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Original Research Article

Macrophyte Biological Index for Rivers estimation in the waters of Pescara Springs by means of SCUBA dive and orthophotos interpretation

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ABSTRACT

Macrophyte Biological Index for Rivers (IBMR) has been applied to the superficial waters of Pescara Springs (Popoli, Italy) according to Water Framework Directive 2000/60/EC. Two complementary and not mutually exclusive methodologies were performed to evaluate possible differences in taxa identification and cover estimation: boat survey and interpretation of orthophotos and images taken during SCUBA (Self-Contained Underwater Breathing Apparatus) dive. Data from submerged macrophytes were analyzed by means of exploratory multivariate analysis to detect vegetation associations and their correlation with some abiotic factors (water current velocity, substrate granulometry, detritus type, water depth). Six homogeneous tracts were observed according to hydromorphological characters and to vegetation associations; IBMR and Ecological Quality Ratio (IBMR-EQR) were calculated for each of them. On an average basis, IBMR was 7.84 (trophic status: very high) and IBMR-EQR was 0.63 (quality class: scarce). A good concordance was registered by means of Bland–Altman plot between values obtained by the two methods: 0.57 ± 3.07 (mean $\pm$ SD) of percent bias. Nevertheless, IBMR failed to correctly describe the trophic status of the surveyed site, possible due to low dissolved oxygen, high pCO_2 and HCO_3 values.

1. Introduction

Macrophyte Biological Index for Rivers (*Indice Biologique Macrophytique en Rivière* – IBMR) has been identified as the official method to assess the Biological Quality Element (BQE) "macrophytes" in Italy, according to Water Framework Directive 2000/60/EC (WFD). IBMR has been developed in France, represents an improvement of an earlier system published by the Group of Scientific Interest "Macrophytes of inland waters" (Haury et al., 1996, 2006) and a technical norm has been developed from it by the French Association for Normalization (AFNOR, 2003). IBMR is primary structured to estimate the nutrient inputs of river and it is a relevant indicator of disturbed situations compared to reference macrophyte communities. Therefore and to compute the proper Ecological

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Quality Ratio (EQR), references from unpolluted rivers should be taken into account wherever and as far as they still exist (Haury et al., 2006). IBMR has been already and successfully applied in Italy, though some difficulties in its application have been reported because of the absence of some species from the tables of the method, difficulties in species classification, the influence of abiotic factors other than the trophic status and, in some instances, the imperfect correspondence with the trophic status assessed by ammonia nitrogen (NH_3-N) and orthophosphate phosphorus levels (PO_4-P) (Abati et al., 2010; Azzollini et al., 2010; Ciccarelli et al., 2010; Mezzotero et al., 2010; Tomasella et al., 2010). To date, IBMR has never been applied in rivers of the Abruzzo Region (Central Italy).

The aim of the present survey was to compare two complementary methodologies – *relevés* from boat and from river banks vs combined relieves achieved by means of interpretation from orthophotos and from georeferenced images taken during SCUBA (Self-Contained Underwater Breathing Apparatus) dive – for the IBMR evaluation of the waters of Pescara Springs (*Sorgenti del Pescara*; Popoli, Italy). SCUBA dive has been successfully and widely used in floristic studies in marine ecosystems (Neushul, 1967; Peña and Bárbara, 2008; Schubert et al., 2011), but relatively less and more recently in freshwater and mainly in lentic ecosystems (Azzella et al., 2013; Chappuis et al., 2011). With regard to orthophotos, GIS (geographical information system) supported photointerpretation has been widely used for vegetation classification in land ecosystems (Madden et al., 1999; Bell, 2009). Neither SCUBA dive nor orthophotos interpretation has been ever used before to determine IBMR. Nevertheless, photointerpretation is profitably usable where river tracts are not crossable and/or navigable. During present survey, possible differences in *taxa* identification and cover estimation, in BQE "macrophytes" and IBMR-EQR were assessed. Furthermore, data from submerged macrophytes were analyzed by means of exploratory multivariate analysis (non-metric multidimensional scaling) to detect vegetation associations and their correlation with some abiotic factors. Correspondence with previously applied classifications and monitoring methods was also discussed.

2. Materials and methods

2.1. Site description

Pescara Springs (247 m amsl) lie at the base of "Capo Pescara" hill (425 m amsl), at the administrative south-western limit of Pescara province and are the most southern drainage of the grand regional karst aquifer "Gran Sasso-Sirente" with a stable water flow (from 5.5 to 8.5 $m^3 s^{-1}$, according to season; 7.5 $m^3 s^{-1}$ on average) (Boni et al., 2002). Hydrologically, major anion is HCO_3^- (326 $mg l^{-1}$); also present is SO_4^{2-} (20 $mg l^{-1}$). Major cation is Ca^{2+} (98 $mg l^{-1}$), followed by Mg^{2+} (14 $mg l^{-1}$) (Adinolfi Falcone et al., 2006). Main springs are on the left bank and underwater wells are also present, particularly in the first southern tract. Water flows from South to North forming a voluminous spring basin (*crenon*), and then it turns abruptly to East to converge, after no more than 1200 m from its origin, into Aterno river. Referring to climatology, analysis of Bagnouls and Gaussen climatic (ombrothermic) diagrams according to Tammaro (1971) (thirty years series from 1921 to 1950) and to Pirone et al. (1997) (thirty years series from 1960 to 1990) revealed temperature means of 13.2–13.5 °C, precipitation of 738–688 mm, respectively, with a temperature peak and relative rainfall deficit from half June to half August. According to Rivas-Martinez (2008) phytoclimatic index, data correspond to an upper mesomediterranean thermotype and a lower subhumid ombrotype (Pirone et al., 1997). Geographic coordinates of the studied area were: Northern extreme, 42°10′10.69″ N–13°49′22.68″ E; Southern extreme, 42°09′40″ N–13°49′17.40″ E; Eastern extreme, 42°10′10.57″ N–13°49′24.91″ E; Western extreme, 42°10′06.04″ N–13°49′14.15″ E.

2.2. Macrophytes identification

Macrophytes were identified by means of proper identification keys. In particular, pteridophytes and phanerogames classification was performed according to Pignatti (1982) and previously published reports (Pirone et al., 1997). Bryophytes were classified, to genus level, according to Cortini Pedrotti (2001) and Smith (2004). Algae and cyanophytes were classified according to John et al. (2005), Bellinger and Sigee (2010), by means of microscopic observation.

2.3. IBMR evaluation

IBMR was assessed according to its technical norm (AFNOR, 2003). Macrophyte sampling and cover estimation were performed from the river banks and by boat survey. Moreover, as a possible complementary method, IBMR was calculated from data obtained by interpretation of orthophotos and of images and samples taken during SCUBA dive. In spite of the adopted method, photograph and specimens sampling were adequately georeferenced. Sampling site was divided into homogeneous tracts according to hydromorphological characters and to vegetation associations; IBMR was calculated for every homogeneous tract. For each uniform tract, assuming river width uniform for each length segment connecting each georeferenced point, real photographically sampled and vegetation covered areas were determined. More in detail and for each homogeneous tract, area (in m^2) determined by means of orthophoto interpretation was divided for the total length of each tract, and then multiplied for each length segment connecting each georeferenced point, in order to estimate the area of the single sample site. It should be stressed that the number of sampling points exceeded the number of the homogeneous tracts; namely more than a sample was taken for each homogeneous tract. In particular, and referring to SCUBA dive, 75 sampling points (relevés) were performed: first tract, 15 (on average, one sampling point each 8.4 m of length); second tract, 19 (on average, one sampling point each 10.7 m of length); third tract, 9 (on average, one sampling point each 13.4 m of length); fourth tract, 10 (on average, one sampling point each 35.7 m of length); fifth tract 15 (on average, one sampling point each 15.9 m of length); sixth tract, 7 (on average, one sampling point each 10.3 m of length). Cover estimations expressed as m^2 were then relativized with respect to total river wet area in order to get real percentage covers. Percentage estimations were then transformed in the respective IBMR cover classes. Data referring to helophytes (e.g. *Phragmites australis*) and floating hydrophytes (e.g. *Lemna* spp.) have been obtained by means of photointerpretation of orthophoto of the sampled area. In particular, color orthophotos (images taken from 01.08.2010 to 31.08.2010; projection, NUTM33; datum, WGS84; coordinate type, UTM; scale, 1:10,000; courtesy of Abruzzo Region, authorization n. 3061 on 02.04.2012, confirmed on 12.08.2014) were analyzed by means of an image processing package (Fiji; http://fiji.sc/wiki/index.php/Fiji; last access 29.06.12), distribution version of ImageJ (Fiji is Just ImageJ), core 1.46j (Rasband, 1997–2012). In particular grayscale and color thresholding (“Threshold Color” ImageJ plugin, 1.12; http://www.dentistry.bham.ac.uk/landinig/software/software.html) were applied in order to estimate total river wet area, helophytes (practically *P. australis*) and floating hydrophytes (practically *Lemna trisulca* and *Lemna minor*) covers. Moreover and thanks to water clarity, limited depth (maximal depth 3.6 m) and the natural contrast between the clear bottom substrate and the vegetation, estimation of covered and uncovered river bottom was also performed. Estimated data were confirmed by means of direct observation during SCUBA dive surveys. Further methodological details are available in Manera (2012).

In order to determine the IBMR-EQR, macrophyte biological community expected in conditions of minimal anthropogenic impact was determined, according to WFD. Sampling site insists in a Mediterranean geographic area according to Pirone et al. (1997) and was classified as a macrophyte “Ma” type according to Tab. 4.1/b, annex 1 of Italian Environmental Minister Act 260/2010 and referring to WFD river classification (Buffagni et al., 2006) and hydro-ecoregion approach (Wasson et al., 2006). Corresponding IBMR reference value is 12.5.

Referring to Mediterranean geographic area and according to Tab. 4.1.1/e, annex 1 of Italian Environmental Minister Act 260/2010, quality classes are: IBMR-EQR $>$ 0.90, high; 0.80 $<$ IBMR-EQR $\leq$ 0.90, good; 0.65 $<$ IBMR-EQR $\leq$ 0.80, moderate; 0.50 $<$ IBMR-EQR $\leq$ 0.65, scarce; IBMR-EQR $\leq$ 0.50, bad.

2.4. Abiotic parameters measuring

During boat sampling following abiotic water parameters were recorded in georeferenced sites: water temperature (°C), pH and dissolved oxygen (mg l^{-1}) by means of a multiparametric probe (MultiLine F/SET-3, WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Moreover, during SCUBA dive following abiotic parameters were recorded for each of the georeferenced sampled point: water depth, water current velocity (operator perceived ordinal scale, Manera (2012)), substrate granulometry of the river bottom (from a modified Wentworth (1922) ordinal scale, Manera (2012)), detritus degradation (ordinal scale, Manera (2012)). The presence/absence of underwater springs was also annotated for each of the georeferenced sampled point.

2.5. Statistical analysis

IBMR and IBMR-EQR values obtained by means of the two aforementioned methods were assessed for their concordance by means of Bland–Altman plot, a method used to analyze the agreement between two different assays (Bland and Altman, 1986). GraphPad Prism® 4 was adopted as statistical package (GraphPad Software Inc., San Diego, CA, USA). Cover data of submersed hydrophytes were analyzed by means of non-metric multidimensional scaling (NMDS), a statistical ordination method in which reduction in dimensionality is achieved by means of a non-metric way. This method was chosen because it is independent from the assumption of normality of data and permits the use of heterogeneous data or data ordinate along arbitrary or discontinuous scales (Kruskal and Wish, 1978; Clarke, 1993). Environmental data were introduced in the model as ordinal data relativized by maximum, to detect their relationships with the ordination axis. Presence/absence of underwater springs was introduced as qualitative variable. Sørensen (Bray and Curtis) distance measure was adopted, because, though originally applied to presence-absence data, it works equally well with quantitative data (Roberts, 1986). PC-ORD was used as multivariate analysis software.

3. Results

Macrophytes distribution varied in relation to the plant life-form, according to Raunkiær system, and in relation to hydromorphological characters. Helophytes (practically *P. australis*) dominated the river banks, particularly the right one. *Schoenoplectus lacustris*, *Typha latifolia*, *Carex riparia* and *Iris pseudacorus* were also focally present. With regard to floating hydrophytes, *L. trisulca* was recorded in proximity to the river banks partially substituted by *L. minor* in the widest, mainly lentic tract of the river. Floristic table with percentage cover obtained by means of *relevés* from boat and from river banks is reported in Table 1.

Basing on hydromorphological characters and to vegetation associations six homogeneous tracts could be observed following river current (Fig. 1). In the first tract, with highest water current and coarsest substrate, the bryophyte *Palustriella commutata* and *Apium nodiflorum fo. submersum* prevailed, with a discrete presence of *Ceratophyllum demersum* and focal presence of *Potamogeton pectinatus*. A second tract followed and was dominated by *C. demersum* and *P. pectinatus* with the presence of the cyanophyceans *Oscillatoria* sp. and *Lyngbya* sp. In the third tract the xanthophycean *Vaucheria* sp. appeared as subglobose aggregates (so named “pad” form). *Myriophyllum spicatum* prevailed in fourth and fifth tracts, which were characterized by slow water current, low water depth ($<$2 m), finer, partially anaerobic detritus and the highest insolation. The fourth tract was completely dominated, at seasonal peak of insolation, by a thick ($>$1 m) felt of *Vaucheria* sp. The fifth tract differed from the fourth one because of the presence of river bottom tract depleted from

Table 1
Floristic table with percentage cover of macrophytes (pteridophytes and bryophytes) obtained by means of *relevés* from boat and from river banks. Total cover, moss layer and algal layer covers were also shown.

	1	2	3	4	5	6	7	8	9	10	11	12	13
Apium nodiflorum	21	26	27	14	0	0	0	0	0	0	0	0	0
Carex riparia	6	0	0	2	0.1	0	0	0	0	0	0	5	0
Ceratophyllum demersum	11	19	18	11	18	26	25	0	0	16	32	14	15
Juncus species	0.5	0	0	0	0	0	0	0	0	0	0	0	0
Lemna trisulca	0.5	3	5	0.5	0.1	3	0	0	0.1	0	4	0	0.5
Nasturtium officinale	0.5	0	0	0	0	0	0	0	0	0	0	0	0
Phragmites australis	14	19	17	14	18	13	20	42	35	32	18	32	18
Sparganium erectum	0.5	0.5	0.1	0	0	0	0	0	0	0	0	0	0
Veronica anagallis-aquatica	0.1	0.5	0	0.1	0	0	0	0	0	0	0	0	0
Palustriella commutata	18	3	9	1	0	0	0	0	0	0	0	0	0
Potamogeton pectinatus	0	4	14	24	21	10	0	0	0	0	0.1	0	18
Equisetum palustre	0	0	0.1	0.1	0	0	0	0	0	0	0	0	0
Iris pseudacorus	0	0	0.1	0.5	0.1	0.1	0	0	0	0	0	0	0
Mentha aquatica	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0
Potamogeton pectinatus	0	0	0	0.1	0	0	0	0	0	0	0	0	0
Schoenoplectus lacustris	0	0	0	2	0	0	0	0	0	0	0	0	0
Lemna minor	0	0	0	0	0	3	0	0	0.5	0	0	5	2
Myriophyllum spicatum	0	0	0	0	0	1	5	0	50	16	37	0	0
Solanum dulcamara	0	0	0	0	0	0	0.1	0	0	0	0	0.1	0
Typha latifolia	0	0	0	0	0	0	0	0	0	0	0	0.1	0
Potamogeton natans	0	0	0	0	0	0	0	0	0	0	0	0	7
Cover total	72	75	91	70	60	65	100	70	91	80	91	92	70
Cover moss layer	18	3	9	1	0	0	0	0	0	0	0	0	0
Cover algae layer	0	0	0	1	3	9	50	28	5	16	10	36	11

With modification from Manera (2012).

vegetation and because of a relative lower presence of *Vaucheria* sp. ("felt" form) and because of a relative higher prevalence of *M. spicatum*. The last tract was characterized by higher water current and depth (compared to the immediately precedent ones), and the occurrence of *P. pectinatus* and *C. demersum*. *Potamogeton natans* was also present.

Results of non-metric multidimensional scaling are graphically summarized in Fig. 2 as a two-axis solution biplot (axis one, $R^2 = 0.21$; axis three, $R^2 = 0.45$), though the best NMDS ordination result was achieved with three axis (cumulative $R^2 = 0.81$). *M. spicatum* and *Vaucheria* sp. appeared to share the same habitat characterized by fine substrate and highly degraded detritus. On the contrary, *P. commutata* and *A. nodiflorum* prefer a coarser substrate, with not degraded or without detritus. Interestingly, the lowest IBMR values were appreciated in tracts with the lowest water depth and current velocity, the finest substrate and highest detritus degradation with evidences of anaerobic metabolism.

Physico-chemical values of superficial water were (mean ± SD): °C, 12.3 ± 0.2; pH, 7.46 ± 0.07; O_2 mg l^{-1}, 4.03 ± 1.92. A South to North gradient was appreciable for dissolved oxygen and temperature values and pH. Lowest values were detected near water springs, the highest at the end of the studied river tract.

IBMR values according to river tracts and the extension of the latter are reported, respectively, in Table 2 and Fig. 1. A South to North gradient of IBMR reduction is apparent, according to river flow (the studied river tract flows South to North). A good concordance was observed by means of Bland–Altman plot between the two adopted methods, with a bias of 0.57 ± 3.07 (mean ± SD) and the following 95% limit of agreement −5.45 to 6.59 (percent difference).

4. Discussion

Vegetation associations were typical of cold spring and varied according to water depth, current velocity and physico-chemical parameters as previously reported (Polunin and Walters, 1985; Pirone et al., 1997). On average, IBMR and IBMR-EQR values correspond, respectively, to a "very high" trophic status and a "scarce" quality class. Such river classification, according to the adopted BQE "macrophytes" evaluation, is only partially confirmed by a precedent classification of the same area. In particular, a peak in July 2001 was recorded for $N\text{-}NH_4^+$ (1.03 mg l^{-1}) and $N\text{-}NO_3^-$ (1.30 mg l^{-1}). Moreover, Extended Biotic Index (EBI) quality classes ranging from "lightly polluted" to "polluted" were observed (Turin et al., 2003). An oscillating range in total phosphorus values, from below the detection value to 1.80 mg l^{-1} and very low dissolved oxygen level (an exception within the monitored spring tracts) have been reported for the same area in a 12 years study (Giansante et al., 2011). The role of *P. pectinatus* as indicator of poor superficial water condition has been recently reported some kilometers downstream the same river basin (Testi et al., 2010). Actually, only a partial concordance of the proposed ranking mean values with previously reported nutrient peaks is appreciable, though the influence of abiotic factors other than nutrients on the trophic status (e.g. current velocity, insolation, substrate nature and granulometry) and, thus, on IBMR values should be considered. Relationship with abiotic factors has been clearly evidenced by means of the ordination results during present survey, and the effect of abiotic factors on the trophic status, in general, and on IBMR classification has been reported in literature (AFNOR, 2003; Haury et al., 2006). Moreover, particular attention has to be paid in the

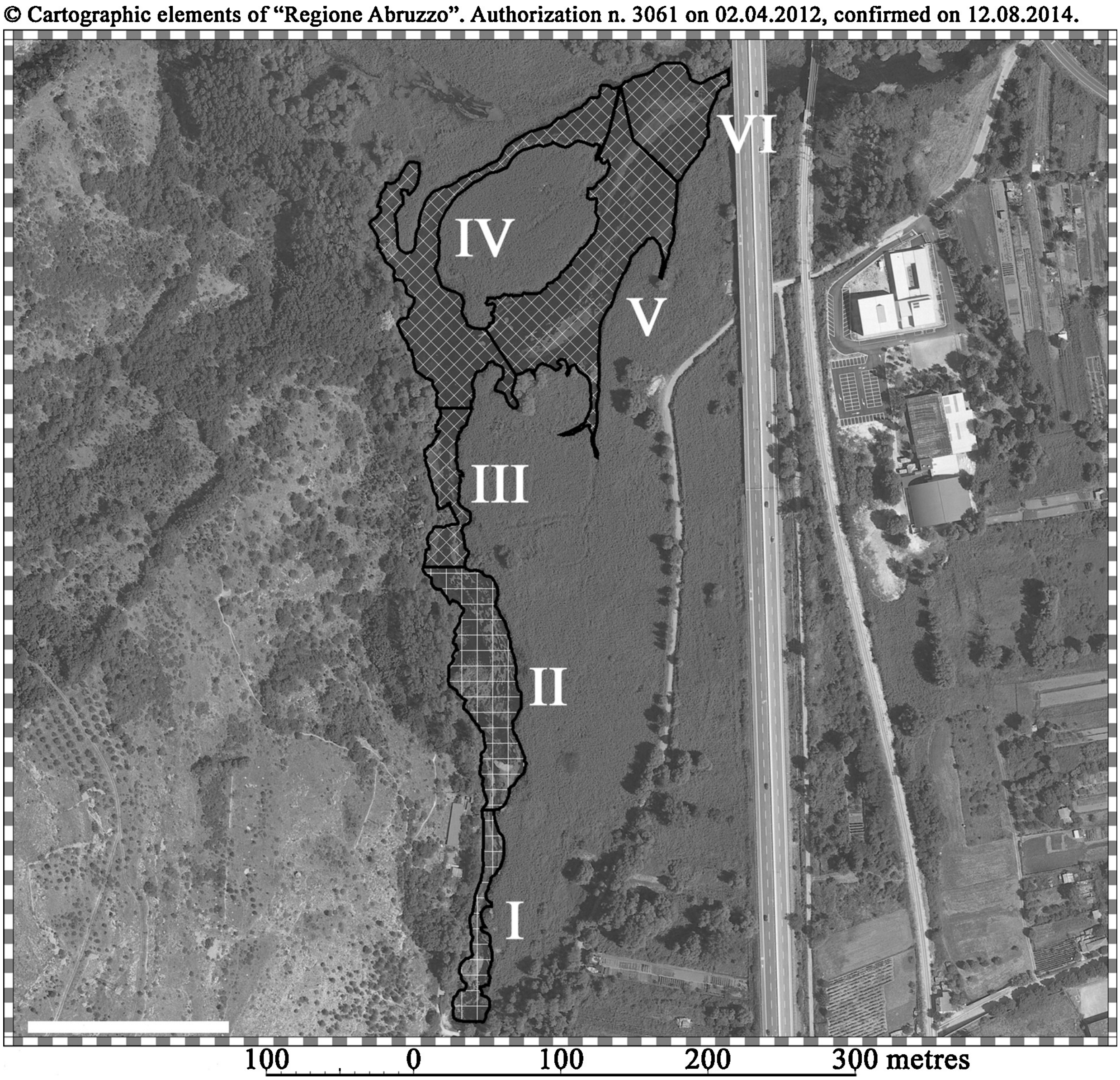

Fig. 1. Georeferenced orthophoto of the studied area. Six homogeneous tracts are appreciable according to hydromorphological characters and to vegetation (submerged hydrophytes) associations. IBMR trophic status and quality classes are graphically summarized as follows: white crossed lines: trophic status "high"; quality class "moderate"; white diagonal crossed lines: trophic status "very high"; quality class "scarce".

application of a classification metric method outside the country where the same was developed, in order to avoid over- or underestimation due to vegetation differences between different countries (Schneider, 2007). IBMR has been successfully applied in Italy, though some difficulties in its application have been reported due to lack of species in the original list, misclassification of some taxa, the influence of abiotic factors other than nutrients on the trophic status and due to the somewhat imperfect correspondence with the trophic status determined by means of N-NH_4^+ and, particularly, P-PO_4^{3-} (Abati et al., 2010; Azzollini et al., 2010; Ciccarelli et al., 2010; Mezzotero et al., 2010; Tomasella et al., 2010). Recently criticism has been posed toward the extensive use of river macrophyte indices within the scope of Water Framework Directive, with particular regard to the lack of ecological meaning, problems in determining the precision of the indices, the large uncertainties and the low explanatory power, which preclude the reliable applicability at specific sites. With specific regard to IBMR, this method has been claimed to be a better indicator of pH and/or carbon availability both as HCO_3 and pCO_2, rather than an indicator of soluble reactive phosphorus and/or NH_4^+ (Demars and Trémolières, 2009; Demars et al., 2012).

Referring to algae, the reciprocal dominance relationships between tracheophytes and algae are complex and

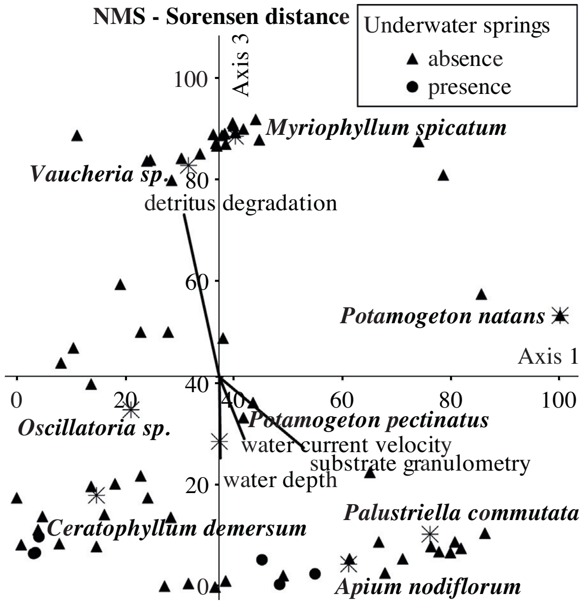

Fig. 2. Non-metric multidimensional scaling ordination plot. Asterisks represent species centroids and vectors represent abiotic parameters.

not limited to variations in the trophic status and/or N/P ratio (Doyle and Smart, 1998; Walstad, 1999; Chen et al., 2007). The inverse correlation between algae and tracheophytes observed during present survey, in particular in the fourth and fifth tract, has been already described in springs and represents a form of competition (Hauxwell et al., 2004; Frazer et al., 2006a,b; Jacoby et al., 2007). Recently decline in the dissolved oxygen in karst springs in Florida has been claimed to influence the proliferation of benthic filamentous algae, acting as a limiting factor for grazer organisms. Dissolved oxygen decline was ascribed to hydrological variation caused to climatic changes and anthropic pressure leading to the recruitment of deeper karst aquifer water (Heffernan et al., 2010). Pescara Springs are the most southern drainage of the grand regional karst aquifer "Gran Sasso-Sirente". The aquifer is characterized by water mixing from different levels according to season, which influences the physico-chemical properties of water (Di Sabatino et al., 2005). In particular, a basal water flow through old rock prevails during the dry seasons (summer and autumn), whereas mixing with new water filtering through younger rock prevails during the rain seasons (winter and spring) (Barbieri et al., 2005).

Interestingly, submerged chalk springs display typically high pCO_2, this feature providing favorable conditions for completely submerged plant growth (Kohler et al., 1973; Sand-Jensen et al., 1992; Carbiener et al., 1995; Sand-Jensen and Frost-Christensen, 1998; Demars and Trémolières, 2009). Moreover, as pCO_2 equilibrates toward the air-equilibrium along river course, a competition occurs between submerged plants depending upon water dissolved CO_2 and plants able to take it by means of aerial leaves, or to take HCO_3 from the water (Madsen and Sand-Jensen, 1987, 1991; Vadstrup and Madsen, 1995). Effectively, during present survey *A. nodiflorum fo. submersum*, the submerged form of a normally terrestrial plant was detected only in the first tract of the spring, where submerged springs prevailed, whereas *P. pectinatus*, *C. demersum* and *M. spicatum*, known HCO_3 users, dominated the second and the third tract the first two, and the fourth and the fifth the last one, where submerged springs were smaller or absent. The abovementioned relationship with submerged springs is clearly depicted in the ordination plot (Fig. 2).

5. Conclusions

Interpretation of orthophotos and images taken during SCUBA dive – methods which are profitably usable where river tracts are not crossable and/or navigable – appeared to be a reliable, complementary method in IBMR evaluation compared to boat survey. Nevertheless, IBMR failed to correctly describe the trophic status of the surveyed site, possible due to low dissolved oxygen, high pCO_2 and HCO_3 values and to the influence of other abiotic factors. Further investigations are needed to fully elucidate the ecology of macrophytes of this unique habitat and to adopt with confidence IBMR for WFD classification purpose.

Conflict of interest

None declared.

Table 2
IBMR and IBMR-EQR according to river tracts and survey methodology.

Tract	SCUBA and orthophoto				Boat			
	IBMR	Trophic[a] status	IBMR-EQR	Quality class[b]	IBMR	Trophic[a] status	IBMR-EQR	Quality class[b]
I	9.18	High	0.73	Moderate	9.06	High	0.72	Moderate
II	8.69	High	0.70	Moderate	8.37	High	0.67	Moderate
III	7.48	Very high	0.60	Scarce	7.48	Very high	0.60	Scarce
IV	7.69	Very high	0.62	Scarce	7.45	Very high	0.60	Scarce
V	7.73	Very high	0.62	Scarce	7.73	Very high	0.62	Scarce
VI	6.47	Very high	0.52	Scarce	6.79	Very high	0.54	Scarce
Mean	7.87	Very high	0.63	Scarce	7.81	Very high	0.63	Scarce

With modification from Manera (2012).
[a] Very low > 14, 12 < low ≤ 14, 10 < medium ≤ 12, 8 < high ≤ 10, very high ≤ 8. Boundary levels according to AFNOR (2003).
[b] High > 0.90, 0.80 < good ≤ 0.90, 0.65 < moderate ≤ 0.80, 0.50 < scarce ≤ 0.65, bad ≤ 0.50. Boundary levels referred to Mediterranean geographic area and according to Tab. 4.1.1/e, annex 1 of Italian Environmental Minister Act 260/2010.

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References

Abati, S., Castorina, M., Iberite, M., Minciardi, M.R., Pelliccioni, I., Spada, C.D., 2010. Utilizzo di macrofite come bioindicatori nelle acque interne dell'Agro Pontino (Lazio meridionale). In: Bottarin, R., Schirpke, U., Tappeiner, U., Oggioni, A., Bolpagni, R. (Eds.), Macrofite & Ambiente. EURAC Book 58, vol. III. Eurac Research, Bolzano, Italy, pp. 119–131.

Adinolfi Falcone, R., Falgiani, A., Petitta, M., Tallini, M., 2006. Characteristics of the Gran Sasso INFN laboratory groundwater (inferred from 1996–1998 spot sampling data) to fine-tune the conceptual model of water–rock interaction in carbonate aquifers. LNGS/GEO-01/06. Istituto Nazionale di Fisica Nucleare – Laboratori Nazionali del Gran Sasso, Assergi (AQ), Italy.

Azzella, M.M., Ricotta, C., Blasi, C., 2013. Aquatic macrophyte diversity assessment: validation of a new sampling method for circular-shaped lakes. Limnologica 43, 492–499.

Azzollini, R., Gerbaz, D., Isabel, S., Vicquéry, L., Minciardi, M.R., Rossi, G.L., Concita Daniela Spada, C.D., 2010. Uso di macrofite acquatiche per il monitoraggio di corsi d'acqua alpini: le applicazioni in Valle d'Aosta. In: Bottarin, R., Schirpke, U., Tappeiner, U., Oggioni, A., Bolpagni, R. (Eds.), Macrofite & Ambiente. EURAC Book 58, vol. III. Eurac Research, Bolzano, Italy, pp. 91–104.

AFNOR, 2003. Qualité de l'eau: Détermination de l'indice biologique macrophytique en rivière (IBMR). NF T90-395.

Barbieri, M., Boschetti, T., Petitta, M., Tallini, M., 2005. Stable isotope (^{2}H, ^{18}O and ^{87}Sr/^{86}Sr) and hydrochemistry monitoring for groundwater hydrodynamics analysis in a karst aquifer (Gran Sasso, Central Italy). Appl. Geochem. 20, 2063–2081.

Bell, S.A.J., 2009. Vegetation and floristics of Columbey National Park, lower Hunter Valley, New South Wales. Cunninghamia 11 (2), 241–275.

Bellinger, E.G., Sigee, D.C., 2010. Freshwater Algae. Identification and Use as Bioindicators. Wiley-Blackwell, Chichester, West Sussex, UK.

Bland, J.M., Altman, D.G., 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 327, 307–310.

Boni, C., Pianelli, A., Pierdominici, S., Ruisi, M., 2002. Le grandi sorgenti del fiume Tirino (Abruzzo). Boll. Soc. Geol. Ital. 121, 411–431.

Buffagni, A., Munafò, M., Tornatore, F., Bonamini, I., Didomenicantonio, A., Mancini, L., Martinelli, A., Scanu, G., Sollazzo, C., 2006. Elementi di base per la definizione di una tipologia per i fiumi italiani in applicazione della direttiva 2000/60/EC. Notiziario dei Metodi Analitici (Istituto di ricerca sulle Acque – CNR) 1, 2–19.

Carbiener, R., Trémolières, M., Muller, S., 1995. Vegetation of running waters and water quality: thesis, debates and prospects. Acta Bot. Gall. 142, 489–531.

Chappuis, E., Gacia, E., Ballesteros, E., 2011. Changes in aquatic macrophyte flora over the last century in Catalan water bodies (NE Spain). Aquat. Bot. 95 (4), 268–277.

Chen, C., Yin, D.Q., Yu, B., Zhu, H.K., 2007. Effect of epiphytic algae on photosynthetic function of *Potamogeton crispus*. J. Freshw. Ecol. 22, 411–420.

Ciccarelli, E., Notargiacomo, T., Charavgis, F., Cingolani, L., 2010. L'utilizzo delle macrofite per il controllo della qualità dei corsi d'acqua umbri: applicabilità e problemi. In: Bottarin, R., Schirpke, U., Tappeiner, U., Oggioni, A., Bolpagni, R. (Eds.), Macrofite & Ambiente. EURAC Book 58, vol. III. Eurac Research, Bolzano, Italy, pp. 133–143.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Austral Ecol. 18, 117–143.

Cortini Pedrotti, C., 2001. Flora dei muschi d'Italia, vol. III. Antonio Delfino Editore, Roma, Italy.

Demars, B.O.L., Trémolières, M., 2009. Aquatic macrophytes as bioindicators of carbon dioxide in groundwater fed rivers. Sci. Total Environ. 407, 4752–4763.

Demars, B.O.L., Potts, J.M., Trémolières, M., Thièbaut, G., Gougelin, N., Nordmann, V., 2012. River macrophyte indices: not the Holy Grail! Freshw. Biol. 57, 1745–1759.

Di Sabatino, V., Manetta, M., Sciannamblo, D., Spizzico, M., Tallini, M., 2005. Caratterizzazione del circuito delle acque sotterranee degli acquiferi carsici tramite lo studio del contenuto in Radon delle sorgenti del Gran Sasso (Italia centrale). G. Geol. Appl. 2, 445–452.

Doyle, R.D., Smart, R.M., 1998. Competitive reduction of noxious *Lyngbya wollei* mats by rooted aquatic plants. Aquat. Bot. 61, 17–32.

Frazer, T., Notestein, S., Jacoby, C., Littles, C., Keller, S., Swett, R., 2006a. Effects of storm-induced salinity changes on submersed aquatic vegetation in Kings Bay, Florida. Estuar. Coasts 29, 943–953.

Frazer, T.K., Notestein, S.K., Pine, W.E., 2006b. Changes in the Physical, Chemical and Vegetative Characteristics of the Homosassa, Chassahowitzka and Weeki Wachee Rivers. Southwest Florida Water Management District, Tampa, FL, USA.

Giansante, C., Scortichini, G., Melai, V., Pelini, S., Ferri, N., 2011. Quality of surface fresh waters needing protection or improvement in order to be support fish life: 12 years of monitoring pursuant to European Union Directive 78/659/EEC. Vet. Ital. 47, 191–204.

Haury, J., Peltre, M.-C., Muller, S., Trémolières, M., Barbe, J., Dutartre, A., Guerlesquin, M., 1996. Des indices macrophytiques pour estimer la qualité des cours d'eau français: premières propositions. Ecologie 27, 233–244.

Haury, J., Peltre, M.-C., Trémolières, M., Barbe, J., Thièbaut, 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.

Hauxwell, J., Osenberg, C.W., Frazer, T.K., 2004. Conflicting management goals: manatees and invasive competitors inhibit restoration of a native macrophyte. Ecol. Appl. 1, 571–586.

Heffernan, J.B., Liebowitz, D.M., Frazer, T.K., Evans, J.M., Cohen, M.J., 2010. Algal blooms and the nitrogen-enrichment hypothesis in Florida springs: evidence, alternatives, and adaptive management. Ecol. Appl. 20, 816–829.

Jacoby, C., Frazer, T., Swett, R., Keller, S., Notestein, S., 2007. Kings Bay vegetation evaluation. Final report. Southwest Florida Water Management District, Brooksville, FL, USA.

John, D.M., Whitton, B.A., Brook, A.J., 2005. The Freshwater Algal Flora of the British Isles. Cambridge University Press, Cambridge, UK.

Kohler, A., Wonneberger, R., Zeltner, G., 1973. Die Bedeutung chemischer und planzlicher "Verschmutzungindikatoren" im Fliessgewässersystem Moosach (Münchener Ebene). Arch. Hydrobiol. 72, 533–549.

Kruskal, J.B., Wish, M., 1978. Multidimensional Scaling. Sage Publications, Beverly Hills, CA.

Madden, M., Jones, D., Vilchek, L., 1999. Photointerpretation key for the Everglades vegetation classification system. Photogramm. Eng. Rem. Sens. 65 (2), 171–177.

Madsen, T.V., Sand-Jensen, K., 1987. Photosynthetic capacity, bicarbonate affinity and growth of *Elodea canadensis* exposed to different concentrations of inorganic carbon. Oikos 50, 176–182.

Madsen, T.V., Sand-Jensen, K., 1991. Photosynthetic carbon assimilation in aquatic macrophytes. Aquat. Bot. 41, 5–40.

Manera, M., 2012. L'immersione scientifica subacquea nelle acque delle "Sorgenti del Pescara". Manera M., Notaresco, Italy.

Mezzotero, A., Minciardi, M.R., Spada, C.D., Gallo, L., Lucadamo, L., 2010. Prima caratterizzazione delle comunità a macrofite acquatiche in alcuni corsi fluviali della Provincia di Cosenza. In: Bottarin, R., Schirpke, U., Tappeiner, U., Oggioni, A., Bolpagni, R. (Eds.), Macrofite & Ambiente. EURAC Book 58, vol. III. Eurac Research, Bolzano, Italy, pp. 145–156.

Neushul, M., 1967. Studies of subtidal marine vegetation in western Washington. Ecology 48 (1), 83–94.

Peña, V., Bárbara, I., 2008. Maërl community in the north-western Iberian Peninsula: a review of floristic studies and long-term changes. Aquat. Conserv.: Mar. Freshw. Ecosyst. 18 (4), 339–366.

Pignatti, S., 1982. Flora d'Italia, vol. I, II, III. Edagricole, Bologna, Italy.

Pirone, G., Frattaroli, A.R., Corbetto, F., 1997. Vegetazione, cartografia vegetazionale e lineamenti floristici della riserva naturale "Sorgenti del Pescara" (Abruzzo – Italia). Comune di Popoli, Popoli, Italy.

Polunin, O., Walters, M., 1985. A Guide to Lire Vegetation of Britain and Europe. Oxford University Press, Oxford, UK.
Rasband, W.S., 1997–2012. ImageJ. U.S. National Institutes of Health, Bethesda, MD, USA. , http://imagej.nih.gov/ij/ (last access 29.06.14).
Rivas-Martinez, S., 2008. Global Bioclimatics (Clasificación Bioclimática de la Tierra), http://www.globalbioclimatics.org/book/bioc/global_-bioclimatics-2008_00.htm#srm (last access 29.06.12).
Roberts, D.W., 1986. Ordination on the basis of fuzzy set theory. Vegetatio 66, 123–131.
Sand-Jensen, K., Frost-Christensen, H., 1998. Photosynthesis of amphibious and obligately submerged plants in CO_2-rich lowland streams. Oecologia 117, 31–39.
Sand-Jensen, K., Pedersen, M.F., Nielsen, S.L., 1992. Photosynthetic use of inorganic carbon among primary and secondary water plants in streams. Freshw. Biol. 27, 283–293.
Schneider, S., 2007. Macrophyte trophic indicator values from a European perspective. Limnologica 37, 281–289.
Schubert, H., Feuerpfeil, P., Marquardt, R., Telesh, I., Skarlato, S., 2011. Macroalgal diversity along the Baltic Sea salinity gradient challenges Remane's species-minimum concept. Mar. Pollut. Bull. 62 (9), 1948–1956.
Smith, A.J.E., 2004. The Moss Flora of Britain & Ireland. Cambridge University Press, Cambridge, UK.
Tammaro, F., 1971. La flora delle Gole di Popoli (Pescara). G. Bot. Ital. 105, 49–93.
Testi, A., D'Angeli, D., Fanelli, G., Gomarasca, S., Guidotti, S., Castigliani, V., Cristaldi, M., De Antonio, M., Ieradi, L., Martella, G., Salvatori, M., 2010. La qualità degli habitat fluviali attraverso l'applicazione di indici a due scale di indagine. In: Bottarin, R., Schirpke, U., Tappeiner, U., Oggioni, A., Bolpagni, R. (Eds.), Macrofite & Ambiente. EURAC Book 58, vol. III. Eurac Research, Bolzano, Italy, pp. 157–176.
Tomasella, M., Pizzul, E., Zanut, E., 2010. Le macrofite come indicatori biologici per la valutazione della qualità del bacino del fiume Stella (Friuli Venezia Giulia, Nord-Est Italia). In: Bottarin, R., Schirpke, U., Tappeiner, U., Oggioni, A., Bolpagni, R. (Eds.), Macrofite & Ambiente. EURAC Book 58, vol. III. Eurac Research, Bolzano, Italy, pp. 105–118.
Turin, P., Ruggeri, L., Colcera, C., Zanetti, M., D'Eramo, A., 2003. Il monitoraggio e la prima classificazione delle acque ai sensi del D, Lgs. 152/99. Regione Abruzzo. Direzione Territorio – Servizio gestione e tutela della risorsa acqua superficiale e sotterranea, L'Aquila, Italy.
Vadstrup, M., Madsen, T.V., 1995. Growth limitation of submerged aquatic macrophytes by inorganic carbon. Freshw. Biol. 34, 411–419.
Walstad, D., 1999. Ecology of the Planted Aquarium. Echinodorus Publishing, Chapel Hill, NC, USA.
Wasson, J.G., Garcia Bautista, A., Chandesris, A., Pella, H., Armanini, D.G., Buffagni, A., 2006. Approccio delle idro-ecoregioni europee e tipologia fluviale in Francia per la direttiva quadro sulle acque (2000/60/EC). Notiziario dei Metodi Analitici (Istituto di ricerca sulle Acque – CNR) 1, 20–38.
Wentworth, C.K., 1922. A scale of grade and class terms for clastic sediments. J. Geol. 30, 377–392.