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RESEARCH PAPER

Longitudinal variation of macroinvertebrate communities in a Mediterranean river subjected to multiple anthropogenic stressors

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River impoundments and waste water discharge are a serious threat to the integrity and biodiversity of river ecosystems, especially in central Italy. Benthic macroinvertebrates were sampled in autumn and summer along the Aniene River to assess the cumulative biological effect of the numerous dams and sewage treatment plants that affect its middle and lower course. We hypothesized that (i) increasing habitat impairment would promote the formation of nestedness in species assemblage, where species poor locations support only a sub-set of organisms from richer sites; (ii) specific life-history traits would confer sensitivity to habitat degradation. Patterns of macroinvertebrate richness and diversity along the river tracked the distribution of dams and sewage treatment plants. Partial Mantel test showed that dissimilarity in assemblages increased with the number of dams and treatment plants between reaches after controlling for longitudinal distance. Assemblages were significantly nested, and nestedness appeared related to both water quality gradients (phosphorous, turbidity) that reflected anthropogenic inputs, and to natural gradient in altitude. Reaches with nested assemblages (supporting a sub-set of the species pool) were characterized by greater representations of taxa with shorter life cycles, while, in contrast, species rich sites supported taxa with longer life cycles and lower dispersal ability. These results suggest that the cumulative effect of dams and sewage treatment plants promoted the formation of nested subsets in species distribution. Moreover, it appeared that certain functional traits that conferred sensitivity also dictated the progressive non-random loss of taxa in face of multiple anthropogenic stressors. These findings have conservation implications in the regions, but need to be considered preliminary since anthropogenic and natural factors co-varied systematically along the study river precluding the identification of single factor effects.

Keywords:
Dams / Multiple stressors / Nestedness / River quality / Species traits

1 Introduction

Rivers worldwide are increasingly threatened by the synergic effects of local and regional stressors that impair ecosystem integrity both directly, from alteration of flow and thermal regimes, sediment release, pollution, impoundment and introduced species, and indirectly via increasing catchment land-use [1–4].

In Mediterranean regions with high population density, as well as agricultural and industrial activities, dam construction for water withdrawal and inputs from urban and industrial waste water treatment plants (hereafter WWTP) represent a serious threat to their biological integrity and diversity [5–7]. For example, river regulation

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by dams disrupt the hydrological continuum, thereby impeding fish migration, and alter natural flow and temperature regimes, sediment transport and channel form, with far-reaching ecological consequences [8, 9]. Similarly, domestic and industrial sewage effluents can deteriorate water quality, with effects particularly severe in Mediterranean systems where water is scarce and dilution capacity is generally low [5]. Ecological effects of WWTP are generally linked to increased nutrient concentration, which can lead to shifts in community composition and reduced diversity of benthic organisms, while effects on benthic density appear highly variable [7, 10].

However, in most cases, especially in central Italy [11, 12], rivers are affected by multiple dams and WWTP along their courses, while catchments are increasingly converted for agriculture and settlements. The Aniene River exemplifies such condition where industrial and domestic sewage effluents, as well as numerous dams have significantly deteriorated its biological integrity, especially in its lower reaches where agricultural development is also higher than upstream areas. These factors represent a serious threat to its biodiversity and conservation, but information on the ecological effects is surprisingly scarce. A previous work in the Aniene River [7], observed large longitudinal changes in water quality and taxonomic composition of the invertebrate assemblages among upper, middle, and lower reaches. Despite this, the shape of the size structure appeared remarkably consistent across reaches [7]. We therefore expanded on this approach by investigating longitudinal trends in specific life-history traits, which could help in providing a mechanistic understanding of biological responses that is transferable across geographical regions [13–15]. Moreover, identification of traits conferring sensitivity has clear conservation implications where, for instance, species with sensitive traits are systematically lost (filtered-out) along gradients of environmental impairment [16, 17].

To this end, investigations on species loss in face of environmental change can benefit from the assessment of “nestedness” in species composition. Nestedness occurs when species in depauperate assemblages are subsets of those in more diverse assemblages [18]. Although, nestedness in species assemblages can develop naturally from extinction-colonization processes in fragmented habitats and over broad spatio-temporal scales [19], it can also reflect habitat alteration and anthropogenic disturbance [16, 20–22]. Nonetheless, studies combining biological traits and nestedness in predicting effects of anthropogenic modification on river biodiversity and conservation are rare (e.g., [17]), especially in Mediterranean systems.

In this study we assessed the response of macroinvertebrate assemblages to multiple anthropogenic alterations (namely dams, WWTP, and increasing anthropogenic land-use) along a Mediterranean river combining both structural and trait-based approaches. In particular we hypothesize that, since species differ in their sensitivity to habitat modification, a non-random loss of taxa with sensitive traits in impacted reaches will likely produce a nested subset pattern in species distribution. In particular, according to previous studies (e.g., [3, 7, 17, 23]), we expected taxa with longer life cycles and low larval dispersal ability to be negatively affected by river degradation. Our sampling design was not intended for assessing the individual effect of dams, WWTP, and land use, but rather to investigate overall trends in benthic assemblages along the river course in relation to the main natural and anthropogenic features.

2 Methods

2.1 Study area

The Aniene River (Fig. 1), in central Italy, is a Mediterranean watercourse within the calcareous HydroEcoRegion (HER) 13 (*sensu* [24]). The Aniene is the most important tributary of the Tiber River and drains a catchment of 1466 km^2, which is characterized by woodland and pasture land-use in its upper course and by agriculture and urbanization in its lower course [7].

2.2 Macroinvertebrate collection

Surveys were conducted in October 2008 and in June 2009 in nine reaches (Fig. 1) along the river over an altitudinal range of 29–753 m a.s.l. No major droughts or floods occurred between the two sampling periods, which are assumed to represent typical autumn and early summer conditions.

Macroinvertebrates were sampled from riffles using a Surber sampler (0.16 m^2; 0.44 mm mesh size) from the most frequent microhabitats, defined as in Hering et al. [25]. Large riffles were scarce in the most downstream study reach (site I) and were mostly composed of fine material (sand and fine gravel).

2.3 Macroinvertebrate identification and biological traits

Macroinvertebrates were sorted in field and identified in laboratory to species (Ephemeroptera), genus (Plecoptera and Trichoptera), and family level (others). The trait composition of all macroinvertebrate samples was defined using 28 categories of six biological traits (Table 1) using the available trait data from literature [26–29]. Trait information was collected for a total of 52 taxa mostly at the genus level including Ephemeroptera, Plecoptera, and Thricoptera, Gastropoda, and Crustacea. Family level was

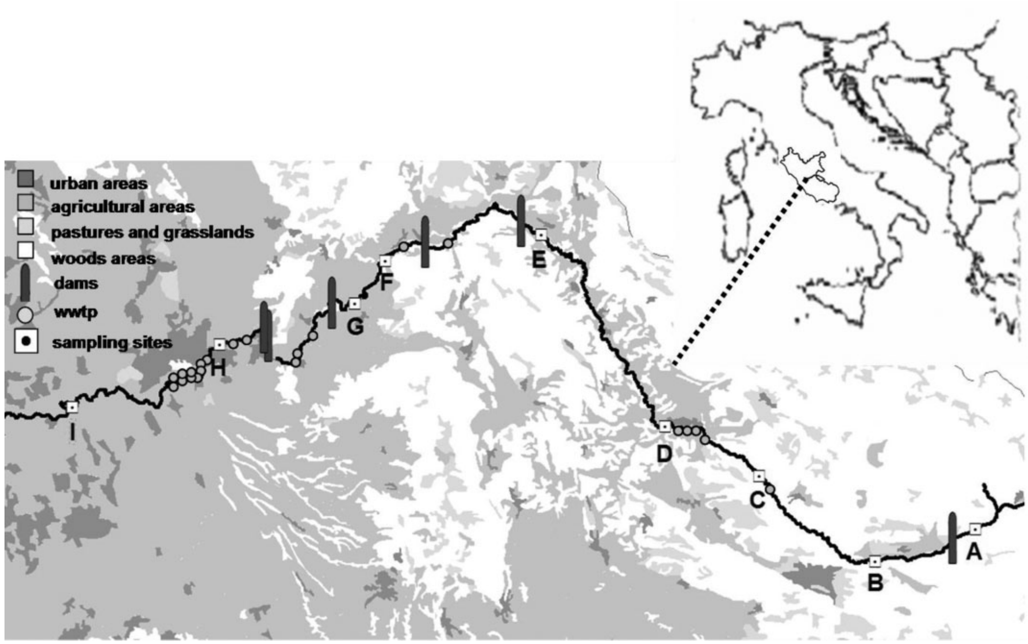

Figure 1. Location of the investigated reaches on the Aniene River (central Italy) in October 2008 and June 2009. Common names and geographic coordinates (Latitude; Longitude, UTM) for A, Filettino (33T 358 488 m E; 4 637 280 m N); B, Trevi (33T 352 780 m E; 4 635 426 m N); C, Jenne (33T 346 117 m E; 4 635 426 m N); D, Subiaco (33T 340718.20 m E; 4 643 137 m N); E, Anticoli Corrado (33T 333 595 m E; 4 654 173 m N); F, Vicovaro (33T 324 656 m E; 4 652 680 m N); G, Castel Madama (33T 322 936 m E; 4 650 229 m N); H, Tivoli (33T 315 203 m E; 4 647 864 m N); I, Lunghezza (33T 306713.15 m E; 4 644 258.15 m N). On the map: dams, real number of wastewater treatment plants (wwtp) and land-use characterization.

used for the remaining taxa (see Table S2 in Supporting Information). Detailed trait information at the species level is very scarce. However, a flexible fuzzy coding approach was used to determine the affinity of each taxon to each category, thus accounting for intra-genus variation [30]. Affinity scores ranged between 0 and 3 or 0 and 5, and reflected the relative strength of association of a taxon for a given trait category [31]. Affinity scores were rescaled as proportion so that their sum for a given species and a given trait was 1. Trait category values were then multiplied by the relative abundance for each taxon in order to obtain a traits-by-site array weighted by the relative abundance of taxa in each site (see also [17, 32], for similar approach).

2.4 Surveyed environmental features

To measure the extent of anthropogenic landscape modification around each sampled location, land-use data for a circular buffer of 1 km (see [11]) were obtained from the CORINE (Co-ordination of Information on the Environment) land-cover database. A geographical information system (ArcGis, ver. 9.3) was used to quantify land cover at each site. Available land use data were summarized into four categories: Agriculture, Urban, and Industrial, Pasture (including meadows, bush areas, scrub, and olive grove) and Woodland. In order to synthesize land-use development, the different categories of land use were combined into an Anthropogenic Index (AI, [33]), which was used as independent variable and calculated as follows:

$$\mathrm{AI} = \sum k_i p_i$$

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 [11, 33]. The k values attributed to the land use categories were: woodland = 1; pasture = 2; agriculture = 3; urban and industrial = 4. The index ranged between 1 (minimum modification) and 4 (maximum modification). The AI was calculated with such buffer distance to reflect both local riparian features and larger scale patterns, and to allow comparison with previous studies in the region [11, 33]. Also, larger buffers would have resulted in overlapping information for neighboring sites.

Position of dams, urban, and industrial WWTP along the river course were retrieved from geo-referenced data of the Lazio Region.

Dissolved oxygen (O_2), conductivity (C), and pH were measured in situ by a multi-parameter field probe WTW Multi340i (WTW, Weilheim). Water samples were

Table 1. Biological traits and categories considered in the present study

Trait	Categories
Maximal size (mm)	<2.5
	2.5–5
	5–10
	10–20
	20–40
	40–80
	>80
Life-cycle duration (y = year)	<1 y
	>1 y
No. of potential generation per year	<1
	1
	>1
Adult longevity (d = day)	1–10 d
	10–30 d
	30–90 d
	>90 d
Larval dispersion (m)	<10 m
	10–100 m
	100–1000 m
	>1000 m
Feeling habits	Tegument
	Deposit feeders
	Shredder
	Scraper
	Filterer
	Piercer
	Predators
	Parasite

transferred to the lab and analyzed for ammonium (NH_4^+–N), nitrates (NO_3^-–N), total phosphorus (TP), and chemical oxygen demand (COD) by spectrophotometry WTW MPM 3000 (WTW, Weilheim). In addition, we recorded for each sites: altitude (ALT); water velocity (Vel) with a mechanical flowmeter General Oceanics 2030 (General Oceanics, Miami; FL); water turbidity (Tu) according to an empirical scale ranging from 0 to 2, and water depth (De).

2.5 Statistical Analysis

Some of the measured environmental parameters (e.g., altitude, land-use intensity, phosphorous concentration) were strongly ($r_s > |0.7|$) inter-correlated. This is expectable when the anthropogenic impact systematically increases along the river continuum. For this reason we opted not to arbitrarily remove some variables, but we presented all results (e.g., correlations) showing the exact p values.

Biological classification of reaches based on macroinvertebrates was formulated according to the STAR_ICM (Intercalibration Common Metric) index using the dedicated ICMeasy software [34, 35]. The ICM is a multimetric index developed during the intercalibration exercise between member states of EU [35]. It is based on numerical combinations of community metrics calculated at family level identification.

The following parameters were derived to describe benthic assemblages: taxon richness (R) and Shannon index [36] for the whole assemblage (H) and for Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa (H EPT). Values of R, H, H EPT, and STAR_ICM along the river course were plotted to visually inspect longitudinal trends in relation to dams and WWTP.

Detrended Correspondence Analysis (DCA; [37]) was used to characterize the main variation in invertebrate assemblages among reaches and to assess the influence of the measured environmental variables. This ordination arranges samples based on taxonomic composition onto orthogonal axes that can be used in subsequent analyses [38]. Ordinations were performed combining samples across seasons, since ordination patterns were near identical for both seasons. Species abundance data were log-transformed prior to the analyses to down weight the influence of dominant taxa.

Partial Mantel tests [39] were used to test the effects of dams and WWTP on both the whole assemblage and EPT taxa separately. First, matrices reporting the number of dams and/or WWTP between each pair of sites were constructed. Then, since the number of dams and WWTP increased with increasing longitudinal distance along the river, a matrix of longitudinal distances was also constructed to be used as covariate. Finally, Partial Mantel tests were used to assess the influence of dams and WWTP on stream assemblages (using Bray–Curtis distance matrix) eliminating the influence of longitudinal distance [40]. Significance level for the Mantel correlation (r_M) were based on 5000 Monte-Carlo permutations.

Trait diversity (TD) in each sample for both surveys was calculated as the average Shannon (H) diversity across all traits (e.g., [17, 41]):

$$TD = H = \sum -(n_j \log n_j)$$

with n_j = relative abundance of trait category j. The average of H across all traits was calculated to account for the lack of independence among traits (e.g., [41]). This index was intended to represent the functional diversity of the community.

TD values were plotted to see longitudinal variation along the river course. Correlations between TD, community parameters, and measured environmental variables were examined with Pearson rank correlation.

To assess patterns of nestedness in species distribution we used the binary-matrix nestedness temperature calculator BINMATNEST [42]. This method is relatively insensitive to matrix size, and correlates well with other nestedness indices, therefore facilitating comparison. BINMATNEST re-order the presence/absence matrix, rearranging rows and columns to maximize matrix nestedness to calculate a matrix temperature (T_M) ranging over 0 (perfect nested) to 100°C (totally random) reflecting the matrix deviation from an ideal nested structure. The statistical significance of the observed temperature was assessed using a Monte-Carlo approach involving comparison with simulated temperatures of 400 random generated matrices. The algorithm use to generate the random matrices was the more conservative null-model III, where the chance of a cell being occupied equals the average probability of occupancy of its row and column [42]. The new ranking of sites in the maximally packed matrix can then be correlated with independent variables to assess potential driver of nestedness (e.g., [17]). Here, to appraise whether nestedness in taxonomic assemblages was dictated by their biological traits, site ranking by BINMATNEST was correlated with the proportion of each trait category (Pearson-rank correlation). To avoid spurious correlations to be observed, we corrected alpha values (=0.05) by dividing by the number of categories within each trait (e.g., if a given trait has six categories, $\alpha = 0.008$).

All the statistical analyses were performed by using Statistica 7 Stat. Soft., PAST package vers. 1.94b [43] and *R* [44].

3 Results

Macroinvertebrate density across reaches ranged between 2628 and 106 769 m^{-2} in October and 4543 and 55 344 m^{-2} in June for a total of 79 Taxa (73 in October and 60 in June) (Table S2 in Supporting Information). The highest taxonomic richness was observed in Anticoli Corrado (E) in October (50) and in Jenne (C) in June (36). The lowest richness was observed in Lunghezza (I) (eight taxa both in October and June). The most abundant taxa were *Baetis rhodani* (12% in October and 8% in June) and two Diptera families: Simuliidae (40% in October and 39% in June) and Chironomidae (9% in October and 14% in June).

Correlations between richness, diversity, EPT diversity, ICM index, TD, and abiotic variables are shown in Table 2. Overall macroinvertebrate richness and diversity appeared lower in the lower reaches were land-use modification, nutrient load, and temperature were higher.

Plot of the study reaches over the two seasons on the first two axes of the DCA is shown in Fig. 2.

Axis one (explaining 22% of variation in assemblage) clearly reflected the longitudinal distribution of reaches along the river, which formed three groups corresponding to upper, middle, and lower sections (Fig. 2). Correlation between the first axis and the measured abiotic parameters are shown in Table 2. Such grouping of sites along the first axis was maintained over the two seasons, while axis two (explaining 10% of variation) clearly

Table 2. Coefficients of the significant Spearman correlations between the first DCA axis (two seasons combined), community parameters, and environmental variables

	DC1	*H*		H EPT		STAR_ICM		*R*		TD	
	October–June	October	June	October	June	October	June	October	June	October	June
pH	−0.82***	–	0.69*	0.71*	–	–	0.76*	–	–	–	–
O_2	−0.59**	–	–	–	–	–	–	–	–	–	–
C (μS/cm)	0.90***	–	–	−0.81**	−0.77*	−0.70*	–	−0.69*	−0.78*	–	–
COD (mg/L)	–	–	–	–		–	–	–	–	–	–
TP (mg/L)	0.63**	−0.69*	–	−0.97***	−0.68*	−0.89**	–	−0.75*	−0.74*	–	–
NO_3^-–N (mg/L)	0.57*	–	–	−0.60*	−0.69*	−0.66*	–	–	−0.71*	–	–
NH_4^+–N (mg/L)	–	–	–	−0.75*	–	–	–	–	–	–	–
ALT (m)	−0.97***	–	0.83**	0.83**	0.78*	0.71*	0.85**	–	0.88**	–	–
Depth	0.53*	–	–	–	–	–	–	–	–	–	–
Tu	0.65**	–	–	−0.73*	–	−0.73*	–	−0.73*	–	–	–
AI	0.84***	−0.68*	−0.88**	−0.68*	−0.73*	–	−0.88**	–	−0.85**	–	−0.67*

H, macroinvertebrate diversity; H EPT, Ephemeroptera, Plecoptera and Trichoptera Shannon index; STAR_ICM, Intercalibration Common Multimetric index; *R*, taxon richness; TD, traits diversity; O_2, dissolved oxygen; C, conductivity; COD, chemical oxygen demand; TP, total phosphorus; NO_3^-–N, nitrates; NH_4^+–N, ammonium; ALT, altitude; Tu, turbidity; AI, anthropogenic index. Statistical significance: –, not significant.

* $p<0.05$.

** $p<0.01$.

*** $p<0.001$.

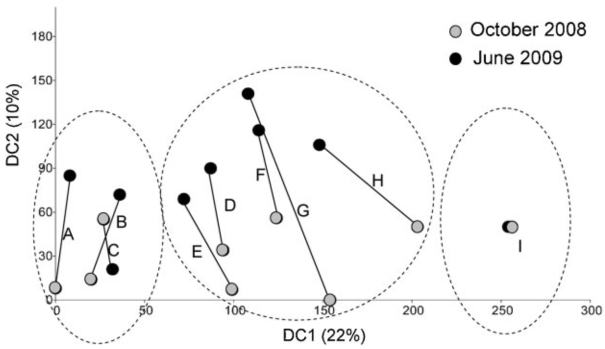

Figure 2. Position of the study reaches (A, Filettino; B, Trevi; C, Jenne; D, Subiaco; E, Anticoli Corrado; F, Vicovaro; G, Castel Madama; H, Tivoli; I, Lunghezza) on the first factorial plane of the Detrended Correspondence Analysis combining data from October (Oct) 2008 and June (Jun) 2009. Variance explained by each axes is shown in parenthesis. From left to right, circles group sites in the upper, middle, and lower river section, respectively.

reflected the seasonal change in assemblages and appeared correlated with COD ($r_s = 0.52$; $p<0.05$), which was systematically higher in June (Table S1 in Supporting Information).

Trends of macroinvertebrate diversity (*H* and H EPT), taxon richness (*R*), STAR_ICM index, and TD along the river course are shown in Fig. 3. Dams and WWTP appeared to negatively affect each of these parameters.

In June, Partial Mantel test (i.e., accounting for longitudinal distance) showed significant correlations between macroinvertebrate composition and the presence of dams ($r_M = 0.59$, $p<0.05$), wastewater treatment plants ($r_M = 0.64$, $p<0.05$) and, more strongly, with both stressors combined ($r_M = 0.71$, $p<0.05$). In October, significant correlations were observed only between EPT taxa composition and the presence of WWTP ($r_M = 0.73$, $p<0.01$) and both dams and WWTP combined ($r_M = 0.72$, $p<0.05$).

Communities showed significant nestedness in species distribution in both seasons (October: matrix temperature = 21.1°, $p<0.01$; June: matrix temperature = 22.5°, $p<0.01$). However, correlation between site ranking in the maximally packed matrix (species × site matrix sorted to emphasize nestedness) and environmental variables differed between the two seasons (Table 3). In October, nestedness was directly correlated with total phosphorus concentration ($r_s = 0.86$; $p<0.01$) and turbidity ($r_s = 0.73$; $p<0.05$); in June, nestedness appeared correlated with the Anthropogenic Index ($r_s = 0.87$; $p<0.01$), as well as with pH and altitude ($r_s = -0.68$ and $r_s = -0.78$, respectively; $p<0.01$). These results suggest that a combination of both anthropogenic and natural factors were potential causes of the nesting patterns in species distribution. In

Table 3. Coefficients of the significant Spearman correlations (r_s) between environmental variables and the ranking of sites ($n = 9$) in the maximally packed species matrix (Nestedness rank) of the Aniene River

	Nestedness rank	
	r_s (October)	r_s (June)
pH	–	-0.68^{*}
C (μS/cm)	–	–
COD (mg/L)	–	–
TP (mg/L)	0.86^{**}	–
NO_3^-–N (mg/L)	–	–
ALT (m)	–	-0.78^{*}
Tu	0.73^{*}	–
AI	–	0.87^{**}

C, conductivity; COD, chemical oxygen demand; TP, total phosphorus; NO_3^-–N, nitrates; ALT, altitude; Tu, turbidity; AI, anthropogenic index. Statistical significance: –, not significant.
* $p<0.05$.
** $p<0.01$.

October, reaches that were less nested (i.e., that had lower phosphorus concentration and turbidity) were characterized by species with lower larval dispersal ability (<10 m, $r = -0.69$, $p<0.01$), while in June, less nested reaches supported communities characterized by higher representation of longer life cycles (i.e., <1 potential generation per year, $r = -0.85$, $p<0.01$) and, conversely, reaches at the nested end of the matrix (i.e., at lower altitude and with higher AI) were characterized by higher representation of shorter life cycles (>1 potential generation for year; $r = 0.98$, $p<0.01$). Similar correlations were observed using EPT taxa only with trait information at genus level (see Table S3 in Supporting Information).

In other words, it appears that the formation of nested patterns in species distribution was related to changes in the representation of specific biological traits. Surprisingly, traits related to feeding modes did not appear to be related with the formation of nested subsets.

4 Discussion

As expected, the combined effects of multiple anthropogenic stressors along the river continuum strongly affected macroinvertebrate richness and community structure. Also, results support the hypothesis that specific life-history traits were involved in the progressive loss of taxa in more impacted reaches, with consequences for macroinvertebrates conservation. Nonetheless, nestedness in

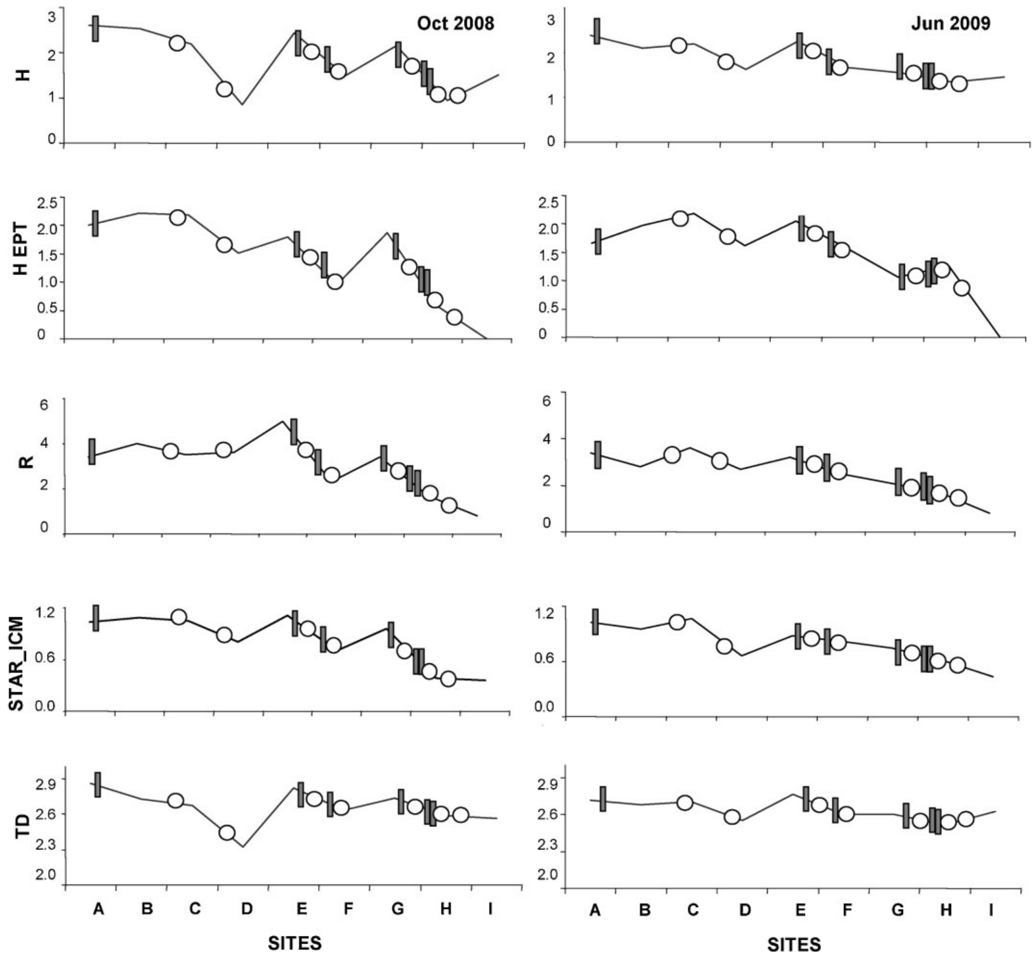

Figure 3. Trends in community parameters along the Aniene River (A, Filettino; B, Trevi; C, Jenne; D, Subiaco; E, Anticoli Corrado; F, Vicovaro; G, Castel Madama; H, Tivoli; I, Lunghezza) in October (Oct) 2008 and June (Jun) 2009. *H*, Shannon index; H EPT, Ephemeroptera, Plecoptera and Trichoptera Shannon index; *R*, taxon richness; STAR_ICM, Intercalibration Common Multimetric index; TD, trait diversity. Dams and wastewater treatment plants are showed as bars and circles, respectively.

species assemblage was also related to natural factors such as altitude and pH that also varied systematically along the river continuum (see below). Our results, however, clearly show that both dams and WWTP cumulatively influenced community structure.

As previously observed by Solimini et al. [7], patterns in macroinvertebrate assemblages in both seasons suggest that the Aniene River can be divided into three longitudinal sectors: upstream, middle, and downstream, which parallel changes in dominant geology and especially anthropogenic land-use and urbanization. Strong correlations between the first DCA axis and main abiotic variables confirm this longitudinal trend. The upstream sector is characterized by good water quality and habitat integrity with only minor anthropogenic influences and where reaches supported a diverse macroinvertebrate community. The middle sector is characterized by a stepped pattern in invertebrate richness and diversity that appeared related to the positioning of dams and WWTP. It is worth noticing how richness and diversity appeared to increase between reach D and E and between F and G, where no dam or WWTP were present. This trend was more evident in October, perhaps as a consequence of increased water flows that were able to dilute excess nutrients from upstream treatment plants, allowing natural community recovery [45]. It is possible that, for the same reason, according to partial Mantel test the effects of stream fragmentation and point pollution on assemblages were stronger in June, whereas in October only sensitive EPT taxa appeared influenced. Overall these results show that, in the Aniene River, assemblage dissimilarity increased with the number of dams and WWTP between reaches. These results parallel those of Grenouillet [40], who also observed a cumulative effect of river fragmentation on aquatic communities. Comparison with previous findings, however, is problematic, since the majority of studies addressing biological effects of dams or point source pollution [46, 47], focussed on direct upstream versus downstream comparison, providing limited insight into their cumulative effects.

Finally, the downstream section of the Aniene is heavily impacted by urbanization and by numerous water treatment plants. The excessive nutrient load resulted in a poor macroinvertebrate community dominated by few tolerant taxa such as gammarids and chironomids (Table S2 in Supporting Information).

In both seasons, species distribution showed a nested pattern, with species poor locations hosting only a subset of the species found in richer sites. The degree of nestedness and its potential drivers are similar to what reported in previous studies with stream invertebrates [48]. Nestedness has been often observed across communities of different organisms [49, 50] and has been related to both natural (habitat isolation, area) and anthropogenic (e.g., habitat quality) factors [17, 48]. Indeed, the challenge is to disentangle the relative importance of the different mechanisms, but this is complicated where natural and anthropogenic factors systematically covary, as it is often the case in river systems. Unfortunately, to date there is little information on natural nestedness patterns along minimally impacted rivers, which limits our ability to draw firm conclusions about potential anthropogenic drivers. Nonetheless, nestedness analysis is increasingly used in conservation studies since it provides information on the mechanisms affecting not only species richness but also composition. In particular, nestedness reveals conservation risks by assessing whether rare species are restricted to a small number of species-rich locations, and whether species loss increases predictably along gradients of environmental change or degradation.

Macroinvertebrate nestedness in June appeared to partially follow the longitudinal gradient of the river, with a progressive downstream loss of taxa as land-use modification also increased; in October, nestedness was related to water quality parameters (phosphorous, turbidity), which also tended to increase downstream, likely reflecting the growing anthropogenic influence. In these circumstances the use of life-history traits can aid in the identification of the biological features that makes certain taxa more prone to local extinction. Our results suggest that merovoltin taxa (with less than one generation per year) and taxa that are poor disperser (e.g., *Perla*, *Nemoura*, *Dinocras*, *Wormaldia*) were progressively disfavored in downstream degraded reaches with lower water quality, which, in contrast, mostly harbored taxa with shorter life cycles (e.g., *Asellus*, Chironomidae, Naididae, Tabanidae). This is unsurprising and confirms that the representation of traits conferring rapid population growth and resilience tend to increase in assemblages exposed to intensive land use and nutrient enrichment [13]. In addition to this, however, our results also suggest that the biological traits that conferred sensitivity to land-use and habitat and water quality degradation (longer life cycles, limited dispersal) dictated a progressive non-random loss of taxa. Similar traits appeared to mediate the formation of nested structure in invertebrate assemblages in Welsh (UK) catchment converted to agriculture [17]. This parallel from a different eco-region suggests that habitat impairment affects aquatic communities via similar ecological processes, and confirms the potential of functional (traits) approaches to provide consistent measures across regions with different taxonomic pools.

These findings have important conservation implications if sensitive taxa with longer life cycles and limited dispersal ability are systematically lost in face of widespread river impairment. This is particularly relevant in Central Italy where most catchments share similar patterns in land-use modification and anthropogenic pressures [11, 51, 52]. Nonetheless results must be interpreted with caution considering the limits of the present study. First, the focus on one river system and the limited sample size provide little support for the generality of our findings. More importantly however, the exact mechanisms behind the observed biotic responses need further assessing, since comparative surveys alone cannot identify single factor effects when these vary in concert along the river system. In the Aniene River, macroinvertebrate communities likely reflect the synergistic and multivariate effects of both natural and anthropogenic gradients. However, parallel trends in macroinvertebrate traits are evident elsewhere in modified river catchments [16, 17], suggesting the similar mechanisms may be involved.

5 Conclusions

This case study focused on the Aniene River as a model system for many Italian Apenninic Rivers. By combining nestedness and species life-history traits, we integrated three aspects of community ecology often examined separately such as species richness, composition, and functional attribute in one comprehensive analysis. To our knowledge, this is the first study of this kind in Italy and showed the potential of such integrated approach for predicting the effects of habitat modification and for identifying biological attributes and taxa potentially at risk of local extinction. Further studies in the region are certainly needed to appraise the generality of these findings.

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