Co-occurrence between macrophytes and macroinvertebrates: towards a new approach for the running waters quality evaluation?

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ABSTRACT

Several studies on concordance between macrophyte and macroinvertebrate communities were carried out for decades while any investigation on co-occurrence of single pair of taxa of these two groups was never addressed. Our main aim was to verify the existence of co-occurrence of single macrophyte-macroinvertebrate pair in a Mediterranean river of central Italy. We compared the co-occurrence frequencies of different plant and animal taxa with those expected by chance through the null model approach. Specifically, we proposed two main co-occurrence patterns of macrophyte and macroinvertebrate taxa in terms of aggregation (when groups co-occur more frequently than expected) and segregation (when groups co-occur less frequently than expected). This approach was supported by correlations and analysis of covariance in order to identify the most significant co-occurring pairs. In this study we collected a total of 123 taxa (50 macrophytes+73 macroinvertebrates) in October 2008 and 102 (42+60) in June 2009. We obtained a total of 379 significantly correlating macrophyte-macroinvertebrate pairs. Amongst all these correlating pairs, 83.7% of them correlated in October session while 90.3% in June. All the correlating pairs showed a high relationships with human alterations in both the sampling sessions. Our results confirmed that direct correlations there are between macrophytes and macroinvertebrates, highlighting how these two biological groups co-occur mainly following the aggregative model in both sampling sessions. Therefore, it was possible to observe significant relationships between macrophyte and macroinvertebrate pairs along an entire river ecosystem. It allows us to demonstrate that there are some prospective for trying to increase the knowledge on macrophytes-macroinvertebrates co-occurrence and for suggesting to use only single pair of co-occurring taxa in the monitoring running water quality. Aware that more investigations ought to be performed for confirming our proposal, the findings of the present study may be suggested as a new monitoring tool since it represents an investigation approach easy in the application, feasible and rapid in the execution, and within the European Water Framework Directive freshwater management policies. In addition, a such tool does not need a detailed taxonomical expertise since its applicability is restricted only to the few taxa indicated as representatives of a certain water quality condition.

Key words: Aquatic plant, aquatic invertebrate, assemblage structure, null models, central Italy.

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INTRODUCTION

Human alteration represents today a well-known phenomenon of disturbance mainly for freshwater habitats (Dynesius and Nilsson, 1994; Malmqvist and Rundle, 2002; Dudgeon *et al.*, 2006). Land-use and urban development are responsible for altering species composition, food web structure, nutrient cycling, and ecosystem functioning (Johnes, 1996; Wootton *et al.*, 1996; Jansson *et al.*, 2000; Vörösmarty *et al.*, 2000). This seems to be particularly worrying for catchments within semi-arid regions, such as a Mediterranean one (Hermoso *et al.*, 2010).

The more and more detrimental condition of the river habitats stimulated the European Community to propose new guidelines for the aquatic monitoring by issuing the Water Framework Directive (WFD; European Council, 2000). Among the main goals of the WFD is to propose a new river monitoring system based on an integrated biological assessment by using different taxonomic groups (Hering *et al.*, 2006). Within the WFD line, several studies on concordance of assemblages amongst different aquatic taxa (diatoms, macrophytes, macroinvertebrates, fishes) were carried out (Hering *et al.*, 2004; Heino, 2010; Larsen *et al.*, 2012; Traversetti *et al.*, 2013; Mazzini *et al.*, 2014). A such data collection, although useful and functional, needs a great effort in terms of fund, time, and expertise. Therefore further approaches ought to be promoted as the WFD suggested. In this context, investigations on co-occurrence of single pairs of macrophyte and macroinvertebrate taxa (hereafter called pairs) may represent an alternative way in the river monitoring activities.

Here we propose for the first time a new viewpoint based on the co-occurrence of the single pairs to provide new water quality descriptors. Then we investigate the cooccurrence between pairs at level of taxon (and not at community) in terms of aggregation and/or segregation by the null model approach (Gotelli, 2000). Null models represent one of the most effective ways to test patterns



of taxa co-occurrence in a community (Gotelli, 2000). Although the use of this method was debated in the past (Colwell and Winkler, 1984), to date it seems provide important information on taxa co-occurrence patterns. In addition, despite some aspects of the algorithms suggested by Gotelli (2000) and Gotelli and Entsminger (2001) have been criticized (Sanderson, 2000; Hausdorf and Hennig, 2007), their good statistical properties have been demonstrated (Gotelli, 2000; Gotelli and McCabe, 2002), leading to a substantial increase in community studies in the last decade (Krasnov et al., 2006, 2010; Dörr et al., 2012). Specifically, in the null model approach, the observed frequency of co-occurring species in a natural community is compared with a set of simulated communities with randomly generated species assemblages. Briefly, if species co-occur more often than expected by chance the assemblage is structured aggregately, throughout interspecific facilitation or shared preferences (Krasnov et al., 2006). When species co-occur less frequently than expected by chance, the assemblage is structured segregatively, mainly due to competition or different microhabitat preferences.

Our study is aimed to i) verify the co-occurrence pattern between macrophytes and macroinvertebrates, and ii) demonstrate a grade of non-randomness for specific pairs. Possible reflections on running water monitoring activities and river status evaluation are discussed in the last section.

METHODS

Study area and data collection

We collected both macrophytes and macroinvertebrates in the same 50 m length river segment of 11 sites of the River Aniene (central Italy, Fig. 1). Sites were distributed along the whole river from the source to its confluence within the River Tiber and are separated by no less than 8 km, according to Lloyd et al. (2006). This river could be subdivided into three sections. Particularly it flow within highly natural areas characterized by woodlands and pastures (sites A-C) in the first section. The second (sites D-G) is characterized by the presence of rare anthropic activities and structures like agriculture and roads, this structures increasing in the last section (sites H-K), when the river flow within some towns, Rome inclusive (site K). The samplings were carried out according to the national protocols of sampling for macrophytes (APAT, 2007) and macroinvertebrates (CNR-IRSA, 2007). Both protocols were partially modified for a better local adaptation of the sampling procedures (Ceschin et al., 2010; Larsen et al., 2012; Manfrin et al., 2013; Traversetti and Scalici, 2014). In order to assess eventual seasonal differences in the macrophyte-macroinvertebrate assemblage, each site was sampled twice: in October 2008 and June 2009. For the macrophyte collection, at each sampling site, we listed all species with their coverage value according to the Braun-Blanquet (1964): +, sporadic species; 1, species with coverage <5%; 2, 5-25%; 3, 25-50%; 4, 50-75%; 5, >75%.

Different collection methods were used for each macrophyte group: floating masses of filamentous macroalgae were collected by means of a 25 μ m mesh plankton net; epilithic macroalgae and cyanobacteria by scraping stones; bryophytes from boulders and cobbles in the riverbed and along the riverbanks by scalpel; vascular plants by direct observation *in situ* or collection of samples using a grappling iron. All macroalgae and cyanobacteria were fixed in formalin (approximately 4% final concentration). Taxo-



Fig. 1. Location of the sampling sites within the study area. 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.

nomical determination and nomenclature were based on scientific literature and taxonomic guides by John et al. (2002), Bourrelly (1981, 1985, 1990), and Ettl and Gärtner (1995) for algae and cyanobacteria, Cortini Pedrotti (2001, 2005) and Aleffi et al. (2008) for bryophytes, Pignatti (1982) and Conti et al. (2005) for vascular plants. The macroinvertebrate collection was performed only in riffles following the multi-habitat scheme (Buffagni et al., 2005). A microhabitat was sampled when it covered at least 10% of the investigated portion of the riverbed (Supplementary Tab. 1). A total of 10 sample units (reach) were collected in each site by using a Surber sampler (area 0.05 m²; mesh size 0.5 mm) by a standard collection protocol just performed in central Italy (Pace et al., 2011). Macroinvertebrates were grossly sorted in field, preserved with 95% ethanol, and then identified in laboratory to family level (except for Trichoptera and Plecoptera at genus, and Ephemeroptera at species), such as the most fine level based on literature and available taxonomic guides (Consiglio, 1980; Belfiore, 1983; Moretti, 1983; Sansoni, 1988; Campaioli et al., 1999). The most representative macrophyte and macroinvertebrate specimens are deposited at the Herbarium and the Museum of Comparative Anatomy and Zoology of University of Roma Tre.

Analysis design

According to the Dörr *et al.* (2012) protocol, we provided a way of testing patterns of co-occurring pairs within a community by comparing the observed frequencies of cooccurrences of macrophytes and macroinvertebrates along the upstream-downstream trend (*i.e.*, across the river sites) with those expected by chance. To quantify structure patterns of the macrophyte and macroinvertebrate assemblages, we organized a single presence-absence (1-0) data matrix (McCoy and Heck, 1987), where each row represents a river sampling site while each column a taxon.

After the matrix organization, we calculated the two indices C-score (checkerboard score, sensu Stone and Roberts, 1990) and V-ratio (variance ratio, sensu Schluter, 1984) which are single numbers measuring patterns for an entire presence/absence matrix. C-score is calculated as the average number of the matrix squares (i.e., checkerboard units) that are found for each pair of taxa (i.e., indicating the co-presence of the two taxa). In a competitively structured community, the C-score should be significantly larger than expected by chance (Gotelli, 2000). The V-ratio is the ratio between the variance in taxa richness and the sum of the variance in taxa occurrence. When the value of the ratio equals 1, taxa are distributed independently. It is smaller or greater than 1 in the case of negative or positive covariance between pairs, respectively (Gotelli, 2000).

We used both indices based on two different matrix structures (namely average co-occurrence and average co-

variance, respectively) because they have shown to be statistically powerful and robust to minor changes in community structure (Gotelli, 2000). Mathematical and statistical details, and properties and performance in null model tests of the metrics can be found in Gotelli and Rohde (2002) and Krasnov et al. (2006, 2010). The Vratio was calculated both including and excluding checkerboard units without the investigated taxa from the presence-absence matrix (WI, with empty checkerboard units; WO, without empty checkerboard units) to investigate their effect on the results (Gotelli and Rohde, 2002). Since they may potentially affect the null model analyses, we choose to study both models and to examine their effect on this analysis. Empty sites may be interpreted in two ways: 1) sampling sites not available for colonization; 2) sampling sites exploitable but not colonized by chance.

For each matrix we compared the observed index (O) with that simulated by 999 Monte Carlo random permutations [i.e., expected (E) by chance] (Gotelli and Entsminger, 2001). This number of permutations ensures that algorithm biases are avoided (Lehsten and Harmand, 2006). Observing a C-score value to be smaller than expected by chance $(O_C \le E_C)$ means aggregation of taxa. In this case we expect that the V-ratio is larger than expected by chance $(O_V \ge E_V)$. On the contrary, when taxa show segregation, we expect the C-score to be larger and the V-ratio to be smaller than expected by chance $(O_C \ge E_C, O_V \le E_V)$ (Gotelli and Rohde, 2002). Non-random differences were assumed setting always α =0.05 (Gotelli and Graves, 1996). We used two null algorithms for each comparison: fixed-fixed (ff) and fixedequiprobable (fe). The ff null model treats the empty units of the matrices as unusable, whereas the fe null model treats the empty units as random absences. In the fe null model, empty units in the real data sets may be occupied in the null communities, whereas in the ff null model, empty units in the real data sets remain empty in the null communities. The *ff* algorithm maintains the differences among units in the number of investigated taxa. Differently, the fe algorithm does not constrain the number of taxa that inhabits a site. This last model suggests no differences in the probability to support a particular number of macrophytes and macroinvertebrates among sampling sites. This statistic approach cannot be used for V-ratio because the latter is determined by marginal totals of the matrix rather than by taxa co-occurrence pattern (Gotelli, 2000). So we used both these algorithms only for C-score. All tests performed for evaluating the macrophyte-macroinvertebrate co-occurrence were carried out for the two sampling sessions separately (October 2008 and June 2009). Since the null models did not show what are the pairs participating to the nonrandomness, we performed a series of Spearman's correlations (setting α =0.05) by using data on abundances across site for both macrophytes and macroinvertebrates in order to identify the plant-animal pairs participating to the co-occurrence. Also in this case, analyses were performed per sampling session separately.

Once performed all the correlation tests, we carried out an analysis of covariance (ANCOVA) per sampling session for demonstrating that the investigated pairs may provide information on the environmental status. To do so, we attributed a value ranging from 1 (very sensitive) to 5 (very tolerant) to the correlating taxa on the base of the sensitivity classes (see below) (Tabs. 1 and 2). The sensitivity grade to human alterations (*i.e.*, water pollution and hydromorphological alterations) was defined exploiting information from literature (Tachet *et al.*, 2002; AFNOR, 2003) and depending on the authors' expertise. In particular, we inserted each collected taxon in one of five sensitivity classes, proposed as follows: i) very sensitive; ii) sensitive; iii) generalist (taxa with wide ecological behaviour); iv) tolerant; v) very tolerant.

ANCOVA was performed using the mean sensitivity class values of pairs per site as dependent variables, the sampling sites as independent categorical variable, and the anthropogenic index (AI) as covariate. AI was calculated on the basis of land use information for a 1 km radius around each site as follows:

$AI = \sum k_i p_i$

where k_i is the specific coefficient for each land-use category and p_i is the relative frequency of each category inside the 1-km buffer. The following k values were attributed to the respective CORINE land use categories: 1, natural woods; 2, pastures, meadows, bush areas, scrub and olive grove; 3, agricultural areas and urban green areas; 4, urban and industrial areas. The 1 km buffer was chosen since in this area macrophytes (*personal observation*) and macroinvertebrates (Mancini *et al.*, 2005) appeared influenced by land use at this scale. The index therefore represents a good surrogate of anthropogenic disturbance (Larsen *et al.*, 2010). Here, we set the confidence level at 0.05 to attribute a significance to the covariate value.

All statistical analyses were performed with Statistica 8 Stat. Soft. and SPSS ver. 16.0.

RESULTS

We collected a total of 137 taxa (58 macrophytes + 79 macroinvertebrates) (Supplementary Tab. 2), 123 (50+73) in October 2008 and 102 (42+60) in June 2009. We observed both abundant (*e.g., Micrasema*, Simuliidae, *Potamogeton pectinatus* and *Plathypnidium riparioides* in both seasons) and very rare (*e.g. Habrophlebia eldae* and *Nostoc punctiforme* in both seasons) taxa. Macrophyte and macroinvertebrate taxa showed different co-occurrence patterns, mainly showing aggregation.

As general output, the co-occurrence analyses showed the same results in both October and June. In particular, we observed a general accordance with the V-ratio results when sites without the investigated taxa were included or excluded from the analyses. Considering the C-score, nonrandom community structures were significantly highlighted by the *ff* model (*i.e.*, among different sites differences in the number of co-occurring taxa) (Tab. 3) while *fe* model never showed significant outputs. Our results indicate that the community is non-randomly structured, with the major part of tests indicating aggregation. In the *ff* model the C-score showed that there is a significant nonrandom co-occurrence in the *macrophyte vs macroinvertebrate* matrix than expected by chance [P(O_C<E_C)<0.05],



Fig. 2. Examples of positive (above) and negative (below) correlations of some taxa pairs in October 2008 and in June 2009. For the sampling sites acronyms see Fig. 1 caption. Bit, Bithynidae; Cst, *Callitriche stagnalis*; Elm, Elminthidae; Hel, *Habrophlebia eldae*; Mic, *Micrasema*; Npu, *Nostoc punctiforme*; Ppe, *Potamogeton pectinatus*; Ptr, *Potamogeton trichoides*; Rhy, *Rhyacophila*; Sim, Simuliidae.



Tab. 2. Macrophyte-macroinvertebrate correlations in June 2009. White point, 0.66<r²<0.74, LC; grey point, 0.75<r²<0.89, MC; black point, r²>0.90. Macrophyte and macroinvertebrate

and this is confirmed by the V-ratio tests both including and excluding empty sites [$P(O_V > E_V) < 0.05$].

All the correlating taxa allowed to obtain a total of 379 significantly correlating pairs. Amongst all these pairs, 83.7% of them are significantly correlated in October (Tab. 1) while 90.3% in June (Tab. 2). Finally, 9.5% and 24.7% of the pairs were negatively correlated in October and June, respectively. Examples of correlating pairs in both sampling seasons are shown in Fig. 2.

The ANCOVA test showed a high relationships between the AI values of the sampling sites and the sensitivity class values of the correlating taxa pairs in both October (F=6.93; P<0.05) and June (F=8.05; P<0.05) (Fig. 3).

DISCUSSION

The first result of our study highlighted how macrophytes and macroinvertebrate taxa co-occur following a non-random model, as C-score and V-ratio values indicated. In addition, where co-occurrence was confirmed, the used statistical tests indicated aggregation, while no significant segregation was recorded. Just in the last case, it was driven by randomness, since we observed non significant values for both C-score for $O_C>E_C$, and V-ratio $O_V<E_V$ in segregation (Tab. 3). Therefore, it was possible to observe some kinds of aggregative significant relationships between macrophyte and macroinvertebrate distribution pattern along the river ecosystem.

The results of null model analysis may depend on whether non colonized sites (*empty checkerboard units*) are included in or excluded from the input matrices (Gotelli and Rohde, 2002). As for the C-score, our findings were significant only for the ff model, while non significant for the fe one. This result suggest that empty sites have same kind of ecological meaning and they ought to be considered as not exploitable. Indeed, when empty sites are considered random absences, the test did not restitute any significant outputs. Additionally, inclusion or exclusion of checkerboard units did not affect values of V-ratio. These results agree with findings of Krasnov et al. (2006, 2010), who stated that outcomes of these two runs of analyses were essentially the same. Although the null model approach allows to compare the observed occurrence frequencies of running water dwelling taxa across river sites with those expected by chance, this tool cannot help for indicating the responsible mechanism for non-randomness (Rohde, 2005). However, we tried to provide some types of interpretations, aware that they may be inferred as speculative, since many synergic (and often unquantifiable) features may affect both habitat preference and river distribution of the taxa. Although different studies show that aquatic macrophytes contribute to diversify macroinvertebrate spatial niche, and consequently promote the increasing of aquatic macroinvertebrate abundances (Orth et al., 1984; Hemminga and Duarte,

эвріінуні appissi espionad nmaridae 0 0 0 dark grey, tolerant; black, very tolerant. 26mico1021mas o c прілахи . vjiydosváy sndo.nuəəʎio Janarudae snuppodojny, unassojuopo grey, generalist; DINU2 บนเอรชมวาม nuoisonida ну баторлі на на vjosiajás g taxa classified into five sensitive classes: white, very sensitive; light grey, sensitive; . . snəijnu 'g *x*์*เลา*doเนอก 1 asbioitidu atiomyidae sychodidae рлпшәиозол, yralidae əppipin 0 asbiinomi snjiudəuun vajono vjuedo: Tydroptilidae 0 lydraenidae sebibiqm sebidinina orbiosity asbixi0 lephancendae 00. snuidip ; 0 Suthomylidae

2000; Mykra *et al.*, 2008), there is a less knowledge about the existence of significant correlations between macrophyte-macroinvertebrate taxa pairs. Anyway, among the possible explanations for correlations between macrophyte-macroinvertebrate taxa pairs, direct biotic interactions may be evoked (Hering *et al.*, 2006). For example, Hansen *et al.* (2011) showed the existence of relationships between macroinvertebrate taxa and the occurrence of different macrophyte species. In particular, they showed that the increasing eutrophication due to human activities induced changes in the vegetation composition, generally rising to the proliferation of more structurally complex species and it can result in an increased abundance of macroinvertebrates. Trophic interactions may also occur between macrophytes and macroinvertebrates but, although this kind of interactions have been long evidenced from controlled experiments (Cooper *et al.*, 1992), this sort of explanation has yet to be confirmed through field studies. Also the organism size may affect the level of the taxa assemblage. In fact, some authors have hypothesized in some studies on concordance (Allen *et al.*, 1999; Paszkowski and Tonn, 2000) that a strong degree of cooccurrence should occur among taxa of similar size. However, findings on specific co-occurring plant-animal pair are completely missing. Another interpretation may be provided. Our results support the idea that the observed

Tab. 3. Summary of the null model analyses of co-occurrence through sites using the indices C-score and V-ratio: $O \le E$, observed value of the index was significantly lower than the expected by chance ($P \le 0.05$); $O \ge E$, observed value of the index was significantly greater than the expected by chance ($P \le 0.05$). Significant probability values are underlined. The taxon numbers are reported within parentheses.

							V-ratio		
October 2008 (123)	aggregation segregation aggregation segregation	ff ff fe fe	$\begin{array}{l} p \; (O_{C} < E_{C}) \\ p \; (O_{C} > E_{C}) \\ p \; (O_{C} < E_{C}) \\ p \; (O_{C} > E_{C}) \end{array}$	0.025 0.784 0.812 0.554		WI WI WO WO	$\begin{array}{l} p \; (O_V > E_V) \\ p \; (O_V < E_V) \\ p \; (O_V > E_V) \\ p \; (O_V < E_V) \\ \end{array}$	0.021 0.742 0.015 0.543	
June 2009 (102)	aggregation segregation aggregation segregation	ff ff fe fe	$\begin{array}{l} p \; (O_{C} < E_{C}) \\ p \; (O_{C} > E_{C}) \\ p \; (O_{C} < E_{C}) \\ p \; (O_{C} > E_{C}) \end{array}$	0.041 0.623 0.758 0.887		WI WI WO WO	$\begin{array}{l} p \; (O_V > E_V) \\ p \; (O_V < E_V) \\ p \; (O_V > E_V) \\ p \; (O_V < E_V) \end{array}$	0.035 0.663 0.015 0.411	



Fig. 3. Diagrams obtained by using the mean value of the sensitivity classes of the correlating pairs (macrophytes in grey, and macroinvertebrates in black), and anthropogenic index (AI, dotted line) per each site, divided per sampling session (October 2008 and June 2009). 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.

co-occurrence can be justified also by different types of relation occurring between single pairs of macrophytes and macroinvertebrates. For example, the use of macrophytes by macroinvertebrates can be as a refuge to escape predators, or to ambush its prey, or to direct trophic relationships related to species of herbivorous macroinvertebrates (Hansen *et al.*, 2011).

Besides the relevant ecological issues discussed above, some applicative aspects may be argued. Specifically, the classification of the correlating taxa within the 5 sensitivity classes contributed to highlight how plants and animals may provide similar information on the human alteration grade of the watercourse. This statement was supported by the analysis of covariance outputs which showed the relationship between the anthropogenic index (as a descriptor of the human alteration) and the mean sensitivity class values along the whole river.

CONCLUSIONS

Our study demonstrates the existence of several correlations between macrophyte and macroinvertebrate taxa. It allows us to highlight different macrophytemacroinvertebrate pairs co-occurring into freshwater ecosystems, and how these pairs are linked to the human alterations. It points out the potentialities in using few taxa to perform an assessment of the running water quality. This may be realizable when the specific pairs are considered as representatives of the entire habitat status.

Clearly, authors are aware that i) this study was conducted on only one watercourse and ii) macroinvertebrate taxa were identified mainly to genus and family levels, the latter topic being the one of the most issue of concern in the riverine quality assessment (since no guides exist in literature for the invertebrate species identification). Indeed, as for the macroinvertebrate taxonomic level, a more detailed macroinvertebrate identification can strengthen obtained results. Only performing further studies (increasing the sampling sites number) we may provide strong statistical outputs helping to detect the co-occurring pairs.

Once solved these problem of the sampling sites number, our findings could be used as a new monitoring tool. The latter represents an investigation approach easy in the application, feasible and rapid in the execution, and within the WFD freshwater management policies. In addition, a such tool does not need a detailed taxonomical expertise since its applicability is restricted only to the few taxa indicated as representatives of a certain water quality condition.

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