harvested surface = 0.28 m^2), every 200 to 500 m, according to the local extension and homogeneity of the stands; in correspondence, a GPS point and the colonization depth was recorded. The operation was carried out by the same operator to minimize the error source (Johnson and Newman, 2011); plants were kept wet and refrigerated during the transport.

In laboratory, morphological traits and biomass were measured. For each shoot, maximal stem and root length (cm), number of stems (stems $shoot^{-1}$) and number of roots (roots $shoot^{-1}$) were measured. For each sample, shoots were counted for estimating shoot density (shoots m^{-2}). Finally, plants were sorted for above- (stems) and belowground (roots) parts and dried at 70°C for at least 72 h until stabilized dry weight. Total biomass was obtained by the sum of above- and belowground dry weight (g_{DW} m^{-2}); root to shoot ratio (R:S) was calculated as the ratio of the below- and aboveground dry weight. Also, additional data obtained along unpublished studies were included in the dataset for the comparison against sedimentary resuspension. Those data were collected at different seasons between late summer 2013 and late summer 2016 following the same protocol described above.

At the office, sonar recordings were read by Humminbird PC and transferred to a GIS system (ArcGIS 10.2 platform, 2017). Verification of the stand boundaries were made on each profile through the program HumViewer. Transects and GPS points were then geolocated and polygons were manually reconstructed to produce distribution maps. From biomass measurements, the mean biomass value (3<*n*<5) was associated to each shape in the distribution map.

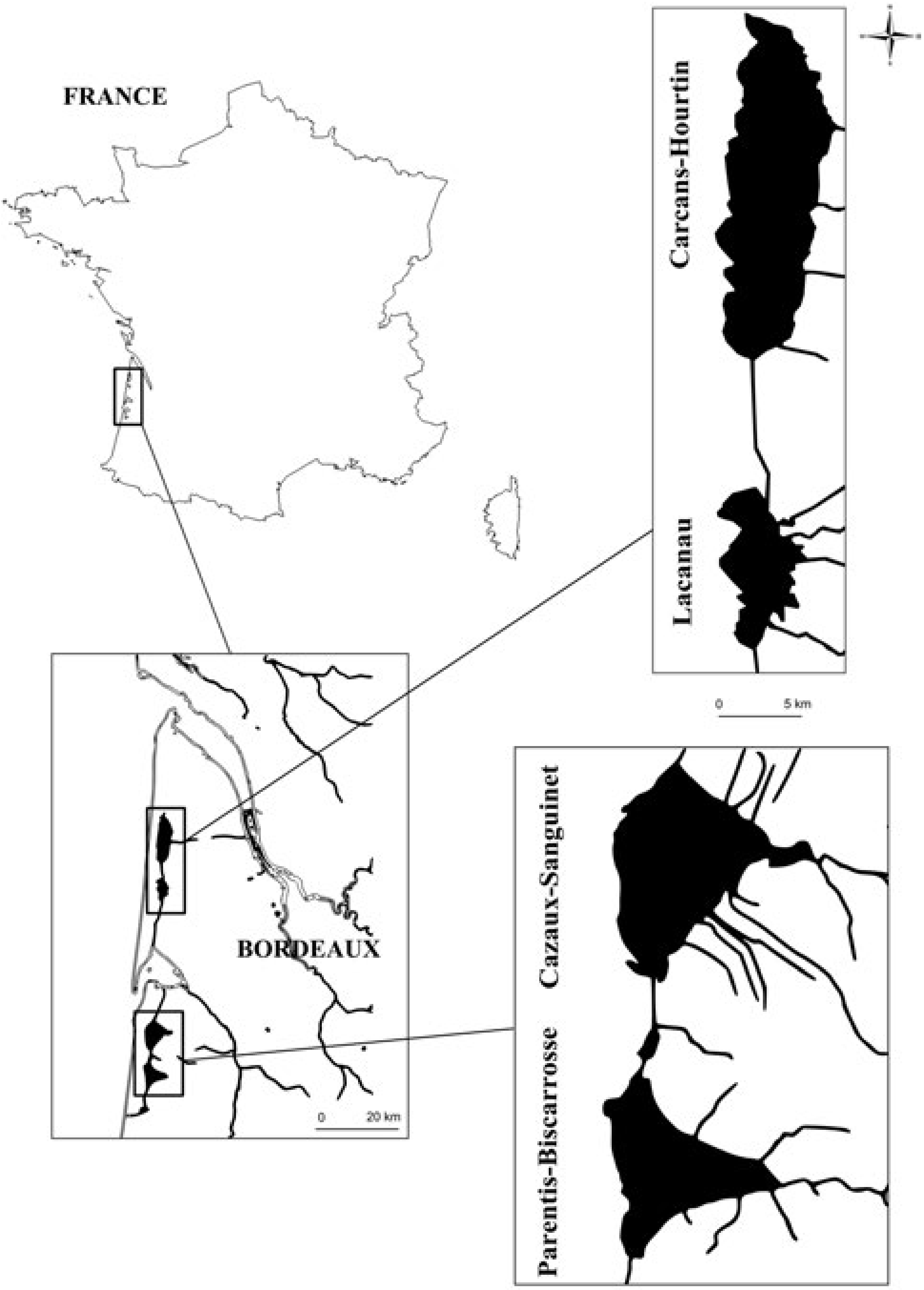

Fig. 1. Geographic location of French Atlantic Lakes.

Sediment characterization

Concomitantly to biomass harvesting, sediment samples were collected by means of a Van Veen grabber (volume = 2.5 L) and, after homogenization of the whole sample, transferred in triplicates into 40 mL plastic vials; samples were kept in a cooled box and immediately frozen at the laboratory. Several sediment samples were also collected in bare areas of the prospected shores. Analyses of sedimentary bulk density, porosity, water content and organic matter (OM) content were performed according to standard procedures, as described in Ribaudo *et al.* (2017).

Bathymetry and sediment resuspension probability

A rasterized georeferenced bathymetric map was provided by the Adour-Garonne Water Agency. Each open-water raster cell had a resolution of 10 m. The probability of sediment resuspension was based on wind data collected at a weather station located in Cap-Ferret (44°37'54"N, 1°14'54"W, 9 m above chart datum; wind measurement height above the ground: 10 m). The velocity ($m\ s^{-1}$) and direction (°) of the wind, averaged each 10 min, were available for this station. We used the maximum daily values of both parameters provided by the French climate normals (Météo France) obtained between November 2012 (one year before the first sampling campaign) and November 2016 (date of the last sampling campaign), for a total of 1451 days.

The wind is able to generate waves and currents depending on the water depth and the fetch (*i.e.*, the length of open water without any obstacle in a distinct direction). Wave formation is therefore influenced by the size and the shape of the lake, as well as by its exposure to the prevailing winds (Keddy, 1982; Schutten *et al.*, 2005). In this study, we calculated the fetch on the all grid cells for each wind compass direction (0-360 in 10-degree increments) as described in the U.S. Geological Survey Wind Fetch Model (Rohweder *et al.*, 2012) with ArcGIS 10.2 platform. We also calculated the probability of sediment resuspension thanks to the Wave Model (Rohweder *et al.*, 2012) with ArcGIS. This model calculates the maximum orbital wave velocity (um, expressed as $m\ s^{-1}$) using water depth from the bathymetric map and the calculated wave length, height and period as described below:

$$\text{um} = \pi \times \text{wave height} / (\text{wave period} \times \sinh \times (2\pi \times \text{water depth} / \text{wave length})) \qquad \text{(eq. 1)}$$

The maximum orbital wave velocity, as it depends on depth, expresses the vertical upward forces acting on the plants, through uprooting (Schutten *et al.*, 2004). For each day and each grid cell, in which the orbital velocity creates sediment resuspension, is attributed the "1" value, whereas days with no sediment resuspension are classified as "0". The model calculates the probability of having a sediment resuspension event during a temporal range for each individual raster cell. Therefore, the final unit of the model is a sediment resuspension probability (0-100%) and is applied to 17 × 17 m map grid cells.

Statistics

Linear regression was employed to test the dependence of the sedimentary OM on sediment resuspension, and the dependence of morphological traits on sediment resuspension probability and OM content. Pairwise t Student test was used to test differences in morphological traits between the two hydrophytes. Pearson correlation test was used to test the relationship between the biomass and the sedimentary OM content. Prior to analysis, all data were transformed as *log (x+1)*. Analyses were performed using R Program (R Development Core Team, 2017).

RESULTS

Prevailing stronger winds between November 2012 and November 2016 blew from the west-northwest; they also came from the north-east but with a minor velocity (Fig. 2). The daily mean wind speed was mainly bounded from 5 to 20 $m\ s^{-1}$ (maximum 26.8 $m\ s^{-1}$, mean 9.1 $m\ s^{-1}$) with only 0.3% of wind-exempted days during the whole investigation period. The resuspension probability reflected a huge range of wave exposure (Fig. 2), with the eastern part of lakes presenting a higher probability of sediment resuspension than the western part.

The distribution of sedimentary OM content varied according to the bathymetry of lakes (Fig. 2). Within each lake, an elevated spatial variability was observed, with fluffy sediments collected at the deep-sheltered bays and clear quartz sands at the shallow wind-exposed shores. On the whole, sedimentary features slightly varied across lakes' shores, with a sandy bottom characterized by a mean bulk density of 1.6±0.3 $g\ cm^{-3}$ (range 0.9-2.3), a porosity of 50±20% (20-98), a water content of 36.1±24.4% (13-93), and OM content of 9.8±18.5% as loss of ignition (LOI, 0.1-85.7) (overall *n*=723). Sedimentary OM was significantly dependent on the resuspension probability ($F_{1,518}$, R^2=0.13, P<0.001).

In 2014, a total of 21 and 82 sonar recordings were performed, for HOU and LAC lakes respectively. In 2015, a total of 41 and 35 sonar recordings were performed, for CAZ and PAR lakes respectively. Some lake areas were not prospected by echo-sounding because no dense stands were observed nearby during the field campaigns. As a consequence, low biomass values (<50 $g_{DW}\ m^{-2}$), corresponding to sparse shoots, were not included in the distribution maps neither in the whole-lake biomass

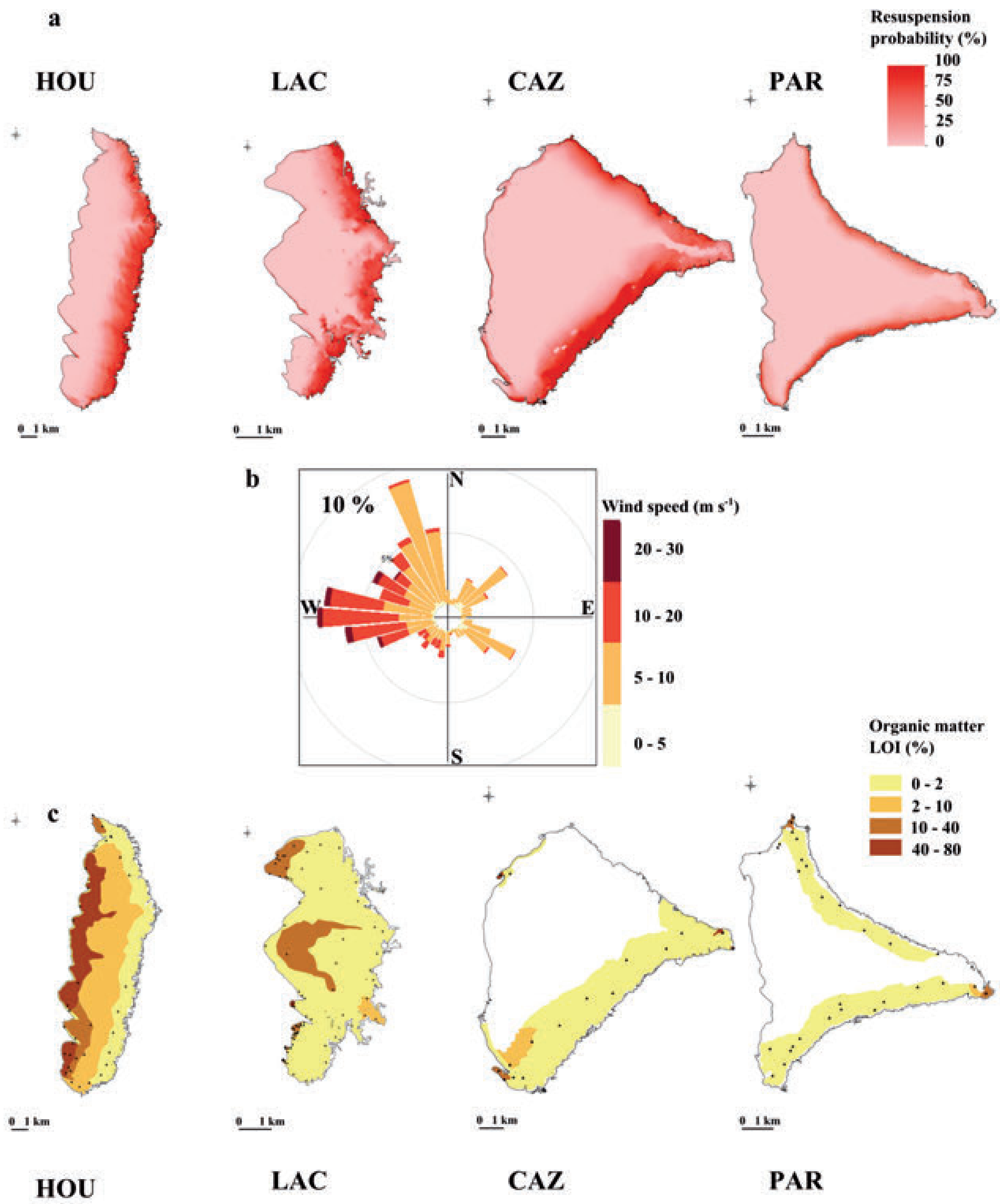

Fig. 2. a) Sediment resuspension probability (0-100%) calculated for the period November 2012-November 2016 in French Atlantic Lakes. b) Windrose elaborated from wind speed daily values for the period November 2012-November 2016. c) Organic matter maps of French Atlantic Lakes obtained in 2014 (HOU and LAC) and 2015 (CAZ and PAR). Triangles indicate sediment collection sites.

assessment for precaution. Whole-lake biomass estimations (2014-2015) were based upon a total of 7, 70, 7 and 15 biomass samplings, for HOU, LAC, CAZ and PAR respectively. Dense stands of both *E. densa* and *L. major* were found in LAC and PAR lakes on extended surfaces (about 7 and 13% of the lake surface, respectively), whereas restricted vegetated areas were observed in HOU and CAZ lakes, and only by *L. major* (Tab. 1 and Fig. 3). Both LAC and PAR presented the highest biomass values and coverage for both hydrophytes. Given the presence of only *L. major* in HOU and CAZ, and given the low occurrence of dense stands in those lakes, we decided to analyse vegetation data and to show results only for LAC and PAR in the next part of this study.

On the whole, the minimum plant colonization depth was 0.4 m, with sparse shoots found at a maximum of 8 m in LAC. At the lake scale, dense vegetated stands (monospecific + mixed stands) were mainly located at intermediate depths, from 1 to 5 m, with 72% of vegetated grid cells located between 2 and 4 m deep (Fig. 4). The main proportion of dense stands (87% of vegetated grid cells) was located in areas with sediment resuspension probability minor than 25%. When considering monospecific stands only, it appeared that 2.5-3.5 m was the optimal depth for the development of *E. densa* stands, while *L. major* seemed to cover indistinctively a large zone situated between 2 and 4 m. In addition, the maximum depth for *L. major* dense stands was 1 m greater than for mono-

Tab. 1. Lakes features (http://adour-garonne.eaufrance.fr) and colonized surfaces (expressed in km^2) and biomass (expressed in tons) by aquatic weeds in French Atlantic Lakes. Total biomass for each lake is calculated on dense stand surfaces only.

	Unit	HOU	LAC	CAZ	PAR
Lake surface	km^2	57.6	16.2	48.9	31.9
Mean and max depth	m	4, 15	2, 7	9, 24	7, 22
Secchi depth	m	2.1±0.2	1.9±0.2	4.6±0.7	2.3±0.8
Sparse shoots ($1<x<50$ g_{DW} m^{-2})	km^2	0.55	1.36	0.15	2.31
Dense stands ($x>50$ g_{DW} m^{-2})	km^2	0.94	1.19	0.17	4.17
L. major monospecific stands	km^2	0.94	0.37	0.17	0.81
	tons	200	47	45	41
E. densa monospecific stands	km^2	0.00	0.57	0.00	0.95
	tons	0	270	0	932
Mixed stands	km^2	0.00	0.25	0.00	2.41
	tons	0	83	0	1812

HOU, Lake Carcans-Hourtin; LAC, Lake Lacanau; CAZ, Lake Cazaux-Sanguinet; PAR, Lake Parentis-Biscarrosse.

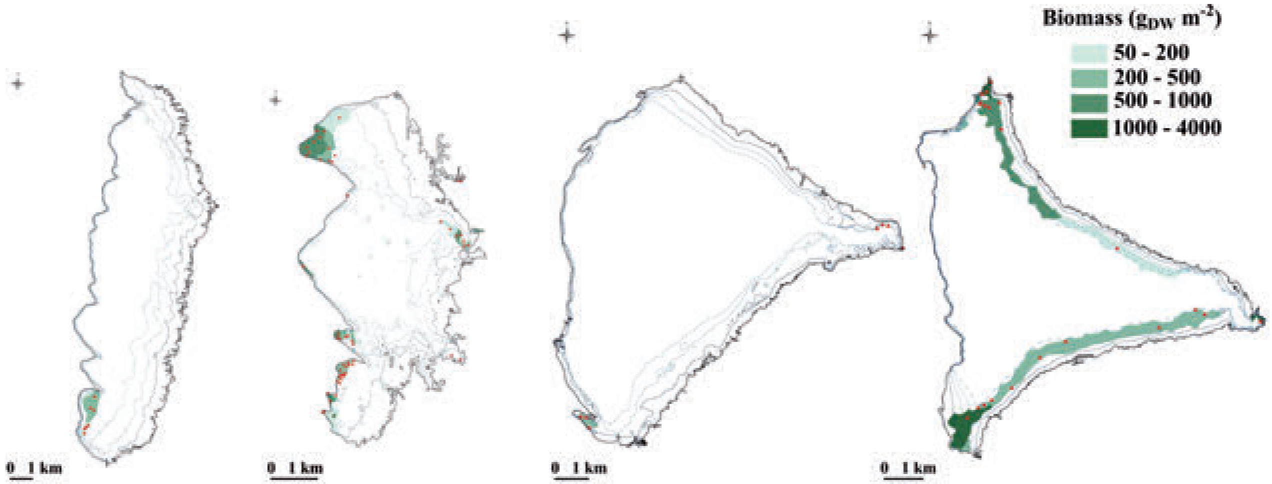

Fig. 3. Distribution and biomass maps of aquatic weeds (*E. densa* and *L. major*) obtained by echo-sounding and biomass harvesting in 2014 (HOU and LAC) and 2015 (CAZ and PAR). Triangles indicate biomass harvesting sites; isobaths are reported until 3 m deep.

specific *E. densa* stands. With regards to sediment resuspension, the distribution of monospecific *E. densa* stands linearly decreased with the increase of the exposition to water movement, whereas *L. major* seemed to be less affected by resuspension. Hydrophyte biomass and sedimentary OM content appeared positively correlated at all lakes but one (r Pearson=0.62, 0.53 and 0.54 for HOU, LAC and CAZ, respectively; PAR = not significant).

Biomass, shoot density and morphological traits varied across lakes and species (Tab. 2); *E. densa* showed significantly higher values than *L. major* for all parameters at both LAC and PAR lakes (*t*-test, P<0.001), except

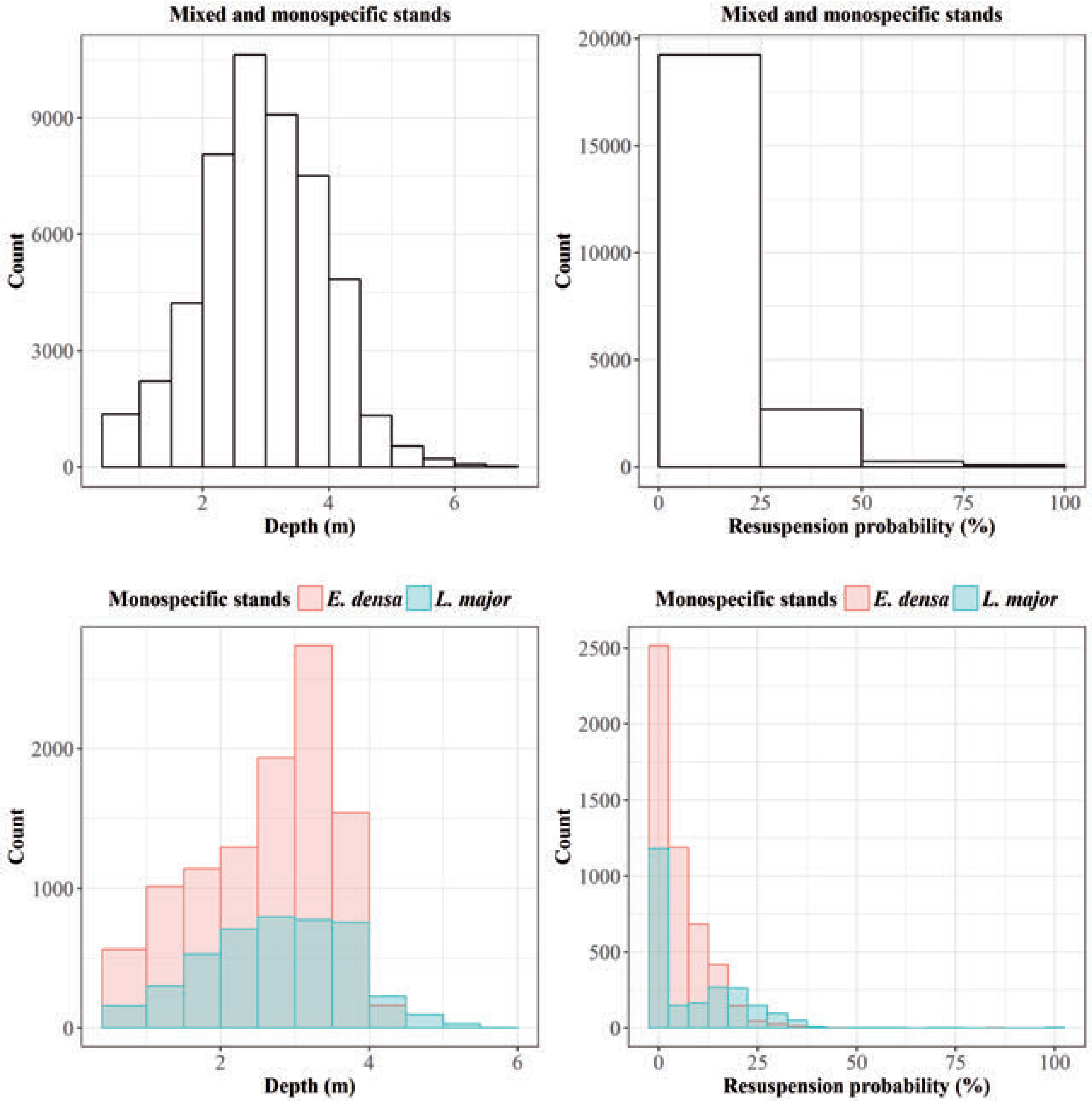

Fig. 4. Occurrence of dense mixed and monospecific stands of two aquatic weeds (*L. major* and *E. densa*) according to the depth (*n*=50,029, on the top left) and resuspension probability (*n*=22,246, on the top right); occurrence of dense monospecific stands according to the species, the depth (*n*=14,769, on the bottom left) and resuspension probability (*n*=7381, on the bottom right). Count is based on the occurrence of grid cells corresponding to dense vegetated stands.

for shoot density at PAR lake. The occurrence of one hydrophyte at elevated density or biomass was inversely correlated to the occurrence of the other one (Fig. 5). Morphological traits in function of sediment resuspension showed different patterns according to the species and the lake (Fig. 6). The influence of resuspension was however not systematic, with many cases where morphological traits were not affected at all. No significant relationship between R:S and resuspension probability was pointed out except in LAC for *L. major*. In the same way, roots

Tab. 2. Biomass, shoot density and morphological traits measured on *Lagaroshiphon majo*r (*L. major*) and *Egeria densa* (*E. densa*) colonizing French Atlantic Lakes. Mean values ±SD, range values and number of replicates (in square brackets) are reported.

		Biomass	Shoot density	RAM		MSL	MRL	R:S
Lake		**(g_{DW} m^{-2})**	**(shoots m^{-2})**	**(stems shoot^{-1})**	**(roots shoot^{-1})**	**(cm)**	**(cm)**	
HOU	*L. major*	149±102 [7] (88–375)	- -	- -	- -	72±27 [70] (32–148)	- -	0.01±0.01 [7] (0.01–0.02)
	E. densa	0 0	0 0	0 0	0 0	0 0	0 0	0 0
LAC	*L. major*	154±135 [45] (1–1060)	98±46 [32] (14–141)	2±1 [8] (1–3)	11±4 [8] (5–17)	64±28 [202] (10–180)	33±7 [8] (24–46)	0.10±0.09 [40] (0.02–0.27)
	E. densa	470±376 [76] (3–1989)	189±136 [39] (42–594)	5±3 [132] (1–15)	16±11 [116] (2–56)	102±38 [367] (8–236)	65±16 [116] (27–98)	0.13±0.22 [69] (0.01–0.50)
CAZ	*L. major*	154±106 [7] (6–337)	38±21 [7] (14–71)	3±1 [13] (1–4)	14±10 [13] (5–38)	73±22 [13] (43–112)	35±8 [13] (25–57)	0.08±0.05 [7] (0.01–0.14)
	E. densa	0 0	0 0	0 0	0 0	0 0	0 0	0 0
PAR	*L. major*	562±559 [22] (1–1505)	84±50 [17] (14–184)	3±1 [36] (1–5)	10±7 [21] (3–39)	137±54 [51] (43–255)	29±11 [22] (8–57)	0.03±0.02 [16] (0.01–0.08)
	E. densa	1942±1820 [35] (6–6177)	127±102 [32] (14–340)	4±3 [133] (1–17)	16±14 [64] (1–77)	205±65 [119] (41–321)	52±22 [76] (9–102)	0.06±0.04 [32] (0.01–0.09)

RAM, ramifications; MSL, max stem length; MRL, max root length; R:S, root to shoot ration; HOU, Lake Carcans-Hourtin; LAC, Lake Lacanau; CAZ, Lake Cazaux-Sanguinet; PAR, Lake Parentis-Biscarrosse.

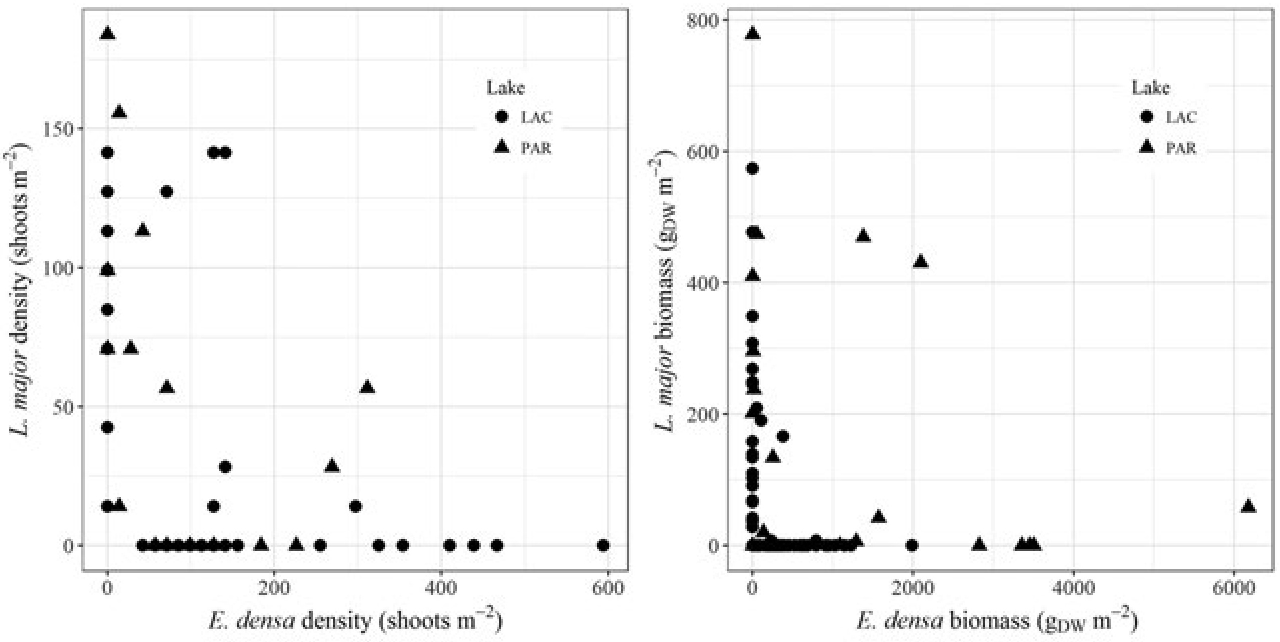

Fig. 5. Relation between the density (on the left) and the biomass (on the right) of the two hydrophytes *L. major* and *E. densa*.

length significantly decreased in PAR for *E. densa* when resuspension probability increased ($F_{1,74}$, R^2=0.12, P<0.01). No significant relationship was evidenced between density and resuspension probability at all lakes. The parameter appearing to best respond to the impact of sediment resuspension was the maximum stem length, which was significantly higher for both species in PAR, while it was not correlated for *L. major* in LAC.

DISCUSSION

Plant distribution

This study documents the massive presence of two rooted aquatic weeds in French Atlantic Lakes. *E. densa* and *L. major* formed extensive dense stands (up to 4.17 km^2), with standing crops varying from 45 to 2785 tons of dry biomass per lake (from 0.05 to 6.18 kg_{DW} m^{-2}), which covered from 1.6 to 13.0% of the total surface of the lakes. In many areas, plant biomass and vegetated patches largely overpassed values reported for invasive free-floating plants (up to 2.5 kg_{DW} m^{-2} and 2 km^2) (Center and Spencer, 1981; Nieder *et al.*, 2004). Though echo-sounding surveys did not allow prospecting the full lake surface, it resulted to be a reliable method for delimiting the stand boundaries (Wells *et al.*, 1997; Zajac, 2008). We acknowledge that some portions of the lakes where sparse shoots exist could not be included in this survey; for this reason, the lake-scaled biomass estimation only refers to dense stands. Also, interannual variability in biomass might have affected our evaluation; anyway, our results highlight the magnitude of the plant colonization and constitute a first biomass assessment.

When looking at the comparison between the two hydrophytes, *E. densa* appeared to be a major potential threat when compared to *L. major*, both in terms of biomass and density. Indeed, while *L. major* maximum stem length and biomass resulted similar or even lower than those reported in other studies and other sites (Clayton, 1982; Dutartre and Oyarzabal, 1993; Wells *et al.*, 1997; Bickel and Closs, 2008), the opposite happened for *E. densa* biomass, which reached much higher values (maximum 6.2 kg_{DW} m^{-2} in PAR) than those reported in tropical waters (maximum 0.5 kg_{DW} m^{-2} and 1.2 kg_{DW} m^{-2} in South-American reservoirs) (Pelicice *et al.*, 2005; Carrillo *et al.*, 2006).

Both *L. major* and *E. densa* appeared to be able to colonize the whole range of depth in French Atlantic Lakes, until 8 m deep for sparse shoots, corresponding to the maximum depths observed in tropical and not light-limited environments (Coffey and Wah, 1988; Carrillo *et al.*, 2006). Dense stands however preferentially developed between 2 and 4 m deep, showing a possible interplay between light availability and hydrodynamics preferences. Indeed, the majority of the dense stands were located at low sediment resuspension areas, which indicates that calm waters constitute a preferential habitat for dense mats development. However, even areas characterized by low to medium sediment resuspension (10-45%) were colonized by an elevated plant density.

The depth zonation of the two hydrophytes did not result as distinct as in other studies on native species, which showed that different species of *Potamogeton* can occupy distinct colonization depth within one lake (Lehmann *et al.*, 1997). Nevertheless, our survey showed that the two hydrophytes rarely occur together at elevated biomass or density. On the whole, our results show that *E. densa* and *L. major* prefer deep-sheltered areas in Atlantic shallow lakes, with a tendency for *L. major* to colonize deeper and more exposed areas than *E. densa*. This result is in line to

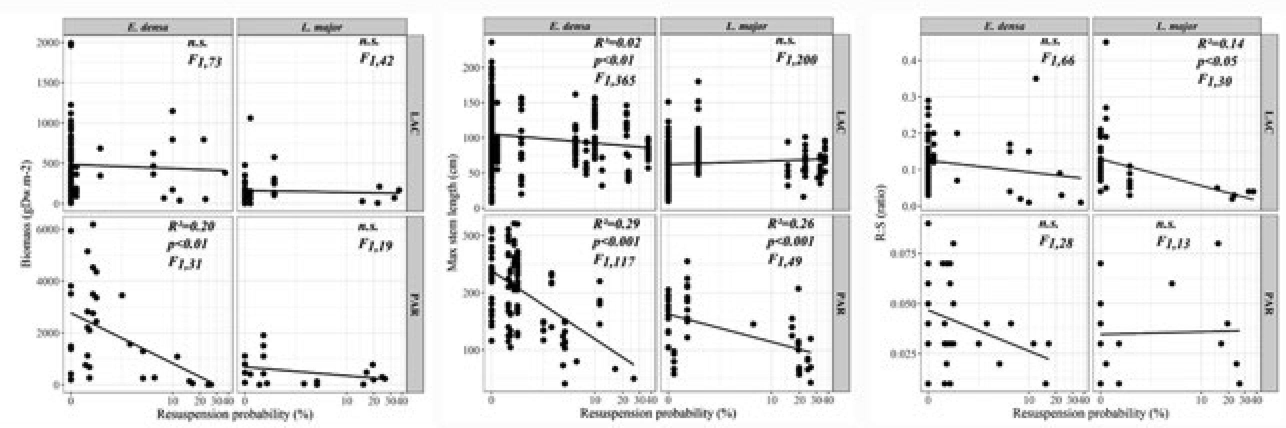

Fig. 6. Relation between the biomass (on the left), maximum stem length (on the middle) and root to shoot ratio (on the right) of the two hydrophytes *L. major* and *E. densa*.

what Ellawala *et al.* (2011) and Riis *et al.* (2012) found in experimental conditions and highlights a species-specific response to hydrodynamic forces.

Plants morphological traits and hydrodynamics

Both *E. densa* and *L. major* produce dense canopies with long stems (maximum 321 cm for *E. densa* in PAR), which drag at the water surface during summer period. This type of vegetative development could reduce wave tolerance, increasing plant breakage and morphology modifications, unlike meadow-forming species which lie closer to the sediment surface when the current velocity is elevated (Koch, 2001). The response of morphological traits to sediment resuspension here reported matches indeed with investigations previously carried out on *E. densa* (Ellawala *et al.*, 2011) and other hydrophytes such as *Myriophyllum spicatum* L. (Strand and Weisner, 2001) and *Vallisneria spiralis* L. (Doyle, 2001) in experimental conditions. Anyway, the relationships we found were not systematic and often lake-dependent and species-specific. These results in general did not confirm our initial hypotheses. An increase of the root length would have been expected with the increase of sediment resuspension, as a phenotypic adaptation for assuring a better anchorage to the plant. Other studies report that belowground biomass plasticity according to wave exposure and sediment granulometry has been found to be often inconsistent (Koch, 2001). Moreover, no relationship was observed in some morphological traits such as the number of stems and roots per shoot. Different responses of the relationship traits-hydrodynamics can be attributable to the different lake sizes. PAR lake surface is two times larger than LAC; as a consequence, the fetch lengths are potentially higher in PAR than in LAC. The orbital velocity of waves is function of the wind direction and velocity, and also depends on the fetch length required to calculate the wave height, length and period (Rohweder *et al.*, 2012). Even if the resuspension probability is always binary (1 = resuspension, or 0 = no resuspension) and identifies the occurrence of a disturbance, it does not give the intensity of the force induced by waves. Wave disturbance in PAR has probably a more important impact on plant morphological features, as reported by some authors for large lakes (Schneider *et al.*, 2015; Schutten *et al.*, 2004). In further research, it would be thus interesting to include the maximum orbital velocity in the model.

Aquatic weeds as species engineers

Our research highlights the importance that dense vegetated stands take on in shallow lakes productivity. French Atlantic Lakes are characterized by slow-growing vegetation typical of acidic conditions and by low pelagic production (Cellamare *et al.*, 2012; Ribaudo *et al.*, 2017). If we assume a carbon content of 39.5% in plant tissues (Carvalho *et al.*, 2005) and consider the total measured biomass and the colonized surface on the four lakes, we can estimate that those two hydrophytes may fix from 84 to 264 g C m^{-2} (for HOU and PAR lakes, respectively). This value is comparable to the C sequestration capacity of other hydrophytes of the same lakes (Ribaudo *et al.*, 2017), yet the proportion of vegetated areas is much different. Indeed, when considering the colonized areas by *E. densa* and *L. major*, we can estimate a productivity ranging between 18 and 1100 tons of C lake^{-1} at their standing crop (for CAZ and PAR lakes, respectively). This budget lacks, however, of the assessment of the carbon release due to respiration processes in dense vegetated stands at the lake scale. A definitive assessment of the net ecosystem metabolism would take into account the quota of carbon decomposed and released *in situ* at the senescence of the plants, that having potential cascading effects on local oxygenation and on the enhancement of anaerobic processes such as methanogenesis (Cunha-Santino and Bianchini, 2004; Carvalho *et al.*, 2005; Urban *et al.*, 2009; Ribaudo *et al.*, 2014).

E. densa and *L. major* are two rooted hydrophytes able to grow on a wide spectrum of substrata and depths (Riis *et al.*, 2010), commonly found on silty, mesotrophic sediments (Bini *et al.*, 1999; Martin and Coetzee, 2014; Matsui, 2014). As a consequence, we would not expect to find dense stands on very organic-poor sediments (<0.3% as LOI, at PAR lake). We can hypothesize that we are dealing with an initial phase of the settlement, where the barrier formed by elevated shoot densities possibly triggers a positive feedback on water flow decrease and favors fine particles sedimentation (Barko *et al.*, 1991; Madsen *et al.*, 2001). We found indeed a significant correlation between the quantity of biomass and the degree of OM content, that indicating an interaction between a substrate preference by the plant and the effect of the canopy on fine particles sedimentation. Here, the release of nutrients from fluffy sediments likely contributes to sustain plant growth under oligotrophic conditions (Anderson and Kalff, 1986; Bolpagni *et al.*, 2015). An aliquot of the organic matter produced in dense stands may be transferred to deeper layers of the lakes and support oxidative degradation (Siffedine *et al.*, 2011).

Implication for management purposes and biomonitoring

The recreational use of French Atlantic Lakes for touristic purposes has historically matched with a management aiming at a low environmental impact (Ghelardoni, 1990). Recently, negative human perception increased against aquatic weeds, due to a spread of lake's recreational uses (sealing, motor boating, hunting and fishing). Concurrently, those activities have a probable positive feedback on the settlement of the two non-native hydrophytes through fragments dispersal (Bruckerhoff *et al.*, 2015). Management actions for removing biomass from harbours accounted for several tens of thousands euros between 2010 and 2015 (SIAEBVELG and Géolandes local authorities); those actions were, however, spatially restrained in order to limit negative impacts caused by nutrients regeneration from sediments (van Nes *et al.*, 2002).

CONCLUSIONS

Our study assesses the current distribution of two aquatic weeds in French Atlantic Lakes, that making easier for managers to plan harvesting interventions. In addition, as we highlighted the link between plant distribution and sediment resuspension, we alert to the risks induced by the creation of artificial calm-water zones (*e.g.*, marinas, harbours, canalizations) that could favour the onset of dense stands. Taking into account artificial hydromorphologic modifications would be convenient for improving biomonitoring approaches, which are at present focused on the detection of nutrient and organic pollution only (Kolada *et al.*, 2014). Our results could be also used to identify suitable areas for potential colonization by *E. densa*, which is still currently not present in two of the four lakes. Both lakes HOU and CAZ are highly vulnerable to be colonized by *E. densa* in the near future, due to their accessibility to human activities through the presence of public boat launches and some navigation ways linking the lakes. Beyond the capacity of predicting the occurrence of invasive organisms at a multiple-systems scale, we believe that it is now necessary to inform managers on the potential distribution of a likely new invasive plant at the local scale (*e.g.*, lake or a specific part of a lake) in order to help them in management decision.

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REFERENCES

Anderson MR, Kalff J, 1986. Regulation of submerged aquatic plant distribution in a uniform area of a weedbed. J. Ecol. 4:953-961.

Barko JW, Gunnison D, Carpenter SR, 1991. Sediment interactions with submersed macrophyte growth and community dynamics. Aquat. Bot. 41:41-65.

Barrat-Segretain MH, 2001. Biomass allocation in three macrophyte species in relation to the disturbance level of their habitat. Freshwater Biol. 46:935-945.

Bertrin V, Boutry S, Dutartre A, Lambert E, 2013. [Communautés de Characées des lacs médocains (Sud-Ouest de la France). Eléments d'écologie et de distribution].[Article in French]. Acta Bot. Gallica. 160:131-140.

Bickel TO, Closs GP, 2008. Fish distribution and diet in relation to the invasive macrophyte *Lagarosiphon major* in the littoral zone of Lake Dunstan, New Zealand. Ecol. Freshw. Fish. 17:10-19.

Bini LM, Thomaz SM, 2005. Prediction of *Egeria najas* and *Egeria densa* occurrence in a large subtropical reservoir (Itaipu Reservoir, Brazil-Paraguay). Aquat. Bot. 83: 227-238.

Bini LM, Thomaz SM, Murphy KJ, Camargo AF, 1999. Aquatic macrophyte distribution in relation to water and sediment conditions in the Itaipu Reservoir, Brazil. Hydrobiologia 415:147-154.

Bolpagni R, Laini A, Soana E, Tomaselli M, Nascimbene J, 2015. Growth performance of *Vallisneria spiralis* under oligotrophic conditions supports its potential invasiveness in mid-elevation freshwaters. Weed Res. 55:185-194.

Bornette G, Puijalon S, 2011. Response of aquatic plants to abiotic factors: a review. Aquat. Sci. 73:1-14.

Bruckerhoff L, Havel J, Knight S, 2015. Survival of invasive aquatic plants after air exposure and implications for dispersal by recreational boats. Hydrobiologia. 746:113-121.

Brundu G, 2015. Plant invaders in European and Mediterranean inland waters: profiles, distribution, and threats. Hydrobiologia 746:61-79.

Carrillo Y, Guarín A, Guillot G, 2006. Biomass distribution, growth and decay of *Egeria densa* in a tropical high-mountain reservoir (NEUSA, Colombia). Aquat. Bot. 85:7-15.

Carvalho P, Thomaz SM, Bini LM, 2005. Effects of temperature on decomposition of a potential nuisance species: the submerged aquatic macrophyte *Egeria najas* Planchon (Hydrocharitaceae). Braz. J. Biol. 65:51-60.

Celesti-Grapow L, Alessandrini A, Arrigoni PV *et al.*, 2010. Non-native flora of Italy: Species distribution and threats. Plant Biosyst. 144:12-28.

Cellamare M, Morin S, Coste M, Haury J, 2012. Ecological assessment of French Atlantic lakes based on phytoplankton, phytobenthos and macrophytes. Environ. Monit. Assess. 184:685-708.

Center TD, Spencer NR, 1981. The phenology and growth of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) in a eutrophic north-central Florida lake. Aquat. Bot. 10:1-32.

Chambers PA, 1987. Nearshore occurrence of submersed aquatic macrophytes in relation to wave action. Can. J. Fish. Aquat. Sci. 44:1666-1669.

Chambers PA, Prepas EE, Hamilton HR, Bothwell ML, 1991.

Current velocity and its effect on aquatic macrophytes in flowing waters. Ecol. Appl. 1:249-257.

Clayton JS, 1982. Effects of fluctuations in water level and growth of *Lagarosiphon major* on the aquatic vascular plants in Lake Rotoma, 1973-80. New Zeal. J. Mar. Fresh. 16:89-94.

Coffey B, Wah CK, 1988. Pressure inhibition of anchorage-root production in *Lagarosiphon major* (Ridl.) Moss: a possible determinant of its depth range. Aquat. Bot. 29:289-301.

Cunha-Santino MB, Bianchini Jr I, 2004. Oxygen uptake during mineralization of humic substances from Infernão Lagoon (São Paulo, Brazil). Braz. J. Biol. 64:583-590.

Doyle RD, 2001. Effects of waves on the early growth of *Vallisneria americana*. Freshwater Biol. 46:389-397.

Dutartre A, Capdevielle P, 1982. [Répartition actuelle de quelques végétaux vasculaires aquatiques introduits dans le Sud-Ouest de la France].[Article in French], p. 390-393. In: J.J. Symoens, S.S. Hooper and P. Compère (eds.), Studies on Aquatic vascular plants. Royal Botanical Society of Belgium.

Dutartre A, Oyarzabal J, 1993. [Gestion des plantes aquatiques dans les lacs et les étangs landais].[Article in French]. Hydroécol. Appl. 5:43-60.

Dutartre A, Haury J, Jigorel A, 1999. Succession of *Egeria densa* in a drinking water reservoir in Morbihan (France), p. 243-247. In: J. Caffrey, P.R.F. Barrett, M.T. Ferreira, I.S. Moreira, K.J. Murphy and P.M. Wade (eds.), Biology, ecology and management of aquatic plants: Proceedings 10th Int. Symp. on Aquatic Weeds, European Weed Research Society.

Ellawala KC, Asaeda T, Kawamura K, 2011. The effect of flow turbulence on plant growth and several growth regulators in *Egeria densa* Planchon. Flora 206:1085-1091.

Foote AL, Kadlec JA, 1988. Effects of Wave Energy on Plant Establishment in Shallow Lacustrine Wetlands. J. Freshwater Ecol. 4:523-532.

Ghelardoni P, 1990. Tourist planning along the coast of Aquitaine, France, p. 191-197. In: P. Fabbri (ed.) Recreational uses of coastal areas. The Geojournal Library.

Hussner A, 2012. Alien aquatic plant species in European countries. Weed Res. 52:297-306.

Johnson JA, Newman RM, 2011. A comparison of two methods for sampling biomass of aquatic plants. J. Aquat. Plant Manage. 49:1-8.

Keddy PA, 1982. Quantifying within-lake gradients of wave energy: interrelationships of wave energy, substrate particle size and shoreline plants in axe lake, Ontario. Aquat. Bot. 14:41-58.

Koch EW, 2001. Beyond light: physical, geological, and geochemical parameters as possible submersed aquatic vegetation habitat requirements. Estuaries 24:1-17.

Kolada A, Willby N, Dudley B, Nõges P, Søndergaard M, Hellsten S, Mjelde M, Penning E, van Geest G, Bertrin V, Ecke F, Mäemets H, Karus K, 2014. The applicability of macrophyte compositional metrics for assessing eutrophication in European lakes. Ecol. Indic. 45:407-415.

Lehmann A, Castella E, Lachavanne JB, 1997. Morphological traits and spatial heterogeneity of aquatic plants along sediment and depth gradients, Lake Geneva, Switzerland. Aquat. Bot. 55:281-299.

Madsen JD, Chambers PA, James WF, Koch EW, Westlake DF, 2001. The interaction between water movement, sediment dynamics and submersed macrophytes. Hydrobiologia 444:71-84.

Martin GD, Coetzee JA, 2014. Competition between two aquatic macrophytes, *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) and *Myriophyllum spicatum* Linnaeus (Haloragaceae) as influenced by substrate sediment and nutrients. Aquat. Bot. 114:1-11.

Matsui A, 2014. Relationship between distribution and bottom sediment of submerged macrophytes in the Seta River, Shiga Prefecture, Japan. Landsc. Ecol. Eng. 10:109-113.

Nieder WC, Barnaba E, Findlay SE, Hoskins S, Holochuck N, Blair EA, 2004. Distribution and abundance of submerged aquatic vegetation and *Trapa natans* in the Hudson River Estuary. J. Coastal Res. 45:150-161.

Pelicice FM, Agostinho AA, Thomaz SM, 2005. Fish assemblages associated with *Egeria* in a tropical reservoir: investigating the effects of plant biomass and diel period. Acta Oecol. 27:9-16.

Puijalon S, Léna JP, Rivière N, Champagne JY, Rostan JC, Bornette G, 2008. Phenotypic plasticity in response to mechanical stress: hydrodynamic performance and fitness of four aquatic plant species. New Phytol. 177:907-917.

Redekop P, Hofstra D, Hussner A, 2016. *Elodea canadensis* shows a higher dispersal capacity via fragmentation than *Egeria densa* and *Lagarosiphon major*. Aquat. Bot. 130:45-49.

Ribaudo C, Bertrin V, Dutartre A, 2014. Dissolved gas and nutrient dynamics within an *Egeria densa* Planch. bed. Acta Bot. Gallica. 161:233-241.

Ribaudo C, Bertrin V, Jan G, Anschutz P, Abril G, 2017. Benthic production, respiration and methane oxidation in Lobelia dortmanna lawns. Hydrobiologia. 784:21-34.

Riis T, Hawes I, 2003. Effect of wave exposure on vegetation abundance, richness and depth distribution of shallow water plants in a New Zealand lake. Freshwater Biol. 48:75-87.

Riis T, Olesen B, Clayton JS, Lambertini C, Brix H, Sorrell BK, 2012. Growth and morphology in relation to temperature and light availability during the establishment of three invasive aquatic plant species. Aquat. Bot. 102:56-64.

Rohweder J, Rogala JT, Johnson BL, Anderson D, Clark S, Chamberlin F, Runyon K, 2012. Application of wind fetch and wave models for habitat rehabilitation and enhancement projects - 2012 Update. U.S. Geological Survey Open-File Report 2008-1200, 43pp.

Sand-Jensen K, 2003. Drag and reconfiguration of freshwater macrophytes. Freshwater Biol. 48:271-283.

Schneider B, Cunha ER, Marchese M, Thomaz SM, 2015. Explanatory variables associated with diversity and composition of aquatic macrophytes in a large subtropical river floodplain. Aquat. Bot. 121:67-75.

Schutten J, Dainty J, Davy A, 2004. Wave-induced hydraulic forces on submerged aquatic plants in shallow lakes. Ann. Bot. London. 93:333-341.

Schutten J, Dainty J, Davy A, 2005. Root anchorage and its significance for submerged plants in shallow lakes. J. Ecol. 93:556-571.

Sifeddine A, Meyers PA, Cordeiro RC *et al.*, 2011. Delivery and deposition of organic matter in surface sediments of Lagoa do Caçó (Brazil). J. Paleolimnol. 45:385-396.

Strand J, Weisner SB, 1996. Wave exposure related growth of epiphyton: implications for the distribution of submerged macrophytes in eutrophic lakes. Hydrobiologia 325:113-119.

Strand J, Weisner SB, 2001. Morphological plastic responses to water depth and wave exposure in an aquatic plant (*Myriophyllum spicatum*). J. Ecol. 89:166-175.

Tastet JP, Lalanne R, Maurin B, Dubos B, 2008. Geological and archaeological chronology of a late Holocene coastal enclosure: The Sanguinet lake (SW France). Geoarchaeology 23:131-149.

Urban RA, Titus JE, Zhu WX, 2009. Shading by an invasive macrophyte has cascading effects on sediment chemistry. Biol. Invasions 11:265-273.

Van Nes EH, Scheffer M, Van Den Berg MS, Coops H, 2002. Aquatic macrophytes: restore, eradicate or is there a compromise? Aquat. Bot. 72:387-403.

Wells RD, De Winton MD, Clayton JS, 1997. Successive macrophyte invasions within the submerged flora of Lake Tarawera, central North Island, New Zealand. New Zeal. J. Mar. Fresh. 31:449-459.

Yarrow M, Marin VIH, Finlayson M, Tironi A, Delgado LE, Fischer F, *et al.*, 2009. The ecology of *Egeria densa* Planchon (Liliopsida: Alismatales): A wetland ecosystem engineer. Rev. Chil. Hist. Nat. 82:299-313.

Zajac RN, 2008. Challenges in marine, soft-sediment benthoscape ecology. Landscape Ecol. 23:7-18.

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Aquatic vegetation in deep lakes: Macrophyte co-occurrence patterns and environmental determinants

Mattia M. AZZELLA,[1]* Mariano BRESCIANI,[2] Daniele NIZZOLI,[3] Rossano BOLPAGNI[3]

[1]Via G. Bellucci 94, 00156 Rome; [2]Institute for Electromagnetic Sensing of the Environment, National Research Council (IREA-CNR), Via Bassini 15, 20133 Milan; [3]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Viale G.P. Usberti 33/A, 43124 Parma, Italy
*Corresponding author: mattia.azzella@gmail.com

ABSTRACT

Our aims were to test the hypothesis that in deep lakes the co-occurrence patterns of macrophytes are not random, and to compare the relative contribution of the main environmental determinants (light, water and sediment parameters, phytoplankton) in structuring aquatic vegetation. We collected data from five deep *Chara*-dominated lakes in Central Italy along gradients of depth (33 to 165 m), dimension (1.7 to 114.5 km^2) and water trophic conditions (12.4 to 41.3 µg L^{-1} of total phosphorous). Twenty-five sampling plots *per* lake were randomly selected at five predetermined depths (1.5, 3.0, 6.0, 12.0 and 20.0 m; n=5) within homogenous littoral sectors. Data were explored by a null model analysis using the checkerboard score (C-score) index, and Canonical Correspondence Analysis. Our data verify the not random co-occurrence patterns of macrophyte' communities in deep lakes. However, present data suggested that C-scores are strictly dependent on lake' trophic status: low nutrient loads, in both water and sediments, seemed to be reflected in a not random co-occurrence zonation of macrophytes. Summarizing, it is fundamental evaluate the local effects of lake trophy on the macrophyte community dynamics both in time and space before inquiring about mutual links. If it fails to assess macrophyte co-occurrence patterns, it may be not possible to identify the determinants of the spatial arrangement of macrophytes and, in turn, the conservation status or the ongoing dynamics of lakes.

Key word: Aquatic plants; C-score index; co-occurrence analysis; environmental drivers; Chara-dominated lakes; Volcanic lakes; central Italy.

INTRODUCTION

Ecological studies generally assume that the co-occurrence patterns of plants are non-random. However, the type of response of plant species and/or communities to environmental gradients is still strongly debated, with a range of evaluation models having been proposed (*e.g.*, null models, nested or checkerboard patterns; Presley *et al.*, 2010; Logue *et al.*, 2011; Ulrich and Gotelli, 2013). In this context, the large amount of plant community studies have generally failed to investigate whether the structure of communities is non-random before analyzing their environmental determinants (Duberstein *et al.*, 2014).

With regard to macrophyte communities, the majority of researchers have not taken into account the potential causality or randomness of their co-occurrence (Penning *et al.*, 2008a), and very few works have evaluated the factors that may influence aquatic vegetation itself (Boschilia *et al.*, 2008; Logue *et al.*, 2011; Chmara *et al.*, 2013). In any case, we exclude the possibility that macrophyte communities respond randomly to environmental gradients. This is in agreement with the observations about the non-random distribution of trait performance within macrophyte communities (Fu *et al.*, 2014). However, in specific situations (*e.g.*, under anthropogenic stress), we hypothesize that macrophyte co-occurrence patterns may be unstable, which would result in unclear spatial gradients, affecting the identification of environmental determinants and limiting the use of macrophytes, for example, as bioindicators (Bolpagni *et al.*, 2016a).

A non-random structure in macrophyte communities is based on the assumption that one or more factors contribute to its spatial pattern. As reported by Boschilia *et al.* (2008), these factors can be of either a competitive or environmental type, and lead to a checkerboard pattern produced by pairs of species with mutually exclusive ranges, as occurs among terrestrial plant communities (see Diamonds, 1975). Some previous works have generally concluded that the non-random distribution patterns of macrophyte communities tend to be driven by environmental factors rather than biotic interactions (Boschilia *et al.,* 2008), a finding that is consistent with those of many other studies (Keddy, 1983; Wilson and Keddy, 1986). The main environmental factors that influence the structure of macrophyte communities are light availability, water and sediment parameters, and hydro-morphological determinants (Bornette and Puijalon, 2011 and references therein). Furthermore, their influence also varies markedly depending on the spatial scale at which they are analyzed as well as on the heterogeneity of the aquatic ecosystems being investigated (Lacoul and Freedman, 2006; Bornette and Puijalon, 2011). The listed variables can assume a greater or lesser importance according to the type of aquatic ecosystems (*e.g.*, lakes, streams, lagoons, wetlands). In general, several studies have suggested that there is a direct dependence of macrophytes distribution on the simultaneous variation in light availability and temperature regime along the depth gradient (Azzella *et al.*, 2014). The progressive worsening in the quality of lake water and of the sediment leads to a reduction in light availability, and a strong cascade effect on macrophytes presence and distribution (Genkai-Kato and Carpenter, 2005). On the other hand, when conditions in a lake improve, a new equilibrium in macrophyte communities' distribution is achieved. Focusing on deep lakes, we generally hypothesized that macrophyte communities may display local random distribution patterns under worsening (*e.g.*, increased nutrient content or turbidity) or improving (*e.g.*, increased transparency) water and sediment parameters, and that macrophytes distribution is not random exclusively in the presence of stable physical and chemical states, both in time and space. As a result, it is possible to identify the ecological determinants of macrophyte richness and community dynamics, as well as the significance of macrophyte indices (Melzer, 1999; Penning *et al.*, 2008b; Kolada, 2010), only when physical and chemical conditions remain stable over time.

Within this context, a further critical aspect is the overestimation of the role of water quality compared to that of sediment or other not trophic factors (Carignan and Kalff, 1980). To fill this gap in our knowledge, in the present work we conducted a detailed investigation of both the sediment parameters and water in relation to the structure and composition of macrophyte communities. We hypothesized that the concentration of nutrients in surficial sediments (especially phosphorous) can shed light on differences that are to be expected between different lakes. Furthermore, our data could be used to discuss the significance of the presence or absence of a non-random co-occurrence pattern in macrophyte distribution for the purposes of an ecological assessment of a lake.

Bearing all this in mind, and considering that failure to assess community structure when macrophyte co-occurrence is analyzed significantly reduces the explicative power of the environmental determinants, this paper was to evaluate the macrophyte co-occurrence patterns by considering a homogeneous set of five natural deep lakes characterized by a gradient in trophic status. A second aim was to assess the role of non-random macrophyte co-occurrence patterns in affecting the analysis of edaphic factors (*i.e.*, environmental determinants).

METHODS

Study area

The study was conducted in the Italian volcanic lake system on five deep lakes (lakes Bolsena, Bracciano, Martignano, Nemi, and Vico) with a mean depth and area of 91 m and 37.7 km^2, respectively (Fig. 1; Tab. 1). The lakes investigated are oligo-mesotrophic, with total phosphorous (TP) and nitrogen (TN) mean concentrations of 24.9 and 600 µg L^{-1}, respectively (Tab. 1). The lakes have a mean conductivity value of 429 µS cm^{-1}, and mean Secchi disk transparency of 6.6 m (summer-winter mean value for the period 2006-2011). These characteristics support the classification of these lakes as belonging to the *Chara*-dominated lakes (Jensen, 1979), as is confirmed by the fact that their littoral vegetation is generally dominated by stoneworts (*Chara globularis* Thuillier 1799, *C. polyacantha* A. Braun in Braun, Rabenhorst & Stizenberger 1859, *C. tomentosa* Linnaeus 1753) from a depth of three to 20 meters. Indeed, these lakes may be considered a European hotspot of stonewort biodiversity and hosted about the 20% of the European stoneworts diversity (Azzella,

Tab. 1. Morphometric characteristics and hydrochemical features (summer-winter mean values for the period 2010-2011) of the five lakes sampled.

Lake	Morphometric characteristics					Hydrochemical features				
	D (m)	A (km^2)	Alt (m asl)	Z$_{max}$ (m)	Vol (m^3 10^6)	TP (µg L^{-1})	TN (µg L^{-1})	Cond (µS cm^{-1})	pH	SD (m)
Bolsena	151	114.5	305	146	8922.0	15.8	710	523	7.4	7.5
Bracciano	165	57.5	164	160	4950.0	12.4	510	519	7.9	8.7
Martignano	60	2.5	207	54	71.2	37.7	410	392	7.4	6.5
Nemi	33	1.7	318	34	32.5	41.3	900	340	7.9	5.0
Vico	48	12.1	507	50	268.0	17.1	660	371	7.6	5.5

D, depth; A, area; Alt, altitude; Z$_{max}$, maximum depth; Vol, volume; TP, total phosphorous; TN, total nitrogen; Cond, conductivity at 20°C; SD, Secchi disk transparency.

2014). Only the first few meters (a depth of 1 to 4 m) could be dominated by vascular species [*e.g.*, *Myriophyllum spicatum* L., *Phragmites australis* (Cav.) Trin. ex Steud., *Potamogeton pectinatus* L., *P. perfoliatus* L.].

Experimental design and macrophyte characterization

A homogenous littoral sector, with a low to moderate slope of the bottom, was selected in each lake according to previous macrophyte surveys (Azzella *et al.*, 2013). Each sector was characterized by the absence of rocky outcrops or other morphological peculiarities that might affect the presence of macrophytes. A GIS program (ESRI's ArcGIS 10.0) was used in each sector to generate a grid with a 50-m mesh net on a one km-long stretch of coast (Fig. 2). Twenty-five squares of the grid were then selected at five different predetermined depths, with plots being drawn randomly from among plots that intercepted a bathymetric reference level. The predetermined depths were chosen based on a logarithmic increase between one

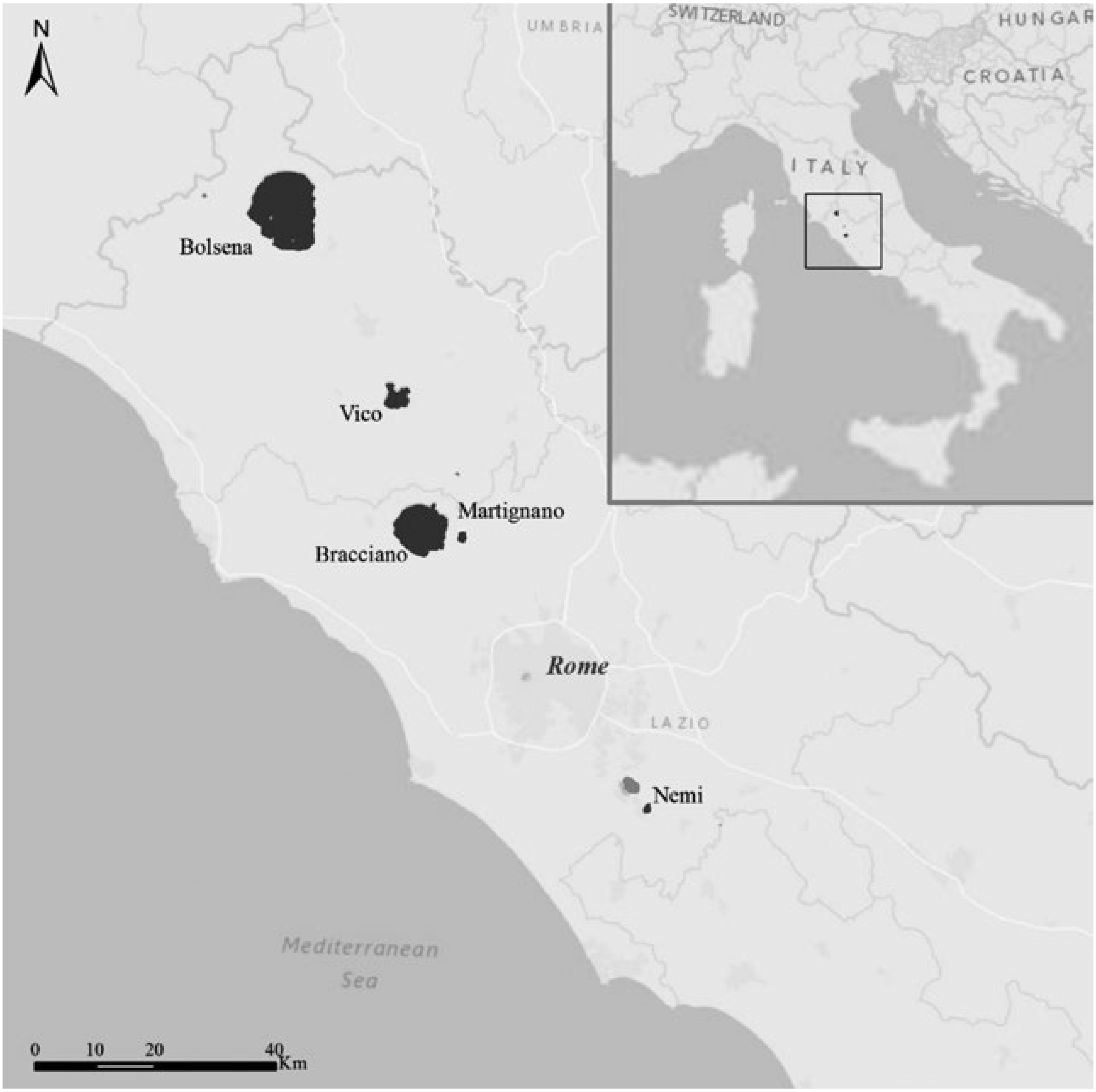

Fig. 1. Study area showing the location of the five lakes investigated.

depth and the next (1.5, 3.0, 6.0, and 12.0 m) with the exception of the greatest depth which was set at 20.0 m. This method was adopted to prevent the sample plots from overlapping the thresholds between the different macrophyte communities previously recorded (Azzella *et al.*, 2013; Azzella, 2014), and to ensure that they fell within each of the characteristic belts of macrophyte communities, according to the following general zonation:

i) 1.5 m depth corresponds to the high diversity emergent vegetation belt dominated by helophytes [*e.g.*, *P. australis, Schoenoplectus lacustris* (L.) Palla] and vascular hydrophytes (*e.g.*, *M. spicatum, P. perfoliatus*);
ii) 3.0 m depth corresponds to the high diversity *Chara aspera*-dominated belt;
iii) 6.0 m depth corresponds to the low diversity and high biomass *C. polyacantha* or *C. tomentosa*-dominated belts;
iv) 12.0 m depth corresponds to the very low diversity *C. globularis*-dominated belt;
v) 20.0 m depth corresponds to the rather monospecific *Nitella opaca*-dominated belt or to bare sediment.

We thus obtained five sample plots for each depth in each lake, which corresponds to 25 plots *per* lake and a total of 125 plots. All the plots were sampled in summer 2013. During the field activities, each sample plot was reached using a Trimble GPS GeoXM and the depth measured with a depth gauge (±0.5 m). If the predetermined depth (sample plot) and the measured depth *in situ* corresponded, we proceeded with the sampling activities; if they did not correspond, we moved perpendicularly to the coastline until the desired depth was reached. A square whose sides measured 1 m was lowered on the sampling plot, and all the species present and the relative cover (expressed as %) were recorded by visual assessment within the square using an underwater camera or by scuba diving.

Water and sediment physical and chemical characterization

At each sampling plot, temperature (T), conductivity (Cond), pH and dissolved oxygen (DO) were measured directly *in situ* just above the canopy of the macrophyte com-

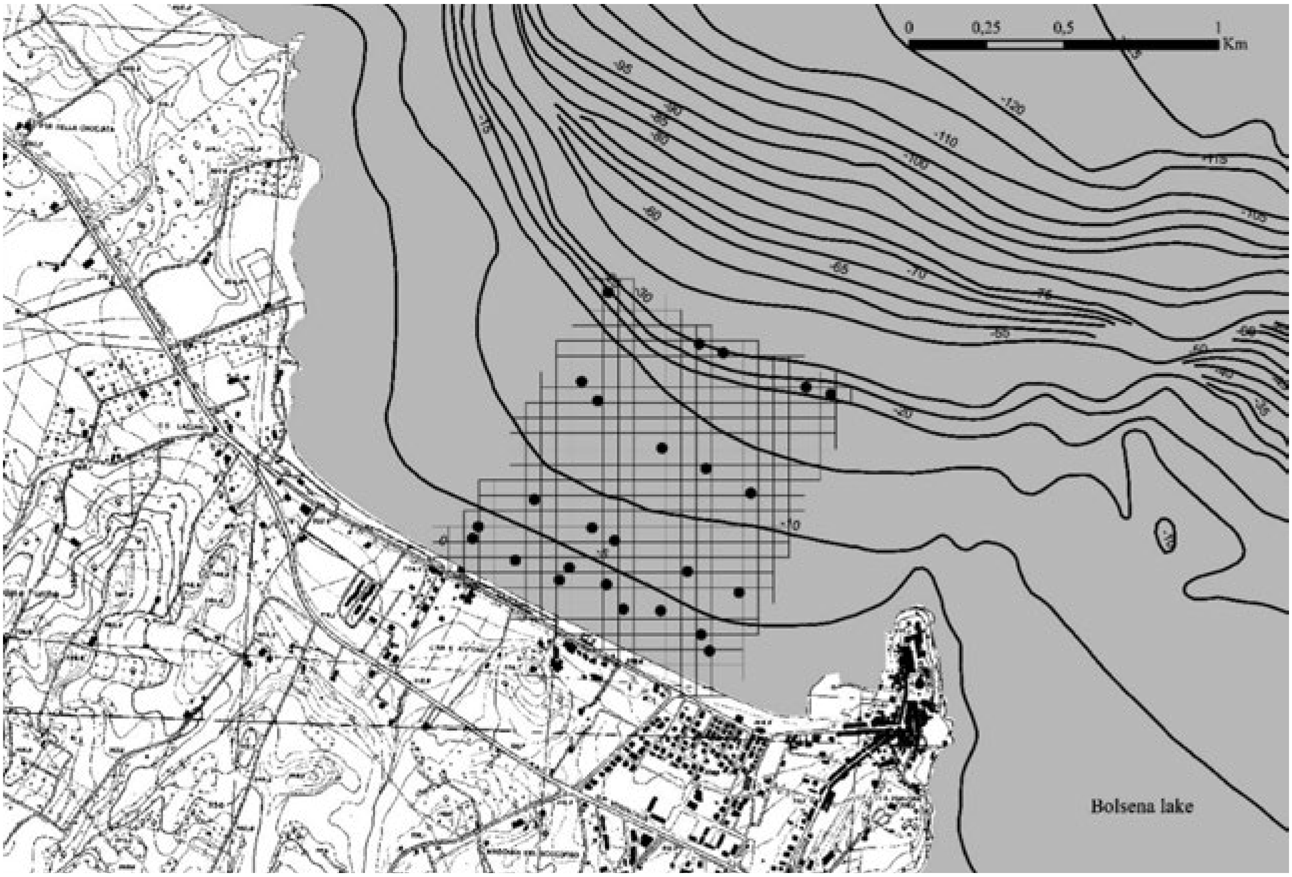

Fig. 2. Example map of the spatial arrangement of the sampling plots (black points) along an experimental homogenous littoral sector, in the present case the map refers to the Bolsena Lake.

munities or the bare sediment (±0.5 m) using a multiple probe (Hanna Instruments, HI 9828). At the same time, water samples were collected using a Ruttner bottle (1 L) and immediately processed and kept in cold storage at around 4°C for subsequent analysis: 100 ml were filtered with GF/C glass-fiber filters (Whatman, Maidstone, UK) for NH_4^+, NO_3^-, soluble reactive phosphorous (SRP) determinations and 40 ml of unfiltered water were collected for TP and TN determination. All analyses were performed using standard spectrophotometric methods (APHA, 1998). Three sediment cores were simultaneously collected using Plexiglass core tubes (20 x 4 i.d. cm). After collection, the cores were kept on ice and returned to the laboratory within six hours. Upon reaching the laboratory, the first five cm of each core were extruded and each sediment slice was immediately homogenized and samples collected using cut-off 10 mL syringes for the determination of organic matter (OM) and total phosphorus (TPsed) content. OM was determined as dry weight loss after ignition at 450°C for 2 h of 0.5 g of dry sediment, while TPsed was determined on ignited sediment according to the acid extraction method (Aspila *et al.*, 1976).

A fluorimeter cyclops-7 equipped with probes for the characterization of the Phycocyanin, Chlorophyll a and Phycoerythrin pigments was used to detect their relative algal fluorimetric units as well as to estimate the colored dissolved organic matter (CDOM). At the same time, the amount of light radiation (LR) that reaches the canopy was measured using a Li-cor detector (PAR LI-192SA Underwater Quantum Sensor). Starting from the Li-cor data, we derived the vertical diffuse attenuation coefficient (K_d) according to Kirk's equation (1994), based on the photosynthetically active radiation (PAR) at a given depth (z) and at the water surface (water-atmosphere interface).

Checkerboard score and null model analysis

To test the non-random co-occurrence of macrophytes, a null model analysis was performed. The analysis was applied to the species x depth zones matrix, using the SIM9 algorithm (Gotelli, 2000) according to Boschilia *et al.* (2008). The randomized matrix generates casual distribution (the so-called null models). The software used (EcoSim 700; Gotelli and Entsminger, 2002) calculated an index to evaluate the species segregation in the observed and expected matrices. If communities have a not randomly co-occurrence pattern, the index of the observed matrix will be significantly different from the mean of the randomized matrices. In this context, we used the checkerboard score (C-score) index to reveal the existence of competition-based differences among communities (Stone and Roberts, 1990), supporting the outputs of the Canonical Correspondence Analysis (CCA) method that is devoted to understand distributions based on abiotic variables. To evaluate the presence of a non-random pattern, driven by the environmental factors reported in previous studies, we used the total matrix of 125 plots (10,000 randomizations). To evaluate the randomness of the distribution in each lake, we tested the null models on the matrices of single lakes (25 plots, 10,000 randomizations).

CCA and null model analysis

A CCA was performed using species and environmental data matrices to analyze the influence of environmental variables on specie distribution and to explain the variability detected within and between lakes. All the analyses were conducted using the vegan package in the R-software (R core team, 2017). Data were normalized by applying a logarithmic transformation to the dataset. A Pearson's correlation analysis was first conducted to determine the univariate relations between all the study environmental variables to avoid the use of dependent (*i.e.*, covariates) factors in the CCA. We set the threshold at R=0.6 in order to detect any correlation between two significant environmental variables avoiding collinearity (Guareschi *et al.*, 2015). As a result, the following variables were selected for the subsequent analysis: water TP, TN, NO_3^-, T, Cond, LR (expressed as $\mu E\ m^{-2}$), CDOM, Chla, sediment OM and TPsed.

Unlike many other authors who used depth as an ecological parameter, we excluded the use of depth data from the analysis in advance for two reasons. First, because both T and LR, as well as other variables, were closely correlated with the depth gradient; second, since we used depth as a key factor in selecting sample plots, the use of this variable may have altered the statistical analysis outputs or masked the relative importance of the other environmental determinants. By excluding the depth data, we wished to avoid any tautological entailment. Nevertheless, by means of the function "ordisurf" of the "vegan" package in R, we fitted the depth into ordination diagram. Ordisurf draws the surface of an environmental determinant into ordination diagram using a GAM model.

To evaluate the influence of randomness on the proportion of total variance explained by the environmental variables, the original dataset was split into two different lake groups according to the C-score performance: lakes with a non-random (group A) and those with a random macrophyte distribution (group B). A CCA was then performed by considering each of the two groups separately to determine whether the total variance explained by the environmental variables of the non-random group A is significantly higher than the total variance explained in the first CCA performed by considering all 125 sample plots. When the number of samples in a matrix is reduced, the total variance decreases whereas the total variance explained increases. To avoid this problem and to determine whether the increase was significant as opposed to being related to the lower number of sampling plots considered in the analysis, the results obtained were compared with those obtained by using simulated macrophyte distributions (10,000 randomizations) created with a random extraction of plots from the original matrix. It is thus possible to consider the effect due to the change in the sample size and the true improvement in the variance explained. All the sample plots were inserted in the matrix, even if devoid of macrophytes. Consequently, a dummy column with cover values of 1 was inserted to perform the aforementioned analyses.

RESULTS

Physical and chemical variables

In the water layer between 1.5 and 6 m, T, pH and DO were relatively constant and above 23.5°C, pH 8.00 and 100% of saturation, respectively. These parameters dropped in the 1.5-20 m range according to the lake size: the smaller the lake, the greater the reduction. A minimum of 8.7°C associated with low DO (39%) was recorded at a depth of 20.0 m in Lake Vico, whereas a minimum of pH 6.45 was recorded at a depth of 20 m in Lake Martignano. Both lakes Bracciano and Bolsena yielded T values always above 11°C, pH 7.39 and 100% of DO saturation. Cond varied somewhat, ranging between 268 µS cm^{-1} (at Lake Nemi at a depth of 12.0 m) and 541 µS cm^{-1} (at Lake Bolsena at a depth of 1.5 m) (Supplementary Tab. 1). Similarly, NO_3^- and TN varied considerably with values of 20.0-167.2 µg L^{-1} and 50.0-514.89 µg L^{-1}, respectively. By contrast, SRP and TP values varied less, with values of 1.2-16.24 µg L^{-1} and 5-38.9 µg L^{-1}, respectively (Supplementary Tab. 1).

Relative algal and CDOM fluorimetric units exhibited similar patterns. The highest values were recorded at the greatest depths (between 12.0 and 20.0 meters). Phycocyanin, Chla and Phycoerythrin pigments peaked in Lake Vico at a depth of 20.0 m, with Relative fluorimetric unit (RFU) mean values (±standard deviation=SD) of 582±199, 720±163 and 238±62, respectively. CDOM yielded its maximum value, equal to 267±4 (SD) RFU, in Lake Nemi at a depth of 20.0 m (Supplementary Tab. 1). For *Kd*, the collected values reflected comparable conditions. The highest values were recorded in Lakes Vico and Nemi, above all in the first meters of depth, thus indicating a rapid extinction of underwater radiation. In Lake Nemi, K_d ranged between -0.53±0.06 (mean ± SE) and -0.51±0.17, at a depth of 1.5 and 3.0 m, respectively (Supplementary Fig. 1).

Sediment OM content ranged between 0.2 and 36.2%, with the highest values being recorded in lakes Vico (25.2% at 1.5 m of depth) and Nemi (36.2% at 1.5 m of depth). TPsed displayed a similar pattern, with a minimum of 0.2 (recorded in Lake Bolsena) and a maximum of 3.1 mg P g^{-1} (recorded at Lake Nemi) (Supplementary Tab. 1).

Macrophyte characterization

A total of 24 macrophytes were recorded: 10 *Characeae*, one bryophyte (*Fontinalis squamosa* Hedw.) and 13 vascular plants. *M. spicatum* was detected in all the lakes, whereas six species were recorded for a single littoral sector [*Baldellia ranunculoides* (L.) Parl*., F. squamosa and Potamogeton lucens* L. at Lake Bolsena, *Nelumbo nucifera* Gaertn., and *Potamogeton nodosus* Poir. at Lake Nemi, *Potamogeton x nitens* Weber at Lake Bracciano] (Supplementary Tab. 1). As expected, the highest macrophyte diversity (2.9±1.9 species *per* plot, SD) was detected closest to the surface (from 1.5 to 3.0 m), while very poor or monospecific communities were recorded below 3.0 m of depth (1.0±1.37). At 20.0 m of depth, macrophytes were recorded exclusively at Lake Bracciano [dominated by *Nitella opaca* (C. Agardh ex Bruzelius) C. Agardh 1824]. Aquatic vegetation at Lake Nemi instead ceased at a depth of six meters (dominated by *Ceratophyllum demersum* L.). The richest plot, consisting of nine species, was recorded in Lake Bolsena at a depth of 3.0 m.

Macrophyte depth distribution and co-occurrence

In lakes Bracciano, Vico and Martignano there was a clear zonation in aquatic vegetation depending on the depth. As expected, a typical community was identified in each belt investigated. At a depth of between 1.5 and 3.0 m, vascular species emerged as the dominant and constant taxa coupled with a limited number of stoneworts, such as *Chara aspera* Detharding ex Wildenow 1809, *C. vulgaris* Linneaus 1753 and *C. hispida* L. *sensu auct. nonnull*. By contrast, below a depth of 6.0 m, stoneworts represented the dominant and constant species, particularly *C. globularis*, whereas the vascular species became rare and localized. Conversely, at Lake Nemi no clear differentiation between the investigated communities along the depth gradient was observed despite the presence of a clear gradient in the TP, NO_3^- and TN concentrations in the sediment and water, respectively. Lake Bolsena yielded a simplified model in which there was a clear distinction between shallow and deep communities (Supplementary Tab. 1).

The CCA revealed the presence of two main gradients (Fig. 3, above panel). A not trophic gradient within lakes, correlated with LR and T values (which decreased along the depth gradient, Fig. 3, below panel) and Chl-*a* concentrations (which increased along the depth gradient), and a second gradient between lakes, correlated with TPs and OM availability. In general, the increased availability of nutrients (such as water TN, sediment OM and TPsed) promoted the dominance of vascular species at shallow depths (*M. spicatum*, *P. nodosus* and *Najas minor*); while at deep depths are favored stoneworts (*C. globularis* and

Nitellopsis obtusa) in conjunction of low T and LR values. By contrast, *B. ranunculoides, P. lucens,* and *P. perfoliatus* were dominant in plots with low concentrations of water TN, Chl-*a*, CDOM and sediment OM and TPsed.

The segregation indices calculated for the sampling plots were significantly greater than the average of the indexes based on null models ($P<0.05$) obtained from the overall dataset of 125 plots (Fig. 4). Thus, the pattern of species distribution across lakes was not random. This result supports the idea that species associations within communities are not random and that species are not-randomly co-occurring but they are strictly regulated by the environmental gradient of lakes.

At the lake scale, the C-score was significantly higher than expected only in three out of five cases in analysis. The macrophytes co-occurrence pattern was random at lakes Bolsena and Nemi, which indicates that there was no clear spatial co-occurrence pattern of the species in

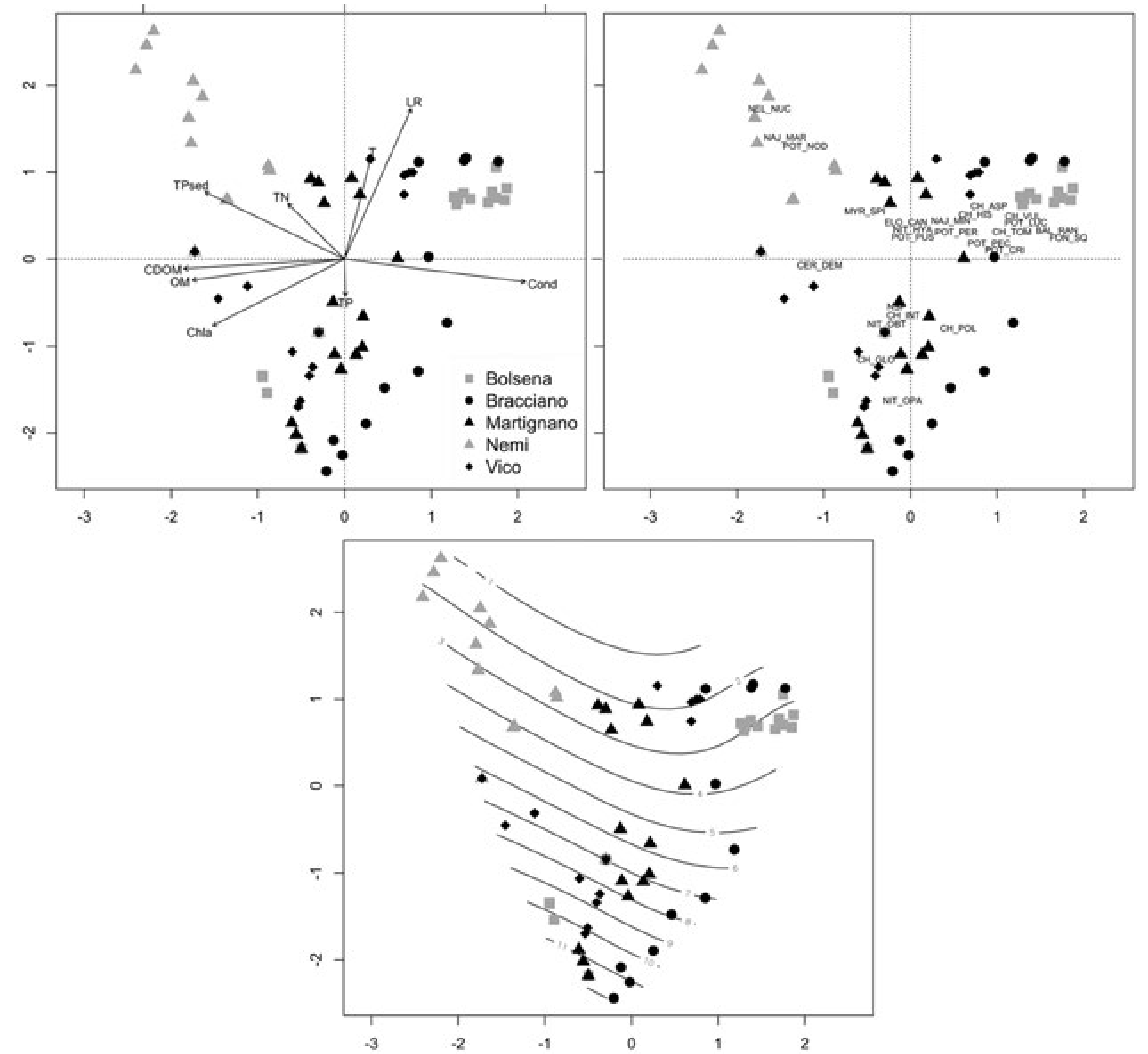

Fig. 3. CCA ordination plots of the ecological gradients obtained by the backward selection for the aquatic vegetation of lakes investigated (above panels). On the left the sampled plots and the environmental drivers, on the right the sampled plots and the species. In the below panel, on the same CCA ordination plot, a projection of depth of the plots onto ordination as a non-linear surface. The total inertia explained is 32%. OM, sediment organic matter; CDOM, colored dissolved organic matter in the water; TPs, total phosphorus in the sediment; TN, total nitrogen in the water; T, water temperature; LR, light radiation measured as $\mu E\ m^{-2}$; Cond, conductivity of the water; TP, total phosphorus of the water; Chla, cholorophyll *a* in the water; for species abbreviation see Supplementary Tab. 1.

these lakes. When the lakes in which the C-score analysis revealed a random co-occurrence pattern of species were excluded from the CCA (group B: Bolsena and Nemi, 50 plots), the variance explained by the environmental parameters increased from 36% to 44%. This increase is significantly correlated with the exclusion of lakes in which a random co-occurrence pattern was detected. This finding is confirmed by the second null model analysis (Supplementary Fig. 2) because the variance explained by the CCA on the plots belonging to lakes Bracciano, Vico and Martignano is significantly higher (P<0.01) than the variance of null models based on 75 plots randomly extracted from the observed matrix.

DISCUSSION

Macrophyte co-occurrence in deep lakes

We demonstrate that macrophyte co-occurrence in deep lakes is structured according to a non-random pattern comparable to the checkerboard scheme (Diamond, 1975). This aspect has previously investigated exclusively

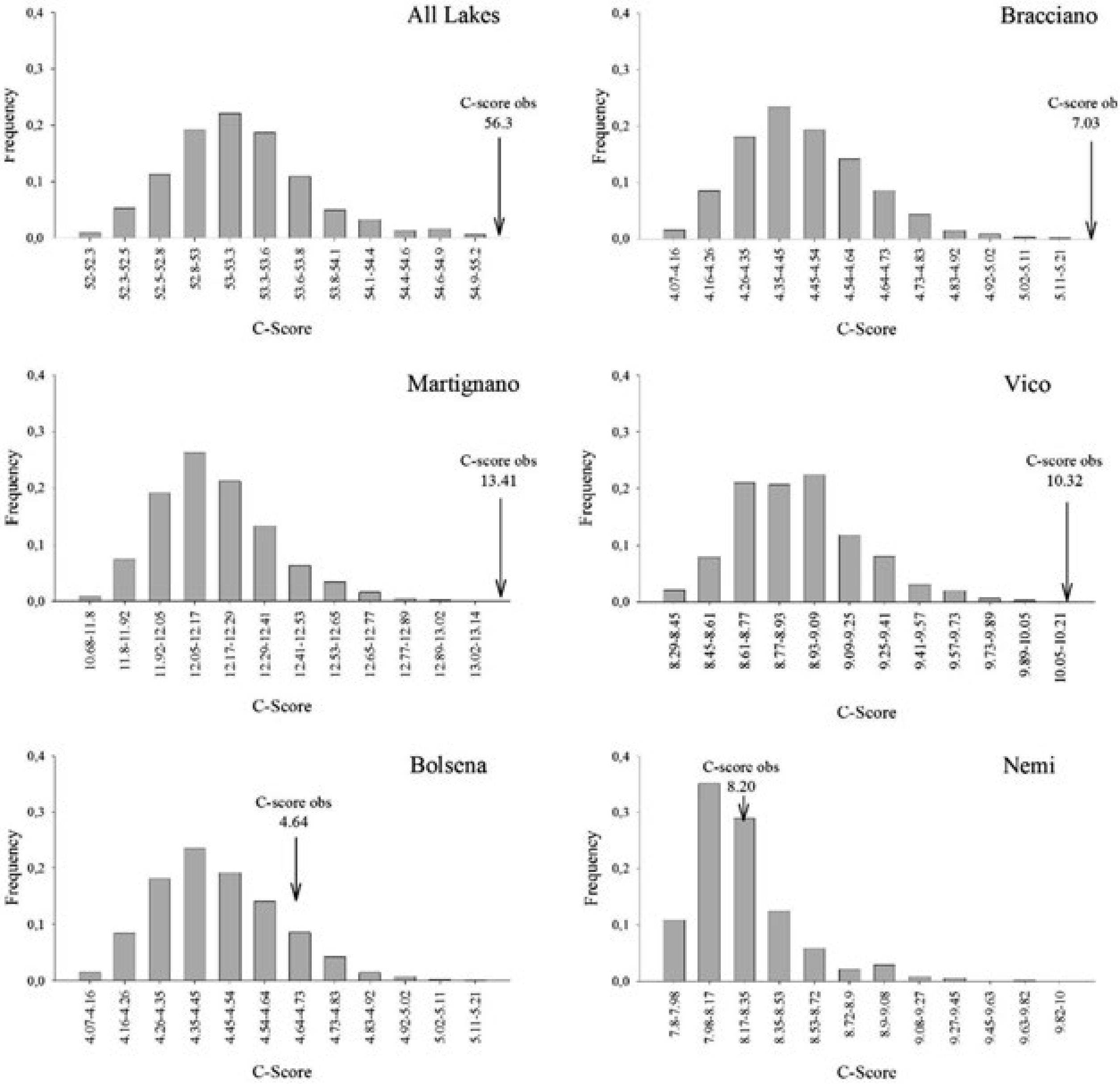

Fig. 4. Histograms of the observed and expected values of the C-score in the whole dataset and at the single lake scale; the arrows correspond to the values obtained for the recorded macrophyte distributions.

in temperate marshes (Shipley and Keddy, 1987), in lacustrine shoreline vegetation (Keddy, 1983), in a heterogeneous set of shallow lakes (Chmara *et al.*, 2013), and in tropical floodplain lagoons (Boschilia *et al.*, 2008). Indeed, the null model analysis we performed showed that deep lake macrophytes interact, as a whole, in a non-random fashion and some explanatory ecological factors drive macrophyte patterns (LR, T, within the lakes; Chl-*a*, Cond and sediment parameters between the lakes).

However, unbundling the analysis at the lake scale, the macrophyte co-occurrence in lakes Bolsena and Nemi, unlike that in lakes Vico, Bracciano and Martignano, seemed to follow a random distribution. A possible explanation is that the environmental determinants of the non-random macrophyte co-occurrence observed for lakes Vico, Bracciano and Martignano are either weaker or are offset by other determinants (*i.e.*, not trophic) in the lakes with a non-significant C-score (lakes Bolsena and Nemi). Indeed, the absence of a clear macrophytes depth gradient in lakes Bolsena and Nemi may be associated with their generally unstable and dynamic water physical and chemical conditions. Lake Nemi changed dramatically at the start of the 20th century (Marchesoni, 1940), went through a hypertrophic crisis in the 1970s (Avena and Scoppola, 1987), and only recently experienced an improvement in water quality and vegetation expansion (Azzella *et al.*, 2014). By contrast, the macrophyte representativeness in Lake Bolsena has decreased dramatically in recent years after a long period of growing floristic richness (Azzella *et al.*, 2014), probably because of local climatic variability (Bolpagni *et al.*, 2016b).

These findings are consistent with those reported by the few authors who have investigated macrophyte co-occurrence models (Boschilia *et al.*, 2008; Logue *et al.*, 2011; Chmara *et al.*, 2013). When Boschilia and colleagues (2008) investigated the C-score at a coarse spatial scale of analysis in several lagoons across the Paraná River floodplain, they found a non-random macrophyte arrangement due to ecological differences. When they analyzed only a portion of the data set, *i.e.*, small lagoons disconnected from the system of the Paraná River, they found a random pattern and suggested that it was due to the emergence of several ecological factors in these situations than in tropical floodplain lagoons. The general instability of these systems, combined with the temporary predominance of competitive interactions between species in search of new euphotic zones to colonize, is likely to underlie these random patterns, thereby contributing to the definition of a new equilibrium. Chmara *et al.* (2013) recorded similar results in shallow lakes in Poland.

Environmental determinants

The CCA analysis confirmed the existence of recurrent macrophyte distribution patterns that were strictly dependent on the trophic status of lakes. However, a clear gradient in macrophyte community patterns was observed as an effect of significant differences in physical and chemical conditions between lakes.

As expected, Cond, Chl-*a* and nutrient concentrations, particularly in the surficial sediments, are the main environmental determinants of differences in macrophyte distribution gradients between lakes. Our results are in keeping with the findings of several previous studies (*e.g.*, Chappuis *et al.*, 2014; Schneider *et al.*, 2015). However, the fact that the macrophyte communities appear to be driven by sediment TPsed to a greater extent than by the TN concentration in water, and above all that the water TP is not significantly related to the macrophyte distribution patterns suggests that sediment parameters play a more important role than water conditions in the spatial arrangement of macrophytes. It is not a complete novelty (see Carignan and Kalff, 1980), but it is generally underestimated (Capers *et al.*, 2010). However, our study lends further support to this evidence and highlights the need for further investigations. Indeed, the majority of both earlier and more recent works on this topic focused predominantly, or even exclusively, on water conditions when analyzing macrophyte patterns, neglecting the potential role of sediment (Kolada, 2010; Alahuhta, 2015; Lukács *et al.*, 2015; Pulido *et al.*, 2015). Furthermore, previous investigations that did analyze the depth distribution of macrophytes often included depth among the environmental determinants tested, thereby limiting the analytical potential of the studies (Azzella *et al.*, 2014 and references therein), or used data mainly from shallow ecosystems (Søndergaard *et al.*, 2013). In this regard, the present dataset based on deep lakes sheds light on a relatively wide range of depths (down to 20.0 m) and provides useful information capable to integrate the data available for shallow lakes. For instance, the present results could allow solving part of the uncertainty highlighted by Capers *et al.* (2010) considering the importance of local and regional processes in driving macrophyte communities. Specifically, the recorded stochasticity rather than depend on the processes of species' colonization and persistence, may result from the underestimation of the sediment's role as well as the influence of the trophic dynamics of the studied lakes.

Implications for ecological study, biomonitoring and lake classification

The majority of recent works on the relationship between macrophytes and the environment, which have generally been performed on very large datasets and have thus been characterized by a very marked variance, did not detect any degree of randomness in macrophyte co-occurrence before the role of expected driving factors was tested (Kolada, 2010; Alahuhta, 2015). Our findings indicate that not testing these factors may result in a misinterpretation of the determinants of macrophyte co-occurrence patterns as well as of their edaphic determinants. Indeed, we may assume that experimental results in numerous previous studies were over-interpreted, generating distorted evaluations of the role of environmental factors in driving macrophyte distribution as well as of the reliability of macrophytes as markers of the ecological status of colonized environments. Indeed, there has been a growing consensus on the existence of weak relationships between changes in "macrophyte dominance" and major environmental variables, usually regarded as the main determinants of aquatic vegetation dynamics (Demars *et al.*, 2012). These authors confirmed the predominance of non-human pressures in driving the variability in river macrophyte indices, suggesting that not-trophic determinants (*e.g.*, temperature, lake area) play an important role in this variability. Furthermore, recent evidence suggests that macrophyte communities that exhibit marked inter-annual fluctuations and stochastic interactions with external disturbance events or weather extremes are characterized by intrinsic high dynamicity (Wiegleb *et al.*, 2014). With respect to these considerations, our approach can complement other types of surveys, especially those aimed at investigating the long-term changes in macrophyte communities using sedimentary macrofossils as verified by Levi *et al.* (2014) in several Mediterranean lakes.

Although the first long-term data were collected for river systems, it is reasonable to presume that lacustrine macrophyte communities are also characterized by alternating phases of establishment and development, as demonstrated by Bolpagni *et al.* (2016b) in the short term (a three-year field survey), that do not appear to be controlled by physical and chemical environmental determinants. For example, in summer Lake Bracciano is frequently affected by intense phenomena of "detachment and emergence" of large portions of the submerged beds of stoneworts, which probably favor the periodical replacement of "aged communities" by new formations. In the early stages of colonization, these new "open patches" are frequently colonized by annual species (such as *Najas marina* L.) before being re-colonized by stoneworts. Synthetic or global assessments generally consider extremely large datasets that cover very long periods of time, which requires the simultaneous analysis of data collected in time intervals spanning more than 10-20 years. The potential distortive effects induced by the underestimation of the role of macrophyte inter-annual fluctuations are generally not considered in this case either.

CONCLUSIONS

Summarizing**,** a non-random model describes the depth co-occurrence of macrophytes in deep lakes under stable trophic conditions and under low human impact. Moreover, given the presence of a clear partitioning of macrophytes, which is demonstrated by the coexistence of vascular-dominated stands at depths closest to the surface (1.5 to 6.0 meters) and stonewort-dominated beds at depths of more than six meters, we believe that significant C-scores are closely related to a trophic stable state of a deep lake. Exclusively low nutrient loads, in both water and sediments, may result in the characteristic macrophyte zonation along the depth gradient. If C-scores are not adequately implemented in ecological investigations, random macrophyte co-occurrence patterns could be used to define the macrophyte determinants largely invalidating their significance.

In comparison with the results obtained by Fu *et al.* (2014), we further put emphasis on the pivotal role of habitat filtering, stressing on the role of sediment conditions, in driving macrophyte community assembly. If it is clear that water depth shaped the macrophyte spatial patterns in close association with nutrients and light, our analyses introduce additional considerations on the importance of the whole-lake trophic status and dynamics in explaining the role and importance of environmental determinants as macrophyte filters. Consequently, any study on the relationship between macrophytes and aquatic environmental conditions, as well studies based on the use of macrophytes in monitoring, must include an assessment of the community structure according to one of the community pattern theories. A failure to do so would lead to an over-estimation of the macrophyte bioindication value, which would in turn seriously compromise any attempt to accurately assess the conservation status of lakes.

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REFERENCES

Alahuhta J, 2015. Geographic patterns of lake macrophyte communities and species richness at regional scale. J. Veg. Sci. 26:564-575.

APHA, 1998. Standard Methods for the Examination of Water and Waste Water. American Public Health Association, Washington DC: 874 pp.

Aspila KI, Agemian H, Chau ASY, 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. Analyst 101:187-197.

Avena GC, Scoppola A, 1987. Features of the hydrophytes and helophytes communities, p. 75-94. In: V. Carunchio (ed.), Evaluation of the environmental situation of Lake Nemi. Università degli Studi di Roma "La Sapienza" and Provincia di Roma.

Azzella MM, 2014. Italian Volcanic lakes: a diversity hotspot and refuge for European charophytes. J. Limnol. 73:502-510.

Azzella MM, Bolpagni R, Oggioni A, 2014. A preliminary evaluation of lake morphometric traits influence on the maximum growing depth of macrophytes. J. Limnol. 73:400-406.

Azzella MM, Ricotta C, Blasi C, 2013. Aquatic macrophyte diversity assessment: Validation of a new sampling method for circular-shaped lakes. Limnologica 43:492-499.

Azzella MM, Rosati L, Iberite M, Bolpagni R, Blasi C, 2014. Changes in aquatic plants in the Italian volcanic-lake system detected using current data and historical records. Aquat. Bot. 112:41-47.

Bolpagni R, Racchetti E, Laini A, 2016a. Fragmentation and groundwater supply as major drivers of algal and plant diversity and relative cover dynamics along a highly modified lowland river. Sci. Total Environ. 568:875-884.

Bolpagni R, Laini A, Azzella MM, 2016b. Short time dynamics of submerged aquatic vegetation diversity and abundance in deep lakes. Appl. Veg. Sci. 19:711-723.

Bornette G, Puijalon S, 2011. Response of aquatic plants to abiotic factors: a review. Aquat. Sci. 73:1-14.

Boschilia SM, Oliveira EF, Thomaz SM, 2008. Do aquatic macrophytes co-occur randomly? An analysis of null models in a tropical floodplain. Oecologia 156:203-214.

Carignan R, Kalff J, 1980. Phosphorus sources for aquatic weeds: water or sediments? Science 207:987-989.

Capers RS, Selsky R, Bugbee GJ, 2010. The relative importance of local conditions and regional processes in structuring aquatic plant communities. Freshwater Biol. 55:952-966.

Carvalho L, Solimini AG, Phillips G, Pietilainen O-P, Moe J, Cardoso AC, Solheim AL, Ott I, Søndergaard M, Tartari G, Rekolainen S, 2009. Site-specific chlorophyll reference conditions for lakes in Northern and Western Europe. Hydrobiologia 633:59-66.

Chappuis E, Gacia E, Ballesteros E, 2014. Environmental factors explaining the distribution and diversity of vascular aquatic macrophytes in a highly heterogeneous Mediterranean region. Aquat. Bot. 113:72-82.

Chmara R, Szmeja J, Ulrich W, 2013. Patterns of abundance and co-occurrence in aquatic plant communities. Ecol. Res. 28:387-395.

Demars BO, Potts JM, Tremolieres M, Thiebaut G, Gougelin N, Nordmann V, 2012. River macrophyte indices: Not the Holy Grail! Freshwater Biol. 57:1745-1759.

Diamond JM, 1975. Assembly of species communities, p. 342-444. In: M.L. Cody and J.M. Diamond (eds.), Ecology and evolution of communities. Harvard University Press.

Duberstein JA, Conner WH, Krauss KW, 2014. Woody vegetation communities of tidal freshwater swamps in South Carolina, Georgia and Florida (US) with comparisons to similar systems in the US and South America. J. Veg. Sci. 25:848-862.

Fu H, Zhong J, Yuan G, Xie P, Guo L, Zhang X, Xu J, Li Z, Li W, Zhang M, Cao T, Ni L, 2014. Trait-based community assembly of aquatic macrophytes along a water depth gradient in a freshwater lake. Freshwater Biol. 59:2462-2471.

Genkai-Kato M, Carpenter SR, 2005. Eutrophication due to phosphorus recycling in relation to lake morphometry, temperature, and macrophytes. Ecology 86:210-219.

Gotelli NJ, 2000. Null model analysis of species co-occurrence patterns. Ecology 81:2606-2621.

Gotelli NJ, Entsminger GL, 2002. EcoSim: null models software for ecology. Ver. 7.0. Acquired Intelligence and Kesey-Bear, Jericho.

Guareschi S, Abellán P, Laini A, Green AJ, Sánchez-Zapata JA, Velasco J, Millán A, 2015. Cross-taxon congruence in wetlands: Assessing the value of waterbirds as surrogates of macroinvertebrate biodiversity in Mediterranean Ramsar sites. Ecol. Indic. 49:204−215.

Jensén S, 1979. Classification of lakes in southern Sweden on the basis of their macrophyte composition by means of multivariate methods. Plant Ecol. 39:129-146.

Keddy PA, 1983. Shoreline vegetation in Axe Lake, Ontario: effects of exposure on zonation patterns. Ecology 64:331-344.

Kirk JTO, 1994. Light and photosynthesis in aquatic ecosystems. University Press, Cambridge: 401 pp.

Kolada A, 2010. The use of aquatic vegetation in lake assessment: testing the sensitivity of macrophyte metrics to anthropogenic pressures and water quality. Hydrobiologia 656:133-147.

Lacoul P, Freedman B, 2006. Environmental influences on aquatic plants in freshwater ecosystems. Environ. Rev. 14:89-136.

Levi EE, Çakiroğlu AY, Bukal T, Odgaard BV, Davidson TA, Jeppensen E, Beklioğlu M, 2014. Similarity between contemporary vegetation and plant remains in the surface sediment in Mediterranean lakes. Freshwater Biol. 59:724-736.

Logue JB, Mouquet N, Peter H, Hillebrand H, Group MW, 2011. Empirical approaches to metacommunities: a review and comparison with theory. TREE 26:482-491.

Lukács BA, Tóthmérész B, Borics G, Várbíró G, Juhász P, Kiss B, Müller Z, G-Tóth L., Erős T, 2015. Macrophyte diversity of lakes in the Pannon Ecoregion (Hungary). Limnologica 53:74-83.

Marchesoni V, 1940. [Il Fitoplancton del Lago di Nemi prima e dopo l'abbassamento del suo livello (1923-1939)].[Article in Italian]. Int. Rev. ges. Hydrobiol. Hydrogr. 40:305-345.

Melzer A, 1999. Aquatic macrophytes as tools for lake management. Hydrobiologia 395:181-190.

Penning WE, Dudley B, Mjelde M, Hellsten S, Hanganu J, Kolada A, van den Berg M, Poikane S, Phillips G, Willby N, 2008a. Using aquatic macrophyte community indices to define the ecological status of European lakes. Aquat. Ecol. 42:253-264.

Penning WE, Mjelde M, Dudley B, Hellsten S, Hanganu J, Kolada A, van den Berg M, Poikane S, Phillips G, Willby N, 2008b. Classifying aquatic macrophytes as indicators of eutrophication in European lakes. Aquat. Ecol. 42:237-251.

Presley JP, Higgins CL, Willing MR, 2010. A comprehensive framework for the evaluation of metacommunity structure. Oikos 119:908-917.

Pulido C, Riera JL, Ballesteros E, Chappuis E, Gacia E, 2015. Predicting aquatic macrophyte occurrence in soft-water oligotrophic lakes (Pyrenees mountain range). J. Limnol. 74:143-154.

R Core Team, 2017. R: A language and environment for statistical computing. R Foundation of Statistical Computing. Vienna, Austria.

Schneider B, Cunha ER, Marchese M, Thomaz SM, 2015. Explanatory variables associated with diversity and composition of aquatic macrophytes in a large subtropical river floodplain. Aquat. Bot. 121:67-75.

Scheffer M, Hosper SH, Meijer ML, Moss B, 1993. Alternative equilibria in shallow lakes. Trends Ecol. E 8:275-279.

Shipley Bvol., Keddy PA, 1987. The individualistic and community-unit concepts as falsifiable hypotheses. Plant Ecol. 69:47-55.

Søndergaard M, Phillips G, Hellsten S, Kolada A, Ecke F, Mäemets H, Mjelde M, Azzella MM, Oggioni A, 2013. Maximum growing depth of submerged macrophytes in European lakes. Hydrobiologia 704:165-177.

Stone L, Roberts A, 1990. The checkerboard score and species distributions. Oecologia 85:74-79.

Ulrich W, Gotelli NJ, 2013. Pattern detection in null model analysis. Oikos 122:2-18.

Vestergaard O, Send-Jensen K, 2000. Alkalinity and trophic state regulate aquatic plant distribution in Danish lakes. Aquat. Bot. 67:85-107.

Wiegleb G, Bröring U, Filetti M, Brux H, Herr W, 2014. Long-term dynamics of macrophyte dominance and growth-form types in two north-west German lowland streams. Freshwater Biol. 59:1012-1025.

Wilson SD, Keddy PA, 1986. Measuring diffuse competition along an environmental gradient: results from a shoreline plant community. Am. Nat. 127:862-869.

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Remote sensing of macrophyte morphological traits: Implications for the management of shallow lakes

Paolo VILLA,[1*] Monica PINARDI,[1,2] Viktor R. TÓTH,[3] Peter D. HUNTER,[4] Rossano BOLPAGNI,[2]
Mariano BRESCIANI[1]

[1]Institute for Electromagnetic Sensing of the Environment, National Research Council, Via Bassini 15, 20133 Milan, Italy; [2]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, V.le Usberti 11/A, 43124 Parma, Italy; [3]Balaton Limnological Institute Centre for Ecological Research, Hungarian Academy of Sciences, Tihany, Hungary; [4]Biological and Environmental Sciences, School of Natural Sciences, University of Stirling, Stirling, United Kingdom
*Corresponding author: villa.p@irea.cnr.it

ABSTRACT

Macrophytes are important elements of freshwater ecosystems, fulfilling a pivotal role in biogeochemical cycles. The synoptic capabilities provided by remote sensing make it a powerful tool for monitoring aquatic vegetation characteristics and the functional status of shallow lake systems in which they occur. The latest generation of airborne and spaceborne imaging sensors can be effectively exploited for mapping morphologically - and physiologically - relevant vegetation features based on their canopy spectral response. The objectives of this study were to calibrate semi-empirical models for mapping macrophyte morphological traits (*i.e.*, fractional cover, leaf area index and above-water biomass) from hyperspectral data, and to investigate the capabilities of remote sensing in supporting macrophyte monitoring and management. We calibrated spectral models using *in situ* reflectance and morphological trait measures and applied them to airborne hyperspectral imaging data, acquired over two shallow European water bodies (Lake Hídvégi, in Hungary, and Mantua lakes system, in Italy) in two key phenological phases. Maps of morphological traits were produced covering a broad range of aquatic plant types (submerged, floating, and emergent), common to temperate and continental regions, with an error level of 5.4% for fractional cover, 0.10 m^2 m^{-2} for leaf area index, and 0.06 kg m^{-2} for above-water biomass. Based on these maps, we discuss how remote sensing could support monitoring strategies and shallow lake management with reference to our two case studies: *i.e.*, by providing insight into spatial and species-wise variability, by assessing nutrient uptake by aquatic plants, and by identifying hotspot areas where invasive species could become a threat to ecosystem functioning and service provision.

Key word: Biomonitoring; fractional cover; LAI; biomass; invasive species; hyperspectral data; APEX.

INTRODUCTION

Macrophytes are important elements of freshwater ecosystems (Jeppesen *et al.*, 1997), performing multiple ecosystem services (*e.g.,* carbon sequestration, habitat provision, nutrient uptake) and fulfilling a pivotal role in the cycling of elements in aquatic ecosystem (*e.g.,* C and gas fluxes; denitrification in sediment) (Wetzel, 1992; Schriver *et al.*, 2005; Bolpagni *et al.*, 2007; Jordan *et al.*, 2011; Boerema *et al.*, 2014; Castaldelli *et al.*, 2015). Under current climate warming scenarios, an alteration in macrophyte ecological status and abundance is predicted in wetlands and shallow lakes (Poff *et al.*, 2002; Dudgeon *et al.*, 2006). In particular, increasing nutrient loading, water consumption and climate change effects are expected to lead to a rapid spread of opportunistic and floating species in macrophyte abundant systems (McKee *et al.*, 2002; Kosten *et al.*, 2011; Bolpagni *et al.*, 2015; Bolpagni and Piotti, 2016). In this context, excessive growth of invasive macrophyte meadows needs to be effectively monitored in order to inform management actions to mitigate negative effects on shallow inland water ecosystems, such as risk of anoxia (Goodwin *et al.*, 2008, Hestir *et al.*, 2008). Furthermore, the multiple anthropogenic uses of water (*e.g.,* water supply, irrigation, fishing, and hydropower) have greatly intensified pressure on freshwater ecosystems (MEA, 2005). This drastic and rapid shift in environmental conditions can have profound effects on macrophyte species resulting in marked changes in the structure and function of ecosystems, increased vulnerability to further perturbation (Steffen *et al.*, 2006; Metzger *et al.*, 2006) and a need for sound, evidence-based ecosystems management. In response, more restrictive chemical and biological monitoring standards for inland waters have been introduced (*e.g.,* the EU Water Framework Directive; European Union, 2000), but the resources available for monitoring programmes, and in particular for *in situ* sampling, are constantly decreasing.

In this context, remote sensing could be a beneficial tool to complement and extend *in situ* measurements, providing frequent, internally-consistent and spatially synoptic observations both for near real-time and retrospective analyses (Adam *et al.*, 2010; Birk and Ecke, 2014). Although many scientific and technological advancements have taken place during recent decades, the potential of remote sensing for ecological applications is still dramatically under-exploited, especially by end-users in charge of environmental monitoring (Bukata, 2005; Schaeffer *et al.*, 2013; Palmer *et al.*, 2015). The biological complexity and high temporal variability of freshwater ecosystems are the main reasons why remote sensing techniques are not yet fully operational in water quality monitoring and in the assessment of aquatic vegetation abundance and phenology. New spaceborne and airborne sensors, either multi- or hyper-spectral, an increasing volume of open and low cost remotely sensed data, and the development of simple straightforward processing approaches are now changing this scene. For instance, the spectral response features of macrophytes have been exploited in freshwater ecosystems for a range of applications (*i.e.,* mapping aquatic plant species and functional groups, Hestir *et al.*, 2008, Hunter *et al.*, 2010; monitoring vegetation status, Bresciani *et al.*, 2009; Villa *et al.*, 2013; assessing plant ecophysiology, Stratoulias *et al.*, 2015; and estimating plant morpho-physiological parameters, Penuelas *et al.*, 1993). In particular, flexible and straightforward approaches based on spectral indices (SIs) have gained scientific and operational recognition for analysing vegetation from remote sensing data. SIs rely on the identification of key spectral wavebands – related to specific physiological and structural characteristics of plants – combined into algebraic indices, which are implemented using empirical or semi-empirical methods to estimate vegetation dynamics and parameters (*e.g.,* vigour or greenness, leaf area index, fractional cover, density, biomass, and the fraction of absorbed photosynthetically active radiation) (Asrar *et al.*, 1989; Goward and Huemmrich, 1992; Penuelas *et al.*, 1993; Gitelson and Merzlyak, 1996; Haboudane *et al.*, 2004). Most of the studies on this topic, and especially on the use of spectral information and SIs to estimate and map vegetation morphological traits, are still based on terrestrial vegetation and agricultural crops (*e.g.*, Rouse *et al.*, 1974; Tucker *et al.*, 1979; Huete, 1988; Gobron *et al.*, 2000; Broge and Leblanc, 2001; Dash and Curran, 2004; Haboudane *et al.*, 2004; Tian *et al.*, 2005; Gitelson *et al.*, 2006; Wu *et al.*, 2009; Hunt *et al.*, 2011; Maccioni *et al.*, 2011).

There is a need to extend the investigation and further test the efficiency of SIs-based models for aquatic plants, which are known to exhibit different spectral and temporal features compared to terrestrial vegetation (Silva *et al.*, 2008; Adam *et al.*, 2010; Villa *et al.*, 2014, 2015). Considering the importance of aquatic vegetation to freshwater biogeochemical processes and the unfavourable conservation status of many inland ecosystems globally, the capabilities of remote sensing for assessing macrophyte characteristics and the functional status of shallow systems dominated by macrophytes are in fact still scarcely exploited or not fully investigated (Ribaudo *et al.*, 2010; Jacobs and Harrison, 2014).

In this study, we focused on filling this gap by demonstrating the great potential of remote sensing for mapping macrophyte morphological traits, by testing and adapting SI-based models that make use of information about canopy spectral features in the visible to near infrared range. To this end, we analysed airborne hyperspectral imaging data acquired over two shallow European water bodies (Lake Hídvégi, in Hungary, and Mantua lakes system, in Italy), hosting abundant and diverse macrophyte communities comprising submerged, floating and emergent species, in two critical phases of their growing season: middle of July and late September, corresponding to the maximum growth peak and the end phase of growth, shortly before the senescence period. The two areas are also representative of two different water management strategies, and represent paradigmatic case studies of macrophyte management: i) Lake Hídvégi is part of the Kis-Balaton Water Protection System, in western Hungary, which serves to retain nutrients and particulate matter carried by the River Zala before it enters Lake Balaton (the largest lake in Central Europe); and ii) the Mantua lakes system, a group of three eutrophic shallow fluvial lakes in Northern Italy, where the abundant vegetation is periodically cut and taken away to minimize the possibility of negative consequences on the ecosystem (*e.g.,* risk of hypoxia, lakes infilling), and promote nutrient removal from the system.

The main objectives of our analysis were: i) to calibrate simple semi-empirical models for estimating and mapping macrophyte morphological traits (fractional cover, leaf area index and above-water biomass) from hyperspectral imaging data over a range of plant types, and ii) to investigate and demonstrate the capabilities of remote sensing data and products in supporting monitoring strategies and shallow lakes management.

METHODS

Study sites

The study sites were two temperate European shallow lakes with connected wetlands and abundant macrophyte cover, mainly consisting of floating and emergent species: Lake Hídvégi (Hungary) and Mantua lakes system (Italy).

Lake Hídvégi (46°38’ N, 17°08’ E; Fig. 1) is part of the Kis-Balaton Water Protection System (~81 km^2; max depth 2 m), a semi-artificial wetland area located immediately upstream of the main inflow into Lake Balaton from the River Zala (which supplies 45% of the water input and 35-40% of nutrient load to Lake Balaton;

Kovács *et al.*, 2010). Kis-Balaton (or “Small Balaton”) was originally the westernmost basin of the lake. Due to intense sediment deposition from the Zala, Kis-Balaton was the shallowest basin, largely dominated by *Phragmites australis* (Cav.) Trin. ex Steud., and other aquatic macrophytes, but with substantial open water areas. Through time, Kis-Balaton was partially drained and later disconnected from Lake Balaton. In 1979, the Kis-Balaton Water Protection System (KBWPS) was built to facilitate sedimentation and nutrient removal from the River Zala (Istvánovics *et al.*, 2007). The KBWPS is composed of two areas: i) an open-water reservoir, Lake Hídvégi (~18 km^2, mean retention time 40 days; water inflow 175 x 10^6 m^3 y^{-1}; Fig. 1) with the main function of retaining inorganic nutrients and total suspended solids carried by the Zala; ii) a marshland with 95% helophyte coverage (*P. australis* and *Carex* ssp. meadows; Tátrai *et al.*, 2000), that plays an important role in the nutrient filtration process as shown by Dömötörfy *et al.* (2003). At the local scale, the shallow eutrophic Lake Hídvégi is colonized by floating-leaved macrophytes, with dense *Trapa natans* L. beds, as well as *Nuphar lutea* (L.) Sm., and *Nymphaea alba* L. populations (Dömötörfy *et al.*, 2003; Dinka *et al.*, 2008), and by submerged species in some littoral zones (*Ceratophyllum demersum* L., and *Najas marina* L.).

The Mantua lakes system is a fluvial system (45°10’ N, 10°47’ E; Fig. 1) composed of three eutrophic shallow lakes and two protected wetland areas, surrounding the city of Mantua, in Northern Italy. The Vasarone dam, built in 1190 A.D. along the River Mincio, and other downstream weirs determined the formation of the three Mantua Lakes (~6.1 km^2; mean depth ~3.5 m) and of the “Valli del Mincio” (~12.7 km^2) and “Vallazza” (~5.0 km^2) wetlands, located upstream and downstream, respectively. Common reed dominates the shorelines and a large portion (40% of total surface) of the “Valli del Mincio” wetland (Tomaselli *et al.*, 2000), and dense meadows of *N. lutea*, *T. natans*, and *Nelumbo nucifera* Gaertn. are present in littoral and open water areas during the vegetative period (April-October). Less frequent but still present in localized littoral areas are *N. alba* and *Ludwigia hexapetala* (Hook. & Arn.) Zardini, H.Y. Gu & P.H. Raven stands. Free-floating (*e.g., Azolla filiculoides* Lam. and *Lemna* spp.) and submerged macrophytes (mainly *C. demersum*) can seasonally colonize littoral zones and areas where the water flow is low. In the last few decades, the progressive eutrophication of the fluvial lake system has favoured a marked proliferation of opportunistic primary producers (phytoplankton and emerging or floating macrophytes) with effects also on the benthic system (*e.g.,* lake infilling,

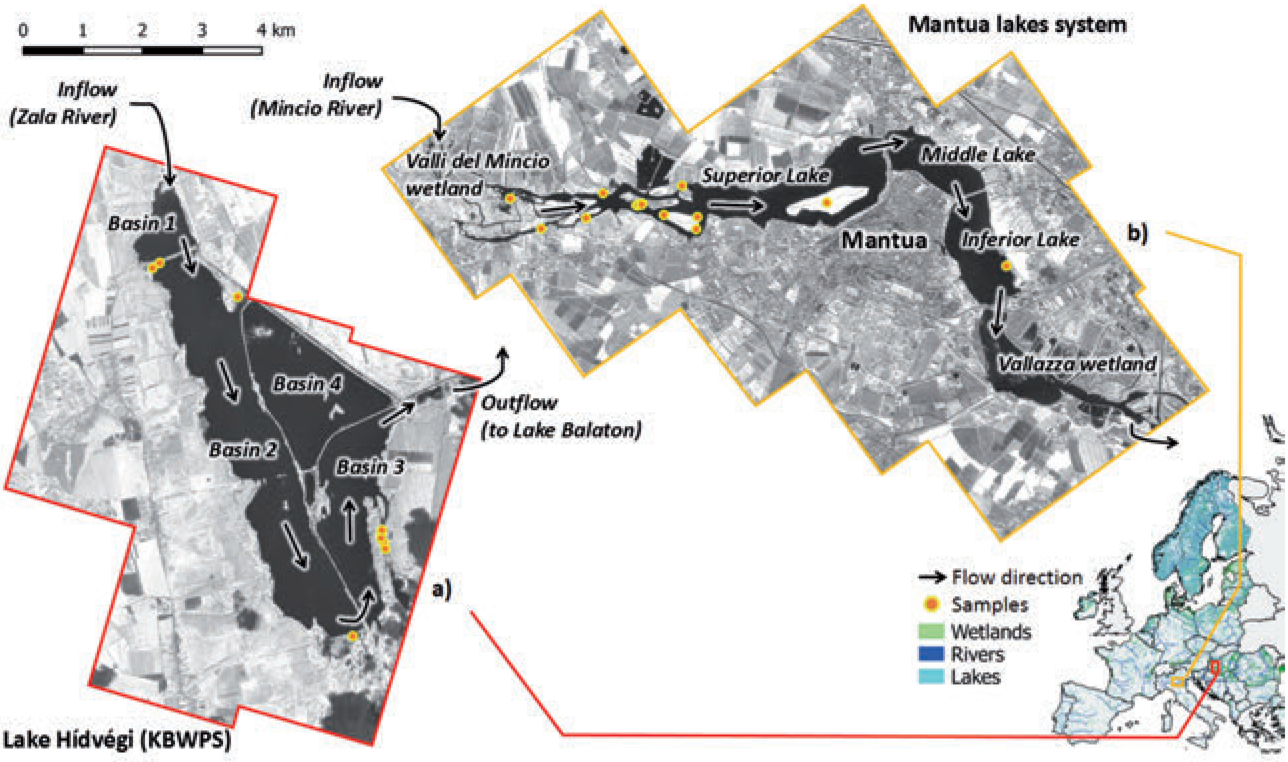

Fig. 1. Study sites and location of macrophyte plot sampled. a) Lake Hídvégi. b) Mantua lakes system.

hypoxia) (Pinardi *et al.*, 2011). In particular, the development of invasive meadows of emerging and floating-leaved macrophytes (*e.g., N. nucifera* and *T. natans*) in periods of high water temperature, nutrient availability and stable weather conditions, has required management intervention by local authorities to control plant growth and to preserve ecosystem services, including water utility (Pinardi *et al.*, 2011, 2015).

In situ and laboratory data

In situ data were collected during boat-based surveys in Lake Hídvégi (16-18 July 2014) and Mantua lakes system (26 June and 23 September 2014, 16 and 31 July 2015), over a total of 26 plots covering 7 species (*C. demersum* - CD, *N. marina* - NM, *A. filiculoides* - AF, *N. lutea* - NL, *N. alba* - NA, *T. natans* - TN, *N. nucifera* - NN). Each plot represents an area of minimum 10 × 10 m homogeneous vegetation cover around the sampling locations, represented by a dominant species. Tab. 1 lists the main characteristics and parameters collected from each sampled macrophyte plot.

In situ macrophyte canopy spectral response data were acquired using portable high-resolution spectroradiometers (ASD FieldSpec Pro FR and Spectral Evolution SR3500). Ten replicate measurements were acquired *per* plot from adaxial surfaces at nadir. Measurement height above the plot was approximately 50 cm and the instrument field of view was 25° (*i.e.,* sampling an area of 20-25 cm diameter). Reflectance spectra were obtained by calculating the ratio between radiance recorded from each sample and radiance acquired for a white reflectance standard (Spectralon white

Tab. 1. Summary of the *in situ* data on macrophyte abundance and morphological traits collected at the two study sites: fractional cover (fC), leaf area index (LAI) and biomass (Biom_{dry}).

Study site	Sample date	Sample ID	Species	Functional group	Coordinates (Lat, Lon)	Morphological traits
Lake Hídvégi (KBWPS)						
	16 Jul 2014	CD1	*Ceratophyllum demersum*	Submerged	46.6154N, 17.1678E	fC* (LAI*), Biom_{dry}*
	16 Jul 2014	NA1	*Nymphaea alba*	Floating-leaved	46.6146N, 17.1672E	fC, LAI, Biom_{dry}
	16 Jul 2014	NA2	*Nymphaea alba*	Floating-leaved	46.6141N, 17.1676E	fC, LAI, Biom_{dry}
	16 Jul 2014	NL1	*Nuphar lutea*	Floating-leaved	46.6149N, 17.1674E	fC, LAI, Biom_{dry}
	18 Jul 2014	NM1	*Najas marina*	Submerged	46.6589N, 17.1238E	fC* (LAI*), Biom_{dry}*
	16 Jul 2014	TN1	*Trapa natans*	Floating	46.5998N, 17.1593E	fC, LAI, Biom_{dry}
	16 Jul 2014	TN2	*Trapa natans*	Floating	46.6146N, 17.1671E	fC, LAI, Biom_{dry}
	18 Jul 2014	TN3	*Trapa natans*	Floating	46.6525N, 17.1425E	fC, LAI, Biom_{dry}
	18 Jul 2014	TN4	*Trapa natans*	Floating	46.6586N, 17.1235E	fC, LAI, Biom_{dry}
Mantua Lakes system						
	26 Jun 2014	AF1	*Azolla filiculoides*	Free-floating	45.1589N, 10.7235E	fC, LAI, Biom_{dry}
	23 Sep 2014	CD1	*Ceratophyllum demersum*	Submerged	45.1636N, 10.7440E	fC* (LAI*), Biom_{dry}*
	26 Jun 2014	NA1	*Nymphaea alba*	Floating-leaved	45.1572N, 10.7138E	fC, LAI, Biom_{dry}
	26 Jun 2014	NL1	*Nuphar lutea*	Floating-leaved	45.1619N, 10.7074E	fC, LAI, Biom_{dry}
	23 Sep 2014	NL2	*Nuphar lutea*	Floating-leaved	45.1608N, 10.7342E	fC, LAI, Biom_{dry}
	16 Jul 2015	NL3	*Nuphar lutea*	Floating-leaved	45.1608N, 10.7343E	fC, LAI, Biom_{dry}
	31 Jul 2015	NL4	*Nuphar lutea*	Floating-leaved	45.1608N, 10.7342E	fC, LAI, Biom_{dry}
	26 Jun 2014	NN1	*Nelumbo nucifera*	Emergent	45.1626N, 10.7270E	fC, LAI, Biom_{dry}
	23 Sep 2014	NN2	*Nelumbo nucifera*	Emergent	45.1610N, 10.7748E	fC, LAI, Biom_{dry}
	16 Jul 2015	NN3	*Nelumbo nucifera*	Emergent	45.1593N, 10.7399E	fC, LAI, Biom_{dry}
	16 Jul 2015	NN4	*Nelumbo nucifera*	Emergent	45.1569N, 10.7472E	fC, LAI, Biom_{dry}
	16 Jul 2015	NN5	*Nelumbo nucifera*	Emergent	45.1590N, 10.7475E	fC, LAI, Biom_{dry}
	26 Jun 2014	TN1	*Trapa natans*	Floating	45.1609N, 10.7351E	fC, LAI, Biom_{dry}
	23 Sep 2014	TN2	*Trapa natans*	Floating	45.1608N, 10.7353E	fC, LAI, Biom_{dry}
	23 Sep 2014	TN3	*Trapa natans*	Floating	45.1510N, 10.8130E	fC, LAI, Biom_{dry}
	16 Jul 2015	TN4	*Trapa natans*	Floating	45.1607N, 10.7354E	fC, LAI, Biom_{dry}
	31 Jul 2015	TN5	*Trapa natans*	Floating	45.1608N, 10.7356E	fC, LAI, Biom_{dry}

fC, LAI* and* Biom_{dry}** for submerged species refer to the part of plants reaching the water surface.*

panel with near Lambertian properties). Spectral measurements with excessive environmental noise (values exceeding ±2 standard deviation from the mean) due to atmospheric variation or presence of water were excluded from the analysis and the mean reflectance spectra *per* macrophyte plot was calculated. A variation on this protocol was used to collect data from *C. demersum* and *N. marina* stands whereby spectra were collected for the above surface water portion of the plant at height of 20 cm above the plot; the plot mean was then computed without filtering for noise. During the sampling campaigns, georeferenced photos (coordinates were acquired using GPS; Trimble GeoXM) from nadir position (at approximately 1 m above canopy) of macrophyte plots were acquired with a compact RGB camera (Sony DSC-HX60).

Macrophyte fractional cover (fC), *i.e.*, the percentage of a horizontal surface occupied by vegetation as seen in nadiral direction, were derived from *in situ* collected georeferenced photos. During the fieldwork, for each macrophyte plot sampled we took three photos from nadir, approximately 1 m above the canopy, framing a square plot of 1 m x 1 m. Pixels of each photo were classified based on their RGB values in five different clusters by applying an ISODATA algorithm (Tou and Gonzalez, 1974), and each of five clusters were labelled as vegetation or water. fC was finally recorded as the average percentage of plot area (1 m^2) covered by vegetation over the three photos of each macrophyte sampling site. For submerged species (CD and NM), only the portion of plant canopy reaching the water surface was considered.

Macrophyte leaf area index (LAI, m^2 m^{-2}) was derived from the same photos used for fractional cover. For each nadiral image, the areal size of each leaf (considering the overlapping of multiple leaves) falling within the framed square plot was calculated by manual digitization. This method directly returns measures close to the actual LAI for floating and floating-leaved species (*e.g., T. natans*, *N. lutea*), the leaves of which lie on the water surface, while it underestimates the actual LAI for species with emerging leaves (*e.g., N. nucifera*). For this species in fact, it is not possible to directly measure from canopy nadiral takes the leaf surface, but only the size of its projection onto the horizontal plane. However, given the slightly concave shape and the almost horizontal arrangement of the circular peltate leaves of *N. nucifera*, the actual LAI underestimation is considered to be limited for the scope of this study. For submerged species (CD and NM), only the portion of plant canopy reaching the water surface was considered, thus resulting in LAI scores which are the same as fractional cover. LAI for each sample was calculated as the mean total leaf area falling within the 1 m^2 plot over the three photos of each macrophyte sampling site.

For measuring macrophyte biomass different approaches were used, depending on the macrophyte type. The number of leaves was counted from photos taken over a 1 m^2 plot randomly placed (3 replicates) over the floating and emergent macrophytes (NA, NL, and NN) beds. Intact leaves for NA, NL, and NN were collected (n=3 *per* plot) and brought back to the laboratory for biomass estimation. The number of *T. natans* rosettes was determined from photos taken over a 1.0 m^2 plot and intact rosettes were collected, as previously described for floating plants. To measure biomass of submerged and free-floating species (CD and AF, respectively), plants were collected inside a 0.09 m^2 and 19.6 cm^2 frame, respectively, randomly placed (3 replicates) over the plant beds. For all species, fresh biomass samples were dried in oven at 70°C and then weighed on a precision balance. Data presented as areal density of above-water biomass (Biom$_{dry}$, kg m^{-2}) was obtained by multiplying leaf (NA, NL, NN) or plant (TN) dry weight for the number of leaves or plants *per* square meter. AF biomass (kg m^{-2}) was obtained dividing the dry weight by the sampling frame area (19.6 cm^2) and multiplying by 10,000 cm^2 to convert the data to mass *per* m^2. For submerged species, above-water biomass was calculated by assuming that approximately 10% of total plant biomass is reaching the water surface in peak of growth conditions (such as the ones observed on sampling dates). CD and NM total biomass (kg m^{-2}) was obtained dividing the dry weight by the area (900 cm^2) of the sampling frame used, and multiplying by 10,000 cm^2 to convert the data to mass *per* m^2. Above-water biomass was finally calculated as 10% of total biomass sampled. After weighing, selected leaves were ground to a powder and analysed according to Aspila *et al*. (1976) for determining total phosphorus (P) content.

In addition to morphological trait data, in the Mantua lakes system we recorded georeferenced field observations of the presence of *L. hexapetala* and *Lemna* spp.

Airborne hyperspectral data

Airborne hyperspectral data were acquired from the Airborne Prism Experiment (APEX) imager (Schaepman *et al.*, 2015). APEX images were acquired on 19 July 2014 over Lake Hídvégi (three flight lines, 11:50-12:10 local time, orientation ~30° azimuth) and on 27 September 2014 over the Mantua lakes system (five flight lines, 13:30-14:00 local time, orientation ~50° azimuth), with ground spatial resolution of 5 m. Additional APEX data acquired over Mantua lakes system on 21 September 2011 (five flight lines, 16:00-16:30 local time, orientation ~50° azimuth) were used for comparison with 2014 data.

The APEX data were radiometrically calibrated by the APEX Calibration Home Base (CHB) hosted at DLR Oberpfaffenhofen, Germany (Gege *et al.*, 2009), and georeferenced based on sensor's GPS/IMU, including boresight correction. The atmospheric correction of the data was performed by with the MODTRAN4 radiative transfer code following the algorithms optimized for water targets (De Haan *et al.*, 1991) and taking into account smile effects. For the atmospheric parameters, *in situ* sunphotometer observations (Microtops II on Lake Hídvégi, EKO MS-120 on the Mantua lakes system) simultaneous with APEX acquisitions were used. The reflectance spectra were smoothed using EOSap_Smoothing IDL routine (https://sourceforge.net/p/enviidlcodelibr/wiki/EOSap_ Smoothing/).

Only the visible and near-infrared bands (426-906 nm) were retained for further processing, resulting in a data cube of 98 spectral bands with 3-10 nm spectral resolution.

Estimation of macrophyte morphological traits

Macrophyte canopy morphological traits (MTs): fractional cover (fC), leaf area index (LAI) and above-water biomass (Biom$_{dry}$) were estimated from spectral data through semi-empirical regression modelling based on spectral vegetation indices. A range of 27 spectral indices focusing on the visible to near infrared spectral range (420-800 nm), developed and documented in scientific literature (related references for each index are cited in Tab. 2) as sensitive to vegetation density and morphology, was tested in order to identify those with potential for retrieval of information on macrophyte morphological traits investigated.

High resolution macrophyte canopy reflectance data collected *in situ* were spectrally resampled to match APEX visible and near-infrared bands (in the range 420-800 nm), and then used to derive the narrowband SIs listed in Tab. 2, corresponding to each plot sampled during fieldwork. The Pearson (r) correlation coefficient between morphological trait scores and calculated SIs was used as indicator of goodness of fit to inform the selection of the five best SIs for estimating macrophyte fC, LAI and Biom$_{dry}$ through linear regression models (*i.e.,* the SIs scoring highest r values for each morphological trait). For each parameter, linear regression models were then fitted using the five best performing SIs from resampled *in situ* canopy spectra. The resulting models were also tested by using as input the spectral reflectance data derived directly from APEX pixels corresponding to macrophyte plots sampled within some days from the APEX flights (3-4 days, 14 plots). The APEX spectra were extracted from a 3 x 3 windows centred around the location of each sampled plot on georeferenced images, following a maximum vegetated pixel approach. The approach consists in selecting among the 9 pixels the one with highest vegetation coverage, *i.e.,* the pixel with highest reflectance in the NIR range (780 nm), and retaining the corresponding full reflectance spectra to be compared with macrophyte morphological traits measured. This approach allows to partially correct for the fact that *in situ* sampling can be frequently biased towards higher vegetation density patches, even within a relatively homogeneous area, and was therefore preferred to taking the mean reflectance over 3 x 3 window.

Model errors were assessed in terms of Mean Absolute Error (MAE) and relative Root Mean Square Error (rRMSE):

$$\mathrm{MAE} = \frac{1}{N}\sum_{i=1}^{N}|f_i - y_i| \qquad \text{(eq. 1)}$$

$$\mathrm{rRMSE} = \frac{\sqrt{\frac{\sum_{i=1}^{N}(f_i-y_i)^2}{N}}}{\sum_{i=1}^{N} y_i} \qquad \text{(eq. 2)}$$

where f_i is the estimated parameter, y_i the parameter measured *in situ*, N the number of observations. Among the linear regression models calculated from five best *in situ* SIs, for each morphological trait we finally selected the linear model which scored lower estimation error (MAE and rRMSE) when using APEX pixel spectra. The three retained models were applied to the entire APEX image cubes for producing maps of macrophyte fC, LAI, and Biom$_{dry}$ of Mantua lakes system and Lake Hídvégi areas at the time of APEX data acquisition.

Regression line slope, P-value (*F*-test), and correlation coefficient (Pearson's r) between MT measured *in situ* and estimated with best performing semi-empirical linear model applied to APEX data were calculated in order to assess and quantify the macrophyte morphological trait mapping performance over the two study areas.

RESULTS

Morphological traits modelling using spectral indices

For each macrophyte morphological trait investigated, the best performing spectral proxy was selected among the SIs tested (Tab. 2). Tab. 3 summarizes the results of SIs performance assessment using reflectance spectra derived from the *in situ* and APEX data respectively. The error level of atmospherically corrected APEX data was quantified as lower than 5% reflectance over macrophyte canopy across the 420-800 nm spectral range (see Supplementary Fig. 1 for details), with some deviations outside the 5% error margin only for some samples (*i.e.*, *N. lutea* in Lake Hídvégi, and *N. nucifera* in Mantua lakes system).

Macrophyte canopy fractional cover (fC) was estimated with high consistency (r>0.84) and low error (MAE<6.6%) using four different background adjusted SIs with VIS-NIR bands (GSAVI, SAVI, EVI, and WAVI), as well as using RDVI. GSAVI scored the highest correlation (r=0.88) and lowest estimation error (MAE=5.4%, rRMSE=0.10, using APEX spectra) among these indices, and was selected for estimating fC through linear regression, using equation 3:

$$\text{fC (\%)} = 105.0(\text{GSAVI}) + 23.3 \qquad \text{(eq. 3)}$$

Range: [0–100%]

Similarly to fC, macrophyte canopy LAI was consistently (r>0.87) and accurately (MAE<0.16 m^2 m^{-2}) estimated by three background enhanced VIS-NIR SIs (EVI, GSAVI and WAVI), and by RDVI. Comparable performance was achieved with $MCARI_{705}$, which includes spec-

Tab. 2. Spectral indices sensitive to vegetation features tested in the study.

Name	Acronym	Formula	Spectral range, type	Reference
Canopy Chlorophyll Content Index	CCCI	$\dfrac{\frac{\rho780-\rho710}{\rho780+\rho710}}{\frac{\rho780-\rho670}{\rho780+\rho670}}$	RE-NIR	Barnes *et al.*, 2000
Chlorophyll Green	Chl_{green}	$\dfrac{\rho550}{\rho780}$	VIS-NIR	Gitelson *et al.*, 2006
Chlorophyll Index Green	CI_{green}	$\dfrac{\rho780}{\rho550}-1$	VIS-NIR	Hunt *et al.*, 2011
Chlorophyll Index RedEdge	CIre	$\dfrac{\rho780}{\rho710}-1$	RE-NIR	Hunt *et al.*, 2011
Chlorophyll Index RedEdge 750	$CIre_{750}$	$\dfrac{\rho750}{\rho710}-1$	RE-NIR	Wu *et al.*, 2009
Chlorophyll vegetation index	CVI	$\rho780\dfrac{\rho670}{\rho550^2}$	VIS-NIR	Hunt *et al.*, 2011
Enhanced Vegetation Index	EVI	$2\dfrac{\rho780-\rho670}{\rho780+6\rho670-7.5\rho470+1}$	VIS-NIR, background adjusted	Huete *et al.*, 2002
Green Leaf Index	GLI	$\dfrac{2\rho550-\rho670-\rho550}{2\rho550+\rho670+\rho550}$	VIS	Gobron *et al.*, 2000
Green Normalized Difference Vegetation Index	GNDVI	$\dfrac{\rho780-\rho550}{\rho780+\rho550}$	VIS-NIR	Gitelson and Merzlyak, 1994
Green Soil Adjusted Vegetation Index	GSAVI	$1.5\dfrac{\rho780-\rho550}{\rho780+\rho550+0.5}$	VIS-NIR, background adjusted	Tian *et al.*, 2005
Maccioni	Maccioni	$\dfrac{\rho780-\rho710}{\rho780+\rho680}$	VIS-RE-NIR	Maccioni *et al.*, 2001
Modified Chlorophyll Absorption in Reflectance Index	MCARI	$[(\rho700-\rho670)-0.2(\rho700-\rho550)]\dfrac{\rho700}{\rho670}$	VIS-RE	Haboudane *et al.*, 2004
Modified Chlorophyll Absorption in Reflectance Index 1	MCARI1	$1.2[2.5(\rho800-\rho670)-1.3(\rho800-\rho550)]$	VIS-NIR, background adjusted	Haboudane *et al.*, 2004
Modified Chlorophyll Absorption in Reflectance Index 2	MCARI2	$1.5\dfrac{2.5(\rho800-\rho670)-1.3(\rho800-\rho550)}{\sqrt{(2\rho800+1)^2-6\rho800+5\sqrt{\rho670}-0.5}}$	VIS-NIR, background adjusted	Haboudane *et al.*, 2004
Modified Chlorophyll Absorption in Reflectance Index 705,750	$MCARI_{705}$	$[(\rho750-\rho705)-0.2(\rho750-\rho550)]\dfrac{\rho750}{\rho705}$	VIS-RE-NIR	Wu *et al.*, 2009
MERIS Terrestrial Chlorophyll Index	MTCI	$\dfrac{\rho754-\rho709}{\rho709+\rho681}$	VIS-RE-NIR	Dash and Curran, 2004
Modified Triangular Vegetation Index 1	MTVI1	$1.2(\rho800-\rho550)-2.5(\rho670-\rho550)$	VIS-NIR	Haboudane *et al.*, 2004
Modified Triangular Vegetation Index 2	MTVI2	$1.5\dfrac{1.2(\rho800-\rho550)-2.5(\rho670-\rho550)}{\sqrt{(2\rho800+1)^2-6\rho800+5\sqrt{\rho670}-0.5}}$	VIS-NIR, background adjusted	Haboudane *et al.*, 2004
Normalized Difference Aquatic Vegetation Index	NDAVI	$\dfrac{\rho780-\rho470}{\rho780+\rho470}$	VIS-NIR	Villa *et al.*, 2014
Normalized Difference RedEdge Index	NDRE	$\dfrac{\rho780-\rho710}{\rho780+\rho710}$	RE-NIR	Barnes *et al.*, 2000
Normalized Difference Vegetation Index	NDVI	$\dfrac{\rho780-\rho670}{\rho780+\rho670}$	VIS-NIR	Rouse *et al.*, 1974
Renormalized Difference Vegetation Index	RDVI	$\dfrac{\rho800-\rho670}{\sqrt{\rho800+\rho670}}$	VIS-NIR	Chen, 1996
Soil Adjusted Vegetation Index	SAVI	$1.5\dfrac{\rho780-\rho670}{\rho780+\rho670+0.5}$	VIS-NIR, background adjusted	Huete, 1988
Simple Ratio	SR	$\dfrac{\rho780}{\rho670}$	VIS-NIR	Tucker *et al*, 1979
Transformed Normalized Difference Vegetation Index	TNDVI	$\sqrt{\dfrac{\rho780-\rho670}{\rho780+\rho670}+0.5}$	VIS-NIR	Bannari *et al.*, 1995
Triangular Vegetation Index	TVI	$0.5[120(\rho750-\rho550)-200(\rho670-\rho550)]$	VIS-NIR	Broge and Leblanc, 2001
Water Adjusted Vegetation Index	WAVI	$1.5\dfrac{\rho780-\rho470}{\rho780+\rho470+0.5}$	VIS-NIR, background adjusted	Villa *et al.*, 2014

tral information in the red edge range (around 705 nm). Highest correlation (r=0.91) and lowest error (MAE=0.10 m^2 m^{-2}, rRMSE=0.18, using APEX spectra) were scored by EVI, which was selected for estimating LAI using the linear regression, using equation 4:

LAI (m^2 m^{-2}) = 1.566(EVI) + 0.057 (eq. 4)
Range: [0.0-1.8 m^2 m^{-2}]

Above-water biomass ($Biom_{dry}$) was found to be better correlated with SIs that include spectral bands centred in the red edge range (around 705-710 nm), and the five best performing indices for this parameter were MTCI, $CIre_{750}$, $MCARI_{705}$, CIre, and Maccioni (r>0.82, MAE 0.08 kg m^{-2}). All these SIs showed a strong saturation effect with increasing biomass and a severe loss of sensitivity for $Biom_{dry}$ values higher than 0.5 kg m^{-2}. For this reason, the semi-empirical linear model for estimating $Biom_{dry}$ was calibrated using samples with biomass measured *in situ* not exceeding this threshold, *i.e.,* excluding two *T. natans* samples collected in Mantua lakes system site during 2015. Among the best SIs, CIre scored the highest correlation (r=0.83) and lowest estimation error using APEX spectra (MAE=0.06 kg m^{-2}, rRMSE=0.42), and was selected for estimating above-water biomass by linear regression, using equation 5:

$Biom_{dry}$ (kg m^{-2}) = 0.147(CIre) – 0.007 (eq. 5)
Range: [0.0–0.5 kg m^{-2}]

Macrophyte morphological trait maps

Maps of canopy morphological traits were produced by applying the semi-empirical models described by equation 3 (fC), equation 4 (LAI) and equation 5 ($Biom_{dry}$) to APEX images: on 19 July 2014 for Lake Hídvégi site, and on 27 September 2014 and 21 September 2011 for Mantua lakes system. For both study sites, morphological traits maps were produced only for the areas covered by floating and emergent macrophytes, which are isolated from other vegetation and different land cover types by using a binary raster mask produced by thresholding aquatic vegetation index scores (WAVI>0) for APEX image pixels falling within the water body area delineated by official cartography.

Figs. 2 and 3, respectively, show the macrophyte fractional cover map of Lake Hídvégi (19 July 2014) and macrophyte above-water biomass of Mantua lakes system (27 September 2014) produced by applying the calibrated models to APEX data. Selected macrophyte beds (12 for Lake Hídvégi, 18 for Mantua) are delineated and highlighted in yellow over Figs. 2 and 3, comprising those surveyed during 2014 field campaigns (8 for Lake Hídvégi, 9 for Mantua) and some additional stands covered by the most relevant plant species present.

Macrophyte morphological traits mapping performance (Fig. 4) using APEX data was tested with respect to *in situ* measurements taken near contemporaneously (within 4 days) to the flights over both study sites (n=14).

Tab. 3. Selection of best performing linear semi-empirical regression model macrophyte morphological traits using spectral reflectance data (best performing Spectral Index for each parameter using APEX data as input is highlighted in bold).

		Calibration (*in situ*) *n*=26 (fC, LAI) *n*=24 ($Biom_{dry}$)			Testing (APEX) *n*=14		
Morphological trait	**SI**	**r**	**MAE**	**rRMSE**	**r**	**MAE**	**rRMSE**
fC (%)	**GSAVI**	**0.879**	**5.7%**	**0.09**	**0.938**	**5.4%**	**0.10**
	SAVI	0.861	6.3%	0.10	0.925	6.1%	0.11
	RDVI	0.858	6.5%	0.10	0.932	7.0%	0.12
	EVI	**0.852**	**6.2%**	**0.11**	**0.902**	**5.6%**	**0.10**
	WAVI	0.845	6.6%	0.11	0.922	6.6%	0.12
LAI (m^2 m^{-2})	EVI	0.912	0.10	0.13	0.895	0.10	0.18
	RDVI	0.907	0.11	0.14	0.919	0.16	0.25
	GSAVI	0.905	0.12	0.15	0.915	0.14	0.23
	$MCARI_{705}$	0.901	0.12	0.15	0.952	0.14	0.23
	WAVI	0.868	0.13	0.17	0.879	0.16	0.28
$Biom_{dry}$ (kg m^{-2})	MTCI	0.868	0.04	0.26	0.790	0.08	0.53
	$CIre_{750}$	0.836	0.04	0.29	0.777	0.07	0.44
	$MCARI_{705}$	0.830	0.05	0.32	0.780	0.07	0.49
	CIre	**0.827**	**0.05**	**0.29**	**0.777**	**0.06**	**0.42**
	Maccioni	0.817	0.04	0.29	0.797	0.07	0.47

Results showed the good reliability of fC (r=0.94) between estimated and measured scores (Fig. 4a) and LAI (r=0.88) maps, although a tendency towards underestimation for high LAI values (*N. nucifera* sample plot) was observed (Fig. 4b). Biomass estimates (Fig. 4c) showed acceptable results (r=0.55 between estimated and measured scores), but were again biased towards underestimation (regression slope=0.72). All morphological traits estimated were statistically significant with P-value (*F*-test) lower than 0.0011.

Spatial and species-dependant variability

Morphological trait statistical descriptors were extracted in correspondence of selected macrophyte beds (highlighted in Figs. 2 and 3) from Lake Hídvégi and Mantua lakes system maps derived from APEX data for 2014. From these beds, an overall representation of both spatial and species-dependant variability can be derived for the main aquatic plant communities in our study sites. Fig. 5 shows the box plots (5, 25, 50, 75, and 95 percentile) of fractional cover, LAI and above-water biomass for all selected beds (Figs. 2 and 3).

For Lake Hídvégi, the 12 macrophyte stands are divided into two groups, composed by submerged and floating-leaved species. Taking into account only the plant parts reaching the water surface, MTs of submerged plants (CD and NM) showed mean values ($fC<30\%$, $LAI<0.19\ m^2\ m^{-2}$ and $Biom_{dry}<0.06\ kg\ m^{-2}$) lower than those derived for floating-leaved species (Fig. 5 a,c,e). Among all *T. natans*

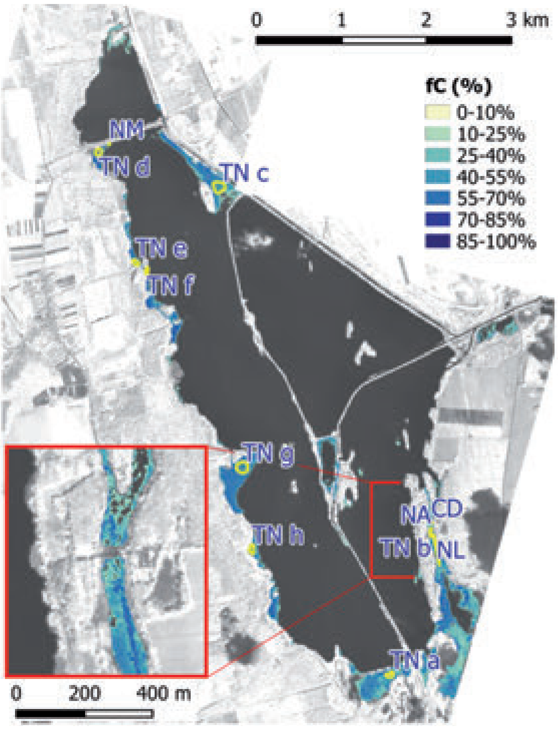

Fig. 2. Map of macrophyte fractional cover for Lake Hídvégi (19 July 2014) derived from APEX data. A discrete color legend is used for visualization of the continuous value fC map. Macrophyte beds over which the discussion focused are highlighted in the figure with their identifier (yellow layers). CD, *Ceratophyllum demersum*; NM, *Najas marina*; TN, *Trapa natans*; NA, *Nymphaea alba*; NL, *Nuphar lutea*.

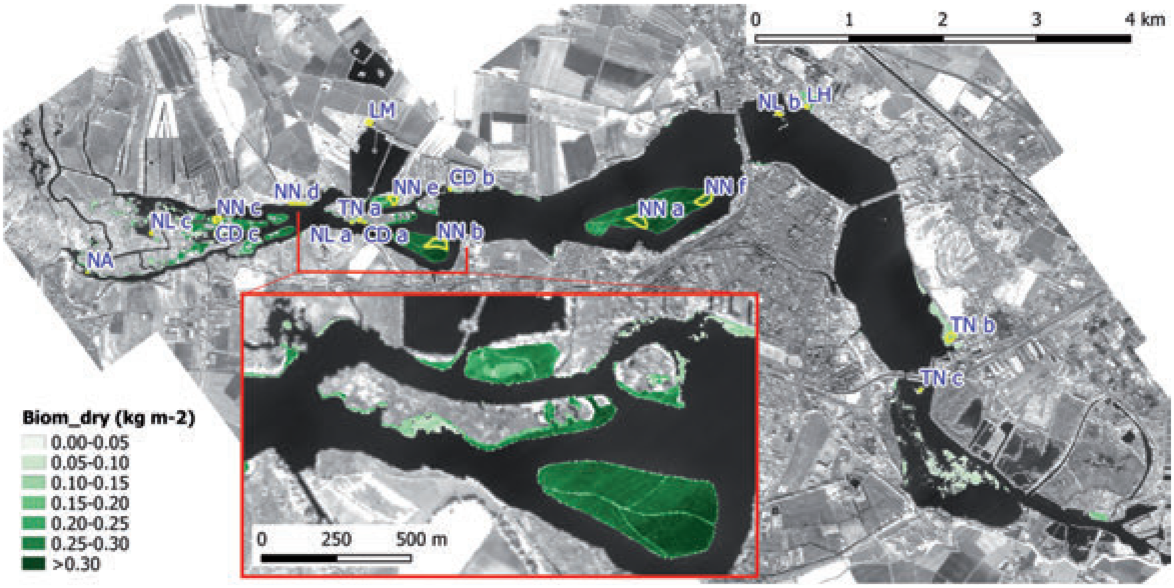

Fig. 3. Map of macrophyte above-water biomass for Mantua lakes system (27 September 2014) derived from APEX data. A discrete color legend is used for visualization of the continuous value Biomdry map. Macrophyte beds over which the discussion focused are highlighted in the figure with their identifier (yellow layers). CD, *Ceratophyllum demersum*; TN, *Trapa natans*; NA, *Nymphaea alba*; NL, *Nuphar lutea*; NN, *Nelumbo nucifera*; LM, *Lemna* spp.; LH, *Ludwigia hexapetala*.

beds, TNb stand, located within a narrow water channel in the eastern portion of Basin 3 (Fig. 1) in the Lake Hídvégi (water depth 0.9-1.1 m under macrophyte beds), had significantly lower MTs mean values (fC=49%, LAI=0.57 m^2 m^{-2} and Biom$_{dry}$=0.11 kg m^{-2}). TNb morphological traits were statistically different (non-parametric Dunn's test, 99% confidence level) from any other TN beds (fC=68±4%, LAI=0.89±0.08 m^2 m^{-2}, and Biom$_{dry}$=0.15±0.02 kg m^{-2}). Instead, *T. natans* beds were located in Basin 2 (water depth 0.50–0.65 m under macrophyte beds) and in particular TNd-h were along the western side of this sub-basin showed similar traits (see Fig. 2). NA and NL beds located in the Basin 3 presented MTs mean values similar to that reported for TNd-h beds (Fig. 5 a,c,e).

From the plots of the Mantua lakes system (Fig. 5 b,d,f), the 18 macrophyte stands can be grouped into four groups: i) submerged (CDa-c); ii) free-floating and floating-leaved (LM, TNa-c, NA, NLa-c); iii) emergent rhizophyte (NNa-f); and iv) floating mat-forming (LH) plants. In September 2014, *A. filiculoides* was not present in the Mantua lakes system due to weather conditions (high precipitation and water discharge) that were unfavourable to plant establishment and persistence and therefore we will not discuss this macrophyte functional group. All MTs values followed a gradient: submerged < floating < emergent/mat-forming (Fig. 5 b,d,f). The high vigour and areal cover of the *N. nucifera* beds, due to the presence of floating-leaved and emergent leaves, were reflected in the highest MTs values (LAI=1.21-1.36 m^2 m^{-2}; Biom$_{dry}$=0.23–0.29 kg m^{-2}), found also for the other invasive species (*i.e.*, *L. hexapetala*). Similarly to floating-leaved species for which fractional cover is the portion of water covered by leaves, for submersed forms the fC is the portion of the plant which reaches the water surface layer. Therefore, it is expected that CD beds showed the lowest fC mean values (18-20%) and NN beds the highest (85-93%) (Fig. 5b). LM and TNb showed the second highest LAI values (0.91 and 0.87 m^2 m^{-2}, respectively) after NNa-f and LH beds (Fig. 5d). TNa, TNb, and TNc beds were located in the Superior Lake, Inferior Lake and Vallazza wetland, respectively. *T. natans* bed in the Inferior Lake (TNb) showed higher fC and LAI values (70% and 0.87 m^2 m^{-2}, respectively) compared to the other two beds (45-46%, and 0.41-0.45 m^2 m^{-2}, respectively, Fig. 5 b-d), probably due to a different phenological status, which was not reflected so clearly by biomass values (Fig. 5f). In fact, in 2014 *T. natans* in the Inferior Lake emerged two-three months later than the other *T. natans* beds, which therefore already reached a more advanced maturation stage in late September. *N. lutea* stands showed that MTs mean values were lower in the Middle Lake (NLb; fC=41%; LAI=0.39 m^2 m^{-2}; Biom$_{dry}$=0.06 kg m^{-2}) compared to the other *N. lutea* stands growth in the Superior Lake (NLa, c), as seen in Fig. 5 b,d,f.

Temporal variability

For the Mantua lakes system, we compared MTs maps derived from APEX data of 27 September 2014 with homologous maps derived from APEX data collected on 21 September 2011. Although the September 2011 maps cannot be validated due to the absence of *in situ* data on macrophyte MTs, a qualitative comparison can be carried out if we assume radiometric consistency and similar atmospheric and geometric acquisition conditions for both APEX flights. An assessment of APEX reflectance spectra matching carried out over targets considered to be radiometrically stable throughout the three years from 2011 to 2014 showed that the relative difference between the two images is under 4%, that is lower than radiometric accuracy of 2014 data *vs in situ* spectra (see Supplementary Fig. 2 for details).

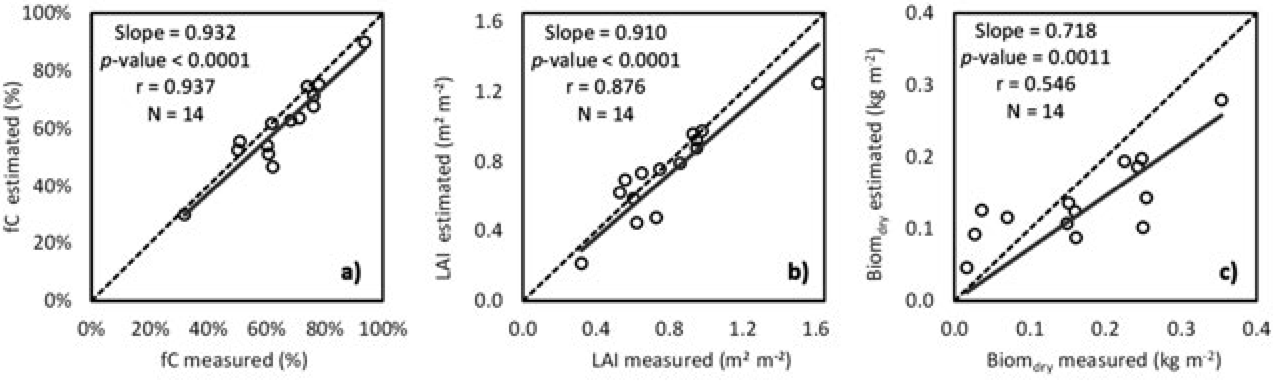

Fig. 4. Comparison of macrophyte canopy morphological traits measured *in situ* with estimates derived from spectral reflectance data using best performing linear model applied to APEX data. a) fC. b) LAI. c) Biom$_{dry}$. Regression line slope, P-value, Pearson's r, and number of samples (N) are superimposed on each graph.

In order to evaluate the difference between the products obtained by APEX 2011 and 2014, we have collected meteorological data of the vegetative period (March-October) of these two years. Meteorological data for both 2011 and 2014 growing seasons were reported in Fig. 6. The amount of precipitation from April to September was comparable between 2011 and 2014 (340 mm and 348 mm, respectively; data source Lombardy Environment Protection Regional Agency), but the seasonal distribution of rainfall varied between years, with a dry spring and rainy start to the summer in 2011 and a wet May followed by dry summer until August when rain was quite abundant in 2014 (Fig. 6a). Air temperature (daily mean) was higher in 2011 compared to 2014, reflecting the heat wave that hit the north of Italy between middle of August to the end of September 2011 (Fig. 6a). Wind velocity was similar between the two years, except from mid-July to mid-August when higher values were measured in 2011 (Fig. 6b). Solar radiation seasonality resembled that of wind velocity, higher from July to middle of August 2011 than what observed in the summer of 2014 (Fig. 6b).

As observed in 2014, the macrophyte MTs maps derived from 21 September 2011 APEX data consistently show a gradient in the canopy density, decreasing from invasive species to floating-leaved native ones (NN > LH > TN+NL; Fig. 8). fC and LAI of *N. nucifera* in 2011 were comparable between Angeli and Belfiore islands (see islands delineation in Fig. 7) in both years, but lower than in 2014 in absolute values (fC, ~85% and ~91%; LAI, ~1.18 and ~1.32 $m^2 m^{-2}$, respectively). *L. hexapetala*

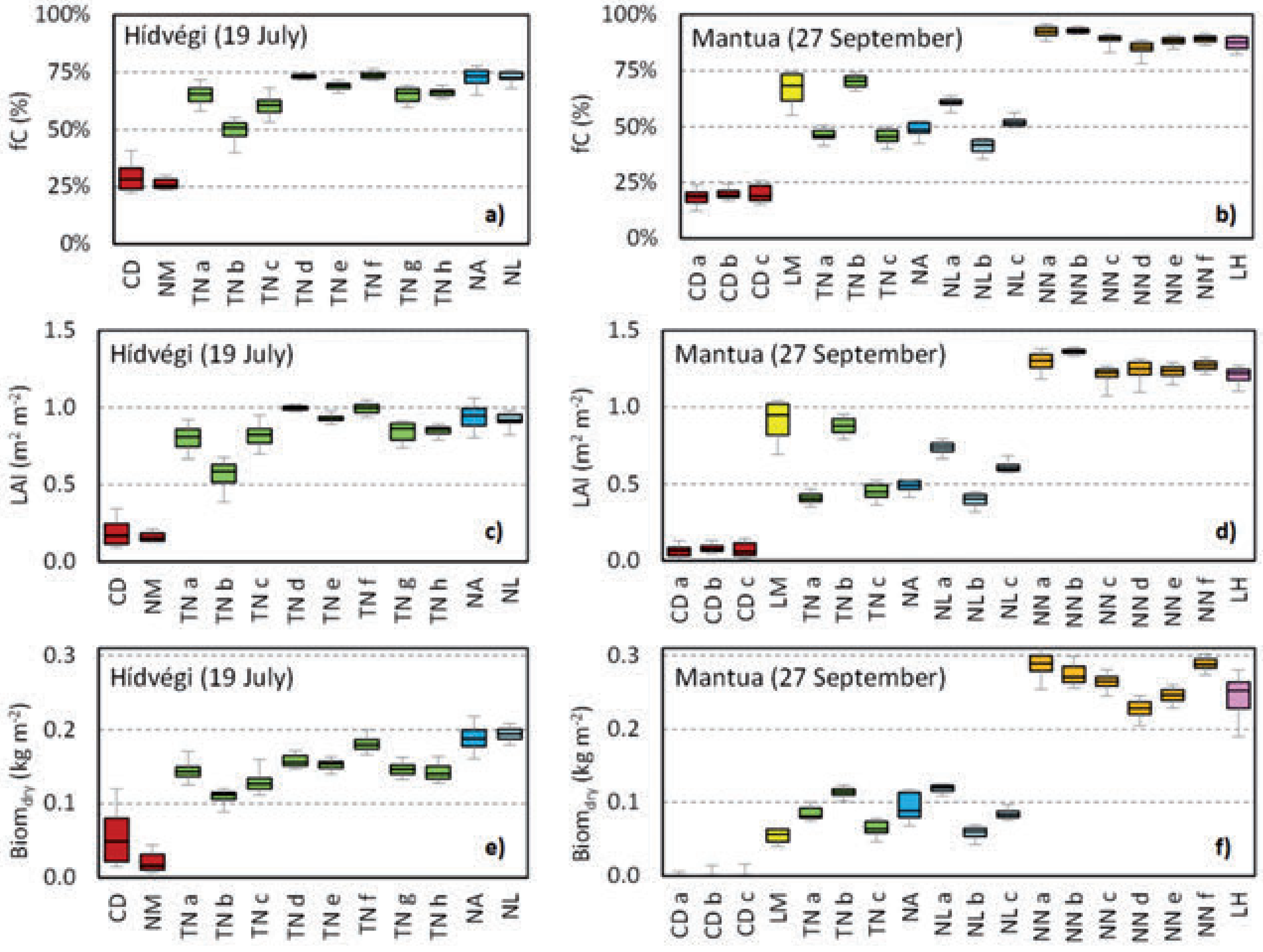

Fig. 5. Box plots (5-25-50-75-95 percentiles) of morphological traits extracted from APEX derived maps for relevant macrophyte beds (see Figs. 2 and 3). a) Fractional cover (Lake Hídvégi, 19 July 2014). b) Fractional cover (Mantua lakes system, 27 September 2014). c) LAI (Lake Hídvégi, 19 July 2014). d) LAI (Mantua lakes system, 27 September 2014). e) Biomass (Lake Hídvégi, 19 July 2014). f) Biomass (Mantua lakes system, 27 September 2014). fC, LAI and Biomdry of submerged species refer to the plant parts reaching the water surface only. CD, *Ceratophyllum demersum*; NM, *Najas marina*; TN, *Trapa natans*; NA, *Nymphaea alba*; NL, *Nuphar lutea*; NN, *Nelumbo nucifera*; LM, *Lemna* spp.; LH, *Ludwigia hexapetala*.

showed fC values similar in both years, while LAI values were higher in 2014 compared to 2011 (0.94 and 0.88 m^2 m^{-2}, respectively) (Figs. 7 and 8b). Instead, slightly lower LH biomass values were measured in 2014 (0.13±0.05 kg m^{-2}), than in 2011 (0.16±0.05 kg m^{-2}). *T. natans* showed a slight increase in biomass from 2011 to 2014 (0.04±0.02 and 0.07±0.03 kg m^{-2}, respectively), while fC and LAI were similar in both years (Fig. 8a-b). fC and LAI of *N.*

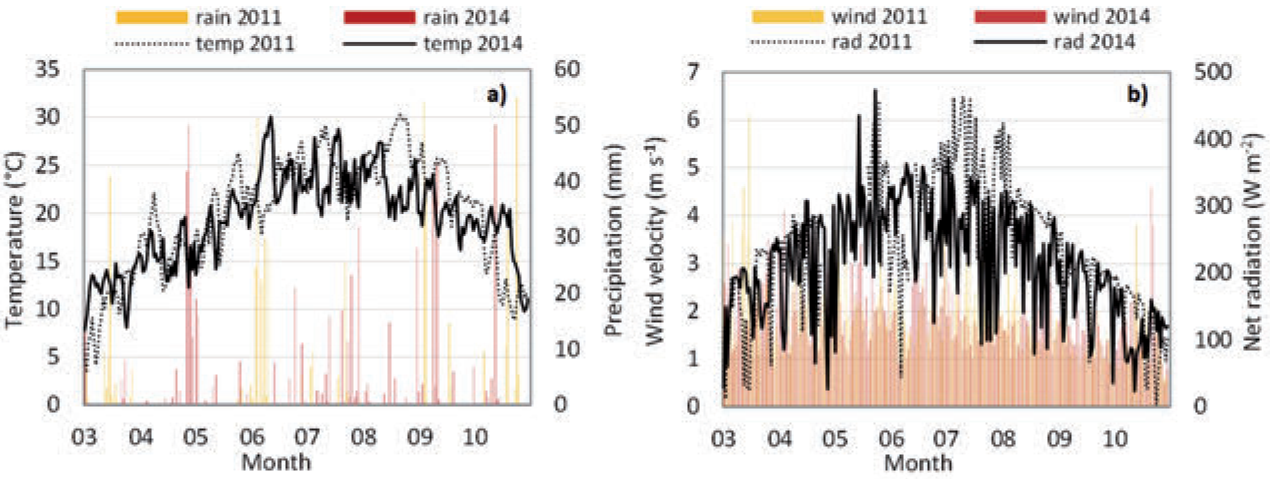

Fig. 6. Meteorological data for Mantua lakes system from March to October in 2011 and 2014. a) Daily mean air temperature and cumulated precipitation. b) Daily mean wind velocity and net radiation.

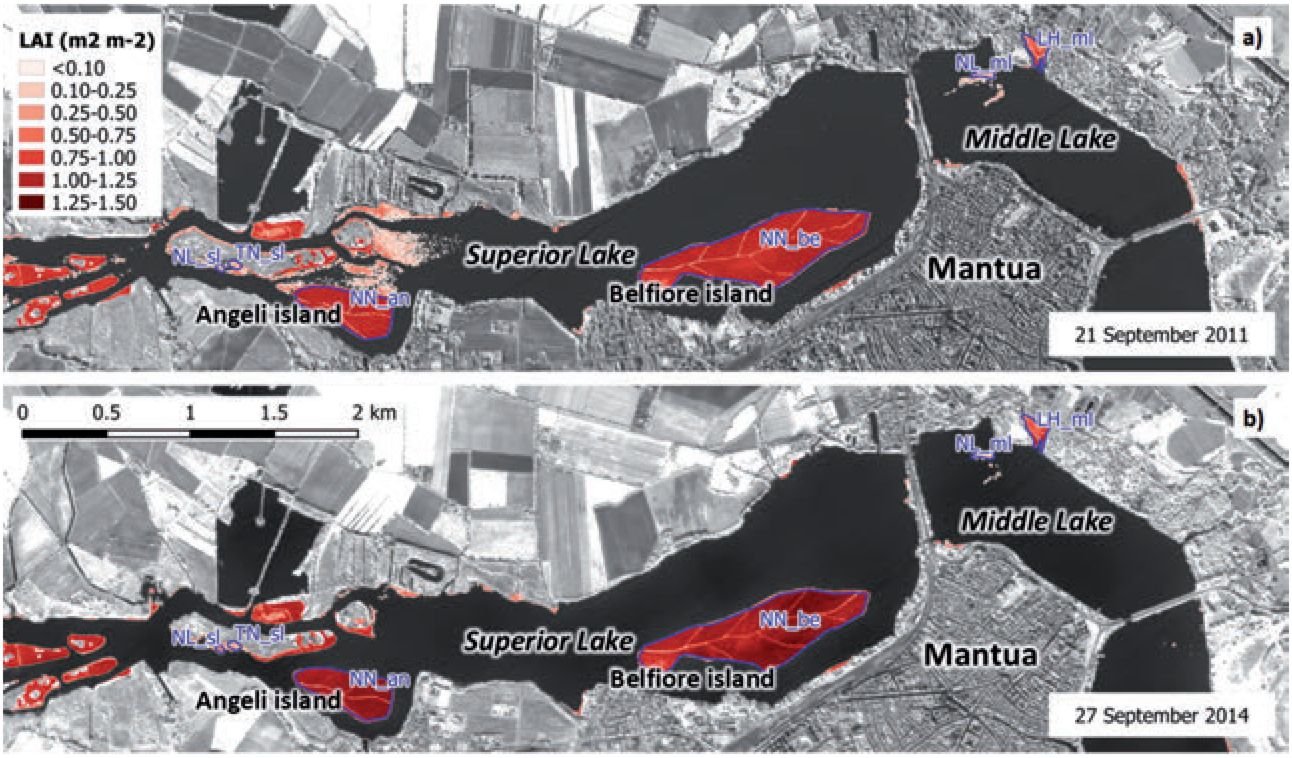

Fig. 7. Comparison of macrophyte LAI mapped using APEX data acquired over Mantua lakes system (detail of Superior Lake and Middle Lake) on 21 September 2011 and on 27 September 2014 over six macrophyte beds, covered by four species of floating and emergent macrophytes. TN, *Trapa natans*; NL, *Nuphar lutea*; NN, *Nelumbo nucifera*; LH, *Ludwigia hexapetala*.

lutea beds in 2011 were lower in Superior Lake (35±9%, and 0.30±0.13 m^2 m^{-2}, respectively) compared to Middle Lake (39±9%, and 0.41±0.14 m^2 m^{-2}, respectively), but biomass values were equivalent (Fig. 8c).

In contrast, MT values for NL stands in 2014 were higher in Superior Lake than in Middle Lake (fC, 50±8 and 36±6%; LAI, 0.55±0.14 and 0.33±0.08 m^2 m^{-2}; Biom$_{dry}$, 0.10±0.02 and 0.05±0.02 kg m^{-2} respectively) (Figs. 7 and 8).

DISCUSSION

Spectral indices as effective proxies of macrophyte morphological traits

The results achieved in this study build upon those previously reported from research on terrestrial plant communities (Asrar *et al*., 1989; Goward and Huemmrich, 1992; Gitelson and Merzlyak, 1996; Haboudane *et al*., 2004) in demonstrating that SIs are straightforward and effective proxies for mapping vegetation morphological traits in aquatic plant communities too. The strong relationships observed between the SIs and plant morphological traits suggest potential for operational monitoring of aquatic plants in lakes over the ranges (*i.e.,* 0-100% for fC, 0.0-1.8 m^2 m^{-2} for LAI, 0.0-0.5 kg m^{-2} for Biom$_{dry}$) and for the species considered in this study. In particular, correlation between SIs and fractional cover or LAI, confirmed the better performance of background adjusted SIs (*e.g.*, GSAVI and EVI) as good linear predictors for canopy density and structure features (Huete *et al*., 2002; Tian *et al*., 2005). For above-water biomass, the best performing SIs were those including spectral bands within the red edge region, confirming that spectral response within this wavelength range (705–710 nm) is particularly sensitive to plant biomass (Hunt *et al*., 2011). Given the high saturation effect at biomass density higher than 0.5 kg m^{-2}, this result may not hold for plant communities with particularly dense canopies, but it is reasonable for most of the macrophyte species which can be found in continental and temperate freshwater ecosystems, such as those studied here.

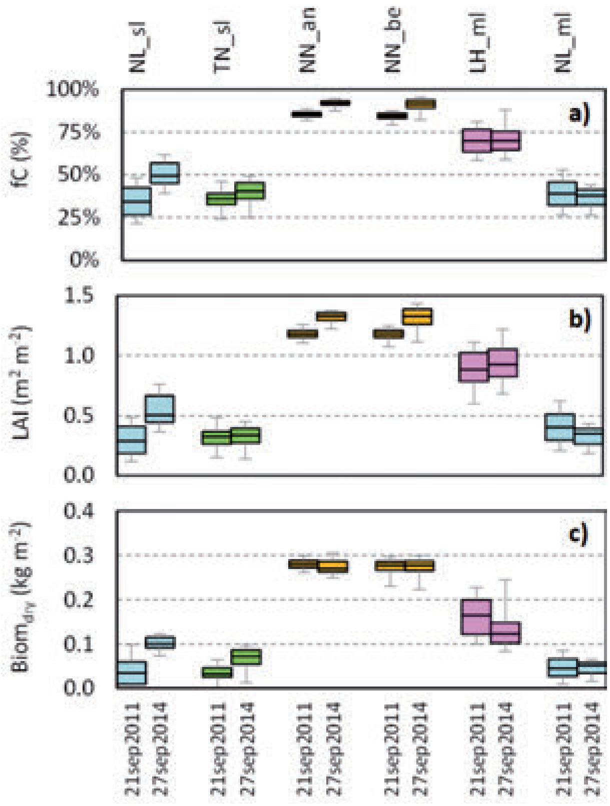

Fig. 8. Box plots (5-25-50-75-95 percentiles) of macrophyte morphological traits mapped from APEX in 2011 and 2014 for the six macrophyte beds identified in Fig. 7: fractional cover (a), LAI (b), and above-water biomass (c). TN, *Trapa natans*; NL, *Nuphar lutea*; NN, *Nelumbo nucifera*; LH, *Ludwigia hexapetala*.

Differently from what observed for fC and LAI, the best performing SI for Biom$_{dry}$ is different when using *in situ* or APEX spectra (Tab. 3). This can be attributed to the slightly different performance of atmospheric correction across different spectral ranges (see Supplementary Fig. 1), being CIre less sensitive to atmospheric effects on canopy reflectance compared to MTCI and the other indices, at least for the species and under the environmental conditions investigated.

Nutrient removal capacity in Lake Hídvégi

In Lake Hídvégi, macrophyte cover was mainly in littoral zones in Basin 2 and 3 (Fig. 1). Basin 4 is closed, with no macrophytes. Among *T. natans* beds, an anomalous behaviour was observed for TNb stand in Basin 3, with fractional cover and biomass around 27% lower than other beds of the same species. *T. natans* growing in the isolated narrow channel area lying in the eastern part of Basin 3 is in fact subject to very different environmental conditions compared to other stands of the same species growing in Basin 2: deeper water, presence of competitor species (*C. demersum* and *N. alba*, mainly) and slower water flow all contribute to TNb stand specific features. *T. natans* stands growing in Basin 2 are instead probably characterized by more homogeneous water chemistry, current and wind reflected in similar MTs values. During field campaigns in July 2014, water in the southern portion of Basin 2 presented ~3 mg L^{-1} of total suspended matter and up to ~130 mg m^{-3} of chlorophyll *a*, which reflect the hypertrophic condition of the Lake Hídvégi (Hatvani *et al*., 2011; Horváth *et al*., 2013).

The macrophyte communities in Lake Hídvégi are a fundamental component of the larger Kis-Balaton wetland

and critical to its ability to promote sedimentation of particulate material and manage nutrient loads carried by the Zala River before it enters Lake Balaton (Istvánovics *et al.*, 2007). Nutrient uptake is performed by submerged macrophytes via leaves and roots, while in floating forms the vast majority of the nutrients are taken up via roots (James *et al.*, 2005; Scheffer and van Nes, 2007). At the end of the vegetative period, following re-mineralization processes in the sediment, most of the nutrients sequestered by macrophytes re-enter the water in a soluble form (Søndergaard *et al.*, 2003; Moss *et al.*, 2005; Jones *et al.*, 2012). The formation of dense macrophytes beds also contributes to the system's filtering capacity by slowing the water flow, favouring the sedimentation of particulate matter (Madsen *et al.*, 2001; Jones *et al.*, 2012). Change in water rheology and oxygen content might result in temporal binding of nutrients (mainly P) in the sediment and reduce its bioavailability (Granéli and Solander, 1988; Søndergaard *et al.*, 2003; Horppila and Nurminen, 2005). In addition, macrophytes can act as substrate for epiphyte communities, which actively assimilate nutrients from the water column (Blindow *et al.*, 2006; Nõges *et al.*, 2010). The accurate mapping of macrophyte morphological traits provides a route to quantifying their capacity for direct nutrient uptake and removal, for example through the conversion of the above-water biomass into an estimate of nutrient storage in leaves or to, at least qualitatively, assess their indirect contribution. The maps derived from APEX show that in July 2014 *T. natans* meadows in Lake Hídvégi covered a total area of 1.05 km^2, corresponding to ~136 t of above-water biomass. In other words, *T. natans* can temporarily store ~3.5 t of nitrogen (N; 2.6% N leaf content, data from Pinardi *et al.*, 2011) and ~0.4 t of P (0.3% P leaf content measured in July 2014, which was similar to those reported in Pinardi *et al.*, 2011). Due to a much lower areal extent, the contribution from the biomass (~3.0 t) of other floating-leaved species (*N. lutea* and *N. alba*) to nutrient storage was negligible compared to *T. natans*. We must point out that the nutrient stock in *T. natans* meadows is likely to be an underestimation of the real nutrient uptake because we only considered above-water biomass (*e.g.,* in July above and below water biomass of TN were 60% and 40% of total biomass; Pinardi *et al.*, 2011) and without leaf turnover during the whole vegetative period (*e.g.,* by doubling the maximum standing stock; Galanti and Topa Esposito, 1996). If we assume that these values are generally representative of the allocation of biomass above and below water, and an equal allocation of nutrients for above and below water plant parts, then we estimate that the total nutrient storage in *T. natans* biomass is up to 11.7 N t and 1.3 P t. According to published works describing nutrient or chemical inflow and water discharge data over time, we calculate a total load for the growing season period (May-October) of 72-206 t N and 6-13 t P (Szilagyi *et al.*, 1990; Pomogyi, 1993; Tátrai *et al.*, 2000; Hatvani *et al.*, 2011; Horváth *et al.*, 2013; Paulovits *et al.*, 2014). The comparison of nutrient content in macrophyte beds derived from APEX maps with nutrient load inflowing by the Zala River evidenced that about 6-16% and 10-21% of N and P, respectively, can be temporarily stored in *T. natans* biomass.

In general, the second half of July, when the standing stock of floating-leaved species is maximum, is an important period to assess the filter capacity of macrophyte stands in Lake Hídvégi. To exploit the capacity of macrophytes to remove nutrients from the inflow it might be appropriate to perform multiple cuttings during the growing season to maximize nutrient removal, and avoid the risk of hypoxia in shallow waters subject to resuspension and rapid infilling. For instance, biomass cutting and removal actions could be undertaken in late July when the standing stock and P loads are maximal and growth rates start to slow down (Hatvani *et al.*, 2011), as this would exploit the maximum nutrient assimilative capacity of plants. The after cutting re-growth phase favours assimilative action of the same plants and consequently the increase in their filtration capacity. For not hindering the regrowth and persistence of the species at the same place, a viable management approach that could be suggested is the partial removal of plants or plant parts during the season.

Management of invasive species in Mantua lakes system

The biomass map of the Mantua lakes system showed higher values in the Superior Lake and in particular in *N. nucifera* meadows around the Angeli and Belfiore islands. In the latter case, the map shows a progressive increase in biomass (from 0.2 to 0.3 kg m^{-2}; Fig. 3) from the western to the eastern portion of the island, probably due to different water circulation around the macrophyte bed, which acts as physical barrier and lowers the water velocity at the end of the island compared to the tip (if wind direction is from west to east; Pinardi *et al.*, 2015). Similar patterns were observed on fC and LAI maps.

Maps derived from remote sensing are useful to identify hotspot areas in terms of invasive species with high biomass or macrophyte cover. For example, possible critical areas include: i) littoral zones with low water circulation, such as the south part of the Angeli and Belfiore stands in the Superior Lake (Pinardi *et al.*, 2015), and the east side of the Inferior Lake, and ii) littoral zones where tributaries enter into the lakes, such as the north bank of the three lakes (*e.g., L. hexapetala* in the Middle Lake). Therefore, local authorities can obtain important information on the localization of critical areas, for example where point sources input might lead to conditions favouring the development of floating macrophyte meadows.

Remote sensing map products can also be used to identify where cutting action has been performed for extended periods (*e.g.,* the canals free of vegetation created in the two largest islands of *N. nucifera* - Angeli and Belfiore - in the Superior Lake). In addition, it is also possible to exploit the spatial information afforded by remote sensing data to obtain insights into the mechanism of invasion and the subsequent spread of macrophyte species through freshwater systems that might be useful in design future management strategies.

In Mantua system, invasive species (*N. nucifera* and *L. hexapetala*) exhibited higher vegetative vigour (in terms of density and biomass) compared to autochthonous floating-leaved species. In order to control invasive species and, in particular, to identify dense macrophyte beds with high biomass, we applied a threshold to LAI map product (LAI > 1 m^2 m^{-2}) of the Mantua lakes system (27 September 2014). The macrophyte stands identified (*N. nucifera*)*,* covered 0.61 km^2 resulting in 161 t of biomass. Therefore, by cutting and removing the whole above-water biomass of *N. nucifera* stands, it would be possible to remove ~4.0 and ~0.3 t of N and P respectively from the system (due to scarcity of literature on nutrient elemental content in *N. nucifera,* we applied 2.5% N and 0.2% P leaf content reported by Longhi *et al.*, 2008 for *N. lutea* in a wetland located in the Province of Mantua). Given the saturation effect on SIs occurring around 0.5 kg m^{-2} (Fig. 4c), we must point out that the model used to calculate biomass tends to underestimate actual above-water biomass. As such, our calculations are an underestimate of the actual amount of nutrients stored in plants.

Multi-temporal analysis (2011 *vs* 2014) over Mantua lakes system

Most of the temporal differences in macrophyte MTs showed by multi-temporal maps derived for Mantua lakes system in 2011 and 2014 can be explained with meteorological features characterizing the two growing seasons (Fig. 6).

Lower fC and LAI observed for *N. nucifera* in 2011, compared to 2014 are probably due to higher wind velocity in 2011 during the period of maximum development of this species (July-August, Fig. 6b). *N. nucifera* biomass, less sensitive to short term wind anomalies, was in fact similar in both years (Fig. 8c). Lower biomass values of *L. hexapetala* mapped in 2014 are instead possibly due to lower temperature at peak of the growing season, which may have inhibited the full development of plants: from beginning of August to middle of September the mean temperature was 22.0°C in 2014, compared to 25.6°C in 2011 (Fig. 6a). On the contrary, increase in biomass from 2011 to 2014 of the *T. natans* in Superior Lake, a species sensitive to wind conditions especially during late growing season, can be due to the presence of an island located north the stand (Fig. 7), which acts as physical barrier and prevent the effect of wind (Fig. 6b).

In both years, the area covered by invasive species (NN+LH), *i.e.*, showing LAI>1 m^2 m^{-2}, and their mean biomass were in agreement (~0.6 km^2 and ~0.27 kg m^{-2}, respectively), resulting in a similar quota of nutrients assimilated by plants. Ultimately, even if the time of year is the same (September), the density of invasive species may be different due to particular meteorological conditions (such as wind velocity and air temperature) characterizing the weeks preceding APEX data acquisition.

Late September is a good period to analyse the status of macrophytes, and in particular of *N. nucifera,* before senescence starts. This allows to plan harvesting of invasive species stands, thus avoiding two critical effects: i) excessive organic matter loads settling on the bottom of the lake, and ii) rapid infilling of the system (Pinardi *et al.*, 2011). While control actions are performed, it is fundamental to guarantee a sufficient water discharge to avoid negative effects on the ecosystem (*e.g.,* high oxygen demand, nutrient release due to sediment resuspension).

CONCLUSIONS

Macrophyte products derived by remote sensing techniques allow to map morphological traits of different species that colonize shallow lakes and wetlands, in terms of above-water biomass and density (fractional cover and LAI), as well as surface area. These mapping products can be an effective tool for efficient and sustainable management of shallow water environments, with relevance to filter capacity assessment (*i.e*., nutrient and particulate matter retention) and invasive species control (*i.e*., harvesting). Reliable biomass estimates at maximum standing stock allow the quantification of the nutrient load assimilated by macrophytes and provide detailed and spatialized input to N and P budgets at watershed level, as we demonstrate here for the Lake Hídvégi case study.

Macrophyte density and LAI maps, as the case of Mantua lakes system, can be used to efficiently identify hotspot areas where invasive species management is most needed (*e.g*., biomass harvesting).

These actions can avoid the establishment of areas characterized by water stagnation, prevent algal blooms, and/or further development of floating or emergent macrophytes. With remotely sensed maps of macrophyte morphological traits it is possible for water management institutions or local authorities to promptly identify the priority areas of action both for conservation and economic purposes. In addition, estimating the amount of biomass that could be removed and destined for reuse is also important in terms of economic value: *e.g.,* for proper planning and energy generation purposes.

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REFERENCES

Adam E, Mutanga O, Rugege D, 2010. Multispectral and hyperspectral remote sensing for identification and mapping of wetland vegetation: a review. Wetl. Ecol. Manag. 18:281-296.

Aspila KI, Agemian H, Chau ASY, 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. Analyst 101:187-197.

Asrar G, Myneni RB, Li Y, Kanemasu ET, 1989. Measuring and modeling spectral characteristics of a tallgrass prairie. Remote Sens. Environ. 27:143-155.

Bannari A, Morin D, Bonn F, Huete AR, 1995. A review of vegetation indices. Remote Sens. Rev. 13:95-120.

Barnes EM, Clarke TR, Richards SE, Colaizzi PD, Haberland J, Kostrzewski M, Moran MS, 2000. Coincident detection of crop water stress, nitrogen status and canopy density using ground based multispectral data. In: Proceedings 5th International Conference on Precision Agriculture, Bloomington, pp. 16-19.

Birk S, Ecke F, 2014. The potential of remote sensing in ecological status assessment of coloured lakes using aquatic plants. Ecol. Indic. 46:398-406.

Blindow I, Hargeby A, Meyercordt J, Schubert H, 2006. Primary production in two shallow lakes with contrasting plant form dominance: A paradox of enrichment? Limnol. Oceanogr. 51:2711-2721.

Boerema A, Schoelynck J, Bal K, Vrebos D, Jacobs S, Staes J, Meire P, 2014. Economic valuation of ecosystem services, a case study for aquatic vegetation removal in the Nete catchment (Belgium). Ecosyst. Serv. 7:46-56.

Bolpagni R, Laini A, Soana E, Tomaselli M, Nascimbene J, 2015. Growth performance of *Vallisneria spiralis* under oligotrophic conditions supports its potential invasiveness in mid-elevation freshwaters. Weed Res. 55:185-194.

Bolpagni R, Pierobon E, Longhi D, Nizzoli D, Bartoli M, Tomaselli M, Viaroli P, 2007. Diurnal exchanges of CO_2 and CH_4 across the water-atmosphere interface in a water chestnut meadow (*Trapa natans* L.). Aquat. Bot. 87:43-48.

Bolpagni R, Piotti A, 2016. The importance of being natural in a human-altered riverscape: role of wetland type in supporting habitat heterogeneity and the functional diversity of vegetation. Aquat. Conserv. 26:1168-1183.

Bresciani M, Stroppiana D, Fila G, Montagna M, Giardino C, 2009. Monitoring reed vegetation in environmentally sensitive areas in Italy. Ital. J. Remote Sens. 41:125-137.

Broge NH, Leblanc E, 2001. Comparing prediction power and stability of broadband and hyperspectral vegetation indices for estimation of green leaf area index and canopy chlorophyll density. Remote Sens. Environ. 76:156-172.

Bukata RP, 2005. Satellite monitoring of inland and coastal water quality: retrospection, introspection, future directions. CRC Press, Boca Raton: 272 pp.

Castaldelli G, Soana E, Racchetti E, Vincenzi F, Fano EA, Bartoli M, 2015. Vegetated canals mitigate nitrogen surplus in agricultural watersheds. Agr. Ecosyst. Environ. 212: 253-262.

Chen JM, 1996. Evaluation of vegetation indices and a modified simple ratio for boreal applications. Can. J. Remote Sens. 22:229-242.

Dash J, Curran PJ, 2004. The MERIS terrestrial chlorophyll index. Int. J. Remote Sens. 25:5403-5413.

De Haan JF, Hovenier JW, Kokke JMM, Van Stokkom HTC, 1991. Removal of atmospheric influences on satellite-borne imagery: a radiative transfer approach. Remote Sens. Environ. 37:1-21.

Dinka M, Ágoston-Szabó E, Urbanc-Berčič O, Germ M, Šraj-Kržič N, Gaberščik A, 2008. Reed stand conditions at selected wetlands in Slovenia and Hungary, p. 1-12. In: J. Vymazal (ed.), Wastewater treatment, plant dynamics and management in constructed and natural wetlands. Springer, Doirdrecht.

Dömötörfy Z, Reeder D, Pomogyi P, 2003. Changes in the macro-vegetation of the Kis-Balaton Wetlands over the last two centuries: a GIS perspective. Hydrobiologia 506:671-679.

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C, Sullivan CA, 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81:163-182.

European Union, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L 327, 22/12/2000, pp. 1-73.

Galanti G, Topa Esposito A, 1996. The invasive capacity of water chestnut as shown in the management of a natural population in Lago di Candia. Lake Reserv. Manage. 2:31-36.

Gege P, Fries J, Haschberger P, Schötz P, Schwarzer H, Strobl P, Suhr B, Ulbrich G, Vreeling WJ, 2009. Calibration facility for airborne imaging spectrometers. ISPRS J. Photogramm. 64:387-397.

Gitelson AA, Keydan GP, Merzlyak MN, 2006. Three-band model for noninvasive estimation of chlorophyll, carotenoids, and anthocyanin contents in higher plant leaves. Geophys. Res. Lett. 33:L11402.

Gitelson AA, Merzlyak MN, 1994. Quantitative estimation of chlorophyll-a using reflectance spectra: Experiments with autumn chestnut and maple leaves. J. Photoch. Photobio B 22:247-252.

Gitelson AA, Merzlyak MN, 1996. Signature analysis of leaf reflectance spectra: algorithm development for remote sensing of chlorophyll. J. Plant Physiol. 148:494-500.

Gobron N, Pinty B, Verstraete MM, Widlowski JL, 2000. Advanced vegetation indices optimized for up-coming sensors: Design, performance, and applications. IEEE T. Geosci. Remote 38:2489-2505.

Goodwin K, Caraco N, Cole J, 2008. Temporal dynamics of dissolved oxygen in a floating-leaved macrophyte bed. Freshwater Biol. 53:1632-1641.

Goward SN, Huemmrich KF, 1992. Vegetation canopy PAR absorptance and the normalized difference vegetation index: an assessment using the SAIL model. Remote Sens. Environ. 39:119-140.

Granéli W, Solander D, 1988. Influence of aquatic macrophytes on phosphorus cycling in lakes. Hydrobiologia 170:245-266.

Haboudane D, Miller JR, Pattey E, Zarco-Tejada PJ, Strachan IB, 2004. Hyperspectral vegetation indices and novel algorithms for predicting green LAI of crop canopies: Modeling and validation in the context of precision agriculture. Remote Sens. Environ. 90:337-352.

Hatvani IG, Kovács J, Kovács IS, Jakusch P, Korponai J, 2011. Analysis of long-term water quality changes in the Kis-Balaton Water Protection System with time-series, cluster analysis and Wilks' lambda distribution. Ecol. Eng. 37:629-635.

Hestir EL, Khanna S, Andrew ME, Santos MJ, Viers JH, Greenberg JA, Rajapakse SR, Ustin SL, 2008. Identification of invasive vegetation using hyperspectral remote sensing in the California Delta ecosystem. Remote Sens. Environ. 112:4034-4047.

Horppila J, Nurminen L, 2005. Effects of different macrophyte growth forms on sediment and P resuspension in a shallow lake. Hydrobiologia 545:167-175.

Horváth H, Mátyás K, Süle G, Présing M, 2013. Contribution of nitrogen fixation to the external nitrogen load of a water quality control reservoir (Kis-Balaton Water Protection System, Hungary). Hydrobiologia 702:255-265.

Huete AR, 1988. A soil-adjusted vegetation index (SAVI). Remote Sens. Environ. 25:295-309.

Huete A, Didan K, Miura T, Rodriguez EP, Gao X, Ferreira LG, 2002. Overview of the radiometric and biophysical performance of the MODIS vegetation indices. Remote Sens. Environ. 83:195-213.

Hunt ER, Daughtry CST, Eitel JU, Long DS, 2011. Remote sensing leaf chlorophyll content using a visible band index. Agron. J. 103:1090-1099.

Hunter PD, Gilvear DJ, Tyler AN, Willby NJ, Kelly A, 2010. Mapping macrophytic vegetation in shallow lakes using the Compact Airborne Spectrographic Imager (CASI). Aquat. Conserv. 20:717-727.

Istvánovics V, Clement A, Somlyódy L, Specziár A, László G, Padisák J, 2007. Updating water quality targets for shallow Lake Balaton (Hungary), recovering from eutrophication. Hydrobiologia 581:305-318.

Jacobs AE, Harrison JA, 2014. Effects of floating vegetation on denitrification, nitrogen retention, and greenhouse gas production in wetland microcosms. Biogeochemistry 119:51-66.

James C, Fisher J, Russell V, Collings S, Moss B, 2005. Nitrate availability and hydrophyte species richness in shallow lakes. Freshwater Biol. 50:1049-1063.

Jeppensen E, Jensen JP, Søndergaard M, Lauridsen T, Pedersen LJ, Jensen L, 1997. Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. Hydrobiologia 342/343:151-164.

Jones JI, Collins AL, Naden PS, Sear DA, 2012. The relationship between fine sediment and macrophytes in rivers. River Res. Appl. 28:1006-1018.

Jordan SJ, Stoffer J, Nestlerode JA, 2011. Wetlands as sinks for reactive nitrogen at continental and global scales: A meta-analysis. Ecosystems 14:144-155.

Kosten S, Jeppesen E, Huszar VLM, Mazzeo N, Van Nes EH, Peeters ETHM, Scheffer M, 2011. Ambiguous climate impacts on competition between submerged macrophytes and phytoplankton in shallow lakes. Freshwater Biol. 56:1540-1553.

Kovács J, Hatvani IG, Korponai J, Kovácsné SzI, 2010. Morlet wavelet and auto-correlation analysis of long term data series of the Kis-Balaton Water Protection System (KBWPS). Ecol. Eng. 36:1469-1477.

Longhi D, Bartoli M, Viaroli P, 2008. Decomposition of four macrophytes in wetland sediments: Organic matter and nutrient decay and associated benthic processes. Aquat. Bot. 89:303-310.

Maccioni A, Agati G, Mazzinghi P, 2001. New vegetation indices for remote measurement of chlorophylls based on leaf directional reflectance spectra. J. Photoch. Photobio. B 61:52-61.

Madsen JD, Chambers PA, James WF, Koch EW, Westlake DF, 2001. The interaction between water movement, sediment dynamics and submersed macrophytes. Hydrobiologia 444:71-84.

Mckee D, Hatton K, Eaton JW, Atkinson D, Atherton A, Harvey I, Moss B, 2002. Effects of simulated climate warming on macrophytes in freshwater microcosm communities. Aquat. Bot. 74:71-83.

MEA - Millennium Ecosystem Assessment, 2005. Ecosystems and human well-being: wetlands and water. World Resources Institute, Washington, DC: 68 pp.

Metzger MJ, Rounsevell MDA, Acosta-Michlik L, Leemans R, Schröter D, 2006. The vulnerability of ecosystem services to land use change. Agr Ecosyst. Environ. 114:69-85.

Moss B, Barker T, Stephen D, Williams AE, Balayla DJ, Beklioglu M, Carvalho L, 2005. Consequences of reduced nutrient loading on a lake system in a lowland catchment: deviations from the norm? Freshwater Biol. 50:1687-1705.

Nõges T, Luup H, Feldmann T, 2010. Primary production of aquatic macrophytes and their epiphytes in two shallow lakes (Peipsi and Võrtsjärv) in Estonia. Aquat. Ecol. 44:83-92.

Palmer SC, Kutser T, Hunter PD, 2015. Remote sensing of inland waters: Challenges, progress and future directions. Remote Sens. Environ. 157:1-8.

Paulovits G, Ferincz Á, Staszny Á, Weiperth A, Tátrai I, Korponai J, Mátyás K, Kováts N, 2014. Long-term changes in the fish assemblage structure of a shallow eutrophic reservoir (Lake Hídvégi, Hungary), with special reference to the exotic *Carassius gibelio*. Int. Rev. Hydrobiol. 99:373-381.

Penuelas J, Gamon JA, Griffin KL, Field CB, 1993. Assessing community type, plant biomass, pigment composition, and photosynthetic efficiency of aquatic vegetation from spectral reflectance. Remote Sens. Environ. 46:110-118.

Pinardi M, Bartoli M, Longhi D, Viaroli P, 2011. Net autotrophy in a fluvial lake: the relative role of phytoplankton and floating-leaved macrophytes. Aquat. Sci. 73:389-403.

Pinardi M, Fenocchi A, Giardino C, Sibilla S, Bartoli M, Bresciani M, 2015. Assessing potential algal blooms in a shallow fluvial lake by combining hydrodynamic modelling and remote-sensed images. Water 7:1921-1942.

Poff NL, Brinson MM, Day JW, 2002. Aquatic ecosystems and global climate change. Pew Center on Global Climate Change, Arlington: 44 pp.

Pomogyi P, 1993. Nutrient retention by the Kis-Balaton water protection system. Hydrobiologia 251:309-320.

Ribaudo M, Greene C, Hansen L, Hellerstein D, 2010. Ecosystem services from agriculture: steps for expanding markets. Ecol. Econ. 69:2085-2092.

Rouse Jr. J, Haas RH, Schell JA, Deering DW, 1974. Monitoring vegetation systems in the Great Plains with ERTS, p. 309-317. In: C.F. Stanley, E.P. Mercanti and M.A. Becker (eds.), Third ERTS-1 Symp. Vol. 1. NASA Special Publication, Washington.

Schaeffer BA, Schaeffer KG, Keith D, Lunetta RS, Conmy R, Gould RW, 2013. Barriers to adopting satellite remote sensing for water quality management. Int. J. Remote Sens. 34:7534-7544.

Schaepman ME, Jehle M, Hueni A, D'Odorico P, Damm A, Weyermann J, Lenhard K, 2015. Advanced radiometry measurements and Earth science applications with the Airborne Prism Experiment (APEX). Remote Sens. Environ. 158:207-219.

Scheffer M, Van Nes EH, 2007. Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. Hydrobiologia 584:455-466.

Schriver P, Bogestrand J, Jeppensen E, Sondergaard M, 2005. Impact of submerge macrophytes on fish-zooplankton interactions: large-scale enclose experiments in a shallow eutrophic lake. Freshwater Biol. 33:255-270.

Silva TS, Costa MP, Melack JM, Novo EM, 2008. Remote sensing of aquatic vegetation: theory and applications. Environ. Monit. Assess. 140:131-145.

Søndergaard M, Jensen JP, Jeppesen E, 2003. Role of sediment and internal loading of phosphorus in shallow lakes. Hydrobiologia 506:135-145.

Steffen W, Sanderson RA, Tyson PD, Jäger J, Matson PA, Moore B III, Oldfield F, Richardson K, Schellnhuber HJ, Turner BL II, Wasson RJ, 2006. Global change and the earth system: a planet under pressure. Springer, Berlin: 336 pp.

Stratoulias D, Balzter H, Zlinszky A, Tóth VR, 2015. Assessment of ecophysiology of lake shore reed vegetation based on chlorophyll fluorescence, field spectroscopy and hyperspectral airborne imagery. Remote Sens. Environ. 157:72-84.

Szilagyi F, Somlyódy L, Koncsos L, 1990. Operation of the Kis-Balaton reservoir: evaluation of nutrient removal rates. Hydrobiologia 191:297-306.

Tátrai I, Mátyás K, Korponai J, Paulovits G, Pomogyi P, 2000. The role of the Kis-Balaton Water Protection System in the control of water quality of Lake Balaton. Ecol. Eng. 16:73-78.

Tian YC, Zhu Y, Cao WX, 2005. Monitoring soluble sugar, total nitrogen and its ratio in wheat leaves with canopy spectral reflectance. Act. Agron. Sin. 31:355-360.

Tomaselli M, Gualmini M, Spettoli O, 2000. [La vegetazione della Riserva Naturale delle Valli del Mincio. Parma (IT)]. [Article in Italian]. Collana Annali Facoltà di Scienze Matematiche Fisiche e Naturali dell'Università di Parma: 90 pp.

Tou JT, Gonzalez RC, 1974. Pattern recognition principles. Addison-Wesley Publishing, London: 378 pp.

Tucker CJ, Elgin JH, McMurtrey JE, Fan CJ, 1979. Monitoring corn and soybean crop development with hand-held radiometer spectral data. Remote Sens. Environ. 8:237-248.

Villa P, Laini A, Bresciani M, Bolpagni R, 2013. Remote sensing approach to monitor the conservation status of lacustrine Phragmites australis beds. Wetl. Ecol. Manag. 21:399-416.

Villa P, Mousivand A, Bresciani M, 2014. Aquatic vegetation indices assessment through radiative transfer modeling and linear mixture simulation. Int. J. Appl. Earth Obs. 30:113-127.

Villa P, Bresciani M, Bolpagni R, Pinardi M, Giardino C, 2015. A rule-based approach for mapping macrophyte communities using multi-temporal aquatic vegetation indices. Remote Sens. Environ. 171:218-233.

Wetzel RG, 1992. Wetlands as metabolic gates. J. Great Lakes Res. 18:529-532.

Wu C, Niu Z, Tang Q, Huang W, Rivard B, Feng J, 2009. Remote estimation of gross primary production in wheat using chlorophyll-related vegetation indices. Agr. Forest Meteorol. 149:1015-1021.

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Earth observation for monitoring and mapping of cyanobacteria blooms. Case studies on five Italian lakes

Mariano BRESCIANI,[1*] Claudia GIARDINO,[1] Rosaria LAUCERI,[2] Erica MATTA,[1] Ilaria CAZZANIGA,[1,3] Monica PINARDI,[1] Andrea LAMI,[2] Martina AUSTONI,[2] Emanuela VIAGGIU,[4] Roberta CONGESTRI,[4] Giuseppe MORABITO[2]

[1]Optical Sensing Group, Institute for Electromagnetic Sensing of the Environment, National Research Council of Italy, via Bassini 15, 20133 Milan; [2]Institute for the Study of Ecosystems, National Research Council of Italy, Largo Tonolli 50-52, 28922 Verbania-Pallanza; [3]Remote Sensing of Environmental Dynamics Lab. DISAT, University of Milano-Bicocca, Piazza della Scienza 1, 20126 Milan; [4]Department of Biology, University of Rome Tor Vergata, Rome, Italy
*Corresponding author: bresciani.m@irea.cnr.it

ABSTRACT

Cyanobacterial blooms occur in many parts of the world as a result of entirely natural causes or human activity. Due to their negative effects on water resources, efforts are made to monitor cyanobacteria dynamics. This study discusses the contribution of remote sensing methods for mapping cyanobacterial blooms in lakes in northern Italy. Semi-empirical approaches were used to flag scum and cyanobacteria and spectral inversion of bio-optical models was adopted to retrieve chlorophyll-a (Chl-a) concentrations. Landsat-8 OLI data provided us both the spatial distribution of Chl-a concentrations in a small eutrophic lake and the patchy distribution of scum in Lake Como. ENVISAT MERIS time series collected from 2003 to 2011 enabled the identification of dates when cyanobacterial blooms affected water quality in three small meso-eutrophic lakes in the same region. On average, algal blooms occurred in the three lakes for about 5 days a year, typically in late summer and early autumn. A suite of hyperspectral sensors on air- and space-borne platforms was used to map Chl-a concentrations in the productive waters of the Mantua lakes, finding values in the range of 20 to 100 mgm^{-3}. The present findings were obtained by applying state of the art of methods applied to remote sensing data. Further research will focus on improving the accuracy of cyanobacteria mapping and adapting the algorithms to the new-generation of satellite sensors.

Key word: Remote Sensing; monitoring; chlorophyll-a; lakes; hyperspectral.

INTRODUCTION

In the past two centuries, human activity has altered the global climate to such an extent that this historical period has been called the Anthropocene (Steffen *et al.*, 2007). The effects of climate change are threatening not only water safety and accessibility but also the quality of aquatic ecosystems, leading to loss of biodiversity (Bálint *et al.*, 2011; Harley, 2011), altered of reproductive cycles, and invasion of allochthonous species (Dukes and Mooney, 1999; Walther *et al.*, 2009). Deteriorating aquatic ecosystems may have a strong economic impact (Landsberg, 2002; Falconer and Humpage, 2005; Backer and McGillicuddy, 2006): Dodds *et al.* (2013) calculated that freshwater ecosystems disruption due to human activity reduced the value of marketable aquatic benefits by ~16% globally, or ~$900 billion. Future climate change scenarios predict rising air and water temperatures, enhanced vertical stratification of aquatic ecosystems, and changing seasonal and annual weather patterns. Climate models foresee more frequent and more intense rainfall events (with storms and floods) alternating with longer periods of drought (Dokulil *et al.*, 2009; Dokulil and Teubner, 2011). Such conditions are ideal for the growth, dominance, persistence, and geographic expansion of several harmful cyanobacteria species (Paerl and Huisman, 2009; Reichwaldt and Ghadouani, 2012). Global climate change and anthropic eutrophication are expected to accelerate the shift to turbid water and cyanobacteria-dominated conditions in aquatic environments (Jöhnk *et al.*, 2008; Paerl and Huisman, 2008; Slim *et al.*, 2014; Rousseaux and Gregg, 2015). The optimal water temperature for the growth of cyanobacteria (>25°C; Robarts and Zohary, 1987; Coles and Jones, 2000) is higher than that of green algae or diatoms (Wetzel, 2001). The density of water drops proportionally to its increase in temperature contributing to its vertical stratification in aquatic ecosystems and promoting cyanobacteria growth in the epilimnion (Salmaso, 2005; Winder and Sommer, 2012). Global warming may prolong the annual period of water stratification (Markensten *et al.*, 2010), increasing the dominance of cyanobacteria and nitrogen fixation (Elliott, 2012; Hense *et al.*, 2013). Cyanobacteria generally thrive on higher nutrient loads (N, P), while nutrient stoichiometry determines interspecific competition between cyanobacteria and other algae (Savadova, 2014). Changes in physical parameters such as surface water temperature over time can even lead to algal species succeeding one another during the same bloom, as reported by Wu *et al.* (2016; Dianchi Lake, China). Recent studies indicate that cyanobacteria have increased far more than other phytoplankton communities since c. 1800, and especially after 1945 (Taranu *et al*., 2015). Coupled with the growing demand for water safety, this trend will probably pose critical environmental and socio-economic problems in the next few years (Paerl and Paul, 2012). Australia, Canada, some European countries and the United States have started state-run toxin monitoring programs (including some for freshwaters) and applied marine fish and shellfish harvesting restrictions, but studies on freshwater harmful algal blooms (HABs), including cyanobacteria and cyanotoxins, lags far behind research on marine HABs and their biotoxins (Carmichael, 2001). Most countries have small research programs on freshwater HABs with small budgets, despite cyanotoxins being considered a priority by the European Water Framework Directive and World Health Organization (Chorus, 2005). The globally increasing frequency of HABs has prompted investigations into environmental monitoring methods and protocols (Lopez *et al*., 2008). Analyses on blooming toxins and their concentrations produce relevant information, but too late for the prevention of health risks, so early-warning tools for continuously monitoring aquatic ecosystems are a strong research priority (Lopez *et al*., 2008; Fadel *et al*., 2014).

The typical dynamics of cyanobacterial blooms make monitoring their quantity and spatial/temporal distribution difficult. Surface blooms can appear within hours and without warning, due not to rapid cell growth but to the upward migration of existing dispersed population. Their onset and severity therefore depend partly on the size of the existing which need not to be particularly large, but becomes much more concentrated as it floats to the surface (Oliver and Ganf, 2000). Processing phytoplankton samples is time-consuming, whereas immediate measurements are often needed to ensure the safe use of water resource. Local agencies monitoring water quality have to combine monitoring programs with faster techniques, which may also be used for a synoptic coverage of their water systems. This is where Earth observation (EO) might provide valuable data (Hestir *et al*., 2015) on sites of algal blooms (Wang and Shi, 2008; Stumpf *et al*., 2012; Matthews and Odermatt, 2015), or their duration across multiple lakes in a given ecoregion. These data are useful for establishing *in situ* monitoring programs, planning *in situ* sampling activities, and identifying environmental factors that can promote cyanobacterial blooms.

Several studies demonstrated the capability of mapping algal blooms with optical radiometers operated from the ground or on to space-borne platforms (Palmer *et al*., 2015; Matthews, 2014; Odermatt *et al*., 2012). Good results were obtained using specific empirical/semi-empirical algorithms for a given site sensor (Matthews *et al*., 2012; Hu *et al*., 2010; Kudela *et al*., 2015; Shi *et al*., 2015), or physically based approaches based on spectral inversion of analytical/semi-analytical models, or neural networks (Doerffer and Schiller, 2008; Riha and Krawczyk, 2011; Wynne *et al*., 2010; Li *et al*., 2013; Mishra *et al*., 2013; Li *et al*., 2015), or hybrid solutions (Carvalho *et al*., 2010; Matsushita *et al*., 2015). The first and most often adopted approach involves studying the spectral shape of a signal reflected by water in the visible- near-infrared (VIS-NIR) spectral range (Gilerson *et al*., 2010; Gurlin *et al*., 2011; Gitelson *et al*., 2008). Accessory photosynthetic pigments make cyanobacteria distinguishable from other phytoplankton communities based on their typical features in water reflectance spectra (Babin and Stramski, 2002; Pozdnyakov and Grassl, 2003; Roy *et al*., 2011). Phycocyanin (PC), the diagnostic pigment most often used to detect cyanobacteria (Dekker *et al*., 1995; Schalles and Yacobi, 2000; Simis *et al*., 2007; Randolph *et al*., 2008; Duan *et al*., 2012; Yacobi *et al*., 2015), has characteristic absorption and reflectance peaks around 620nm and 650nm, respectively (Gons *et al*., 2005; Simis *et al*., 2005). Phycoerythrin (PE), another specific pigment, has absorption and reflectance peaks at 565 nm and 600 nm, respectively (Bresciani *et al*., 2011).

Multispectral sensors (*e.g*., Landsat and more recently Sentinel-2) are generally unable to distinguish between waters dominated by cyanobacteria *vis-à-vis* by other algal species because their spectral band configuration is unsuitable for detecting features of PC-related reflectance or other characteristics unique to cyanobacteria. These sensors might be used in spectral inversion techniques (Dekker *et al*., 1991), however, to map water quality parameters (including Chl-a concentration), and in empirical relations with phytoplankton pigments (Vincent *et al*., 2004). Ocean color sensors (MERIS from 2002 to 2012 and now Sentinel-3), have bands appropriate for identifying spectral features due to Chl-a and both PC (Becker *et al*., 2009; Qi *et al*., 2014; Dash *et al*., 2011) and PE (Westberry *et al*., 2005; Bresciani *et al*., 2011), but not at low concentrations (Kutser *et al*., 2006), or in small lakes (where a 300-m pixel size is not good enough for image analysis). Ground-based observations like those obtained with hyperspectral sensors can provide reference measures for EO data validation (Brando *et al*., 2016; Zibordi *et al*., 2009), mediate between EO, *in situ* and laboratory data (Bresciani *et al*., 2013) and generate monitoring data for areas too narrow for EO data (Hommersom *et al*., 2012). Airborne and space hyperspectral sensors provide a contiguous for identifying key water quality indicators and phytoplankton pigments (Hestir *et al*., 2015). Finally, integration of multi-sensor EO data, such as MERIS and

ASAR imagery (Adamo *et al*., 2013; Bresciani *et al*., 2014) or MERIS and MODIS (Olmanson *et al*., 2011; Shuchman *et al*., 2013; Schaeffer *et al*., 2013) provides further insight on spatial patterns under cloud, or at different times of day. Numerous variables may correlate with aquatic optics data retrievable by remote sensing data, including cyanobacteria cell counts (Hunter *et al*., 2010), biovolumes (Reinart and Kutser, 2006), pigment concentrations measured by fluorimetry (Giardino *et al*., 2010; Seppala *et al*., 2007) and high-performance liquid chromatography (HPLC) (Zimba and Gitelson, 2006). Surface blooms might be identified by mapping Chl-a (Isenstein *et al*., 2014; Kutser, 2004; Moses *et al*., 2012) or PC (Hunter *et al*., 2010; Shi *et al*., 2015) concentrations.

This study presents the first results of an Italian research project called BLASCO (Blending LAboratory and Satellite techniques for detecting CyanObacteria) for monitoring cyanobacteria in lakes based on EO data, and for tracking their blooms. The first section describes the cyanobacterial blooms occurring in Italian lakes in recent years. The second section concerns the contribution of remote sensing to cyanobacterial bloom mapping in four lakes. Satellite data obtained from Landsat-8 (L8) were used to assess the spatial distribution of scum and Chl-a concentrations during surface bloom events. The maximum peak-height (MPH) index (Matthews *et al*., 2012) was obtained from a 2003-2011 MERIS time-series to identify cyanobacterial surface blooms in meso-eutrophic subalpine lakes. Chl-a concentration products obtained for a shallow, turbid hypereutrophic lake were used to identify areas where the strongest blooms were likely to occur, also depending on the morphometric features of the lake basin as this might support an *in-situ* sampling strategy (Kiefer *et al*., 2015).

CYANOBACTERIAL BLOOMS IN ITALIAN LAKES

Toxic cyanobacteria are causing ecological and toxicological problems in Italy. Cyanobacterial blooms have been reported in 71 bodies of water (natural lakes and artificial reservoirs), and this figure probably underestimates the real situation. These events are linked to a general increase in the trophic status of the country's inland waters (Garibaldi *et al*., 1997, 2003; Carollo and Libera, 1992; Cordella and Salmaso, 1992).

Toxic blooms of freshwater cyanobacteria involve several filamentous genera, such as *Aphanizomenon* (Bruno *et al*., 1989), *Chrysosporum* (ex *Aphanizomenon*) (Messineo *et al*., 2009), *Cylindrospermopsis* (Manti *et al*., 2005) *Dolychospermum* (ex *Anabaena*) (Bruno *et al*., 1994) and *Planktothrix* (Pomati *et al*., 2000; Messineo *et al*., 2006), as well as unicellular, colonial *taxa*, such as *Microcystis* (Bruno *et al*., 1989), in which toxin production has been detected in specific populations.

Two species reportedly most often responsible for - *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis and Komárek, and *Microcystis aeruginosa* (Kützing) Kützing (Messineo *et al*., 2006; Salmaso and Mosello, 2010) - both of them produce microcystins (Briand *et al*., 2003), a very common class of cyanotoxins, implicated in human and animal poisoning. *P. rubescens* typically inhabits deep lakes with a stable stratification and a metalimnetic layer in summer where this species adapted to low light and low temperatures can find the ideal growing conditions, as the phycoerythrin pigment gives rise to extremely effective light-capturing mechanisms (Steinberg and Hartmann, 1988), allowing its survival at lower depths than most algae (Davis *et al*., 2003). Many deep lakes and reservoirs in Europe are suitable for *P. rubescens* (Guiry and Guiry, 2011). In Italy, *P. rubescens* blooms have been reported in: Lakes Garda (Salmaso, 2000), Iseo (Garibaldi *et al*., 2003), Maggiore (Morabito *et al*., 2002), Orta (Morabito, 2001), Spino (Viaggiu *et al*., 2003) and Pusiano (Legnani *et al*., 2005) in the northern subalpine region; Lakes Albano and Fiastrone (Viaggiu *et al*., 2003), Nemi (Margaritora *et al*., 2005) and Vico (Manganelli *et al*., 2010) in Central Italy; and Lake Arancio (Naselli-Flores and Barone, 2007) in the South. In some cases, *P. rubescens* has been repeatedly reported as the dominant cyanobacterium in long-lasting bloom events (Viaggiu *et al*., 2004). The physiological mechanism behind *P. rubescens* blooms has been studied extensively in Lake Zurich (Walsby, 2005; Walsby *et al*., 2006): the buoyancy of the filaments is regulated by the balance between carbohydrates production and consumption mediated by the underwater light and controlled by the depth of the mixed layer.

M. aeruginosa is a typical inhabitant of epilimnetic waters, adapted to high light conditions. This species is very common in Italy. In the north, its presence and/or blooms have been reported for Lakes Garda, Iseo, Maggiore, Caldonazzo, Canzolino, Serraia, Pusiano, Como and Monate (Manganelli *et al*., 2014; ISTISAN 35/11). In central and southern Italy, it has been detected in 6 lakes: Massacciuccoli, Trasimeno, Polverina, Castreccioni, Liscione and Cecita. It has also been found in 13 lakes in Sardinia and 3 lakes in Sicily (Manganelli *et al*., 2014; ISTISAN 35/11). Studies on the Sicilian reservoirs found blooms of *Microcystis* spp. associated with variations in water level, occurring common occurrence due to the Mediterranean climate (rainy winters and dry summers) and the island's river network (mainly consisting of temporary streams). In summer, water is drawn from lakes for irrigation and drinking purposes, causing a rapid drop in their le vel, that often prompts a lowering thermocline and disrupted stratification. The consequent marked change of mixing regime can mobilize the nutrients stored in the hypolimnion, boosting *Microcystis* blooms

(Naselli-Flores, 2003, 2014; Naselli-Flores and Barone, 2003, 2005, 2007). Its strong buoyancy also enables *M. aeruginosa* to counteract occasional mixing of surface waters (Salmaso *et al*., 2014b; ISTISAN 11/14).

CASE STUDIES: EXPLOITING REMOTE SENSING DATA

Three case studies were conducted in Lombardy (northern Italy), a region rich in both deep, medium-to-large, and small shallow lakes (Fig. 1). Materials and methods used for the three study cases analyzed are summarized in Tab. 1. Details for each study case are provided in the specific paragraphs.

Landsat-8 OLI for detecting blooms in Como and Pusiano lakes

The Como and Pusiano lakes (Fig. 1) are on the edge of the Landsat-8 OLI (L8) acquisition path, so they can be monitored on an 8-day (instead of the standard 16-day) cycle, which improves the chances of cyanobacterial blooms being identified because they sometimes last only a few days (O'Neil *et al*., 2012). Their spatial mapping with L8 imagery used in this study shows that the satel-

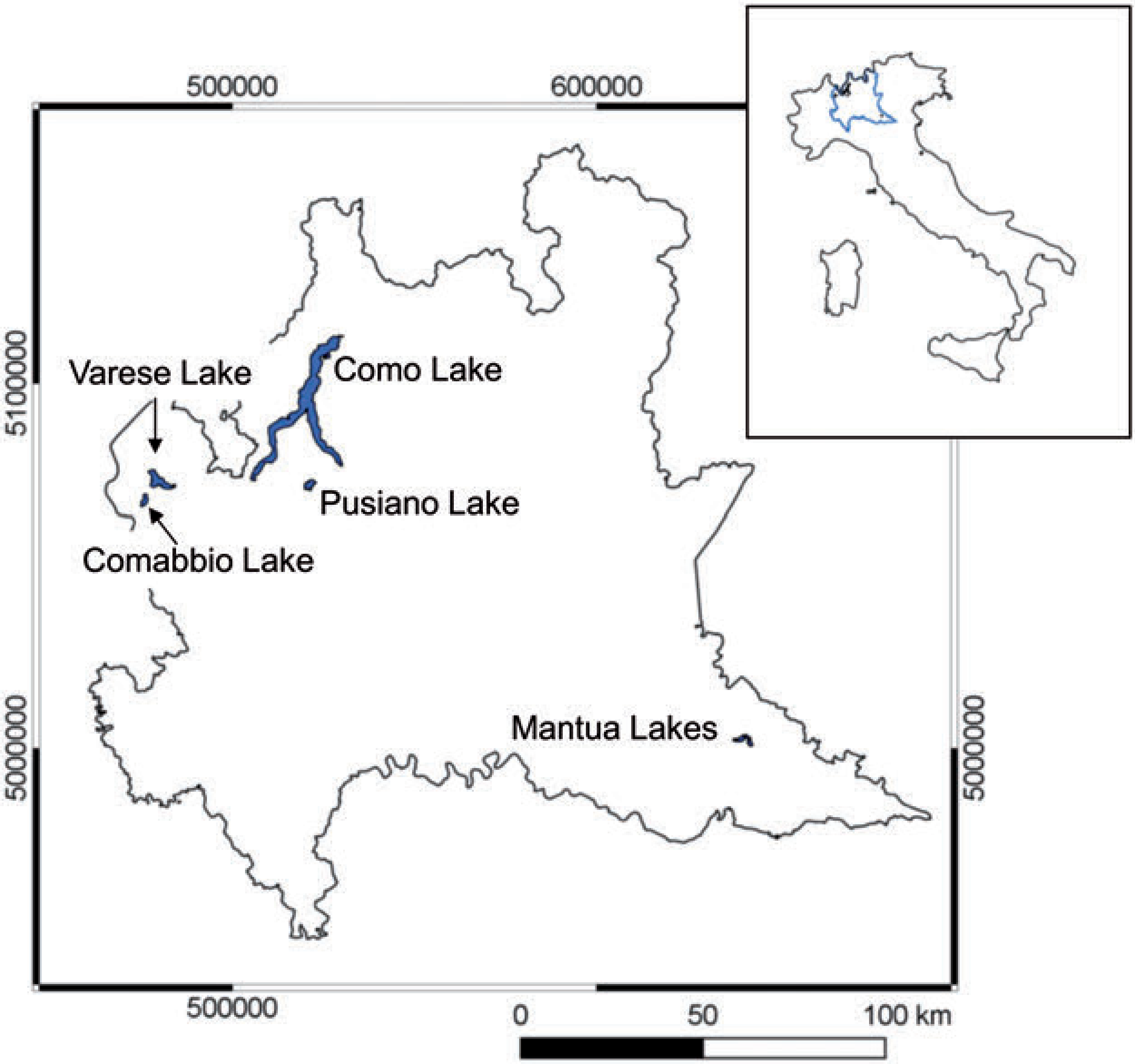


Fig. 1. Map showing the lakes in Lombardy Region (northern Italy) investigated in this study.

lite's 30-m pixel resolution suffices to capture the patchy distribution of cyanobacteria blooms (Fig. 2).

In early August 2013, an anomalous cyanobacterial bloom occurred in Lake Como (a large, deep lake in an oligo-mesotrophic state) that made its waters unsuitable for bathing or drinking for several days. The bloom was caused by *Dolichospermum lemmermannii* (Richter) Wacklin, Hoffmann and Komárek, a cyanobacterial species that produces surface scum. The bloom may be patchy, so L8 images were coupled with *in situ* monitoring, which was particularly challenging given the lake's size (145 km^2) and the bloom's short duration. Five L8 images acquired between the end of July and mid-August 2013 were radiometrically adjusted for water applications (Pahlevan *et al*., 2014), then atmospherically corrected with the 6SV code (Vermote *et al*., 2006). Surface bloom was detected using a band-ratio approach developed for similar purposes (Mayo *et al.*, 1995; Mahasandana *et al.*, 2001). Pixels where all three of the band-ratios i) b3 (561 nm) / b2 (483 nm); ii) b5 (865 nm) / b4 (655 nm); and iii) b3 (561 nm) / b4 (655 nm) higher than 1 were identified as scum. Using this method, numerous pixels revealed scum on the L8 image acquired on 1 August 2013, and none on or subsequent images. Fig. 2 shows the patchy distribution of *D. lemmermannii* at sites distributed all over the lake (total area = 431.8 ha). The satellite map was comparable with *in-situ* measurements obtained a day later, when the surface cyanobacteria concentration at the site in Fig. 2 was 365x10^6 cell L^{-1}. The scum was only mapped on 1 August 2013. It probably appeared as a result of significant rainfall blooming few days earlier. On 29 July 2013 precipitation occurred on Lake Como, 26 mm and 40 mm of 24 h cumulated precipitation, was recorded respectively in Como (south of the lake) and in Gera Lario (north) by ARPA Lombardia stations. A recent investigation (Callieri *et al*., 2014) found that *D. lemmermannii* blooms occasionally recorded in deep subalpine lakes in Italy were supported by nutrient pulses deriving from the mineralization of organic matter deposited along the lakeshore and released by rainfall event.

Nutrients arriving from the lake's catchment area can stimulate phytoplankton growth, especially in oligo-mesotrophic lakes (Morabito *et al*., 2012), and combined with a seasonal increase in water temperature this would facilitate *D. lemmermannii* proliferation (Olrik *et al*., 2012; Salmaso *et al*., 2015).

L8 data acquired on 11 November 2015 captured a

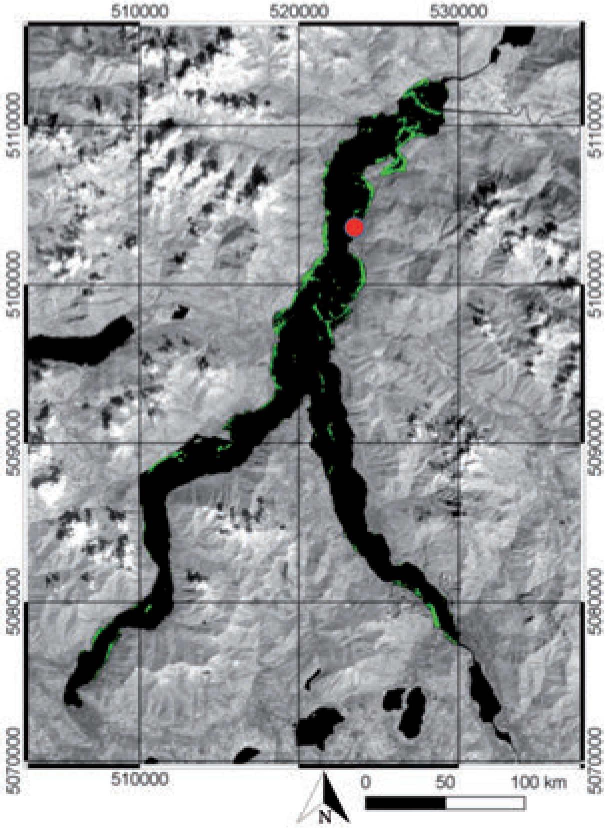


Fig. 2. Map of cyanobacteria scum (in green) in Lake Como from L8 data on 1 August 2013. The red circle marks the site of *in situ* measurements.

Tab. 1. Details on the sensors, number of images, approaches used and products derived for each lake object of this study.

Lake	Sensor	Number of images	Approach	Aim
Como	Landsat 8 - OLI	5	Band ratio	Cyanobacteria blooms identification
Pusiano	Landsat 8 - OLI	1	Bio-optical modeling	Maps of Chl-a concentrations
Comabbio	ENVISAT MERIS-FR	217	MPH	Multi temporal Cyanobacteria occurrence
Pusiano	ENVISAT MERIS-FR	248	MPH	Multi temporal Cyanobacteria occurrence
Varese	ENVISAT MERIS-FR	276	MPH	Multi temporal Cyanobacteria occurrence
Mantua	Hyperspectral (MIVIS-APEX-CHRIS)	7	Semi-empirical algorithms	Maps with zones characterized by high mean Chl-a concentration and high variability

cyanobacterial bloom in Lake Pusiano (a small lake south of Lake Como, in between its two branches). This lake has been hypereutrophic since the 1970s. Its total phosphorus concentrations have been gradually reduced by means of a water treatment plant, although cyanobacterial blooms have been observed again in recent years (Margaritora *et al*., 2006). During the L8 acquisition (was at 10:10 UTC) some stations were visited between 9:00 to 11:00 UTC to collect water samples and measure water reflectance spectra with a WISP-3 (Hommersom *et al*., 2012). The average Secchi disk depth was 1 meter (±20 cm), and no scum was apparent, while the cyanobacterial biomass in the uppermost layers of the water column was observed. The phytoplankton samples analyzed under the inverted microscope (400x magnification) according to Utermöhl (1958) revealed mainly *Woronichinia naegeliana* (Unger) Elenkin, but also *Dolichospermum* (cf. *planctonicum*) (Brunnthaler) Wacklin, L.Hoffmann and Komárek, and *M. aeruginosa*.

Chl-a concentrations were measured by spectrophotometry according to Lorenzen (1967) and HPLC. Photosynthetic pigments for HPLC analysis were extracted in 90% acetone, overnight in the dark, under nitrogen. The extract obtained was used to quantify Chl and its derivatives (in Chl derivatives units, CD) and total carotenoids by spectrophotometry. Individual carotenoids were detected by revers-phase HPLC with an Ultimate 3000 (Thermo Scientific). Specific pigments were identified by ion pairing, reverse-phase HPLC described in Guilizzoni (2011). PC concentrations were quantified with the spectrophotometer (SAFAS UVmc2) in 1 cm path-length cuvettes using the equations of Bennett and Bogorad (1973).

The average Chl-a concentration measured at the pelagic stations with no accumulated surface cyanobacteria (dots in Fig. 3) was 12 mgm^{-3} (±5 mgm^{-3}), while it was significantly higher at the two coastal stations and the other pelagic station where scum was found, at 173 mg m^{-3}, 550 mg m^{-3} and 97.4 mg m^{-3} respectively. The corresponding PC concentration were 490 mg m^{-3} and 5210 mg m^{-3} respectively for two coastal stations. HPLC on two surface samples collected in the pelagic zone revealed high concentrations of two cyanobacteria marker pigments (echinenone and myxoxanthophyll, with mean values of 14.7 mg m^{-3} and 16.4 mg m^{-3}, respectively). L8 data were radiometrically and atmospherically corrected using the same procedure as for Lake Como to compute the Chl-a concentrations and test the ability of L8 to capture blooms. The water reflectances obtained in the first four L8 bands were comparable with the spectra obtained *in situ* (with correlation coefficients of 0.57, 0.72, 0.83 and 0.79 for bands 1, 2, 3 and 4; ***P<0.001 for all four bands). L8-derived water reflectances were converted into Chl-a concentrations by adopting a spectral inversion procedure based on a bio-optical model (Giardino *et al*., 2012, 2014) parameterized with specific inherent optical properties of eutrophic water. For the three pelagic stations, the average Chl-a concentration was 10.7 mg m^{-3} (±1.4). The coastal area was more difficult to assess because the L8 band setting might be too coarse for the very high concentrations involved, but the Chl-a concentrations for the two coastal stations exceeded 30 mg m^{-3} (much higher than at the pelagic stations), consistently with field observations.

As in other inland water ecosystems, the patchy distribution of cyanobacterial blooms seen in Lake Pusiano was due mainly to wind (Webster and Hutchinson, 1994; Zilius *et al*., 2014; Wu *et al*., 2015).

MERIS for monitoring cyanobacterial blooms in meso-eutrophic subalpine lakes

Small lakes south of the Alps are shallow, highly eutrophic, with highly variable Chl-a concentrations. Lake Varese is calcareous of glacial origin, sited to the west of Lake Maggiore. It has a mean depth of 11 m, and a surface area of 14.8 km^2. It is dimictic, with a summer stratification from May to November and an inverse stratification in winter. Lake Comabbio was originally linked to Lake Varese. It is polymictic, with a summer stratification from April to October. It has a mean depth of 4.6 m and a surface of 3.6 km^2.

For lakes Comabbio, Pusiano and Varese, respectively, 217, 248, and 276 MERIS Full Resolution (FR) images obtained from June to November (2003-2011) were processed to assess cyanobacterial blooms.

The MERIS FR Coast-Color level-1b images were pre-processed to correct the Rayleigh effect with the

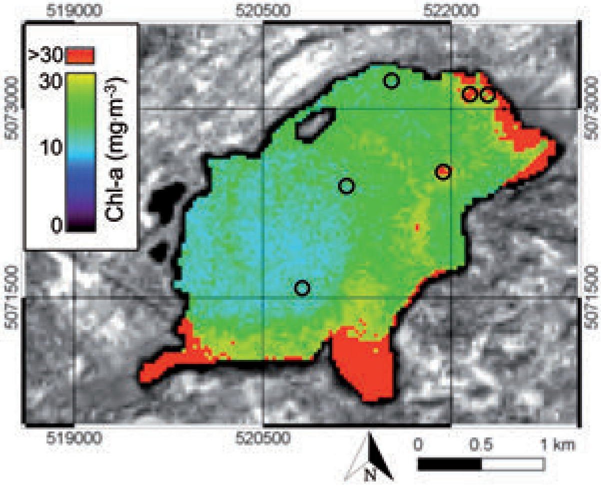


Fig. 3. Chl-a concentrations mapped in Lake Pusiano from L8 on 11 November 2015. The circles indicate the sites of *in situ* stations.

BEAM BRR (Bottom-of-Rayleigh Reflectance) processor. The product was then processed with Maximum Peak-Height (MPH) processor (Matthews *et al*., 2012). MPH exploits the BRR peaks in the red and near-infrared bands above a given baseline, which moves depending on the pigment concentrations. It provides a MPH index that is useful for calculating Chl-a concentrations, as towelled as flags for floating material and for eukaryote or cyanobacteria dominance for each pixel. Cyanobacteria dominance was estimated at 25%, 6%, and 12% on the images of the Comabbio, Pusiano and Varese lakes, respectively. The timing of this phenomenon varied from lake to lake, from season to season, and from to year. It was recorded most frequently in 2008 for Lakes Pusiano and Varese, and in 2011 for Lake Comabbio (Fig. 4). Considering the whole period, October was the month most frequently involved for Lake Comabbio, November for Lakes Pusiano and Varese (Fig. 5). Some of the events have been fully documented, *i.e.*, *P. rubescens* blooms in Lake Pusiano in Autumn 2010 (Salmaso *et al*., 2014a) and in Lake Varese in November 2011.

These results clearly show that cyanobacterial blooms

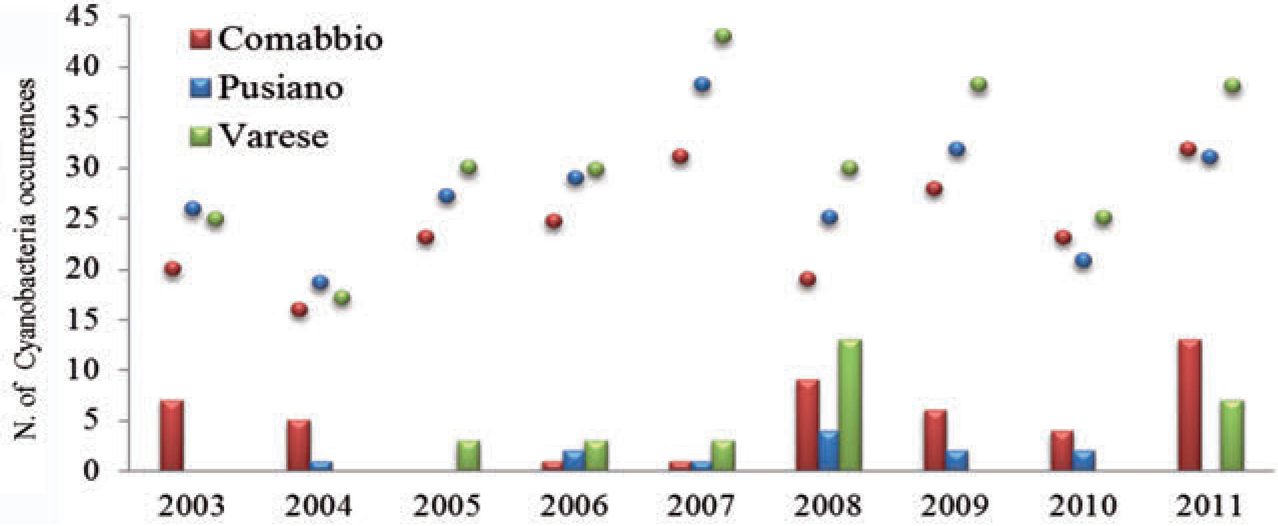
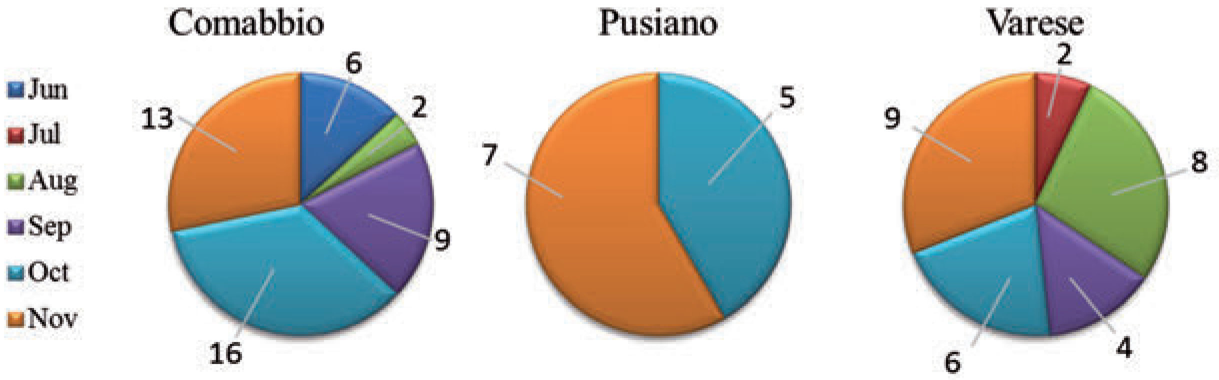


Fig. 4. Number of cyanobacterial blooms thereby year meso-eutrophic subalpine lakes.



Number of cloud-free images per month						
LAKE	Jun	Jul	Aug	Sep	Oct	Nov
COMABBIO	30	51	42	38	28	28
PUSIANO	37	57	53	40	32	29
VARESE	43	67	57	47	33	29

Fig. 5. Number of cyanobacterial blooms by month in the three meso-eutrophic subalpine lakes. In the table the number of cloud-free images per month.

in the subalpine lakes can occur even outside the bathing season, meaning that environmental agencies' typical monitoring programs can underestimate them if sampling is only done in summer. Recreational uses of these lakes often continue into late summer and early autumn, however, carrying a risk of intoxication for people and animals.

Imaging spectrometry for spatial analysis of Chl-a in hypertrophic waters

Lake Superior is the largest of three shallow hypertrophic lakes surrounding the town of Mantua, in northern Italy, with a surface area of 3.67 km^2 and an average depth 3.6 m. It is part of an artificial fluvial lake system created by damming the Mincio River in the 12th century. Water levels in Lake Superior are regulated by the Vasarone dam and Vasarina gate (built in 2015), to ensure a constant 17.5 m asl (Pinardi *et al.*, 2011**,** 2015). Considerable nutrient loads enter the lake from its main tributary (the Mincio River), sustaining a dense phytoplankton community, with recurrent blooms that bring Chl-a concentrations up to about 100 mg m^{-3} (Bolpagni *et al*., 2014).

Seven images of Lake Superior were used to examine the spatial variability of mean Chl-a concentrations over time. All images were acquired from June to September by hyperspectral sensors on airborne platforms, *i.e.* MIVIS (2007-07-26) and APEX (2011-09-21 and 2014-09-27), and the satellite platform Proba-1 CHRIS (2008-06-29, 2008-09-16, 2011-08-28 and 2012-08-06). Chl-a concentrations were measured using the procedures described in Pinardi *et al*. (2015), during the season most associated with phytoplankton blooms, when local authorities, monitor the situation in accordance with the Italian Water Framework Directive guidelines.

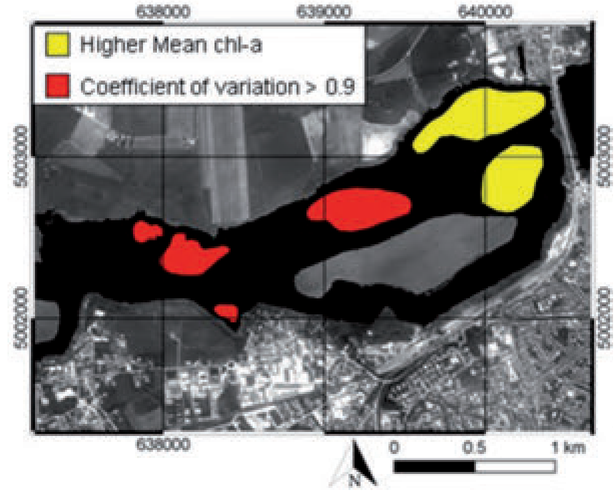


Fig. 6. Spatial analysis of Chl-a in Mantua Lake Superior obtained from hyperspectral images. The red zones had a higher coefficient of variation. The yellow zones had higher mean Chl-a concentrations, and would be appropriate for new *in situ* sampling sites.

The temporal analysis of the seven images was done in a GIS environment, using the same reference system (WGS84) and cartographic projection (UTM Zone 32N). Pixels were aggregated to a 15-m spatial resolution, which is the minimum common pixel size providing the spatially most detailed outcome. The GRASS function (r.series) and raster calculator tool were used to obtain statistics (*e.g.*, mean, standard deviation, coefficient of variation) of the temporal series for each pixel of Mantua Lake Superior. Some zones with particular statistical properties were selected and polygonized (Fig. 6): the yellow polygons identify areas with higher mean Chl-a concentrations (35-45 mg m^{-3}), and relatively lower standard deviations (less than 30 mgm^{-3}; mean coefficient of variation 0.75); the red polygons are areas with a high coefficient of variation (>0.9). The main hydrodynamic events influencing Chl-a distribution related to the combined effects of wind force and riverine current. The red zones identify areas where significant water circulation influenced the Chl-a concentrations, the yellow zones indicate areas where water stagnation favored phytoplankton bloom and accumulation.

CONCLUSIONS

This study aimed to describe the capabilities of remote sensing for mapping cyanobacterial blooms and to highlight the main advantages of such techniques, *i.e.* a synoptic view and frequent acquisitions to track dynamic phenomena. The case studies show that combining remote sensing with *in situ* measurements can help monitor cyanobacterial blooms in Italian lakes. Landsat-8 OLI data provided both the spatial distribution of cyanobacterial blooms in a small eutrophic lake, and the patchy distribution of scum in a large deep subalpine basin. The 10-year-long database of MERIS images enabled a dynamic mapping of cyanobacterial blooms affecting water quality in three small meso-eutrophic lakes, showing that algal blooms occurred for about 5 days a year, typically in late summer and early autumn. Air- and space-borne hyperspectral sensors were tested as a source of data for mapping Chl-a concentrations in Mantua's lakes, revealing that some zones of these lakes have higher Chl-a concentrations due to water circulation, suggesting the need to align *in situ* monitoring programs with the findings on hyperspectral images.

Future research will focus on further developing algorithms to enhance cyanobacterial mapping accuracy by including of semi-empirical and physically-based approaches to secondary pigments in cyanobacteria. The algorithms will also be adapted to new generation satellite sensors like the ESA Sentinel of the EC Copernicus program to access their fully operational EO capacity and improved spatial, radiometric and temporal resolutions. Sentinel-1 is a radar (SAR) instrument that can support scum detection even under cloud, and Sentinel-2 (like Landsat-8) can shed light on water quality. Most importantly, Sentinel-3 will be the successor of MERIS, with many optical bands specifically geared to water quality applications, and will assure continuous data acquisition for the next decades.

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REFERENCES

Adamo M, Matta E, Bresciani M, De Carolis G, Vaiciute D, Giardino C, Pasquariello G, 2013. On the synergistic use of SAR and optical imagery to monitor cyanobacteria blooms: the Curonian Lagoon case study. Eur. J. Remote Sens. 46:789-805.

Babin M, Stramski D, 2002. Light absorption by aquatic particles in the near-infrared spectral region. Limnol. Oceanogr. 47:911-915.

Backer L, McGillicuddy D, 2006. Harmful algal blooms at the interface between coastal oceanography and human health. Oceanography 19:94-106.

Bálint M, Domisch S, Engelhardt CHM, Haase P, Lehrian S, Sauer J, Nowak C, 2011. Cryptic biodiversity loss linked to global climate change. Nature Clim. Change 1:313-318.

Becker RH, Sultan MI, Boyer GL, Twiss MR, Konopko E, 2009. Mapping cyanobacterial blooms in the Great Lakes using MODIS. J. Great Lakes Res. 35:447-453.

Bennett A, Bogorad L, 1973. Complementary chromatic adaptation in a filamentous blue-green alga. J. Cell Biol. 58:419-435.

Bolpagni R, Bresciani M, Laini A, Pinardi M, Matta E, Ampe EM, Giardino C, Viaroli P, Bartoli M, 2014. Remote sensing of phytoplankton-macrophyte coexistence in shallow hypereutrophic fluvial lakes. Hydrobiologia 737:67-76.

Brando VE, Lovell JL, King EA, Boadle D, Scott R, Schroeder T, 2016. The potential of autonomous ship-borne hyperspectral radiometers for the validation of ocean color radiometry data. Remote Sens. 8:150.

Bresciani M, Adamo M, De Carolis G, Matta E, Pasquariello G, Vaiciute D, Giardino C, 2014. Monitoring blooms and surface accumulation of cyanobacteria in the Curonian Lagoon by combining MERIS and ASAR data. Remote Sens. Environ. 146:124-135.

Bresciani M, Giardino C, Bartoli M, Tavernini S, Bolpagni R, Nizzoli D, 2011. Recognizing harmful algal bloom based on remote sensing reflectance band ratio. J. Appl. Remote Sens. 5:053556.

Bresciani M, Rossini M, Morabito G, Matta E, Pinardi M, Cogliati S, Julitta T, Colombo R, Braga F, Giardino C, 2013. Analysis of within- and between-day chlorophyll-a dynamics in Mantua Superior Lake, with a continuous spectroradiometric measurement. Mar. Freshwater Res. 64:303-316.

Briand JF, Jacquet S, Bernard C, Humbert JF, 2003. Health hazards for terrestrial vertebrates from toxic cyanobacteria in surface water ecosystems. Vet. Res. 34:361-377.

Bruno M, Gucci PMB, Volterra L, 1989. [Fioriture algali: rilevabilità della presenza di biotossine].[Article in Italian]. Ambiente Risorse Salute 91:6-7.

Bruno M, Barbini DA, Pierdominici E, Serse AP, Ioppolo A, 1994. Anatoxin-a and a previously unknown toxin in Anabaena planctonica from blooms found in Lake Mulargia (Italy). Toxicon 32:369-373.

Callieri C, Bertoni R, Contesini M, Bertoni F, 2014. Lake level fluctuations boost toxic cyanobacterial "oligotrophic blooms". PLoS One 9:e109526.

Carmichael WW, 2001. Health effects of toxin-producing cyanobacteria: "The CyanoHABs." Hum. Ecol. Risk Assess. 7:1393-1407.

Carollo A, Libera V, 1992. Geographical and characteristics of the main Italian lakes. Mem. Istit. Ital. Idrobiol. 50:29-35.

Carvalho GA, Minnett PJ, Fleming LE, Banzon VF, Baringer W, 2010. Satellite remote sensing of harmful algal blooms: A new multi-algorithm method for detecting the Florida Red Tide (Karenia brevis). Harmful Algae 9:440-448.

Chorus I, 2005. Water safety plans - A better regulatory approach to prevent human exposure to harmful cyanobacteria, p. 201-227. In: J. Huisman, H.C.P. Matthijs and P.M. Visser (eds.), Harmful Cyanobacteria. 3. Springer, Dordrecht.

Coles JF, Jones RC, 2000. Effect of temperature on photosynthesis-light response and growth of four phytoplankton species isolated from a tidal freshwater river. J. Phycol. 36:7-16.

Cordella P, Salmaso N, 1992. Studies on some reservoirs and lakes in North-East Italy. Mem. Ist. Ital. Idrobiol. 50:259-271.

Dash P, Walker ND, Mishra DR, Hu C, Pinckney JL, D'Sa EJ, 2011. Estimation of cyanobacterial pigments in a freshwater lake using OCM satellite data. Remote Sens. Environ. 115:3409-3423.

Davis PA, Dent M, Parker J, Reynolds CS, Walsby AE, 2003. The annual cycle of growth rate and biomass change in *Planktothrix* spp. in Blelham Tarn, English Lake District. Freshwater Biol. 48:852-867.

Dekker A, Malthus T, Hoogenboom HJ, 1995. The remote sensing of inland water quality, p. 123-142. In: F.M. Danson and S.E. Plummer (eds.), Advances in environmental remote sensing. J. Wiley & Sons, Chichester.

Dekker A, Malthus T, Seyhan E, 1991. Quantitative modeling of inland water-quality for high-resolution MSS systems. Ieee T. Geosci. Remote 29:89-95.

Dodds WK, Perkin JS, Gerken JE, 2013. Human impact on freshwater ecosystem services: a global perspective. Environ. Sci. Technol. 47:9061-9068.

Doerffer R, Schiller H, 2008. MERIS Lake Water Algorithm for BEAM-MERIS algorithm theoretical basis document. V1.0, 10 June 2008. GKSS Research Center, Geesthacht, Germany.

Dokulil MT, Teubner K, 2011. Eutrophication and climate change: present situation and future scenarios, p. 1-16. In: A.A. Ansari, S. Singh Gill, G R. Lanza and W. Rast (eds.), Eutrophication: causes, consequences and control. 1. Springer, Berlin.

Dokulil MT, Teubner K, Jagsch A, Nickus U, Adrian R, Straile D, Jankowski T, Herzig A, Padisák J, 2009. The impact of climate change in Central Europe, p. 387-409. In: D.G. George (ed.), The impact of climate change on European lakes. 4. Springer, Dordrecht.

Duan H, Ma R, Hu C, 2012. Evaluation of remote sensing algorithms for cyanobacterial pigment retrievals during spring bloom formation in several lakes of East China. Remote Sens. Environ. 126:126-135.

Dukes JS, Mooney HA, 1999. Does global change increase the success of biological invaders? Trends Ecol. Evol. 14:135-139.

Elliott JA, 2012. Is the future blue-green? A review of the current model predictions of how climate change could affect pelagic freshwater cyanobacteria. Water Res. 46:1364-1371.

Fadel A, Atoui A, Lemaire BJ, Vinçon-Leite B, Slim K, 2014. Dynamics of the toxin cylindrospermopsin and the cyanobacterium Chrysosporum (Aphanizomenon) ovalisporum in a Mediterranean eutrophic reservoir. Toxins 6:3041-3057.

Falconer IR, Humpage AR, 2005. Health risk assessment of cyanobacterial (blue-green algal) toxins in drinking water. Int. J. Environ. Res. Public. Health 2:43-50.

Garibaldi L, Anzani A, Marieni A, Leoni B, Mosello R, 2003. Studies on the phytoplankton of the deep subalpine Lake Iseo. J. Limnol. 62:177-189.

Garibaldi L, Brizzio MC, Galanti G, Varallo A, Mosello R, 1997. [Idrochimica e fitoplancton del Lago d'Idro].[Article in Italian]. Documenta Ist. Ital. Idrobiol. 61:153-172.

Giardino C, Bresciani M, Cazzaniga I, Schenk K, Rieger P, Braga F, Matta E, Brando VE, 2014. Evaluation of Multi-Resolution Satellite sensors for assessing water quality and bottom depth of Lake Garda. Sensors 14:24116-24131.

Giardino C, Bresciani M, Pilkaityte R, Bartoli M, Razinkovas A, 2010. In situ measurements and satellite remote sensing of case 2 waters: first results from the Curonian Lagoon. Oceanologia 52:197-210.

Giardino C, Candiani G, Bresciani M, Lee Z, Gagliano S, Pepe M, 2012. BOMBER: A tool for estimating water quality and bottom properties from remote sensing images. Comput. Geosci. 45: 313-318.

Gilerson AA, Gitelson AA, Zhou J, Gurlin D, Moses W, Ioannou I, Ahmed SA, 2010. Algorithms for remote estimation of chlorophyll-a in coastal and inland waters using red and near infrared bands. Opt. Express 18:24109.

Gitelson AA, Dall'Olmo G, Moses W, Rundquist DC, Barrow T, Fisher TR, Gurlin D, Holz J, 2008. A simple semi-analytical model for remote estimation of chlorophyll-a in turbid waters: validation. Remote Sens. Environ. 112:3582-3593.

Gons HJ, Hakvoort H, Peters SW, Simis SG, 2005. Optical detection of cyanobacterial blooms, pp. 177-199. In: J. Huisman, H.C.P. Matthijs and P.M. Visser (eds.), Harmful Cyanobacteria. Springer, Dordrecht.

Guilizzoni P, Marchetto A, Lami A, Gerli S, Musazzi S, 2011. Use of sedimentary pigments to infer past phosphorus concentration in lakes. J. Paleolimnol. 45:433-445.

Guiry MD, Guiry GM, 2016. AlgaeBase. Accessed on: 15 July 2016. Available from: http://www.algaebase.org

Gurlin D, Gitelson AA, Moses WJ, 2011. Remote estimation of chl-a concentration in turbid productive waters - Return to a simple two-band NIR-red model? Remote Sens. Environ. 115:3479-3490.

Harley CDG, 2011. Climate change, keystone predation, and biodiversity loss. Science 334:1124-1127.

Hense I, Meier HEM, Sonntag S, 2013. Projected climate change impact on Baltic Sea cyanobacteria. Climatic Change 119:391-406.

Hestir EL, Brando VE, Bresciani M, Giardino C, Matta E, Villa P, Dekker AG, 2015. Measuring freshwater aquatic ecosystems: The need for a hyperspectral global mapping satellite mission. Remote Sens. Environ. 167:181-195.

Hommersom A, Kratzer S, Laanen M, Ansko I, Ligi M, Bresciani M, Giardino C, Beltrán-Abaunza JM, Moore G, Wernand M, Peters S, 2012. Intercomparison in the field between the new WISP-3 and other radiometers (TriOS Ramses, ASD FieldSpec, and TACCS). J. Appl. Remote Sens. 6:063615-063615.

Hu C, Lee Z, Ma R, Yu K, Li D, Shang S, 2010. Moderate Resolution Imaging Spectroradiometer (MODIS) observations of cyanobacteria blooms in Taihu Lake, China. J. Geophys. Res. Oceans 115:C04002.

Hunter PD, Tyler AN, Carvalho L, Codd GA, Maberly SC, 2010. Hyperspectral remote sensing of cyanobacterial pigments as indicators for cell populations and toxins in eutrophic lakes. Remote Sens. Environ. 114:2705-2718.

Isenstein EM, Trescott A, Park M-H, 2014. Multispectral remote sensing of harmful algal blooms in Lake Champlain, USA. Water Environ. Res. 86:2271-2278.

Jöhnk KD, Huisman J, Sharples J, Sommeijer B, Visser PM, Stroom JM, 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Glob. Change Biol. 14:495-512.

Kiefer I, Odermatt D, Anneville O, Wüest A, Bouffard D, 2015. Application of remote sensing for the optimization of in-situ sampling for monitoring of phytoplankton abundance in a large lake. Sci. Total Environ. 527:493-506.

Kudela RM, Palacios SL, Austerberry DC, Accorsi EK, Guild LS, Torres-Perez J, 2015. Application of hyperspectral remote sensing to cyanobacterial blooms in inland waters. Remote Sens. Environ. 167:196-205.

Kutser T, 2004. Quantitative detection of chlorophyll in cyanobacterial blooms by satellite remote sensing. Limnol. Oceanogr. 49:2179-2189.

Kutser T, Metsamaa L, Strombeck N, Vahtmae E, 2006. Monitoring cyanobacterial blooms by satellite remote sensing. Estuar. Coast. Shelf Sci. 67:303-312.

Landsberg JH, 2002. The effects of harmful algal blooms on aquatic organisms. Rev. Fish. Sci. 10:113-390.

Legnani E, Copetti D, Oggioni A, Tartari G, Palombo MT, Morabito G, 2005. *Planktothrix rubescens* seasonal dynamics and vertical distribution in Lake Pusiano (North Italy). J. Limnol. 64:61-73.

Li L, Li L, Song K, 2015. Remote sensing of freshwater cyanobacteria: An extended IOP Inversion Model of Inland Waters (IIMIW) for partitioning absorption coefficient and estimating phycocyanin. Remote Sens. Environ. 157:9-23.

Li L, Li L, Song K, Li Y, Tedesco LP, Shi K, Li Z, 2013. An inversion model for deriving inherent optical properties of inland waters: Establishment, validation and application. Remote Sens. Environ. 135:150-166.

Lopez CB, Jewett EB, Dortch Q, Walton BT, Hudnell HK, 2008. Scientific assessment of freshwater harmful algal blooms. Interagency Working Group on Harmful Algal Blooms, Hypoxia, and Human Health of the Joint Subcommittee on Ocean Science and Technology, Washington, USA.

Lorenzen CJ, 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnol. Oceanogr. 12:343-346.

Mahasandana S, Tripathi NK, Honda K, 2009. Sea surface multispectral index model for estimating chlorophyll a concentration of productive coastal waters in Thailand. Can. J. Remote Sens. 35:287-296.

Manganelli M, Scardala S, Stefanelli M, Vichi S, Mattei D, Bogialli S, Ceccarelli P, Corradetti E, Petrucci I, Gemma S, Testai E, 2010. Health risk evaluation associated to Planktothrix rubescens: An integrated approach to design tailored monitoring programs for human exposure to cyanotoxins. Water Res. 44:1297-1306.

Manganelli M, Viaggiu E, Barone R, Buzzi F, Caviglia F, Congestri R, Copetti D, De Angelis R, Godeas F, Guzzella L, Masala E, Naselli-Flores L, Salmaso N, Scardala S, 2014. [Situazione nazionale: corpi idrici interessati da cianobatteri tossici], p. 116-143. In: E. Funari, M. Manganelli and E. Testai (eds.), [Cianobatteri: linee guida per la gestione delle fioriture nelle acque di balneazione].[Report in Italian]. Rapporti ISTISAN 14/20. Istituto Superiore di Sanità, Roma.

Manti G, Mattei D, Messineo V, Bogialli S, Sechi N, Casiddu P, Luglié A, Di Brizio M, Bruno M, 2005. First report of *Cylindrospermopsis raciborskii* in Italy. Harmful Algae News 28:8-9.

Margaritora FG, Cherubini E, Copetti D, Legnani E, Seminara M, Tartari G, Vagaggini D, 2006. Recent trophic changes in Lake Pusiano (northern Italy) with particular reference to the influence of hydrodynamics on the zooplankton community. Chem. Ecol. 22:S37-47.

Margaritora FG, Fumanti B, Alfinito S, Tartari G, Vagaggini D, Seminara M, Cavacini P, Vuillermo E, Rosati M, 2005. Trophic condition of the volcanic Lake Nemi (Central Italy): environmental factors and planktonic communities in a changing environment. J. Limnol. 64:119-128.

Markensten H, Moore K, Persson I, 2010. Simulated lake phytoplankton composition shifts toward cyanobacteria dominance in a future warmer climate. Ecol. Appl. 20:752-767.

Matsushita B, Yang W, Yu G, Oyama Y, Yoshimura K, Fukushima T, 2015. A hybrid algorithm for estimating the chlorophyll-a concentration across different trophic states in Asian inland waters. ISPRS J. Photogramm. 102:28-37.

Matthews MW, 2014. Eutrophication and cyanobacterial blooms in South African inland waters. Remote Sens. Environ. 155:161-177.

Matthews MW, Bernard S, Robertson L, 2012. An algorithm for detecting trophic status (chlorophyll-a), cyanobacterial-dominance, surface scums and floating vegetation in inland and coastal waters. Remote Sens. Environ. 124:637-652.

Matthews MW, Odermatt D, 2015. Improved algorithm for routine monitoring of cyanobacteria and eutrophication in inland and near-coastal waters. Remote Sens. Environ. 156:374-382.

Mayo M, Gitelson A, Yacobi YZ, Ben-Avraham Z, 1995. Chlorophyll distribution in Lake Kinneret determined from Landsat Thematic Mapper data. Remote Sens. 16:175-182.

Messineo V, Bogialli S, Melchiorre S, Sechi N, Lugliè A, Casiddu P, Mariani MA, Padedda BM, Di Corcia A, Mazza R, Carloni E, Bruno M, 2009. Cyanobacterial toxins in Italian freshwaters. Limnologica 39:95-106.

Messineo V, Mattei D, Melchiorre S, Salvatore G, Bogialli S, Salzano R, Mazza R, Capelli G, Bruno M, 2006. Microcystin diversity in a *Planktothrix rubescens* population from Lake Albano (Central Italy). Toxicon 48:160-174.

Mishra S, Mishra DR, Lee Z, Tucker CS, 2013. Quantifying cyanobacterial phycocyanin concentration in turbid productive waters: A quasi-analytical approach. Remote Sens. Environ. 133:141-151.

Morabito G, 2001. Six years' (1992-1997) evolution of phytoplankton communities after recovery by liming in Lake Orta, northern Italy. Lakes Reserv. Res. Manage. 6:305-312.

Morabito G, Oggioni A, Austoni M, 2012. Resource ratio and human impact: How diatom assemblages in Lake Maggiore responded to oligotrophication and climatic variability. Hydrobiologia 698:47-60.

Morabito G, Ruggiu D, Panzani P, 2002. Recent dynamics (1995-1999) of the phytoplankton assemblages in Lago Maggiore as a basic tool for defining association patterns in the Italian deep lakes. J. Limnol. 61:129-145.

Moses WJ, Gitelson AA, Berdnikov S, Saprygin V, Povazhnyi V, 2012. Operational MERIS-based NIR-red algorithms for estimating chlorophyll-a concentrations in coastal waters - The Azov Sea case study. Remote Sens. Environ. 121:118-124.

Naselli-Flores L, 2003. Man-made lakes in Mediterranean semi-arid climate: the strange case of Dr Deep Lake and Mr Shallow Lake. Hydrobiologia 506:13-21.

Naselli-Flores L, 2014. Morphological analysis of phytoplankton as a tool to assess ecological state of aquatic ecosystems. The case of Lake Arancio, Sicily, Italy. Inland Waters 4:15-26.

Naselli-Flores L, Barone R, 2003. Steady-state assemblages in a Mediterranean hypertrophic reservoir. The role of *Microcystis* ecomorphological variability in maintaining an apparent equilibrium. Hydrobiologia 502:133-143.

Naselli-Flores L, Barone R, 2005. Water-level fluctuations in Mediterranean reservoirs: setting a dewatering threshold as a management tool to improve water quality. Hydrobiologia 548:85-99.

Naselli-Flores L, Barone R, 2007. Pluriannual morphological variability of phytoplankton in a highly productive Mediterranean reservoir (Lake Arancio, Southwestern Sicily). Hydrobiologia 578:87-95.

O'Neil JM, Davis TW, Burford MA, Gobler CJ, 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14:313-334.

Odermatt D, Pomati F, Pitarch J, Carpenter J, Kawka M, Schaepman M, Wueest A, 2012. MERIS observations of phytoplankton blooms in a stratified eutrophic lake. Remote Sens. Environ. 126:232-239.

Oliver RL, Ganf GG, 2000. Freshwater blooms, p. 149-194. In: B.A. Whitton and M. Potts M. (eds.), Ecology of cyanobacteria: Their diversity in time and space. Kluwer, Dordrecht.

Olmanson LG, Brezonik PL, Bauer ME, 2011. Evaluation of medium to low resolution satellite imagery for regional lake water quality assessments. Water Resour. Res. 47:W09515.

Olrik K, Oronbergz G, Annadotter H, 2012. Lake phytoplankton responses to global climate changes, p. 173-199. In: C.R. Goldman, M. Kumagai and R.D. Robarts (eds.), Climatic change and global warming of inland waters: impacts and mitigation for ecosystems and societies. J. Wiley & Sons, Chichester.

Paerl HW, Huisman J, 2008. Climate - Blooms like it hot. Science 320:57-58.

Paerl HW, Huisman J, 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environ. Microbiol. Rep. 1:27-37.

Paerl HW, Paul VJ, 2012. Climate change: links to global expansion of harmful cyanobacteria. Water Res. 46:1349-1363.

Pahlevan N, Lee Z, Wei J, Schaaf CB, Schott JR, Berk A, 2014. On-orbit radiometric characterization of OLI (Landsat-8) for applications in aquatic remote sensing. Remote Sens. Environ. 154:272-284.

Palmer SCJ, Kutser T, Hunter PD, 2015. Remote sensing of inland waters: Challenges, progress and future directions. Remote Sens. Environ. 157:1-8.

Pinardi M, Bartoli M, Longhi D, Viaroli P, 2011. Net autotrophy in a fluvial lake: the relative role of phytoplankton and floating-leaved macrophytes. Aquat. Sci. 73:389-403.

Pinardi M, Fenocchi A, Giardino C, Sibilla S, Bartoli M, Bresciani M, 2015. Assessing potential algal blooms in a shallow fluvial lake by combining hydrodynamic modelling and remote-sensed images. Water 7:1921-1942.

Pomati F, Sacchi S, Rossetti C, Giovannardi S, 2000. The freshwater cyanobacterium *Planktothrix* sp. FP1: molecular identification and detection of paralytic shellfish poisoning toxins. J. Phycol. 36:553-562.

Pozdnyakov D, Grassl H, 2003. Colour of inland and coastal waters: A methodology for Its Interpretation. Springer/Praxis, Heidelberg/Chichester: 170 pp.

Qi L, Hu C, Duan H, Cannizzaro J, Ma R, 2014. A novel MERIS algorithm to derive cyanobacterial phycocyanin pigment concentrations in a eutrophic lake: Theoretical basis and practical considerations. Remote Sens. Environ. 154:298-317.

Randolph K, Wilson J, Tedesco L, Li L, Pascual DL, Soyeux E, 2008. Hyperspectral remote sensing of cyanobacteria in turbid productive water using optically active pigments, chlorophyll a and phycocyanin. Remote Sens. Environ. 112: 4009-4019.

Reichwaldt ES, Ghadouani A, 2012. Effects of rainfall patterns on toxic cyanobacterial blooms in a changing climate: Between simplistic scenarios and complex dynamics. Water Res. 46:1372-1393.

Reinart A, Kutser T, 2006. Comparison of different satellite sensors in detecting cyanobacterial bloom events in the Baltic Sea. Remote Sens. Environ. 102:74-85.

Riha S, Krawczyk H, 2011. Development of a remote sensing algorithm for cyanobacterial phycocyanin pigment in the Baltic Sea using neural network approach. In: C.R. Bostater, S.P. Mertikas, X. Neyt and M. VelezReyes (eds.), Remote sensing of the ocean, sea ice, coastal waters, and large water regions. Proc. SPIE 8175, Remote Sensing of the Ocean, Sea Ice, Coastal Waters, and Large Water Regions 2011:817504.

Robarts R, Zohary T, 1987. Temperature effects on photosynthetic capacity, respiration, and growth-rates of bloom-forming cyanobacteria. New Zeal J. Mar. Fresh. 21:391-399.

Rousseaux CS, Gregg WW, 2015. Recent decadal trends in global phytoplankton composition. Glob. Biogeochem. Cycles 29:1674-1688.

Roy S, Llewellyn CA, Egeland ES, Johnsen G, 2011. Phytoplankton pigments: characterization, chemotaxonomy and applications in oceanography. Cambridge University Press, Cambridge: 890 pp.

Salmaso N, 2000. Factors affecting the seasonality and distribution of cyanobacteria and chlorophytes: a case study from the large lakes south of the Alps, with special reference to Lake Garda. Hydrobiologia 438:43-63.

Salmaso N, 2005. Effects of climatic fluctuations and vertical mixing on the interannual trophic variability of Lake Garda, Italy. Limnol. Oceanogr. 50:553-565.

Salmaso N, Capelli C, Shams S, Cerasino L, 2015. Expansion of bloom-forming *Dolichospermum lemmermannii* (Nostocales, Cyanobacteria) to the deep lakes south of the Alps: colonization patterns, driving forces and implications for water use. Harmful Algae 50:76-87.

Salmaso N, Copetti D, Cerasino L, Shams S, Capelli C, Boscaini A, Guzzella L, 2014a. Variability of microcystin cell quota in metapopulations of Planktothrix rubescens: Causes and implications for water management. Toxicon 90:82-96.

Salmaso N, Copetti D, Guzzella L, Manganelli M, Masala E, Naselli-Flores L, 2014b. [Fattori inerenti allo sviluppo di fioriture di cianobatteri tossici con particolare riferimento a eutrofizzazione e cambiamenti climatici], p. 5-36. In: E. Funari, M. Manganelli and E. Testai (eds.), [Cianobatteri: linee guida per la gestione delle fioriture nelle acque di balneazione].[Report in Italian]. Rapporti ISTISAN 14/20. Istituto Superiore di Sanità, Roma.

Salmaso N, Mosello R, 2010. Limnological research in the deep southern subalpine lakes: synthesis, directions and perspectives. Adv. Oceanogr. Limnol. 1:29-66.

Savadova K, 2014. Response of freshwater bloom-forming planktonic cyanobacteria to global warming and nutrient increase. Botanica Lithuanica 20:57-63.

Schaeffer BA, Hagy JD, Stumpf RP, 2013. Approach to developing numeric water quality criteria for coastal waters: transition from SeaWiFS to MODIS and MERIS satellites. J. Appl. Remote Sens. 7:073544.

Schalles JF, Yacobi YZ, 2000. Remote detection and seasonal patterns of phycocyanin, carotenoid and chlorophyll pigments in eutrophic waters. Ergeb. Limnol. 153-168.

Seppala J, Ylostalo P, Kaitala S, Hallfors S, Raateoja M, Maunula P, 2007. Ship-of-opportunity based phycocyanin fluorescence monitoring of the filamentous cyanobacteria bloom dynamics in the Baltic Sea. Estuar. Coast. Shelf Sci. 73:489-500.

Shi K, Zhang Y, Xu H, Zhu G, Qin B, Huang C, Liu X, Zhou Y, Lv H, 2015. Long-term satellite observations of microcystin concentrations in Lake Taihu during cyanobacterial bloom periods. Environ. Sci. Technol. 49:6448-6456.

Shuchman RA, Leshkevich G, Sayers MJ, Johengen TH, Brooks CN, Pozdnyakov D, 2013. An algorithm to retrieve chlorophyll, dissolved organic carbon, and suspended minerals from Great Lakes satellite data. J. Great Lakes Res. 39:14-33.

Simis SGH, Peters SWM, Gons HJ, 2005. Remote sensing of the cyanobacterial pigment phycocyanin in turbid inland water. Limnol. Oceanogr. 50:237-245.

Simis SGH, Ruiz-Verdu A, Antonio Dominguez-Gomez J, Pena-Martinez R, Peters SWM, Gons HJ, 2007. Influence of phytoplankton pigment composition on remote sensing of cyanobacterial biomass. Remote Sens. Environ. 106:414-427.

Slim K, Fadel A, Atoui A, Lemaire BJ, Vinçon-Leite B, Tassin B, 2014. Global warming as a driving factor for cyanobacterial blooms in Lake Karaoun, Lebanon. Desalin. Water Treatm. 52: 2094-2101.

Steffen W, Crutzen PJ, McNeill JR, 2007. The Anthropocene: are humans now overwhelming the great forces of nature. Ambio 36:614-621.

Steinberg CEW, Hartmann HM, 1988. Planktonic bloom-forming Cyanobacteria and the eutrophication of lakes and rivers. Freshwater Biol. 20:279-287.

Stumpf RP, Wynne TT, Baker DB, Fahnenstiel GL, 2012. Interannual variability of cyanobacterial blooms in Lake Erie. Plos One 7:e42444.

Taranu ZE, Gregory-Eaves I, Leavitt PR, Bunting L, Buchaca T, Catalan J, Domaizon I, Guilizzoni P, Lami A, McGowan S, Moorhouse H, Morabito G, Pick FR, Stevenson MA, Thompson PL, Vinebrooke RD, 2015. Acceleration of cyanobacterial dominance in north temperate-subarctic lakes during the Anthropocene. Ecol. Lett. 18:375-384.

Utermöhl H, 1958. [Zur Vervollkommung der quantitative Phytoplankton Methodik].[Article in German]. Mitt. Int. Verein. Limnol. 9:1-38.

Vermote E, Tanré D, Deuzé JL, Herman M, Morcrette JJ, Kotchenova SY, 2006. Second simulation of a satellite signal in the solar spectrum-vector (6SV). 6S User Guide Version 3:1-55.

Viaggiu E, Calvanella S, Melchiorre S, Bruno M, Albertano P, 2003. Toxic blooms of *Planktothrix rubescens* (Cyanobacteria/Phormidiaceae) in three water bodies in Italy. Arch. Hydrobiol. Algol. Stud. 109:569-577.

Viaggiu E, Melchiorre S, Volpi F, Di Corcia A, Mancini R, Garibaldi L, Crichigno G, Bruno M, 2004. Anatoxin-a toxin in the cyanobacterium *Planktothrix rubescens* from a fishing pond in northern Italy. Environ.Toxicol. 19:191-197.

Vincent RK, Qin XM, McKay RML, Miner J, Czajkowski K, Savino J, Bridgeman T, 2004. Phycocyanin detection from LANDSAT TM data for mapping cyanobacterial blooms in Lake Erie. Remote Sens. Environ. 89:381-392.

Walsby AE, 2005. Stratification by cyanobacteria in lakes: a dynamic buoyancy model indicates size limitations met by *Planktothrix rubescens*. New Phytol. 168:365-376.

Walsby AE, Schanz F, Schmid M, 2006. The Burgundy-blood phenomenon: a model of buoyancy change explains autumnal waterblooms by *Planktothrix rubescens* in Lake Zürich. New Phytol. 169:109-122.

Walther G-R, Roques A, Hulme PE, Sykes MT, Pysek P, Kühn I, Zobel M, Bacher S, Botta-Dukát Z, Bugmann H, Czúcz B, Dauber J, Hickler T, Jarosík V, Kenis M, Klotz S, Minchin D, Moora M, Nentwig W, Ott J, Panov VE, Reineking B, Robinet C, Semenchenko V, Solarz W, Thuiller W, Vilà M, Vohland K, Settele J, 2009. Alien species in a warmer world: risks and opportunities. Trends Ecol. Evol. 24:686-93.

Wang M, Shi W, 2008. Satellite-observed algae blooms in China's Lake Taihu. Eos 89:201-202.

Webster IT, Hutchinson PA, 1994. Effect of wind on the distribution of phytoplankton cells in lakes revisited. Limnol. Oceanogr. 35:365-373.

Westberry TK, Siegel DA, Subramaniam A, 2005. An improved bio-optical model for the remote sensing of *Trichodesmium* spp. blooms. J. Geophys. Res.-Oceans 110:C06012.

Wetzel RG, 2001. Limnology: lake and river ecosystems. Academic Press, San Diego: 1006 pp.

Winder M, Sommer U, 2012. Phytoplankton response to a changing climate. Hydrobiologia 698:5-16.

Wu TF, Qin BQ, Brookes JD, Shi K, Zhu GW, Zhu MY, Yan WM, Wang Z, 2015. The influence of changes in wind patterns on the areal extension of surface cyanobacterial blooms in a large shallow lake in China. Sci. Total Environ. 518-519:24-30.

Wu Y, Li L, Zheng L, Dai G, Ma H, Shan K, Wu H, Zhou Q, Song L, 2016. Patterns of succession between bloom-forming cyanobacteria Aphanizomenon flos-aquae and Microcystis and related environmental factors in large, shallow Dianchi Lake, China. Hydrobiologia 765:1-13.

Wynne TT, Stumpf RP, Tomlinson MC, Dyble J, 2010. Characterizing a cyanobacterial bloom in western Lake Erie using satellite imagery and meteorological data. Limnol. Oceanogr. 55:2025-2036.

Yacobi YZ, Koehler J, Leunert F, Gitelson A, 2015. Phycocyanin-specific absorption coefficient: Eliminating the effect of chlorophylls absorption. Limnol. Oceanogr.-Meth. 13:157-168.

Zibordi G, Holben B, Slutsker I, Giles D, D'Alimonte D, Melin F, Berthon JF, Vandemark D, Feng H, Schuster G, Fabbri BE, Kaitala S, Seppälä J, 2009. Aeronet-OC: A network for the validation of ocean color primary products. J. Atmos. Ocean. Tech. 26:634-1651.

Zilius M, Bartoli M, Bresciani M, Katarzyte M, Ruginis T, Petkuviene J, Lubiene I, Giardino C, Bukaveckas PA, de Wit R, Razinkovas-Baziukas A, 2014. Feedback mechanisms between cyanobacterial blooms, transient hypoxia, and benthic phosphorus regeneration in shallow coastal environments. Estuar. Coast Shelf Sci. 37:680-694.

Zimba PV, Gitelson A, 2006. Remote estimation of chlorophyll concentration in hyper-eutrophic aquatic systems: model tuning and accuracy optimization. Aquaculture 256:272-286.

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Barbel species arrangement in a regional Natura 2000 network (Emilia Romagna, Northern Italy): An altitudinal perspective

Federica PICCOLI,[#] Gemma BURGAZZI,[#] Alex LAINI, Claudio FERRARI, Andrea VOCCIA, Laura FILONZI, Rossano BOLPAGNI, Francesco NONNIS MARZANO[*]

Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11/A, I-43124 Parma, Italy
[#]Both authors have contributed equally to this research
*Corresponding author: francesco.nonnismarzano@unipr.it

ABSTRACT

Southern Europe hosts a large number of critical catchments for freshwater biodiversity, including endemic fish species. Unfortunately, these areas are severely threatened due to direct and indirect anthropogenic effects. In this context, with the aim to improve the effectiveness of threatened fish protection, the Life project BARBIE (LIFE13 NAT/IT/001129) started in 2014 and focused on three congeneric species of the genus *Barbus*: two of "priority interest" *sensu* Habitats Directive [*Barbus caninus* (Bonaparte, 1839), and *B. plebejus* (Bonaparte, 1839)], and one alien [*Barbus barbus* (Linnaeus, 1758)]. Our main objective was to assess the contribution of a complex of protected areas included in the Natura 2000 network - located in the provinces of Parma, Piacenza and Reggio Emilia (Norther Italy) - to support the presence of the three barbel species in analysis. Additionally, we explored the role of a set of environmental variables (*i.e.*, physical, chemical, biological, and land-use descriptors) to drive the current conditions of the study sites and the responses of *Barbus* species. As a general rule, the present study confirmed a clear decline of the local native barbel populations, and confirmed the existence of a zonation pattern of the barbel *taxa*. Hence, we observed a strong altitude segregation between native *vs.* alien species, with the exotic *B. barbus* currently limited to plain and only sporadically present in the Apennine areas as genetic introgression. These evidences mirrored the altitudinal gradients of anthropogenic disturbance. The main causes were the progressive disappearance of well-structured riparian stripes, and the intense land use change, ranging from semi-natural patches (mountain and hill sectors) to land clearing for intensive agriculture (lowland sectors). This highlights the need to take into account the spatial dynamics of alien invasive species in programming recovery actions that could have unexpected impacts to the native fish populations, for example the reduction of the hydraulic fragmentation along rivers.

Key word: *Barbus* genus species; inland waters; endangered fish; Habitats Directive; Fluvial Functionality Index; physical and chemical drivers.

INTRODUCTION

Since streams and rivers have become some of the most endangered ecosystems worldwide, there is an urgent demand for comprehensive methodological approaches to evaluate their conservation status, and to monitor their rate of changes (Li *et al.*, 2010). Mediterranean regions represent key areas for freshwater fish endemism and introductions (Leprieur *et al.*, 2008). Indeed, a large portion of the European critical catchments for freshwater biodiversity is located primarily in southern Europe (Carrizo *et al.,* 2017). Unfortunately, these areas are severely threatened because of water scarcity and environmental degradation due to the high anthropic pressure (*e.g.*, hydropower, agriculture, public uses), and to the on-going climate change (Hermoso and Clavero, 2011; Hermoso *et al.*, 2011).

In inland waters, the natural fish stocks are generally over-exploited, and in the developed countries, uncontrolled larvae and juveniles' reintroduction have been carried out for recreational fishery purposes (Lewin *et al.*, 2008). Additionally, in last decades the distributions of many exotic fish species have extended worldwide further impairing the quality of autochtonous fish populations (Carosi *et al.*, 2017). This is especially true for Italy, particularly along the lower stretches of rivers throughout the Apennines (Italian Peninsula) that are characterized by rather warm and slow waters, and scarce quality conditions (Carosi *et al.*, 2017). All this translates into less than ideal conditions for the survival of endemic and threatened fish, and the conservation of the local population diversity.

Since the beginning of 21st century, to counteract the above-mentioned critical issues many actions have been carried out to improve fish population, including mitigation of hydropower development, river fragmentation and hydropeaking (Premstaller *et al.*, 2017). In this context, the Natura 2000 network may represent a strategic tool to preserve both fish genetics and population resources, with the final goal of improving their local and regional survival chances. However, the designation procedures of the Natura 2000 sites often are driven by factors marginally related to ecological criteria, thus reducing their effectiveness (Trochet and Schmeller, 2013). Furthermore, the biotic integrity of biological communities in rivers and lakes is considered a key predictor of colonized water bodies' quality suggesting a strong synergy between the Habitat Directive (HD; European Commission, 1992) and the Water Framework Directive (WFD; European Commission, 2000). However, unfortunately, these two fundamental legal issues proceed in isolation with negative effects on their relative efficiency (Bolpagni *et al*., 2017).

In order to actively and mutually implement the HD and the WFD, and with the aim to improve their effectiveness in terms of threatened fish protection, the Life project BARBIE (LIFE13 NAT/IT/001129) started in 2014, focusing on three congeneric species of the genus *Barbus*. Two of them are of "priority interest" *sensu* HD: *Barbus caninus* (Bonaparte, 1839) and *B. plebejus* (Bonaparte, 1839). This two native species of *Barbus* are charismatic indicators for the estimation of the conservation status of water bodies (Angelini *et al*., 2016). The third is an alien species, *Barbus barbus* (Linnaeus, 1758), that - in the last decades - showed an exceptionally fast spreading capacity within the Po River basin. This invasive species has also hybridized with the endemic *B. plebejus* (Meraner *et al*., 2013), thus resulting in a widespread genetic introgression in the autochthon *Barbus* species.

In this context, to test the distinct distribution of the different species as per our hypothesis, we described the fish community structure in a representative array of 14 of the watercourses included in the Natura 2000 sites - and their surroundings - of the Parma, Piacenza and Reggio Emilia provinces (Emilia Romagna Region, Northern Italy). Our main hypothesis is that the exotic *taxon* (*B. barbus*) may be spatially limited to the lowland sectors, which are those with the highest human disturbance rates. On the contrary, prior barbel *taxa* (*B. caninus* and *B. plebejus*) are expected to be mainly distributed in mountain and/or hill areas. In other words, altitudinal segregation between these species may be hypothesized. To do this, we focused on the presence/absence and representativeness of the *Barbus* species, implementing the current data on their local spatial distribution. In this way, we were also able to assess the contribution of a complex of Natura 2000 sites to support fish communities. Additionally, we collected a set of environmental variables (including physical, chemical, biological, and land-use descriptors) to assess the current conditions of sites colonized by the different species in analysis.

METHODS

Study area

This study covered rivers and streams running along 14 sites of the Natura 2000 network of the Parma, Piacenza and Reggio Emilia provinces (Emilia Romagna region, Northern Italy; Fig. 1 and Supplementary Tab. 1). The Köppen-Geiger classification includes both humid subtropical (cfa; plains and hill sectors) and oceanic climates (cfb; mountain sector), characterized by few extremes of temperature and pronounced precipitation in all months.

The study area includes a complex hydro-system that consists of several streams and rivers across an extended altitudinal range (about 600 m), stretching from the Tuscan-Emilian Apennine ridge to the Po River. Accordingly, the mean annual discharges and the main water chemical and physical conditions are extremely variable. Generally, the investigated water bodies showed a good chemical status, and fall into the sufficient or good quality classes of the ecological status *sensu* WFD (ARPA Emilia Romagna, 2013). The study area covers the entire distribution range of the two autochthonous target species in the Emilia Romagna region: canine barbel (*B. caninus*) inhabits mountain fast-flowing brooks and small rivers while the common barbel (*B. plebejus*) commonly occurs in hilly and lowland streams.

Biological, water quality and morphological characterization

Between July 2014 and December 2015, a total of 305 barbel individuals were collected by electric fishing (Macchio and Rossi, 2014), from 31 localities of the study area. Each animal was measured and fin-clipped and then released in the same location. Measurements included the total length and the weight. Fin fragment were preserved in 70% ethanol until DNA extraction. Starting from the visual based analysis of the fish community, the Index of the Ecological Status of Fish Communities (ISECI) was also calculated in each of the 31 study sites, according with Zerunian (2009) protocol.

Simultaneously with the fish characterization, a water sample was collected by a plastic bottle just below the water surface. Directly *in situ* temperature, pH, conductivity and dissolved oxygen data were collected by a multi-parameter probe (YSI model 556 MPS). Samples for the biochemical oxygen demand (BOD) were transferred in glass bottles. Samples for NH_4^+ (ammonium), NO_3^- (nitrate), and NO_2^- (nitrite) determinations were filtered through Whatman GF/F glass fiber filters (Ø 47 mm, porosity 0.45 μm) and transferred to plastic vials. All water samples were kept to 4°C, and transferred to the laboratory. Total suspended solids (TSS) were measured by filtration through a pre-dried and weighed glass fiber filter GF/F (Whatman, UK, Ø 25 mm and 0.45 μm) (APHA, 2012). NH_4^+, NO_3^-, NO_2^-, were determined with standard spectrophotometric methods APHA (2012), whereas BOD was calculated after incubation at 20±1°C for 5 days (= BOD_5) according to APHA (2012).

Each sampling sites was characterized by the application of the Fluvial Functionality Index (FFI), that is devoted to investigate the functionality of a river stretch in terms of metabolic capacity (*i.e.*, fine and coarse particulate organic matter retention and cycling) (Siligardi *et al.*, 2000). This method is based on the analysis of riverbank vegetation, physical and morphological structure, the extent of the riparian area, the land use impact, the riverbed structure, and the key biological characteristics of river ecosystem. For further details, see Siligardi *et al.* (2000).

Molecular data analysis

Total genomic DNA was extracted from fin tissue using Wizard genomic DNA Purification kit (PROMEGA, Madison, WI, USA). DNA quality and concentration were tested by 1% agarose gel electrophoresis in 1% TAE buffer, by visual comparison with a DNA ladder mix and by spectrophotometry at 260 e 280 nm. The extraction procedure typically yielded not less than 40 ng/mL of HMW (high molecular weight) DNA. For each of 305 specimens we analysed 600 bp long region of the *cytb mtDNA* and was amplified by polymerase chain reaction (PCR) using primer pair CYTB-Thr 5'-ACCTCCGATCTTCGGATTACAAGACCG-3' and CYTB-Glu 5'-AACCACCGTTGTATTCAACTACAA - 3' (Zardoya and Doadrio, 1998). A reaction volume of 25 µl containing 1 U of GoTaq Polymerase (PROMEGA, Madison, WI, USA), Mg^{2+} 1.5 mM and dNTPs 0.2 mM, and 10 pmol of each primer were used. PCR was set as follows: 35 cycles of 45 s at 94°C, 1 min at 47°C, and 2 min at 72°C, after an initial 3 min denaturation step at 94°C and a final extension at 72°C for 10 min. Fragments sequencing was performed by MACROGEN Europe service (Amsterdam, the Netherlands). The obtained sequences were manually corrected using MEGA7.0 and were compared with those available in genomic databases using NCBI BLAST.

Statistical analysis

A principal component analysis (PCA) was performed on chemical and physical data. The interpretation of PCA

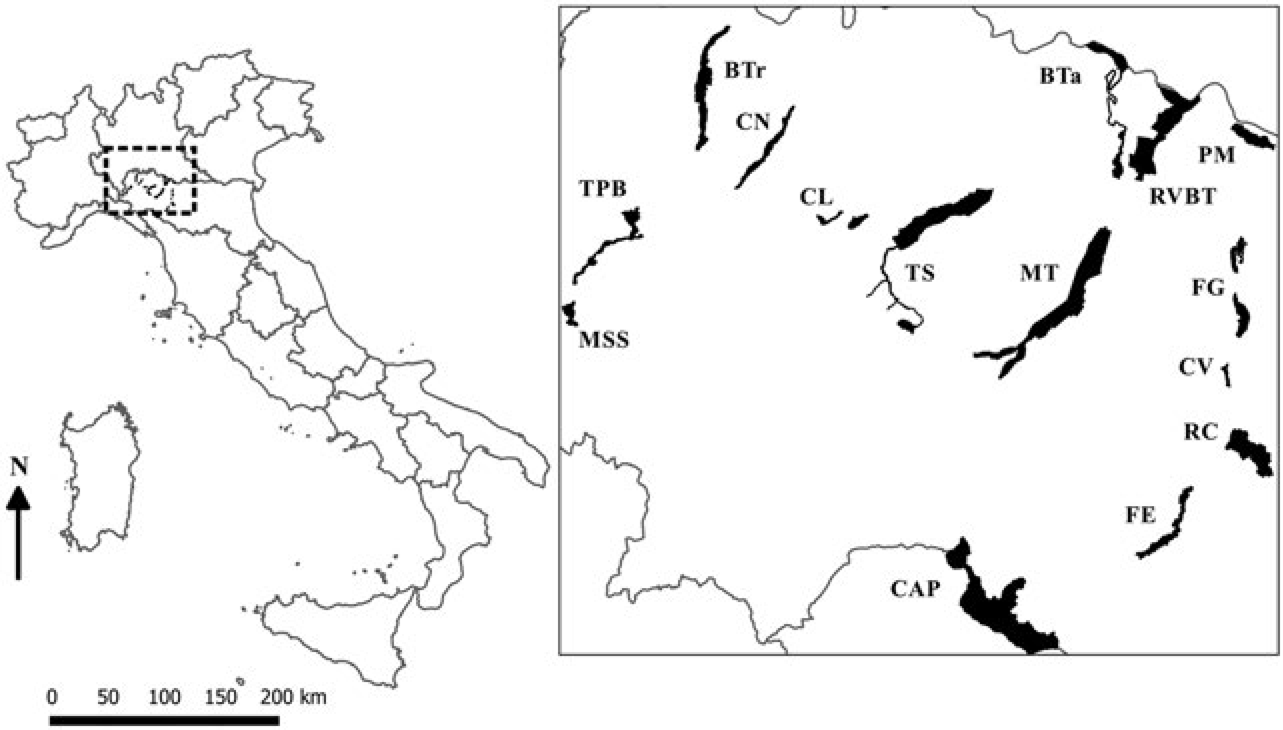

Fig. 1. Study area with the indication of the Natura 2000 network. MSS, Trebbia - Meandri di San Salvatore (IT4010006); TPB, Trebbia River - from Perino to Bobbio (IT4010011); BTr, Low Trebbia River (IT4010016); CN, Nure River - Conoide del Nure and Bosco di Fornace Vecchia (IT4010017); CL, Arada River - Castell'Arquato, Lugagnano Val d'Arda (IT4010008); TS, Stirone Stream (IT4020003); Bta, Low Taro River (IT4020022); RVBT, Lorno River - Aree delle risorgive di Viarolo, Bacini di Torrile, Fascia golenale del Po (IT4020017); PM, Parma Morta (IT4020025); MT, Medium Taro River (IT4020021); FG, Enza River - Fontanili di Gattatico (IT4030023); CV, Enza River - Cronovilla (IT4020027); RC, Cerezzola Stream - Rupe di Campotrera, Rossena (IT4030014); FE, Enza River - from La Mora to Compiano (IT4030013); CAP, Parma - Crinale dell'Appennino parmense (IT4020020).

ordination was limited to variables with loads higher than the vector representing a variable contributing equally to all the dimensions of the PCA space (Borcard *et al.*, 2011). The relationship between PCA ordination and the structure of *Barbus*' populations was assessed by means of the function envfit ("vegan" package) that fits vectors onto a multivariate ordination. Simple regression analysis was used to examine the relationships between FFI and altitude and BOD_5 values.

All the analyses and graphs were performed with the statistical software R (R Core Team, 2015), with base version, ggplot2 (Wickham, 2009) and vegan (Oksanen *et al.*, 2016) packages.

RESULTS

Water quality and morphological characterization of sites

Physical, chemical, and morphological (*i.e.*, FFI outputs) data are reported in Supplementary Tab. 1. Results from water measurements highlight the variability between sites in terms of altitude and human impact gradients. In summer 2015, temperature, pH, dissolved oxygen, and conductivity were in the ranges 14.4-31.0°C, 7.30-8.91, 37-295% saturation, and 190-825 $\mu S\ cm^{-1}$, respectively. Similarly, the BOD_5 and TSS values varied from 0.0 (mountain sites, Trebbia River) to 20.8 $mg\ L^{-1}$ (lowland site, Parma Morta), and from 0.0 to 66.2 $mg\ L^{-1}$, respectively. At the same time, NH_4^+ and NO_2^- concentrations exhibited only small variations, within the range 0.02-0.04 NH_4^+ $mg\ N\ L^{-1}$, and 0.00-0.02 NO_2^- $mg\ N\ L^{-1}$. On the contrary, NO_3^- ranged between 0.08 and 5.00 $mg\ N\ L^{-1}$.

FFI ranged between 102 and 245, from poor/bad to good classes. It showed a clear spatial arrangement with a tendentially significant negative correlation with altitude ($r=0.45$, $P=0.07$; $n=17$). A progressive reduction in FFI values was recorded from mountain/hill sectors to lowlands. On the contrary, no significant relationship between FFI and BOD_5 values was recorded ($r=0.37$, $P>0.1$; $n=17$), although a gradual increase of the biochemical oxygen demand with the progressive loss of the functionality of riparian belt is generally expected.

Fish community and ISECI assessment

The number of individuals detected in each sampling station for the three investigated species is reported in Tab. 1. From a general point of view, considering the population size (expressed in terms of number of individuals), the observed data were quite low in the range 0-29 individuals. Considering species separation, mean values (± standard deviation) were 1.0 (±3.5), 1.8 (±6.1), and 7.1 (±8.4) individuals for alien European barbel, canine, and common respectively. Eight over 31 (26%) study sites showed complete absence of barbel specimens.

A structured population of canine barbel constituted of 24 and 25 specimens were retrieved in the streams Rio Cerezzola (Natura 2000 site IT4030014) and Rio Parmossa (bordering this Natura 2000 site area), respectively. On the contrary, common barbel peaked at Nure and Enza rivers with 29 and 25 individuals, respectively. The alien European barbel species colonized 7 (23%) study sites belonging to Arda, Trebbia, Taro, Ceno and Enza rivers, besides Naviglio channel (Tab. 1). In particular, the alien European barbel showed a representative population in Taro River, with 19 individuals, as a result of the only investigated plain river site.

The ISECI values varied from 0.72 (good ecological quality) at Nure River to 0.20 (poor ecological quality) at lowland site Parma Morta, with a mean value of 0.57 (±0.14) (sufficient ecological quality) (Tab. 1). More specifically, 18 over 31 (58%) sites displayed "good quality" class, 10 (32) "moderate quality", 1 (3%) "poor quality", and 2 (7%) "bad quality".

Molecular analyses provided data on the percentage of different species at sampling site level (Tab. 1). Fragments of 600 bps were analyzed and compared to GenBank sequences. Samples displayed 100% identity with deposited sequences according to different species with alignment values E=0.0 and maximum identity in the range 97-100%. ClustalW assessment among investigated samples showed a total number of 230 polymorphic sites. From a taxonomy point of view, molecular analyses on mtDNA evidenced the following density values: 72% common barbel, 18% canine barbel, and 10% alien European barbel.

In terms of environmental drivers of the observed barbel' species spatial distribution, FFI and oxygen saturation were the variables contributing most to respectively PCA axes 1 and 2, explaining the 88% of the total variance. Alien European barbel was significantly related to the PCA ordination ($R^2=0.67$, $P<0.05$), while this was not true for the other two barbel species: *B. caninus* ($R^2=0.15$, $P>0.05$) and *B. plebejus* ($R^2=0.14$, $P>0.05$) (Fig. 2).

DISCUSSION AND CONCLUSIONS

Our results confirmed a decline of native barbel populations in the study area compared with literature data (Nonnis Marzano *et al.*, 2003; Fish Charts Provinces of Parma, Reggio Emilia, and Piacenza). Barbel populations were historically present in several streams of the three provinces, both in the mid-Apennine and hillsides (Nonnis Marzano *et al.*, 2003). However, a well-structured population of the canine barbel was retrieved only in two sampling stations, highlighting a local high degree of rarity for this species. Additionally, in agreement with our initial hypotheses the present data confirmed the general existence of a clear zonation pattern among the barbel *species* analyzed, although a frequent overlapping of populations limited statistical significance of principal component analysis. A non-negligible altitude segregation between native *vs.* alien species was detected, with the exotic invasive *B. barbus* mainly limited to lowland watercourses. We also detected invasive *B. barbus* DNA in the native populations of the hill areas as a result of genetic introgression. These observations corroborate recent evidence on the rapid expansion of the European barbel in the Po basin (Meraner *et al.*, 2013), and indicate a higher level of vulnerability for the autochthonous fish populations placed at lower altitudes, where the likely presence of alien barbels is wider. It is noteworthy observing that migration of *B. barbus* mitochondrial haplotypes reaching hill and mid-Apennines catchments could be referred to water heating due to global warming and water deprivation.

More specifically, the species spatial distribution mirrored the anthropogenic disturbance gradients. In fact, all the investigated sites are arranged along an altitudinal gradient stretching from the Tuscan-Emilian Apennine (up to 600 m asl) to the Po River (~25 m asl), that overlaps with human pressures, which significantly decreases

Tab. 1. Barbus' population size and ISECI results; for each sampled site, the indication of each watercourse and the Natura 2000 site it belongs, the number of individuals and their % distribution value (in brackets) of Canine (*B. caninus*), Common (*B. plebejus*), and alien European (*B. barbus*) Barbel, the values and quality class of ISECI were reported. In bold the sites without barbels.

Site	Watercourse	Code	Natura 2000	Number of individuals			ISECI	
			site code	Canine	Common	European	Value	Quality class
10006.TR.1	Trebbia	MSS	IT4010006	1 (7.5%)	15 (92.5%)	0	0.61	Good
10008.AR.1	Arda	CL	IT4010008	0	23 (95.8%)	1 (4.2%)	0.61	Good
10011.TR.1	Trebbia	TPB	IT4010011	2 (12.5%)	14 (87.5%)	0	0.64	Good
10016.TR.1	Trebbia	BTr	IT4010016	0	15 (75.0%)	5 (25.0%)	0.72	Good
10016.TR.2	**Trebbia**	**BTr**	**IT4010016**	**0**	**0**	**0**	**0.69**	**Good**
10017.NU.1	Nure	CN	IT4010017	0	29 (100.0%)	0	0.72	Good
20003.ST.1	Stirone	TS	IT4020003	0	13 (100.0%)	0	0.71	Good
20017.LO.1	**Lorno**	**RVBT**	**IT4020017**	**0**	**0**	**0**	**0.66**	**Good**
20020.PR.1	Parma		-	0	3 (100.0%)	0	0.40	Poor
20020.PR.2	Parma		-	1 (11.1%)	8 (88.9%)	0	0.52	Moderate
20020.PR.3	Parma	CAP	IT4020020	0	0	0	0.66	Good
20020.PM.1	Parmossa		-	25 (89.3%)	3 (10.7%)	0	0.66	Good
20020.FA.1	**Fabiola**		**-**	**0**	**0**	**0**	**0.66**	**Good**
20020.MO.1	**Moneglia**		**-**	**0**	**0**	**0**	**0.63**	**Good**
20020.BA.1	Baganza		-	1 (20.0%)	4 (80.0%)	0	0,49	Moderate
20021.TA.1	Taro	MT	IT4020021	0	5 (83.3%)	1 (16.7%)	0.63	Good
20021.NA.1	Naviglio	MT	IT4020021	0	8 (80.0%)	2 (20.0%)	0.52	Moderate
20021.CE.1	Ceno	MT	IT4020021	0	8 (88.9%)	1 (11.1%)	0.68	Good
20022.TA.1	Taro	BTa	IT4020022	0	0	19 (100.0%)	0.30	Mediocre
20025.PR.1	**Parma Morta**	**PM**	**IT4020025**	**0**	**0**	**0**	**0.20**	**Bad**
20027.EN.1	Enza	CV	IT4020027	0	7 (100.0%)	0	0.72	Good
30013.EN.1	Enza	FE	IT4030013	0	25 (100.0%)	0	0.55	Moderate
30013.CE.1	Cedra		-	0	2 (100.0%)	0	0.42	Moderate
30013.CE.2	**Cedra**		**-**	**0**	**0**	**0**	**0.20**	**Bad**
30014.RV.1	Rio Vico	RC	IT4030014	0	6 (100.0%)	0	0.50	Moderate
30014.RC.1	Rio Cerezzola	RC	IT4030014	1	0	0	0.67	Good
30014.RC.2	Rio Cerezzola	RC	IT4030014	24 (100.0%)	0	0	0.59	Moderate
30023.EN.1	Enza	FG	IT4030023	0	21 (91.3%)	2 (8.7%)	0.60	Moderate
30023.EN.2	Enza	FG	IT4030023	0	5 (100.0%)	0	0.53	Moderate
30023.EN.3	Enza	FG	IT4030023	0	5 (100.0%)	0	0.64	Good
30023.EN.4	**Enza**	**FG**	**IT4030023**	**0**	**0**	**0**	**0.63**	**Good**

ISECI, Ecological Status of Fish Communities.

as altitude increases. Hence, a progressive improvement of all monitored physical, chemical and morphological features was detected with the decreasing of elevation. The strong overlap between physical and chemical data reinforces the existence of an altitudinal zonation in term of barbel species representativeness among sites. At the site scale, with decreasing altitude the river functionality (*e.g.*, FFI) varied from good to poor/bad, and BOD_5 from 0.0 to 20.8 mg L^{-1}.

Our results suggest that the progressive disappearance of well-structured riparian stripes, the increase of the riverbed incision - that actually encourages the progressive isolation of the watercourse from the local context surrounding - and the intense land use change, are the main reasons of the observed zonation. Consequently, the present results obtained through a multidisciplinary approach support the idea that the native and threatened priority barbel species were found preferentially in moderate to well-preserved watercourses, characterized by more expanded riparian areas, in terms of greater IFF values. Similar results were modeled for two Minnesota watersheds, where substantial changes in agricultural management, including an expansion of the riparian areas, would be expected to significantly improve local brook trout [*Salvelinus fontinalis* (Mitchill, 1814)] populations, by increasing streams shading up to 50% (Blann *et al.*, 2002). Hence, in lowlands and agricultural settings the destruction of riparian communities leads to a rapid physical and chemical deterioration of watercourses with dramatic effects on fish populations, as highlighted by Lorenzoni *et al.* (2006) for rivers in Central Italy.

Although the small number of sites under consideration in this work, a quite verified preference of the canine and common barbels for higher IFF values was verified, as a proxy of well-developed and structured riparian contexts and higher altitude values. Marginal vegetated belts are able to efficiently offset the nutrient inputs generated at the basin scale, and to control the main local edaphic factors (Siligardi *et al.*, 2000). Hence, in presence of high FFI values, higher shading values, as well as lower water temperatures and reduced day-night temperature fluctuations are expected. Furthermore, watercourses with high FFI levels should have reduced primary producer rates with rather scarce macrophyte cover values, including algal mats. On the other hand, the alien European barbel was positively and significantly related to high levels of dissolved oxygen, which in turn was associated to higher levels of STT and BOD_5. This is not surprising, given that the above-mentioned high dissolved oxygen levels (up to 295% saturation) were due to the hyper-proliferation of microalgae, and were typical of lowland sites of Po plain where riparian belts/zones have been almost completely lost to land reclamation and by agricultural mechanization (Bolpagni and Piotti, 2015, 2016).

All the above clearly remarks the need for more efficiently designed long-term and wide-spatial scales actions to counteract the alien fish expansion, and furtherly focus on the sustainable management of river habitats and water flows. In this context, the reduction of the hydraulic fragmentation of watercourses is an essential paradigm to improve the survival prospects of a very large number of fish species of conservation interest. In fact, the progressive impairment of the longitudinal river continuity causes significant alterations in river dynamic processes and aquatic vegetation (Bolpagni *et al.*, 2016). However, it is of interest, and possibly of considerable practical importance, taking into account the spatial dynamics of the alien invasive species before operating management actions that

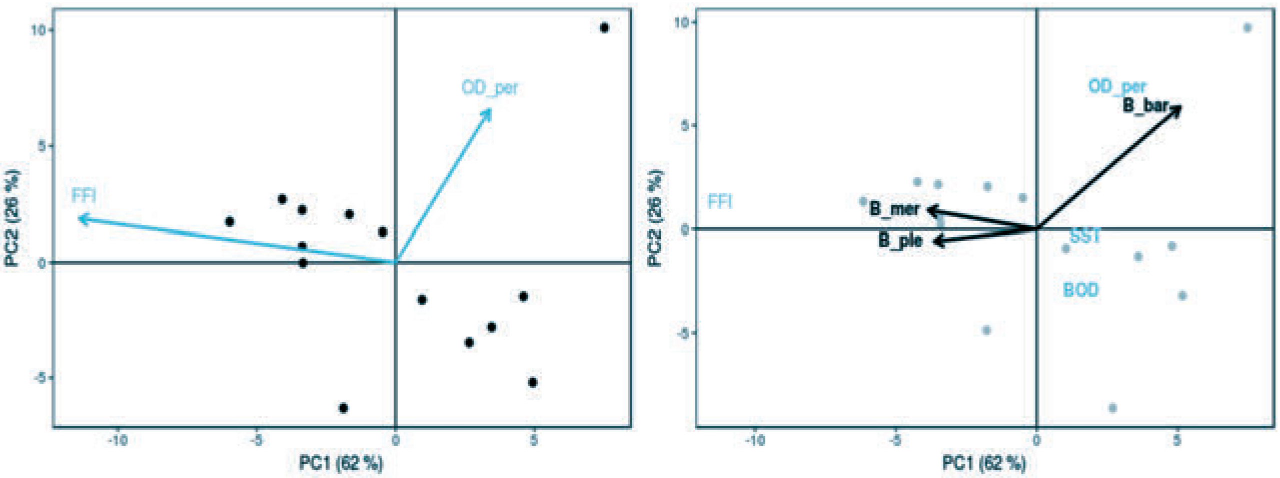

Fig. 2. Results of principal component analysis (left); vectors representing Barbus' population structure.

could later have an impact to the native fish populations. This critical issue must also be further discussed in the light of the on-going climate change that can affect barbel migration along the altitudinal gradient.

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REFERENCES

Angelini P, Casella L, Grignetti A, Genovesi P, 2016. [Manuali per il monitoraggio di specie ed habitat di interesse comunitario (Direttiva 92/43/CE) in Italia: habitat].[Book in Italian]. ISPRA, Serie Manuali e Linee Guida 142.

APHA, 2012. Standard methods for the examination of water and wastewater. 22nd ed. American Public Health Ass., Washington, DC: 1360 pp.

ARPA Emilia Romagna, 2013. [Report sullo stato delle acque superficiali. Triennio 2010-2012].[Book in Italian]. Regione Emilia Romagna: 105 pp.

Blann KL, Nerbonne JF, Vondracek B, 2002. Relationship of riparian buffer type to physical habitat and stream temperature. N. Am. J. Fish. Manage. 22:441-451.

Bolpagni R, Azzella MM, Agostinelli C, Beghi A, Bettoni E, Brusa G, De Molli C, Formenti R, Galimberti F, Cerabolini BEL, 2017. Integrating the Water Framework Directive into the Habitats Directive: analysis of distribution patterns of lacustrine EU habitats in lakes of Lombardy (northern Italy). J. Limnol. 76(Suppl.1):75-83. [Epub 14 Feb 2017].

Bolpagni R, Piotti A, 2015. Hydro-hygrophilous vegetation diversity and distribution patterns in riverine wetlands in an agricultural landscape: a case study from the Oglio River (Po plain, Northern Italy). Phytocoenologia 45:69-84.

Bolpagni R, Piotti A, 2016. The importance of being natural in a human-altered riverscape: Role of wetland type in supporting habitat heterogeneity and vegetation functional diversity. Aquat. Conserv. 26:1168-1183.

Bolpagni R, Racchetti E, Laini A, 2016. Fragmentation and groundwater supply as major drivers of algal and plant diversity and relative cover dynamics along a highly modified lowland river. Sci. Total Environ. 568:875-884.

Borcard D, Gillet F, Legendre P, 2011. Introduction, p. 1-7. In: D. Borcard, F. Gillet and P. Legendre (eds.), Numerical ecology with R. Springer, New York.

Carosi A, Ghetti L, Cauzillo C, Pompei L, Lorenzoni M, 2017. Occurrence and distribution of exotic fishes in the Tiber River basin (Umbria, central Italy). J. Appl. Ichthyol. 33:274-283.

Carrizo SF, Lengyel S, Kapusi F, Szabolcs M, Kasperidus HD, Scholz M, Markovic D, Freyhof J, Cid N, Cardoso AC, Darwall W, 2017. Critical catchments for freshwater biodiversity conservation in Europe: identification, prioritisation and gap analysis. J. Appl. Ecol. 54:1209-1218.

European Commission, 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. OJ L 206, 22/7/1992, p. 7-50.

European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L 327, 22/12/2000, p. 1-73.

Hermoso V, Clavero M, 2011. Threatening processes and conservation management of endemic freshwater fish in the Mediterranean basin: a review. Mar. Freshwater Res. 62:244-254.

Hermoso V, Linke S, Prenda J, Possingham HP, 2011. Addressing longitudinal connectivity in the systematic conservation planning of fresh waters. Freshwater Biol. 56:57-70.

Leprieur A, Beauchard O, Blanchet S, Oberdorff T, Brosse S, 2008. Fish invasions in the world's river systems: When natural processes are blurred by human activities. PLoS Biol. 6:e28.

Lewin W-C, McPhee DP, Arlinghaus R, 2008. Biological impacts of recreational fishing resulting from exploitation, stocking and introduction, p. 75-92. In: Ø. Aas (ed.), Global challenges in recreational fisheries. Blackwell, Oxford.

Li L, Zheng B, Liu L, 2010. Biomonitoring and bioindicators used for river ecosystems: Definitions, Approaches and trends. Procedia Environ. Sci. 2:1510-524.

Lorenzoni M, Mearelli M, Ghetti L, 2006. Native and exotic fish species in the Tiber river watershed (Umbria, Italy) and their relationship to the longitudinal gradient. B. Fr. Peche Piscic. 382:19-44.

Macchio S, Rossi GL, 2014. [Protocollo di campionamento e analisi della fauna ittica dei sistemi lotici guadabili].[Book in Italian]. Manuali e Linee Guida 111/2014, ISPRA: 234 pp.

Meraner A, Venturi A, Ficetola G, Rossi S, Candiotto A, Gandolfi A, 2013. Massive invasion of exotic *Barbus barbus* and introgressive hybridization with endemic *Barbus plebejus* in Northern Italy: where, how and why? Mol. Ecol. 22:5295-5312.

Nonnis Marzano F, Pascale M, Piccinini A, 2003. [Atlante dell'ittiofauna della Provincia di Parma].[Book in Italian]. Regione Emilia Romagna, Stilgraf, Viadana: 127 pp.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H, 2016. vegan: Community Ecology Package. R package version 2.3-5. Available from: https://CRAN.R-project.org/package=vegan

Premstaller G, Cavedon V, Pisaturo GR, Schweizer S, Adami V, Righetti M, 2017. Hydropeaking mitigation project on a multi-purpose hydro-scheme on Valsura River in South Tyrol/Italy. Sci. Total Environ. 574:642-653.

R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available from: https://www.R-project.org/

Siligardi M, Cappelletti C, Chierici E, Ciutti F, Egaddi F, Franceschini A, Maiolini B, Mancini L, Minciardi MM, Monauni C, Rossi GL, Sansoni G, Spaggiari R, Zanetti M,

2000. [I.F.F. Indice di Funzionalità Fluviale].[Book in Italian]. ANPA, Rome: 223 pp.

Trochet A, Schmeller DS, 2013. Effectiveness of the Natura 2000 network to cover threatened species. Nature Conserv. 4:35-53.

Wickham H, 2009. ggplot2: elegant graphics for data analysis. Springer, New York: 212 pp.

Zardoya R, Doadrio I, 1998. Phylogenetic relationships of Iberian cyprinids: Systematic and biogeographical implications. P. Roy. Soc. Lond. B-Biol. 265:1365-1372.

Zerunian S, Goltara A, Schipani I, Boz B, 2009. [Adeguamento dell'Indice dello Stato Ecologico delle Comunità Ittiche alla Direttiva Quadro sulle Acque 2000/60/CE].[Article in Italian]. Biol. Amb. 23:15-30.

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