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Concordance between macrophytes and macroinvertebrates in a Mediterranean river of central Apennine region

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Abstract

The main aim of this study was to improve the knowledge about the concordance among macrophytes and macroinvertebrates to provide complementary information and facilitate the procedures for quality assessment of river ecosystems. Macrophytes and macroinvertebrates were collected in 11 sampling sites along a central Apennine calcareous river in October 2008 and June 2009. The concordance between the two biomonitoring groups was tested according to several environmental parameters. The comparison of data matrix similarities by Mantel test showed differences in the assemblage of macrophytes and macroinvertebrates along the river since correlation values were 0.04, $p>0.05$ in October 2008 and 0.39, $p>0.05$ in June 2009. The study revealed lack of concordance between the two groups, emphasizing that the information provided by macrophytes and macroinvertebrates does not overlap in terms of response to environmental parameters. Indeed, the two different biological groups resulted useful descriptors of different parameters. Together, they could represent a complementary tool to reflect the river environmental quality.

Key words

Central Italy, Concordance, Environmental quality, River biomonitoring, Water framework directive

Introduction

It is well-known that anthropic activities strongly affect aquatic environments resulting in damages to ecosystems integrity. Assessment of human impact on river habitat has resulted in a long history of monitoring procedures using bioindicators (Hellawell, 1986; De Pauw and Hawkes, 1993). For example, the use of macroinvertebrates as bioindicators began in the early 1900s (Kolkwitz and Marson, 1909), then becoming essential in the water quality bioassessment (Bonada *et al.*, 2006), as community is affected by both habitat quality and water chemistry (Theodoropoulos and Iliopoulou-Georgudaki, 2010). Despite the well known indices based on macroinvertebrates, the Water Framework Directive (WFD, European Commission, 2000) recently suggested the use of additional biological groups as bioindicators such as diatoms, macrophytes and fish for biomonitoring of river ecosystem integrity. Although the response of each group to different environmental parameters is well known (Hering *et al.*, 2006), comparative studies are nowadays exploring the suitability and performance of different groups in providing accurate description of environmental conditions. Over the past ten years many studies performed in north-central European countries have compared a concordant variation patterns (concordance) of species composition among different taxonomic groups (Hering *et al.*, 2006; Paavola *et al.*, 2006; Mykrä *et al.*, 2008; Heino, 2010). Unfortunately, only few studies were carried out on concordance among different groups in Mediterranean rivers: macroinvertebrate, diatoms, fish and vegetation (Iliopoulou-Georgudaki *et al.*, 2003); macro invertebrate, fish, macrophytes (Pinto *et al.*, 2006); macro invertebrate, fish, macrophytes and bird (Hughes *et al.*, 2009, 2010); macroinvertebrate and fish (Cheimonopoulou *et al.*, 2011); macro invertebrate and diatoms (Pace *et al.*, 2012). Several methodologies were used to test concordance, including correlations (Mykrä *et al.*, 2008), Procrustes rotation (Paavola *et al.*, 2006), detrended correspondence analysis (Pace *et al.*,

2012) and Mantel tests (Mykrä *et al.*, 2008; Johnson and Hering, 2009). All these methodologies were suitable and results were very similar. Although a certain type of significance emerged amongst groups, the strength of their relationships was low (Heino, 2010).

In light of the above, the present study was carried out to study the relationship of macrophytes and macroinvertebrates in the Mediterranean river of Central Apennine region.

Materials and Methods

Study area and survey design : The River Aniene, of 98 km length, is approximately 1100 to 15 m a.s.l. and with a basin area of 1.453 km^2. A total of 11 sites were selected (Fig. 1) and a mean distance of 10 km was left between the sampling sites and the subsequent one, according to Lloyd *et al.* (2006). Each site was visited during early autumn (October 2008) and late spring (June 2009). Within each site, a homogeneous 50m transect along the river was investigated for both macrophytes and macroinvertebrates.

A study area exploratory overview was performed through geographical information system (GIS), the latter being also used to identify human land pressure elements (urban areas, industrial areas, agricultural landscape units-cultivations and pastures, river habitat modification). The Anthropogenic Index (A_i) was applied in order to quantify human pressures according to Larsen and Ormerod (2010).

The same sampling protocol was applied to all sites. A macrohabitat description of both riparian and river habitats was firstly carried out using the River Functionality Index (IFF; from the Italian Indice di Funzionalità Fluviale; APAT, 2007). IFF consisted of fourteen questions (for main parameters describing the river characteristics), which define the structure of riparian zone, stream channel morphology, and biological condition of the investigated area.

A number of physico-chemical data, relevant to the mesohabitat level, were also analyzed : temperature (T), conductivity (C), dissolved oxygen (O_2), and pH *in situ* by probes for immersion (WTW Multi 340i/SET), while, chemical oxygen demand (COD), nitrates (NO_3^-), nitrites (NO_2^-), ammonium ions (NH_4^+) and total phosphorus (P) was estimated by a field spectrophotometer (WTW Photometer MPM). In addition, the velocity of water was recorded using flowmeter General Oceanics 2030 series, and water turbidity according to an empirical scale attributing the following values: 0 for absent, 1 for low and 2 for high turbidity. Finally, a granulometric microhabitat characterization of the riverbed was carried out. Standard classes of grain size were defined and the microhabitat characteristics were described by the proportional relationship among different classes (Buffagni *et al.*, 2004).

Collection of macrophyte: The sampling procedure regarded inventory of all aquatic macrophytes (macroalgae, cyanobacteria, bryophytes, angiosperms), and assessment of their coverage percentage by using the Braun-Blanquet scale (Braun-Blanquet, 1964). For macroalgae and cyanobacteria, the data on coverage percentage was completed by observing slides under light microscope (Leica DM RB). Different collection methods were used: floating masses of filamentous algae were collected by means of a 25 μm-mesh plankton net; epilithic algae and cyanobacteria by scraping stones; bryophytes from rocks and stones in the riverbed by scalpel and hands; angiosperms by direct observation *in situ* or by collecting samples using a grappling iron. All macroalgae were fixed in formalin (4% was the

Fig. 1 : Location of the sampling sites within the study area of River Aniene, Italy. Marks: A = Filettino; B = Trevi nel Lazio; C = Jenne; D = Subiaco; E = Madonna della Pace; F = Anticoli Corrado; G = Vicovaro; H = Castel Madama; I = Tivoli; J = Lunghezza; K = Roma Nomentana

Table 1 : Values of correlation coefficients between all considered parameters and the first two components of the dentrended correspondence analysis (significant correlations at p<0.05 are in bold)

Physico-chemical parameters	DC1	DC2
EC (μScm^{-1})	0.942	-0.196
COD (mgl^{-1})	0.482	0.228
GS	-0.866	0.356
NH_4^+ (mgl^{-1})	0.478	-0.390
NO_2^- (mgl^{-1})	0.111	-0.186
NO_3^- (mgl^{-1})	0.810	-0.051
O_2 (mgl^{-1})	-0.171	0.112
P (mgl^{-1})	0.873	0.035
pH	-0.432	0.013
T (°C)	0.807	-0.112
TUR	0.913	-0.258
VEL (cms^{-1})	0.032	-0.685
Indices and Metrics		
Ai	0.789	-0.056
EBI	-0.942	0.167
EPT	-0.949	0.084
EPT/C	-0.585	0.079
ICMi	-0.920	-0.008
IFF	-0.927	0.131
Hmh	-0.859	0.061
Hmi	-0.739	-0.037
Hmp	-0.426	0.213

Acronyms: Ai = Anthropogenic Index; C = conductivity; COD = chemical oxygen demand; EPT = number of Ephemeroptera, Plecoptera and Trichoptera families; EPT/C = number of EPT individuals/number of Chironomidae family individuals; GS = main grain size microhabitat; Hmi = macroinvertebrate Shannon index value; Hmh = microhabitat Shannon index value; Hmp = macrophyte Shannon index value; EBI = Extended Biotic Index value; ICMi = Intercalibration Common Multimetric Index value; IFF = River Functionality Index value; NH_4^+ = ammonium; NO_2^- = nitrites; NO_3^- = nitrates; O_2 = dissolved oxygen; P = total phosphorus; T = temperature; TUR = turbidity; VEL = water velocity

approximate final concentration). Taxonomical identification was based on specific literature with regard to algae and cyanobacteria (John *et al.*, 2002), bryophytes (Cortini Pedrotti, 2001) and angiosperms (Pignatti, 1982).

Collection of macroinvertebrate: All microhabitats were proportionally sampled following a multihabitat scheme according to Buffagni *et al.* (2004). In particular, a microhabitat was sampled when it covered at least 10 % of the investigated portion of the riverbed. A total of 10 sample units were collected in each site by using a Surber sampler (area 0.05 m^2; mesh size 0.5 mm) and only riffles were taken into account. Macroinvertebrates were grossly sorted in field (at order or family level), preserved with 95 % ethanol and then identified in laboratory to family level (only Trichoptera and Plecoptera were identified at genus level, and Ephemeroptera at species) based on Tachet *et al.* (2000) taxonomic guide.

Data analysis : To evaluate normality assessment of the dataset, both environmental parameters and biotic data were tested with Kolmogorov-Smirnov test and data were log-transformed, since they did not follow a Gaussian distribution. To assess the spatial separation of sampling sites, an ordination method was performed to summarize biotic variables. In particular, a detrended correspondence analysis (DCA) combining macroinvertebrate and macrophyte data for all sampling periods was used to obtain a two-dimensional plot describing an upstream-downstream gradient. Additionally, a series of Spearman's correlations between the first 2 DC axes (obtained using macrophyte + macroinvertebrate dataset) vs. each one of the environmental parameters were performed to evaluate the driver responses of the macrophyte/macroinvertebrate concordance (Table 1). Correlations were significant at p<0.05. DC axes were correlated also with different biotic indices calculated to better describe the sampling site heterogeneity. Specifically, Shannon Index (H) was calculated for macroinvertebrates (Hmi), macrophytes (Hmp) and microhabitats (Hmh). Four usual metrics for macroinvertebrates were also correlated which are as follows : total number of Ephemeroptera, Plecoptera and Trichoptera families (EPT; Lenat, 1988); ratio between the sum of individual number of EPT and individual number of Chironomidae (EPT/C; Plafkin *et al.*, 1989); Extended Biotic Index (EBI; Ghetti *et al.*, 1997); Intercalibration Common Metrics Index (ICMi; Buffagni *et al.*, 2005).

Table 2 : Pairwise Mantel correlation values above the grey diagonal, and significance (P value) below the same one, divided per sampling season. Significant values are written in bold

	Env 2008	Env 2009	Mac 2008	Mac 2009	Inv 2008	Inv 2009
Env 2008		0.15	0.14	-	**0.57**	-
Env 2009	0.185		-	0.10	-	0.18
Mac 2008	0.164	-		**0.37**	0.04	-
Mac 2009	-	0.299	**0.048**		-	0.39
Inv 2008	**0.001**	-	0.380	-		**0.57**
Inv 2009	-	0.241	-	0.064	**0.007**	

Abbreviations: Env = environmental parameters; Inv = macroinvertebrates; Mac = macrophytes

Sites were also classified into groups through cluster analysis (Ward's method), using biotic data in order to highlight the biological similarity amongst sampling sites. Analysis of these data were carried out following two different approaches in order to verify if differences in concordance might depend on different collection seasons. Particularly, macrophytes and macroinvertebrates data collected during two seasons (October 2008 and June 2009) were analyzed jointly and separately.

Finally, to evaluate the macrophyte/macroinvertebrate concordance, similarity of taxa matrices were compared by Mantel test using Bray-Curtis distance, analogous to Pearson correlation coefficient, ranging from –1 to 1. The respective percentage of significance was evaluated by Monte-Carlo permutation test (999 permutations), according to the procedure analysis used in other similar studies on concordance (e.g. Pinto *et al.*, 2006; Mykrä *et al.*, 2008). Matrix correlations were performed between environmental parameters and two investigated groups. To do this, matrices for macrophytes, macroinvertebrates and environmental parameters were constructed for both seasons. Then, pairwise correlation were run between matrices obtaining concordance values. Correlations were significant at $p<0.05$. Alpha values from case to case were always corrected by Bonferroni test. All statistical analyses were performed with Statistica 7 Stat. Soft. and PAST package ver. 1.94b.

Results and Discussion

A total of 137 taxa (58 macrophytes + 79 macro invertebrates) were collected, particularly 123 (50+73) in October 2008 and 102 (42+60) in June 2009. Some macrophyte and macroinvertebrate taxa occurred in the sampling sites either in one of the two seasons: 34 taxa (18+16) in October 2008 and 13 (6+7) in June 2009.

DCA performed on entire macrophyte+macroinvertebrate dataset produced the diagram shown in Fig. 2. The sum of first 2 eigenvalues (DC1+DC2) explained that 81.97 % of total variance. The diagram showed, a clear double distribution pattern: an upstream/downstream gradient along DC1, and a seasonal trend along DC2, In addition, a clear distinction was obtained between October 2008 and June 2009. Almost all physico-chemical data were significantly correlated with DC1 except for NO_2^-, O_2 and VEL, while only VEL was significantly correlated with DC2 (Table 1).

Cluster analysis based on both macrophytes and macroinvertebrates (Fig. 3) segregated all investigated sites in

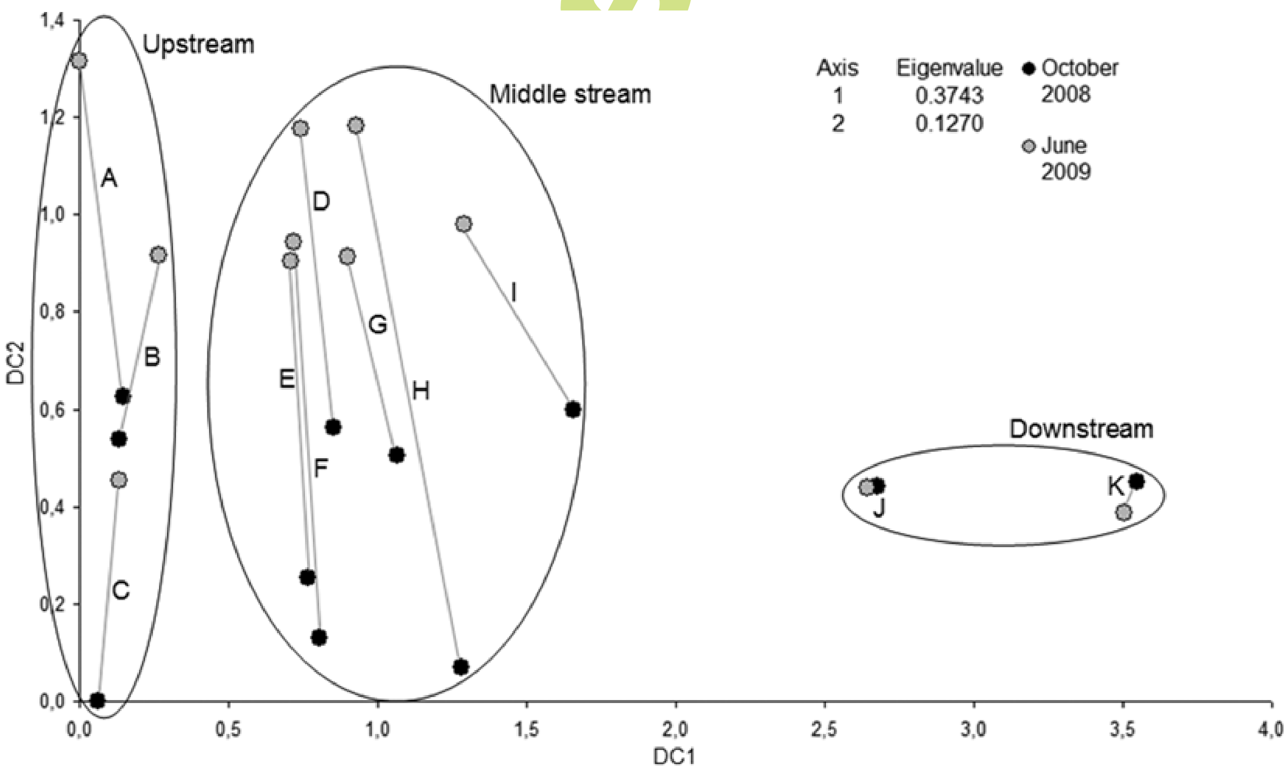

Fig. 2 : Scatter plot of the two first detrended correspondence axis obtained by using all the environmental parameters. Points corresponding to same sampling site for both seasons are joined by grey line

different ways. When both groups were used together, sampling sites seemed to be randomly mixed in October 2008, but well organized in an upstream/downstream gradient in June 2009. This gradient seemed to overlap well with the clusters obtained using only macroinvertebrates in October 2008. When only macrophytes were considered, the upstream/downstream gradient in June 2009 was obtained, but not in October 2008, for which a confused clustering was observed. In all the obtained clusters, the only similar outputs were the two most lowland sites (J and K), being always positioned as out-group of all the remnant sites.

Pair crossed correlations was referred to matrices of the same sampling season session and between the two years of study. Each single matrix of both macrophyte and macro invertebrate groups in 2008 significantly correlated with following sampling season in 2009 (e.g. macrophytes 2008 vs. macrophytes 2009), but result was not obtained by using environmental parameters (Table 2). Considering the correlation between the environmental parameters vs. macrophytes, the significance appeared quite low while correlations between the environmental parameters vs. macroinvertebrates resulted more significant.

The target of this study was approached by comparing different matrices using a multivariate correlation analysis. When macrophytes and macroinvertebrates were compared, they did not correlate in both sampling seasons. This study showed a lack of concordance between macrophytes and macroinvertebrates and these results did not agree with those of the previous studies, where authors argued that assemblage concordance was significant in freshwater systems, especially at small spatial scales (e.g. within-basin) (Paavola *et al.*, 2003). For example, Johnson and Hering (2009) showed a low (but significant) concordance between these two biological groups while Mykrä *et al.* (2008) and Heino *et al.* (2009) showed a high value for the same ones.

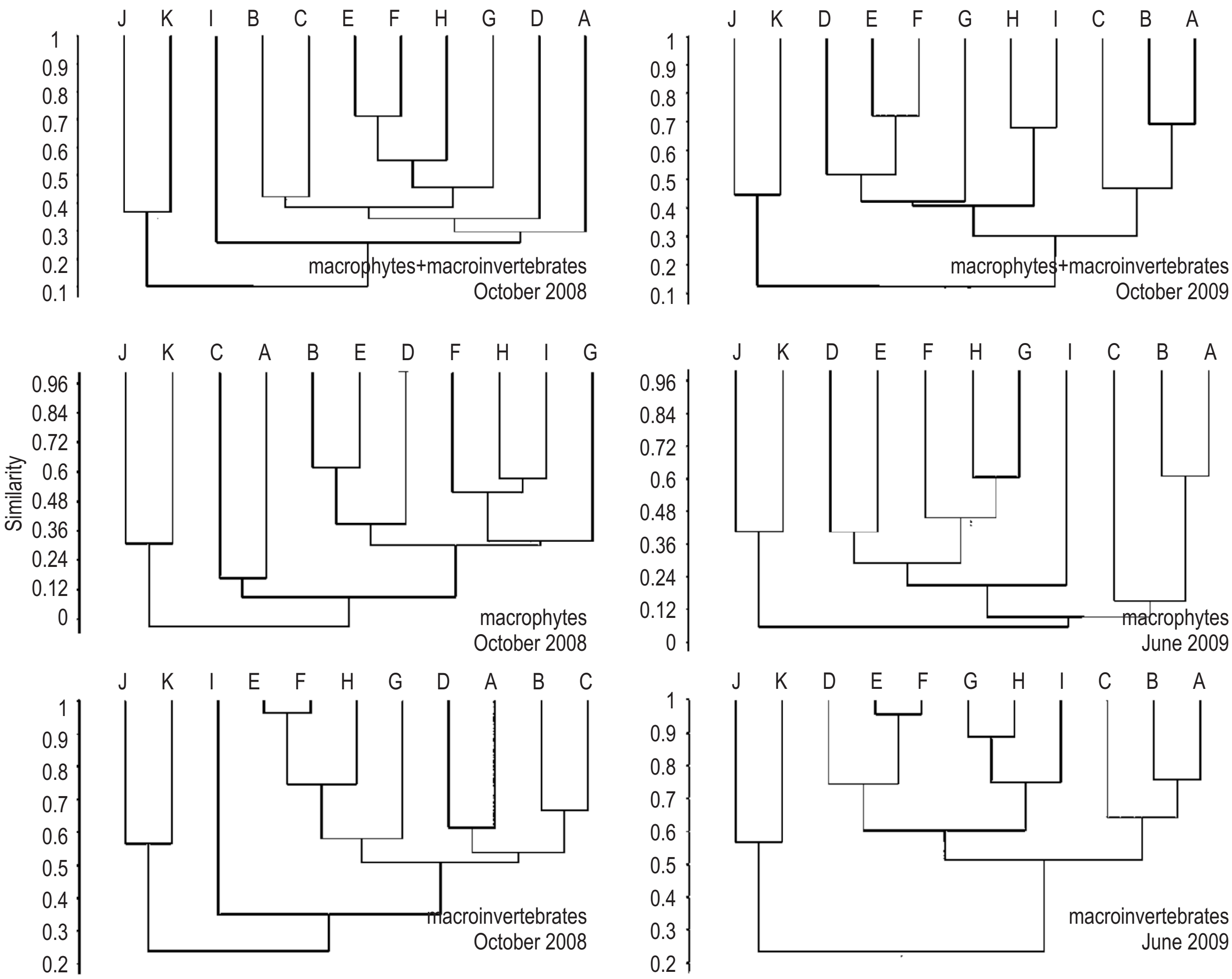

Fig. 3 : Dendrograms based on quantitative data of both macroinvertebrates and macrophytes (analysed singularly and pooled together) divided per sampling season

In light of results, differences between macrophytes and macroinvertebrates could be related to the fact that these two groups exhibited different responses to habitat features at different scales, or to other environmental features not measured in this study. Indeed, composition and distribution of macrophytes is mainly regulated by eutrophication (Egertson *et al.*, 2004) and physico-chemical parameters of water (Abou-Hamdan *et al.*, 2005), whereas macroinvertebrates of water were mainly influenced by hydromorphological alteration of riverbed (Buffagni *et al.*, 2004; Verdonschot, 2009) and water quality (Metcalfe-Smith, 2009). Therefore, lack of concordance suggested that macrophytes and macroinvertebrates provided independent responses to human pressures (Hering *et al.*, 2006), the latter findings being confirmed by matrix correlation analyses. The correct selection of bioindicator(s) depends on the descriptor-type being assessed and the investigative monitoring type.

The differences observed justify the obtained lack of concordance in a highly heterogeneous and anthropized Mediterranean river such as River Aniene. Obviously, these statements deserve to be confirmed by further studies in calcareous Mediterranean basins.

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