

Distribution of the Triclad *Polycelis felina* (Planariidae) in Aezkoa Mountains: Effect of Stream Biotic Features

Raoul Manenti¹, Barbara Bianchi²

¹ Dipartimento di Bioscienze, Università degli Studi di Milano, Via Celoria, 26 20133, Milano, Italy;
E-mail: raoul.manenti@unimi.it

² Comitato per la Difesa delle Bereve, Via Giuseppe Garibaldi, 420836 Briosco, MB, Italy

Abstract: The study of freshwater invertebrates composing macrobenthos communities of stream habitats can be of great importance for both ecological and conservational purposes. In this paper we analyse the distribution of the triclad *Polycelis felina* in western Pyrenees along the Aezkoa Mountains where forests and pastures prevail. We found a relatively wide distribution of the species that was affected mainly by stream biotic characteristics, in particular by fish occurrence and gammarid abundance. These factors in a poorly urbanised area can reflect the importance of the well-structured community for the presence of *P. felina* and its role as a bioindicator.

Keywords: Planaria, freshwater, crustaceans

Introduction

The study of freshwater invertebrates inhabiting streams and watercourses can be of particular importance for ecological and conservational purposes (PAILLEX *et al.* 2013). Generally, macrobenthos communities are recognised as important biological indicators of water quality and aquatic ecosystem structure (BORISOVA *et al.* 2013). They are often included in studies assessing the ecological requirements of aquatic or semiaquatic vertebrates (KOPERSKI 2011) and used to reveal the impact of human activities on the water system. Planarians are typical members of macrobenthos communities in both lotic and lentic aquatic systems. They are small, free-living flatworms that can colonise various types of freshwater biotopes, from springs and streams to ponds, lakes and rivers. Planarians are generally zoophagous organisms feeding mainly on small living invertebrates and decaying organisms occurring in their ecosystem. In particular, they can eat small crustaceans, annelids, insect larvae, crustaceans, leeches, snails and other small herbivores or detritivores. Moreover, they can feed also on bacteria and fungi decomposing leaves and on other submerged organic matter of

vegetal origin. Most of species that live in streams are particularly sensible to organic pollution and water quality as in the case of the genera *Polycelis* Kenk, 1930 or *Crenobia* Kenk, 1930 (see ALONSO, CAMARGO 2011, WU *et al.* 2012). Conversely, there are also species more resistant to water pollution and alterations. *Polycelis felina* (Dalyell, 1814) is sensible to different pollutants and typically inhabits lowly-polluted streams, brooks, slow creeks, and various types of springs (MANENTI 2010, ALONSO, CAMARGO 2011, STUBBINGTON *et al.* 2011). This species has been extensively used for ecotoxicological studies in order to assess its low tolerance to many chemical substances or different experimental conditions involving habitat peculiarities (KALAFATIC, TABORSK 1998, FRANJEVIC *et al.* 2000, HORVAT *et al.* 2005, LAU *et al.* 2007). These studies demonstrated the important role of *P. felina* as a biological indicator and its high potential for the assessment of freshwater habitat characteristics. However, the field studies on the ecology of *P. felina* remain scarce. In the past, it was studied in freshwater habitats of North Wales and in springs of central Pyrenees (WRIGHT 1974,

ROCA *et al.* 1992). Both studies confirmed its occurrence in unpolluted and well preserved slow running freshwater habitats. Recently, an ecological survey of the species in a highly urbanised area of North Italy (MANENTI 2010) revealed that *P. felina* is more versatile than indicated by its biotic score (GHETTI 1997), with some records in conditions of pollution and within non-optimal macrobenthic communities, even if the great percentage of sites inhabited by this triclad species remain in unpolluted waters. The species is associated with streams with higher shelter availability and higher microhabitat occurrence surrounded by woody landscapes (MANENTI 2010).

The aim of this study is to increase the knowledge on the ecology of *Polycelis felina*. We assess the factors affecting its distribution in a mountain area with low density of the human population and characterised by both traditional pastures and old forests.

Material and Methods

Study area

The study area is situated in western Pyrenees between the southern Spanish side (Navarra) and the northern French side (Pyrenneés – Atlantique) in the north Aezkoa Mountain chain (42°59'.39"N, 1°8'48.78"E). The Aezkoa Mountain chain is composed of 14 mountain ridges divided in three ranges. We studied streams in the north range between the mountains Urkulu (1419 m a.s.l.), Mendizar (1323 m a.s.l.), Mendilatz (1337 m a.s.l.), Mozoloak (1255 m a.s.l.), and Lizardoia (1197 m a.s.l.) (Fig. 1). The study area is situated at the western limit of the Irati Forest, an old and protected native beech forest that extends on 17 300 hectares, mainly in the Spanish

side. The forest is surrounded by pastures. All the pasture territories are situated between 500 and 2300 m a.s.l. Our research focused mainly around the Irabia Lake on the central and western side of the Irati Forest as well as westwards, where other patches of beech forest occurred and were surrounded by extended pasture landscapes. The altitude of the streams surveyed ranged between 700 and 1200 m a.s.l. All woodlands within the study area were broadleaved, dominated by beech (Fig. 1).

Surveys and habitat characterisation

We used diurnal samplings to evaluate the presence/ absence of *P. felina*. To maximise the homogeneity of sampling among streams and pools, the same observer performed all surveys. During each survey, we conducted a linear transect (150 m) along streams. In some cases, we considered multiple sampling localities in the same stream, if environmental conditions (*e.g.* landscape, stream morphology) markedly changed along the stream course; the average distance between the sampling localities of the same stream was 2140 m.

The streams were described on the basis of rapid bioassessment protocols (BARBOUR *et al.* 1999). We recorded four characteristics that described stream morphology, water quality and ecosystem functioning and could be hypothetically important for the occurrence of *P. feline*: *i)* We measured substrate heterogeneity on the basis of the percentage of alternation of substrate elements, *i.e.* sand, gravel, stones, and sunken branches (see PETERSEN 1992). Each site was classified using the following rank scale: 1, absence of diversity, only a single substrate element covering almost 100% of the site; 2, poorly diverse, only 2 substrate elements covering >90% of the transect; 3, quite diverse, at least three



Fig. 1. Study area. The non-inhabited sites are in black, the inhabited sites – in white. The map layout has been obtained from Google Earth

elements present in at least 10% of the transect; 4, highly diverse, >90% of the transect presenting an alternation of at least three elements. *ii*) Three parameters describing streams morphology: maximum width, minimum width and average width. *iii*) Four parameters describing the biotic features of sites: fish presence/ absence, richness of amphibians and abundance of gammarids.

We used a fine mesh net to collect *P. felina* and gammarids occurring along a linear transect. For 7 minutes, we turned over the sites substrate collecting the macrobenthos into the net. We estimated the abundance of gammarids (*Gammarus* spp.) considering a three ranking scale: 1 = poorly abundant (1-9 individuals per transect), 2 = discretely abundant (10-20 individuals per transect), and 3 = highly abundant (more than 20 individuals per transect), considering both adults and juvenile specimens.

We also measured, in addition to the site altitude, two variables representing the landscape surrounding each stream: distance from forest and the percentage of wood cover within 100 m from each sampling site (FICETOLA *et al.* 2009). The landscape variables were measured from the 1:25000 SITNA vector map of Navarra (Geoportal de Navarra: <http://sitna.navarra.es/geoportal>). To measure the wood-cover percentage within 100 m, we used ArcView GIS 3.2 (ESRI 1999).

Statistical analyses

We used multiple logistic regression to evaluate the relative role of stream and landscape features on the distribution of *P. felina*. Using stepwise method, we first built a blank model of the occurrence of *P. felina* as a response variable and detected the values of Akaike Information Criterion (AIC) for all the variables considered in our study. Starting from the variables with the lowest AIC, we built models adding one variable for time till the last added was not significant. By removing the latter, we obtained the best available model explaining the distribution of *P. felina*. We then analysed the fit of our best model calculating D² value. We performed all statistical analyses in the R 2.14 environment (R Development Core Team 2012).

Results

We surveyed 30 sites from 21 watercourses, mainly belonging to the catchment basin of the upper Irati River (Fig 1). We found *P. felina* at 12 sites (Fig 1). The elevation of positive sites ranged between 790 and 905 m a.s.l., with a good surrounding wood cover (average 75% of the surface within 100 m from the

Table 1. Characteristics of streams inhabited and non-inhabited by *Polycelis felina* (mean ± standard deviation)

Characteristics	Inhabited	Non-inhabited
Altitude (m a.s.l.)	857±15.87	882±14.49
Substrate heterogeneity	4.66±0.14	3.66±0.22
Maximum width (m)	7.8±1.58	2.7±0.35
Minimum width (m)	2.3±0.47	0.95±0.14
Average width (m)	3.8±0.70	1.69±2.02
Amphibian species richness	2.08±0.35	2.11±0.34
Gammarid abundance	2.08±0.37	0.33±0.18
Distance to forest (m)	38.75±33.09	67.94±30.06
Wood cover (%)	75±11.10	67.9±10.01

Table 2. Statistical characteristics of the variables examined with a view to explaining factors that influence the distribution of *Polycelis felina* in streams in Aezkoa Mountains

Variable	Df	Deviance	AIC
Fish occurrence	1	23.410	27.410
Gammarid abundance	1	24.895	28.895
Maximum width	1	28.296	32.296
Average width	1	30.107	34.107
Substrate heterogeneity	1	30.213	34.213
Minimum width	1	31.351	35.351
Wood cover	1	40.130	44.130
Forest distance	1	40.304	44.304
Amphibian richness	1	40.378	44.378

sampling point) and highly diversified watercourses (Table 1).

The distribution of *P. felina* was mainly affected by stream biotic features and in particular the variables with the lowest AIC value were the fish occurrence ($P=0.012$) and the abundance of gammarids ($P=0.017$) (Table 2). These were the only variables included in the best model obtained through the multiple logistic regression. D² value of this model was 0.62, showing that the model had a high fit and explained more than 60% of variance.

Discussion

P. felina showed an extensive distribution over the study area occurring in most of the main basins of the Northern Aezkoa. This species occurred in highly diversified streams surrounded by woody areas. Our analysis showed that biotic features of streams were the major determinants of the distribution of *P. felina*. In particular, we found that the abundance of

gammarids positively affected the distribution of this species. Gammarids are the most common and effective shredders in temperate streams and they can be associated with both oligotrophic waters of little streams and springs and sites with higher organic content and pollution levels (KUPISCH *et al.* 2012). Gammarids can play important role in the in-stream litter decomposition (KUPISCH *et al.* 2012), in spite of the fact that young specimens may be collectors with preference to smaller-size food particles, while the larger individuals may exhibit cannibalism and predation (KUPISCH *et al.* 2012). Some classical field studies have shown that *Gammarus* spp. populations are highly resource-dependant and their abundance increases or decreases depending on the leaf availability indicating that leaf litter is their highly preferred food resource (FRIBERG, JACOBSEN 1994). They can be suitable prey for *P. felina* and represent an important trophic source for it. The positive role of fish occurrence can be considered a biotic factor indicating the existence of a well structured aquatic ecosystem. Fish can play an important role and have an impact in the ecosystem of streams in which they occur, with some species being indicators of high microhabitats occurrence and the largest predators of macrobenthos invertebrates including planarians (DUBEY *et al.* 2012, PEKARIK *et al.* 2012). The fish recorded were essentially young or adult trout that are considered at the top of the trophic chain in watercourses and that may be regarded as important bioindicators (WILHELM *et al.* 1999, TRIEBSKORN *et al.* 2003), often occurring in habitats with higher macrobenthos richness (ENCINA, RODRIGUEZ-RUIZ 2003). Fish are also associated with the stable traits of watercourses and their co-occurrence with planarians can be linked to that. Also amphibians can be important predictors of stream features and can affect macrobenthos communities (FICETOLA *et al.* 2004, FICETOLA *et al.* 2009, MANENTI *et al.* 2009); moreover, planarians have been recently pointed out as possible important food resources for some salamanders (GILLESPIE 2013). However, we did not de-

tect any significant influence of amphibian richness on *P. felina* occurrence. Studies on the same tricolad in areas characterised by high urbanisation showed that the landscape structure (except the richness of microhabitats in the watercourses) play a major role for the species distribution (MANENTI 2010). In such a landscape, human impact can affect both stream morphology and surrounding landscape composition, with only residual areas that show natural wood cover and stream diversification that remain the most suitable for this tricolad species. The current study area is characterized by a few urbanised areas and extended old forests alternated with traditional grazing pastures. Pastures can be a possible threat for the quality of watercourses because of the high organic content that may represent an important source of pollution (ROSENTHAL *et al.* 2012). Therefore, the occurrence of gammarids and fish can also reflect the low general impact of pastures on those streams.

Macrobenthos is often used as a measure of water quality since the polluted streams have poor macrobenthos communities (MAITLAND 1990, MOSS 1998). Planarians are generally important elements of macrobenthos communities, with various species that have an important role as bioindicators (MIRJANA *et al.* 2003, HARRATH *et al.* 2012). Being mainly zoophagous, they occupy the relatively high levels in the trophic web of the macrobenthic species occurring in streams. Therefore, the study of planarians may improve the knowledge of features that affect the ecosystem functioning in certain lotic habitats. The results of this study increase the knowledge about the distribution of the tricolad *P. felina* in an important area of the Pyrenees. They also reveal the importance of the biotic requirements of this taxon to the habitats and their role in the maintenance of freshwater biodiversity.

Acknowledgments: We acknowledge the comments of an anonymous reviewer that helped to improve a previous version of the manuscript. We are grateful to L. Massobrio for the linguistic comments on the text.

References

- ALONSO A., J. A. CAMARGO 2011. The freshwater planarian *Polyelis felina* as a sensitive species to assess the long-term toxicity of ammonia. – *Chemosphere*, **84** (5): 533-537.
- BARBOUR M. T., J. GERRITSEN, B. D. SNYDER, J. B. STRIBLING 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish. Washington, D.C. (U.S. Environmental Protection Agency, EPA 841-D-99-002.).
- BORISOVA P., E. Varadinova and Y. Uzunov 2013. Contemporary State of the Bottom Invertebrate Communities of the Tundzha River Basin (South-East Bulgaria). – *Acta Zoologica Bulgarica* **65** (1): 75 -87.
- DUBEY V. K., U. K. SARKAR, A. PANDEY, R. SANI, W. S. LAKRA 2012. The influence of habitat on the spatial variation in fish assemblage composition in an unimpacted tropical River of Ganga basin, India. – *Aquatic Ecology*, **46** (2): 165-174.
- ENCINA L., A. RODRIGUEZ-RUIZ 2003. Abundance and distribution of a brown trout (*Salmo trutta* L.) population in a remote

- high mountain lake. – *Hydrobiologia*, **493** (1-3): 35-42.
- FICETOLA G. F., E. PADOA-SCHIOPPA, F. DE BERNARDI 2009. Influence of landscape elements in riparian buffers on the conservation of semiaquatic amphibians. – *Conservation Biology*, **23** 114-123.
- FICETOLA G. F., E. PADOA-SCHIOPPA, A. MONTI, R. MASSA, F. DE BERNARDI, L. BOTTONI 2004. The importance of aquatic and terrestrial habitat for the European pond turtle (*Emys orbicularis*): implications for conservation planning and management. – *Canadian Journal of Zoology*, **82** 1704-1712.
- FRANJEVIC D., A. KRAJNA, M. KALAFATIC, N. LJUBESIC 2000. The effects of zinc upon the survival and regeneration of planarian *Polycelis felina*. – *Biologia*, **55** (6): 689-694.
- FRIBERG N., D. JACOBSEN 1994. Feeding plasticity of 2 detritivore-shredders. – *Freshwater Biology*, **32** (1): 133-142.
- GHETTI P. F. 1997. Indice Biotico Esteso (I.B.E.): Manuale di applicazione. Trento (Provincia Autonoma di Trento).
- GILLESPIE J. H. 2013. Application of Stable Isotope Analysis to Study Temporal Changes in Foraging Ecology in a Highly Endangered Amphibian. – *Plos One*, **8** (1): 10.
- HARRATH A. H., R. SLUYS, A. GHLALA, S. ALWASEL 2012. The first subterranean freshwater planarians from North Africa, with an analysis of adenodactyl structure in the genus *Dendrocoelum* (Platyhelminthes, Tricladida, Dendrocoelidae). – *Journal of Cave and Karst Studies*, **74** (1): 48-57.
- HORVAT T., M. KALAFATIC, N. KOPJAR, G. KOVACEVIC 2005. Toxicity testing of herbicide norflurazon on an aquatic bioindicator species – the planarian *Polycelis felina* (Daly.). – *Aquatic Toxicology*, **73** (4): 342-352.
- KALAFATIC M., S. TABORSKAK 1998. Effects of chromium upon neoblast division in the regenerates of *Polycelis felina*. – *Biologia*, **53** (3): 321-325.
- KOPERSKI P. 2011. Diversity of freshwater macrobenthos and its use in biological assessment: a critical review of current applications. – *Environmental Reviews*, **19** 16-31.
- KUPISCH M., S. MOENICKES, J. SCHLIEF, M. FRASSL, O. RICHTER 2012. Temperature-dependent consumer-resource dynamics: A coupled structured model for *Gammarus pulex* (L.) and leaf litter. – *Ecological Modelling*, **247** 157-167.
- LAU A. H., T. KNAKIEVICZ, D. PRA, B. ERDTMANN 2007. Freshwater planarians as novel organisms for genotoxicity testing: Analysis of chromosome aberrations. – *Environmental and Molecular Mutagenesis*, **48** (6): 475-482.
- MAITLAND P. S. 1990. Biology of fresh waters. Stirling (Kluwer).
- MANENTI R. 2010. The Role of Watercourse Features and of Landscape Structure in the Distribution of Triclad Inhabiting Head Waters: the Example of *Polycelis felina*. – *Revue D Ecologie-La Terre Et La Vie*, **65** (3): 279-285.
- 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.
- MIRJANA E., K. GORAN, I. ZUPAN, D. FRANJEVIC 2003. Diflufenzuron toxicity upon the planarian *Dugesia tigrina* (Gir.). – *Periodicum Biologorum*, **105** (2): 177-180.
- MOSS B. 1998. Ecology of fresh waters. Oxford (Blackwell Science).
- PAILLEX A., S. DOLEDEC, E. CASTELLA, S. MERIGOUX, D. C. ALDRIDGE 2013. Functional diversity in a large river floodplain: anticipating the response of native and alien macroinvertebrates to the restoration of hydrological connectivity. – *Journal of Applied Ecology*, **50** (1): 97-106.
- PEKARIK L., J. KOSCO, M. SVATORA 2012. Reference conditions for fish microhabitat use in foothill streams: A case study on undisrupted carpathian streams. – *River Research and Applications*, **28** (3): 369-376.
- 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.
- ROCA J. R., M. RIBAS, J. BAGUNA 1992. Distribution, Ecology, Mode of Reproduction and Karyology of Fresh-Water Planarians (Platyhelminthes, Turbellaria, Tricladida) in the Springs of the Central Pyrenees. – *Ecography*, **15** (4): 373-384.
- ROSENTHAL G., J. SCHRAUTZER, C. EICHBERG 2012. Low-intensity grazing with domestic herbivores: A tool for maintaining and restoring plant diversity in temperate Europe. – *Tuexenia*, (32): 167-205.
- STUBBINGTON R., P. J. WOOD, I. REID 2011. Spatial variability in the hyporheic zone refugium of temporary streams. – *Aquatic Sciences*, **73** (4): 499-511.
- TRIEBSKORN R., S. ADAM, A. BEHRENS, S. BEIER, J. BOHMER, T. BRAUNBECK, H. CASPER, U. DIETZE, M. GERNHOFER, W. HONNEN, H. R. KOHLER, W. KORNER, J. KONRADT, R. LEHMANN, T. LUCKENBACH, A. OBEREMM, J. SCHWAIGER, H. SEGNER, M. STRMAC, G. SCHURMANN, S. SILIGATO, W. TRAUNSPURGER 2003. Establishing causality between pollution and effects at different levels of biological organization: The VALIMAR project. – *Human and Ecological Risk Assessment*, **9** (1): 171-194.
- WILHELM F. M., B. R. PARKER, D. W. SCHINDLER, D. B. DONALD 1999. Seasonal food habits of bull trout from a small alpine lake in the Canadian Rocky Mountains. – *Transactions of the American Fisheries Society*, **128** (6): 1176-1192.
- WRIGHT J. F. 1974. Some factors affecting the distribution of *Crenobia alpina* (Dana), *Polycelis felina* (Dalyell) and *Phagocata vitta* (Dugès) (Platyhelminthes) in Caernarvonshire, North Wales. – *Freshwater Biology*, **4** 31 – 59.
- WU J.-P., H.-C. CHEN, M.-H. LI 2012. Bioaccumulation and Toxicodynamics of Cadmium to Freshwater Planarian and the Protective Effect of N-Acetylcysteine. – *Archives of Environmental Contamination and Toxicology*, **63** (2): 220-229.

Received: 13.05.2013
Accepted: 26.06.2013

