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Role of Stream Morphology and Predators as Driving Factors for the Abundance of Two Common Ephemeropteran Genera in Rural Hilly Areas

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Abstract: Lotic environments may play an important role for semi-aquatic species. Streams are among the most threatened breeding habitats for some macrobenthos taxa. Ephemeropterans are often considered good bioindicators. Two of the most common genera of mayflies are *Ecdyonurus* and *Baetis*. The species from the first genus are very sensitive toward organic pollution, while the second genus might be quite resistant. The aim of this study was to evaluate the relative importance of stream morphological features on the abundance of *Ecdyonurus* and *Baetis* genera at unpolluted sites upstream an urbanised area. Further, I assessed the relationships between the abundance of the studied mayflies and the common spring and stream predators, i.e. salamander larvae and fish. The substrate complexity, reflecting shelter availability, was the main feature affecting the abundance of both genera. The two genera showed distinct requirements only in terms of stream shading, with *Baetis* preferring sunny sites and *Ecdyonurus* shady sites. *Baetis* showed higher affinity for streams with trout occurrence and *Ecdyonurus* for streams with *Salamandra salamandra* larvae occurrence. This study underlines that when pollution is not a main factor, both sensitive and insensitive common Ephemeroptera genera show similar habitat requirements and that watercourse substrate heterogeneity should be attentively preserved.

Keywords: Macrobenthos, stream, river, *Ecdyonurus*, *Baetis*

Introduction

Stream ecology is multidimensional, with species diversity and occurrence being dependent upon factors and processes at multiple spatial and temporal scales (Chee & Elith 2012, Dudley *et al.* 2015). Many species of insects breed in streams and several studies have documented the important role that macrobenthos community, including aquatic larvae of insects, may play for lotic systems (Koperski 2010, Tachet 2010, Koperski 2011). In particular, the high richness or biomass of stream macrobenthos are an indicator that they play an important role in stream–riparian dynamics (Clarke *et al.* 2010). At the same time, landscape composition and features can strongly affect macrobenthos species distribution (Petersen 1992, Renai *et al.* 2006, Manenti & Bianchi 2014). Numerous studies of stream systems have reported dramatic alterations in the macrobenthos composition with changes in the microhabitat availability in streams. These changes might be a result of urbanisation, intensive agriculture or land fragmentation by humans (Dauba *et al.* 1997, Gupta & Sharma 2005, Koperski 2010). Different studies indicate a consistent loss of invertebrate species and even fish in streams in urbanised watersheds (Gupta & Sharma 2005, Manenti 2010), together with effects on semi-aquatic insects distribution (Lencioni & Rossaro 2005, Miyazono & Taylor 2013). Moreover, the structure of stream communities may be strongly related to the absence or to the presence of fish such as trout, which are potential predators on larvae and potential competitors for macrobenthos prey (Lowe & Bolger 2002, Gillespie 2010). The occurrence of trout is linked both to its natural dispersal upstream and also to its introduction for fisheries purposes. In several pre-Alpine and Apennine stretches of streams which would be naturally fish-free it is a usual practice of local governments and fisheries associations to introduce both juveniles and adults of trout (Mazzotti 1993, Manenti & Pennati 2016). The impact of trout occurrence on freshwater communities is not well studied in stream habitats, while in mountain lakes and ponds it is well known that introduced salmonids may be a great threat to once fishless freshwater ecosystems (Tiberti & von Hardenberg 2012).

Macrobenthos communities are known to be an important biological indicator of water quality and aquatic ecosystem structure (Koperski 2011). Recent studies have provided evidence that Ephemeroptera species assemblages are affected by habitat features and water quality (Grandjean *et al.* 2011, Jandry *et al.* 2014). In this study I evaluated the relative abundances of two common genera of Ephemeroptera occurring in both streams and springs: *Ecdyonurus*, linked to well-oxygenated waters with a scarcity of organic matter, and *Baetis*, which is found in waters with lower oxygen content and higher organic pollution levels. In particular, the relative abundance of these two genera was studied in a still relatively wild area, upstream to major urbanised centres where pollution is still not a major factor (Seu & Borroni 2005).

The aim of this study was twofold. First, we evaluated the relative importance of streams morphological features for the abundance of *Ecdyonurus* and *Baetis* communities. Second, we related the abundance of these two genera to the most common vertebrate predators in our study area such as trout, *Salamandrina perspicillata* and *Salamandra salamandra* larvae occurrence.

Materials and Methods

Study area

The study area was situated in the Northern Apennines, in Liguria Region (Italy) between the Caucaso, Lavagnola and Becco Mountains (44.49 N, 9.17564 E). I surveyed streams and springs in the catchment basins of the Lavagna, Lentro and Trebbia streams (Fig. 1). In the study area, livestock farming was much diffused until the 1960s, and around the villages, terracing with dry stone walls for forage and potato cultivation was much diffused. The landscape around the villages was made up of terraces that are still cultivated or are covered by grass that is periodically cut. Small villages bordered by xerophile broadleaved woods with the prevalence of *Quercus robur* and some *Erica arborea*, while the surrounding landscape was generally covered by mesophile woods and beech forests. The altitude of the surveyed sites ranged between 400 and 800 m a.s.l.

Surveys and habitat characterisation

From early March to late May 2014, I used diurnal removal samplings to evaluate the abundance of *Ecdyonurus* and *Baetis* larvae. two successive samplings with an interval of 5 minutes from each other were performed. The relative abundance was estimated using the algorithm proposed by Chao & Chang (1999). To maximise the homogeneity of sampling among streams, the same observer performed all surveys. During each survey I sampled a linear transect (50 m) along streams; in the case of springs I sampled the whole site for a maximum of 15 m downstream from the resurgence point. I sampled 57 different sites belonging to three main hydrographic networks including 12 distinct springs, 33 different first order streams with four stations in the same second order stream and eight stations, always upstream from urbanised areas, divided in the three different main tributaries. The 12 total multiple sampling localities were included in the surveys when environmental conditions (e.g. landscape, stream morphology) differed markedly along the same stream course; the average distance between sampling localities of the same stream was 1710 m.

I recorded four features, which can be important for the Ephemeroptera, describing stream morphology, quality and ecosystem functioning. I measured site floor heterogeneity, which reflects the availability and number of shelters, using the percentage of alternation of substrate elements (sand, gravel, stones, sunken branches; see Petersen 1992). Each spring or stream transect was classified using the following rank scale: 1. absence of diversification, only a single substrate element covering almost 100% of the site; 2. poorly diversified, only two substrate elements covering > 90% of the transect; 3. quite diversified, at least three elements present in at least 10% of the transect; 4. highly diversified, > 90% of the transect presenting an alternation of at least three elements.

I recorded also the degree of shading, area and maximum depth. Shading degree was assessed measuring the percentage of aquatic site surface covered by shadow using the following scale rank: 1 – shade <10% of the surface; 2 – shade, covering between 10 and 30% of the surface; 3 – shade covering 30 – 50 % of the surface; 4 – shade covering 60-90 % of the surface; and 5 – shade covering > 90% of the aquatic surface.

Moreover, I recorded the presence/absence of three common vertebrate predators in the area: *Salmo trutta fario*, *Salamandra salamandra* larvae and

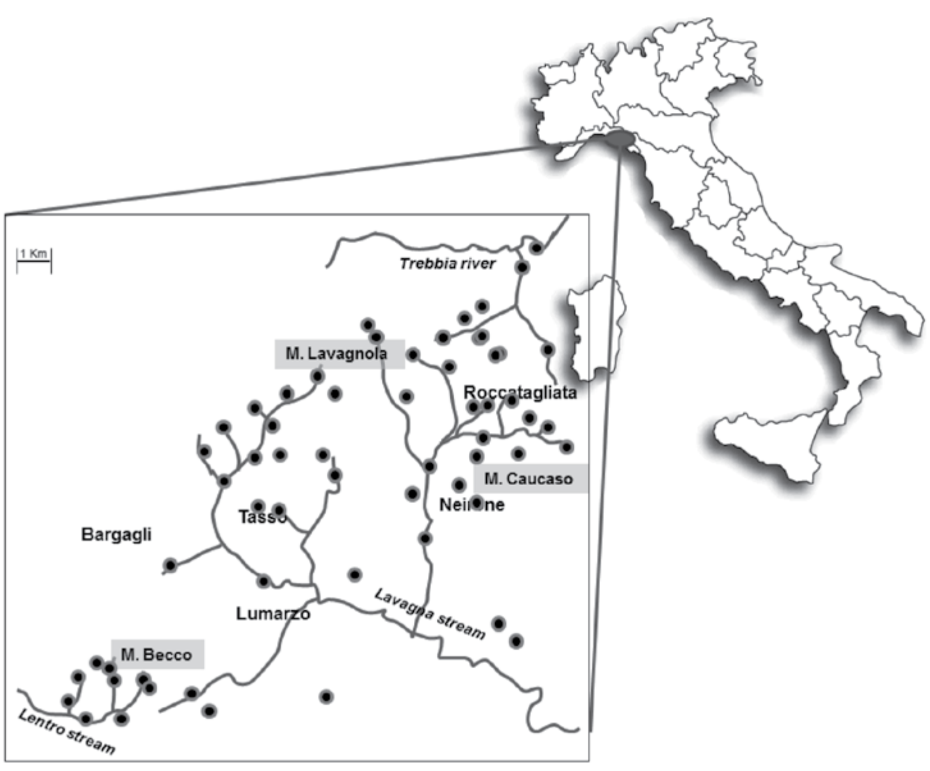

Fig. 1. Study area. Black circles indicate the sampling sites. Due to geographic proximity some of them are superimposed

Salamandrina perspicillata larvae or eggs. Predators' occurrence was assessed taking into account detection probability through two visual encounter surveys performed both during the night and during the day in each site. For all sites, at least one visit was performed in daytime, and at least one visit was performed after dusk, using spotlights to lighten the stream.

Statistical analyses

A site is confidently "occupied" if a species is detected at that site, but the lack of detection of a species during all sampling occasions does not necessarily mean that the species is absent (MacKenzie 2006). This can lead to an underestimation of occupancy and might influence the results of analyses, increasing the risk of data over-interpretation, with type II errors being potentially significant. We used Presence 5.5 (Hines 2006) to assess the probability of detection per visit of the predators and we calculated misdetection probability of each comparing the percentage of sites in which the species were observed with the percentage of occupancy estimated by Presence. As misdetection rate was quite low for *S. salamandra* and *S. perspicillata* (< 3 %) but high for trout (25 %) in the analyses for the two amphibian species I kept as variables the naïve presence/absence data, while for the trout I used the conditional probability of occupancy of each site estimated by Presence. Generalized linear mixed models (GLMMs) were used to relate Ephemeroptera abundance in each stream to the four morphological variables recorded. In the analysis sites' altitude was included as an independent variable. The three main hydrographic basins and the first stream situated downstream to each site were incorporated in the models as random factors in order to account for streams belonging to the same basin and hydrographic network. Even if the number of variables was low I built models representing all possible combinations of independent variables, and the model with the lowest AICc was considered the "best AICc" model (Rolls 2011). Models explaining the highest proportion of variation using the smallest number of predictors have smallest AICc values and are considered to be the "best models". As AICc may select overly complex models, a complex model was preferred only if its AICc was smaller than the AICc of all of its simpler nested models (Richards *et al.* 2011). I calculated the Akaike weights, wi (AICc weights), representing the probability of the different models given the data, and the evidence ratios E = wi / wj to compare the relative support of the different models by the data (Lukacs *et al.* 2007). All the models were checked using the variance inflation factor (VIF). The variance inflation factor quantifies collinearity among the variables in a regression model; only models with a VIF value < 5 were considered. Subsequently, for each variable I summed the AICc weights of all the models in which the variable was included, to obtain the probability for each variable to be included in the best model (Burnham & Anderson 2002). We assessed significance of variables composing the best model using a likelihood ratio test. We performed the analysis using the lme4, MuMIn and car packages.

To assess the relationships between the abundance of *Ecdyonurus* and *Baetis*, I used a constrained redundancy analysis (RDA). RDA is a canonical analysis, combining the properties of regression and ordination techniques, that allows evaluation of how much of the variation of the structure of one dataset (e.g. community composition in a stream; endogenous dataset) is explained by independent variables (e.g. habitat biotic and abiotic features; exogenous datasets) (Borcard *et al.* 2011). I considered as the exogenous matrix one matrix composed of the predators' distribution records and we used the matrices of *Ecdyonurus* and *Baetis* abundance as endogenous. The significance of explained variance was calculated by performing ANOVA-like permutation tests (10,000 permutations) (Borcard *et al.* 2011). I performed RDAs using the vegan package (Oksanen *et al.* 2005). All statistical analyses were done in the R 3.2 environment.

Results

The abundance of the two genera was generally not very high. The average abundance of *Ecdyonurus* was 0.71 ind/m^2 with a maximum of 7.5 ind/ m^2, while the average abundance of *Baetis* was 0.31 ind/ m^2, and the maximum density was of 5 ind/ m^2.

The abundance of *Ecdyonurus* was positively related to shelters availability, shading degree and area (Table. 1). The abundance of *Baetis* was positively related to shelters availability and negatively related to the shading degree of the site (Table 1).

The abundance of Ephemeroptera was related significantly to the distribution of vertebrate predators (permutation test: $P = < 0.0001$). The relationship between the abundance of *Ecdyonurus* and *Baetis*, and vertebrate predators' occurrence explained 37 % of the variation (Fig. 2). In particular, the abundance of *Ecdyonurus* was negatively related to sites where *S. perspicilllata* bres, while positively linked to sites where trout and *S. salamandra* larvae occurred, with a strongest affinity for the latter. The abundance of *Baetis* was negatively related to sites with *S. perspicilllata* and was higher at sites where *S. t. fario* occurred.

Discussion

Our results indicated that the substrate complexity, reflecting shelter availability, was the main factor affecting the abundance of both genera of Ephemeroptera. This fact is particularly interesting as provides indications that, in streams situated in areas where human pollution does not play particular role, streams heterogeneity is important for Ephemeroptera genera with similar reophilous requirements, but very distinct sensitivity toward organic pollution.

Ecdyonurus larvae belong to the family Heptageniidae and are considered very good indicators of water quality having a high biotic score (Gherardi *et al.* 2002). Their prevailing trophic role is of herbivorous and detritivorous, i.e. mainly scraping algae and encrusting organisms, but also collectors of organic matter (Tachet 2010). *Baetis* larvae belong to the family Baetidae and are the most resistant taxon from Ephemeroptera to water organic pollution having a low biotic score (Tachet 2010) and being abundant in several polluted lotic environments. They are considered essentially detritivorous being collectors of organic particles drifted by the flow (Tachet 2010). Secondarily, they may also feed on periphyton and other encrusting organisms (Tachet 2010). Both genera are typically rheophilous (Tachet 2010).

The present finding of higher abundances of both taxa at the sites with higher shelter availability might reflect the impact of possible predators and revealed the importance that stream and spring substratum structure may play for Ephemeroptera. Further, it demonstrated that *Baetis* may be as sensitive as *Ecdyonurus* in unpolluted streams in terms of shelter availability.

The only morphological stream feature discriminating between the two genera was the shading degree. *Ecdyonurus* was more abundant in shady streams, while *Baetis* was more numerous in sunny ones. This may reflect the importance of the vegetation cover

Table 1. Results of GLMMs analysis showing the variables included in the best model selected on the basis of AIC weight explaining the distribution of each species

Species	Variables in the best model	B	X^2	P
Ecdyonurus spp.	Area	0.33	9.98	<0.01
	Shade degree	0.75	4.49	0.03
	Shelters availability	1.08	12.13	< 0.001
Baetis spp.	Shade degree	-0.62	4.38	0.03
	Shelters availability	0.64	5.61	0.01

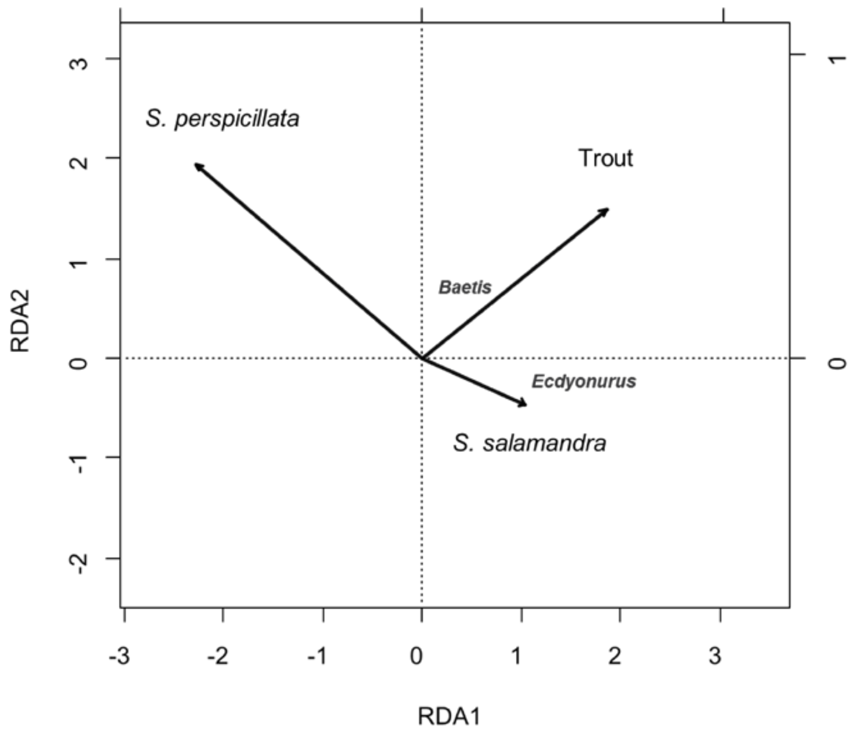

Fig. 2. Results of constrained redundancy analysis showing the relationship between *Ecdyonurus* and *Baetis* abundance and the occurrence of the three vertebrate predators. Constraining variables are represented by grey arrows

typology as Ephemeroptera richness is often affected also by riparian vegetation cover (Koperski 2010) even if *Ecdyonurus* is mainly herbivorous and, thus should in theory benefit from sunnier sites. Our results may be due to the fact that the encrusting organisms that *Ecdyonurus* larvae scrape from the substrate are linked to more close lotic environments but also that in hilly areas the collecting of organic matter deriving from the surrounding woody slopes may prevail.

Some useful insights come from the analysis of the relationships with the most spread vertebrates' predators in the area. Both *Ecdyonurus* and *Baetis* abundances were lower at sites where *S. perspicillata* bred. This species often chooses small pools with low flow (Romano *et al.* 2012). Even this type of site could be favourable due to the low drift risk (Poff *et al.* 1991); in the Apennines these streams may also dry up during summer with only some pools that keep water inside. For this reason these sites may be not favourable for typical rheophilous larvae as those of the considered taxa. *Ecdyonurus* larvae were particularly abundant at the sites used for breeding by the fire salamander.

Salamandra salamandra usually breeds in small shallow stream with high macrobenthos abundance, high shelters availability (Manenti *et al.* 2009) and where fish lack (Denoël & Winandy 2014). Generally the species is linked to highly wooded areas (Ficetola 2012). This may explain the affinity for the shady streams where *Ecdyonurus* abundance is higher. Moreover, *Ecdyonurus* larvae have several behavioural responses to escape predation by fire salamander (Oberrisser & Waringer 2011).

The abundance of *Baetis* was related to trout occurrence even if the relationships were not particularly strong. Fish is often considered as a major predators of macrobenthos and amphibian larvae (Tiberti & von Hardenberg 2012). This affinity may reflect the productivity of these streams, where perhaps the organic matter and the trophic resources for both taxa are more available.

Recent studies have provided evidence that Ephemeroptera may be considered as a very good proxy of several environmental factors considering both water quality and habitat structures (Grandjean *et al.* 2011, Jandry *et al.* 2014). Assessing Ephemeroptera composition and assemblages may give important insights for understanding the requirements also of some endangered species typical of lotic systems (Grandjean *et al.* 2003, Grandjean *et al.* 2011). Our study gives novel data on the role of habitat features on Ephemeroptera abundance and on the relationships with vertebrate predators, often spread in lotic environments. The study area is particularly favourable for detecting the role of morphological lotic habitat features because the valley slopes and structure allow the existence of only rare urban centres that do not alter the pollution level of the studied streams. Streams and springs may be very important determinants for Ephemeroptera population survival in this area. This study confirms that also in unpolluted areas the watercourse substrate heterogeneity should be attentively preserved.

References

Borcard D., F. Gillet & P. Legendre 2011. Numerical Ecology with R. New York. (Springer).

Burnham K. P. & D. R. Anderson 2002. Model selection and multimodel inference: a practical information-theoretic approach. New York. (Springer Verlag).

Chao A. & S. H. Chang 1999. An estimating function approach to the inference of catch-effort models. – *Environmental and Ecological Statistics*, **6** (3): 313-334.

Chee Y. E. & J. Elith 2012. Spatial data for modelling and management of freshwater ecosystems. – *International Journal of Geographical Information Science*, **26** (11): 2123-2140.

Clarke A., R. Mac Nally, N. R. Bond & P. S. Lake 2010. Conserving macroinvertebrate diversity in headwater streams: the importance of knowing the relative contributions of alpha and beta diversity. – *Diversity and Distributions*, **16** (5): 725-736.

Dauba F., S. Lek, S. Mastrorillo & G. H. Copp 1997. Long-term recovery of macrobenthos and fish assemblages after water pollution abatement measures in the River Petite Baise (France). – *Archives of Environmental Contamination and Toxicology*, **33** (3): 277-285.

Denoël M. & L. Winandy 2014. Fire salamander (*Salamandra salamandra*) in Larzac plateau: low occurrence, pond-breeding and cohabitation of larvae with paedomorphic palmate newts (*Lissotriton helveticus*). – *Acta Herpetologica*, **9** (1): 43-49.

Dudley M. P., M. C. Ho & C. J. Richardson 2015. Riparian Habitat Dissimilarities in Restored and Reference Streams are Associated with Differences in Turtle Communities in the Southeastern Piedmont. – *Wetlands*, **35** (1): 147-157.

Ficetola G. F., Manenti, R., De Bernardi, F. & Padoa-Schioppa, E. 2012. Can patterns of spatial autocorrelation reveal population processes? An analysis with the fire salamander. – *Ecography*, **35** 693-703.

Gherardi F., E. Tricarico & M. Ilheu 2002. Movement patterns of an invasive crayfish, *Procambarus clarkii*, in a temporary stream of southern Portugal. – *Ethology Ecology & Evolution*, **14** (3): 183-197.

Gillespie G. 2010. Population age structure of the spotted tree frog (Litoria spenceri): insights into population declines. – *Wildlife Research*, **37** (1): 19-26.

Grandjean F., J. Jandry, E. Bardon, A. Coignet, M. C. Trouilhe, B. Parinet, C. Souty-Grosset & M. Brulin 2011. Use of Ephemeroptera as bioindicators of the occurrence of white-clawed crayfish (Austropotamobius pallipes). – *Hydrobiologia*, **671** (1): 253-258.

Grandjean F., J. Momon & M. Bramard 2003. Biological water quality assessment of the white-clawed crayfish habitat based on macroinvertebrate communities: Usefulness for its conservation. – *Bulletin Francais De La Peche Et De La Pisciculture*, (370-71): 115-125.

Gupta K. & A. Sharma 2005. Macroinvertebrates as indicators of pollution. – *Journal of Environmental Biology*, **26** (2): 205-211.

Hines J. E. 2006. PRESENCE2- Software to estimate patch occupancy and related parameters.

Jandry J., M. Brulin, B. Parinet & F. Grandjean 2014. Ephemeroptera communities as bioindicators of the suitability of headwater streams for restocking with white-clawed crayfish, Austropotamobius pallipes. – *Ecological Indicators*, **46** 560-565.

Koperski P. 2010. Diversity of macrobenthos in lowland streams: ecological determinants and taxonomic specificity. – *Journal of Limnology*, **69** (1): 88-101.

Koperski P. 2011. Diversity of freshwater macrobenthos and its use in biological assessment: a critical review of current applications. – *Environmental Reviews*, **19** 16-31.

Lencioni V. & B. Rossaro 2005. Microdistribution of chironomids (Diptera : Chironomidae) in Alpine streams: an autoecological perspective. – *Hydrobiologia*, **533** 61-76.

Lowe W. H. & D. T. Bolger 2002. Local and landscape-scale predictors of salamander abundance in New Hampshire headwater streams. – *Conservation Biology*, **16** (1): 183-193.

Lukacs P. M., W. L. Thompson, Kendall W.L., W. R. Gould, P. F. Doherty, K. P. Burnham & D. R. Anderson 2007. Concerns regarding a call for pluralism of information theory and hypothesis testing. – *Journal of Applied Ecology*, **44** 456-460.

MacKenzie D. I. 2006. Modeling the probability of resource use: The effect of, and dealing with, detecting a species imperfectly. – *Journal of Wildlife Management*, **70** (2): 367-374.

Manenti R. 2010. The Role of Watercourse Features and of Landscape Structure in the Distribution of Triclads Inhabiting Head Waters: the Example of *Polycelis felina*. – *Revue D Ecologie-La Terre Et La Vie*, **65** (3): 279-285.

Manenti R. & B. Bianchi 2014. Distribution of the Triclad *Polycelis felina* (Planariidae) in Aezkoa Mountains: Effect of Stream Biotic Features. – *Acta Zoologica Bulgarica*, **66** (2): 271-275.

Manenti R., G. F. Ficetola & F. De Bernardi 2009. Water, stream morphology and landscape: complex habitat determinants for the fire salamander *Salamandra salamandra*. – *Amphibia-Reptilia*, **30** (1): 7-15.

Manenti R. & R. Pennati 2016. Environmental factors associated with amphibian breeding in streams and springs: effects of habitat and fish occurrence -*Amphibia-Reptilia*, **37** (2): 237 – 242.

Mazzotti S. 1993. Competizione fra fauna ittica e batracofauna in laghi dell'Appennino settentrionale. – *Quad. Civ. Staz. Idrobiol. Milano.*, **20** (Atti I Convegno Italiano sulla Salvaguardia degli Anfibi. (II). 1993, Ed: Ferri,V): 67-74.

Miyazono S. & C. M. Taylor 2013. Effects of habitat size and isolation on species immigration-extinction dynamics and community nestedness in a desert river system. – *Freshwater Biology*, **58** (7): 1303-1312.

Oberrisser P. & J. Waringer 2011. Larval salamanders and diel drift patterns of aquatic invertebrates in an Austrian stream. – *Freshwater Biology*, **56** (6): 1147-1159.

Oksanen J., R. , R. Kindt, & R. B. O'Hara 2005. Vegan: community ecology package.

Petersen R. C. 1992. The Rce – a riparian, channel, and environmental inventory for small streams in the agricultural landscape. – *Freshwater Biology*, **27** (2): 295-306.

Poff N. L., R. D. Decino & J. V. Ward 1991. Size-Dependent Drift Responses of Mayflies to Experimental Hydrologic Variation – Active Predator Avoidance or Passive Hydrodynamic Displacement. – *Oecologia*, **88** (4): 577-586.

Renai B., S. Bertocchi, S. Brusconi, F. Gherardi, F. Grandjean, M. Lebboroni, B. Parinet, C. S. Grosset & M. C. Trouilhe 2006. Ecological characterisation of streams in Tuscany (Italy) for the management of the threatened crayfish Austropotamobius pallipes complex. – *Bulletin Francais De La Peche Et De La Pisciculture*, (380-81): 1095-1113.

Richards S. A., M. J. Whittingham & P. A. Stephens 2011. Model selection and model averaging in behavioural ecology: the utility of the IT-AIC framework. – *Behavioral Ecology and Sociobiology*, **65** (1): 77-89.

Rolls R. J. 2011. The role of life-history and location of barriers to migration in the spatial distribution and conservation of fish assemblages in a coastal river system. – *Biological Conservation*, **144** (1): 339-349.

Romano A., R. Bartolomei, A. L. Conte & E. Fulco 2012. Amphibians in Southern Appennine: distribution, ecology and conservation notes in the "Appennino Lucano, Val d'Agri e Lagonegrese" National Park (Southern Italy). – *Acta Herpetologica*, **7** (2): 203-219.

Seu A. & M. Borroni 2005. Carta Ittica della Provincia di Genova. Genova (Provincia di Genova – Assessorato Caccia e Pesca). 478.

Tachet H. 2010. Invertébrés d'eau douce : Systématique, biologie, écologie Paris (CNRS). 592.

Tiberti R. & A. von Hardenberg 2012. Impact of introduced fish on Common frog (*Rana temporaria*) close to its altitudinal limit in alpine lakes. – *Amphibia-Reptilia*, **33** (2): 303-307.

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