ORIGINAL ARTICLE

Influence of wastewater discharges on benthic macroinvertebrate communities in a cantabrian-atlantic coastal river

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ABSTRACT

This paper studies the effect of wastewater discharges on benthic macroinvertebrates in the Furnia River (Pontevedra, NW Spain). Semiquantitative surveys were carried out in spring 2008 and 2017 in three different locations, upstream and downstream of a sewage treatment plant built in 2013. Different indexes were calculated based on benthic macroinvertebrate assemblages: abundance, richness, Shannon-Wiener, EPT, IASPT, IBMWP and several physicochemical variables were measured concurrently. Although the indexes values decreased slightly along the water course, the results indicate an optimal water quality of the Furnia River, supporting a very diverse community of aquatic macroinvertebrates.

INTRODUCTION

Organic pollution is one of the most important threats to freshwater ecosystems, bound to wastewater discharge from human activities (cities, farming, agriculture and industry) above all, affecting human health and ecosystems viability (Wen *et al*., 2017). In the first steps, organic pollution from untreated urban sewage contains pathogens that can cause a variety of diseases like diarrhoea (Sirota *et al*., 2013). If prolonged organic pollution keeps up in the river, microbial growth will be observed, leading to oxygen depletion and disturbance of the entire freshwater ecosystems as consequence (Sirota *et al*., 2013).Wastewater discharge from settlements and intensive livestock farms and crops represent the main entry pathway of these pollutants into the rivers, and it is expected that both sources of organic pollutant will increase because of the fast urban population growth in the following decades (Bruinsma, 2003). These pollutants are transported along the river network affecting populations and ecosystems not only near the wastewater discharge points, but also downstream along the river course (Nelson and Murray, 2008).

The self-cleaning capacity of the river is the principal determining factor of downstream impacts extension through natural runoff dilution and microorganisms' degradation (McDonald *et al*., 2011; Milly *et al*., 2005). This kind of pollution is especially important in small rivers, whose flow is very affected by seasonality and climate change effects (Milly *et al*., 2005; McDonald *et al*., 2011). The areas which are experiencing climate wetness reductions are getting doubly affected in the last decades (Milly *et al*., 2005; McDonald *et al*., 2011): on the one hand, their dilution capacity is harshly reduced; on the other hand, their pollution risk is getting increased as well.

Among many other consequences, biodiversity loss is the most important impact that pollution can cause in freshwater ecosystems, which are recognized as the most threatened ecosystems around the world nowadays (Ceballos *et al*., 2017). There are so many evidences that show an incredibly fast biodiversity loss in the last few years (Dudgeon *et al*., 2006). A variety of global changes are driving rates of species extinction which will make us face the sixth mass extinction in 240 years, if the trend continues increasing (Hooper *et al*., 2012). Diversity effects in small-scale may underestimate the impact of these living-beings lost on the functioning of the natural ecosystems (Cardinale *et al*., 2012), for this reason detailed studies are being increasingly important. Freshwater ecosystems are an important fount of life and resources too, with a widespread list of uses, according to Jordaan (2009). It is obvious the reason: water is the main cause of our existence, but recently it is being highly threatened by human activity, and the species inhabiting them are witness to the slow (or not so much) degradation of their

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own habitat (Prenda *et al*., 2006). At least 130,000 animal and vegetal species inhabit fluvial ecosystems, including 25% of vertebrates (Dudgeon *et al*., 2006; Mora *et al*., 2011), and most of them will disappear or they will have to readapt themselves to the new conditions.

In Galicia, Furnia River was chosen as the object of study because previous researches inferred that it was conserved excellently, being one of the most biodiverse Galician rivers (Benetti *et al*., 2012). A sewage treatment plant (STP) was installed in the middle of the river's path in 2013, so the aim of the present study is to analyze the possible impact on the benthic macroinvertebrate communities in relation to the wastewater discharges.

METHODS

Study area

The study was carried out in the Furnia River, one of the most important tributaries of the Minho River by the Spanish margin. It is located entirely within the municipality of Tui (Pontevedra), in the south of the Autonomous Community of Galicia (NW Spain). Furnia waterbody (ES503MAR002300) is included in the Minho basin and it is regulated by the River Basin Authority of Minho-Sil. Furnia River is roughly 9 km length and its water runs in a north-south direction, with a mean altitude of 100 m over the sea level according to the Hydrologic Plan of the River Basin Authority of Minho-Sil. Furnia River features make it belong to the cantabrian-atlantic coastal rivers category (river type 30) (CHMS, 2014).

The study area has particular climatic conditions because the Minho River represents a natural edge between the Atlantic and Mediterranean bio-climate, with warm temperatures and abundant precipitations (Martínez-Cortizas and Pérez-Alberti, 1999; Kottek *et al*., 2006).

Three sampling sites were selected for this study, with a 3 km distance separation between them (Fig. 1). The first one (Ponteliñares) was located in the high course of the river, upstream of the place where the sewage station was

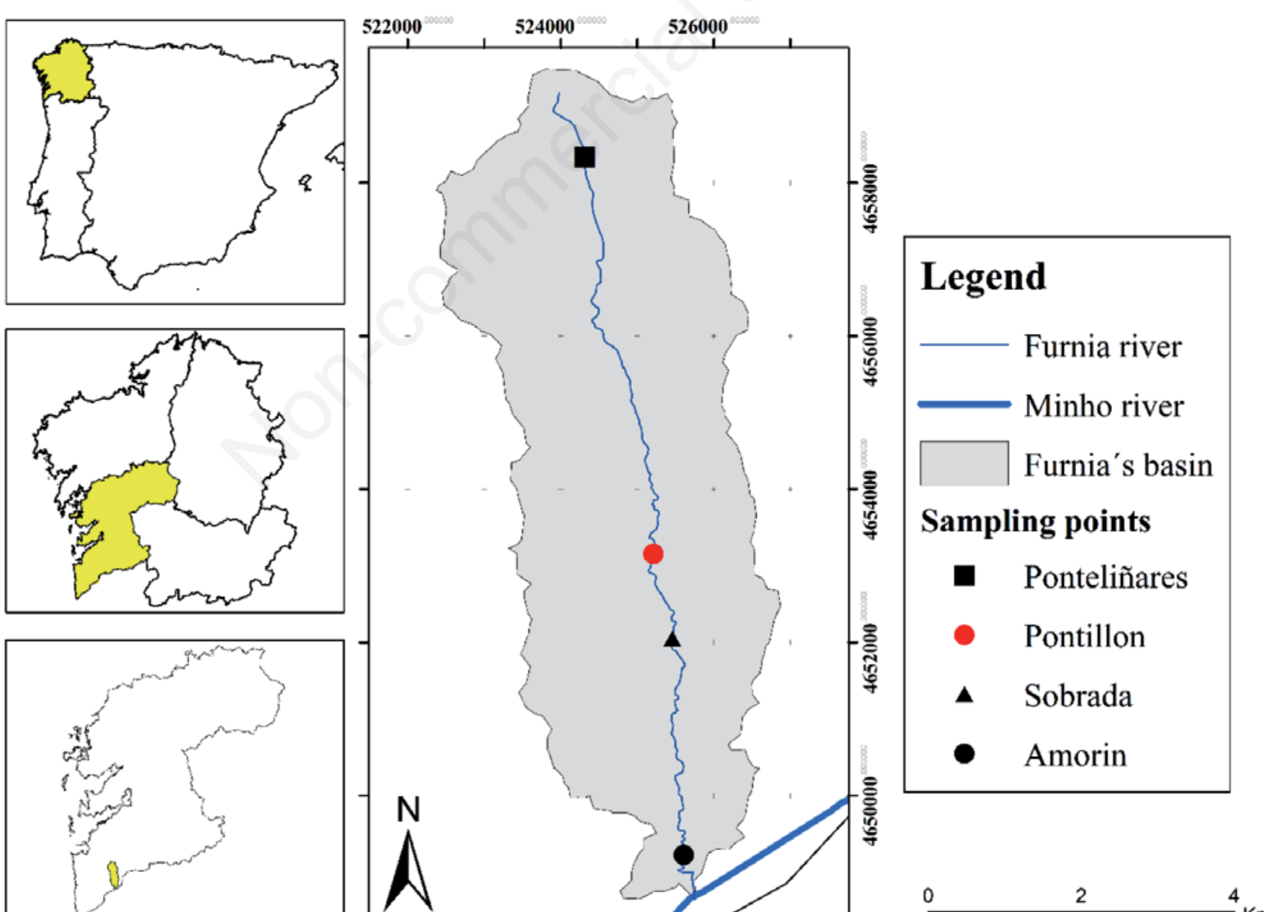

Fig. 1. Furnia River waterbody with the 3 sampling points: F1 (Ponteliñares), F2 (Sobrada) and F3 (Amorín). Also is shown the location of the sewage station (Pontillon).

installed. The river's substrate in this point was mainly composed by rounded boulders of different sizes with abundant macrophyte coverage water (Pérez-Bilbao *et al*., 2012). The second sampling point was downstream of the sewage station, in the medium course of the river with a substrate composed mainly of sand and small boulders, with low coverage of macrophytes. The third sampling site was situated in the lowest course, near the confluence of the Furnia River with the Minho River, and is characterized by a high macrophytes' coverage and a substrate formed mainly by boulders and sand with abundant submerged roots (Pérez-Bilbao *et al*., 2012). The sewage station is located close to the village of Pontillon, between the first and the second sampling points (Fig. 1).

Samples were taken according to the methodology established by the Water Framework Directive (2000/60/EC) (European Communities, 2000), accepted by the River Basin Authority of Minho-Sil (MAGRAMA, 2011; CHMS, 2013). For this study two sampling campaigns were analysed: one in 2008 and another in 2017, both during the spring season. In each sampling point several physical and chemical variables were measured *in situ* by using a multiparameter probe, including water temperature, pH and conductivity. The benthic macroinvertebrates samples were taken with entomological hand nets of 500 µm mesh size. Samples were pooled and saved in 4% formaldehyde solution. All the individuals were identified to family level using a stereomicroscope, as well as different reference works like the taxonomic key of Tachet *et al*. (2003) and the ID-Tax app (Garrido *et al*., 2012).

Data analysis

After the identification, different biological indexes were calculated. The obtained abundances were analysed using the ASTERICS software to calculate the common invertebrate metrics used to measure the ecological quality of the river in each sampling point. On the one hand, the Iberian Biomonitoring Working Party (IBMWP) index (Alba-Tercedor and Sánchez-Ortega, 1988) was calculated to assess the water quality and check the possible differences between the two sampling periods. The obtained score for the IBMWP index was compared to the reference values for the Furnia's River category in order to calculate the Equivalent Quality Ratio (EQR). The average sensitivity of the families found in each site was estimated with the Iberian Average Score per Taxon (IASPT), calculated by dividing the IBMWP score by the family richness in the different sampling points. High values of the IASPT index are indicative of a good quality water bodies containing large numbers of IBMWP's high-scoring taxa (Armitage *et al*., 1983). On the other hand, different diversity indexes were calculated to assess the structure and the composition of the macroinvertebrate communities, so as the possible spatial and temporary variations in them. The applied indexes were richness (S), abundance (N), the EPT (Ephemeroptera, Plecoptera, Trichoptera) index and the Shannon-Wiener diversity index (H'). All of them were calculated through R software. The last index calculated was the rarefied richness (ES), which allows comparing areas where the density of individuals was very different (McCabe and Gotelli, 2000). The values of rarefied richness were calculated for 100 individuals, ES (100), using PAST software. Moreover, and according to Cummins (1974) and Barbour *et al*. (1999), the different families of macroinvertebrates were classified in five trophic groups depending on their feeding strategies (predators, filtering-collectors, gatherers, scrapers and shredders) in order to know if they were in ecological equilibrium.

Relationships between environmental variables, diversity indexes and the macroinvertebrate communities' composition were determined by the Pearson correlation test. Moreover, in order to facilitate comparisons and determine the similarities between sampling points among both years, different management analyses were applied: Principal Component Analysis (PCA) with environmental variables and biological indexes, Non-metric Multidimensional Scaling (NMDS) and Cluster Analysis with biological communities. A direct gradient analysis was not performed due to the lack of joint measurements of biological and environmental data. A permutational univariate analysis of variance (PERMANOVA, one way), based on the Bray Curtis similarity index and 9999 permutations, was used for pairwise testing (for differences between sampling events for total number of individuals, seasonal and annual pattern). All statistical tests were performed using PAST software (Hammer *et al*., 2001).

RESULTS

An amount of 10,859 specimens were identified: 5,229 of them belonging to the 2008 campaign and the remaining 5,630 to the 2017 one. The number of different taxa was substantially higher in 2008 (56 taxa) than in 2017 (31 taxa). In 2008, the insects represented the 89% of the total individuals, being Diptera (47%), Trichoptera (19%) and Ephemeroptera (14%) the most abundant groups, followed to a lesser extent by the crustaceans (9%). Otherwise, in 2017 the insects were more than 94% of the total identified individuals, and the most abundant taxa belonged to the orders of Diptera (47%), Trichoptera (19%) and Ephemeroptera (14%). The relative abundance of the different groups in each year is shown in the Fig. 2 while the different proportion of the trophic groups is shown in Fig. 3.

In terms of richness, in 2008, the best represented order was Trichoptera with individuals from 14 different families, followed by Diptera with 13 families, and Odonata and Ephemeroptera with 4. In 2017, the most diverse group was Ephemeroptera with 6 families, followed by Diptera and

Trichoptera, both represented by 5 families each. In relation to the result of the different index, there is a general decrease in 2017 at all sampling points compared to 2008 (Tab. 1). More specifically, this descent is remarkable in richness (S) and Shannon-Wienner (H') at Sobrada and Amorín, which were the most diverse points in 2008. In fact, in this year Sobrada was the sampling point with highest values for every single index except for abundance and IASPT index. According to the rarefied richness (S) obtained, the highest values ocurred in 2008 samples: Sobrada has the highest rarification value (52.96) followed by Amorin (39.93) and Ponteliñares (36.93). The Shannon-Wiener index (H') revealed that most of the sampling points in Furnia River presented high diversity values

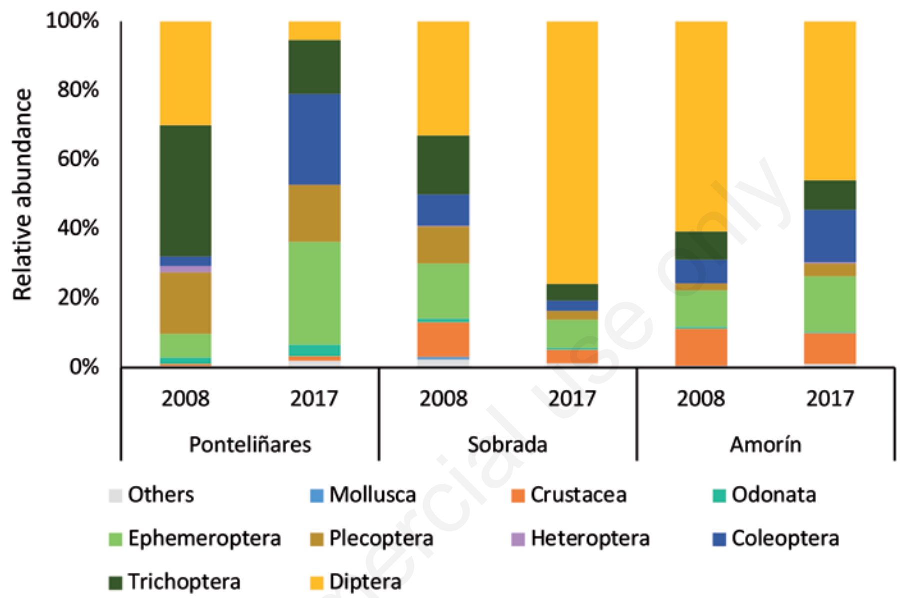

Fig. 2. Relative abundance of the different groups in each sampling point in both years.

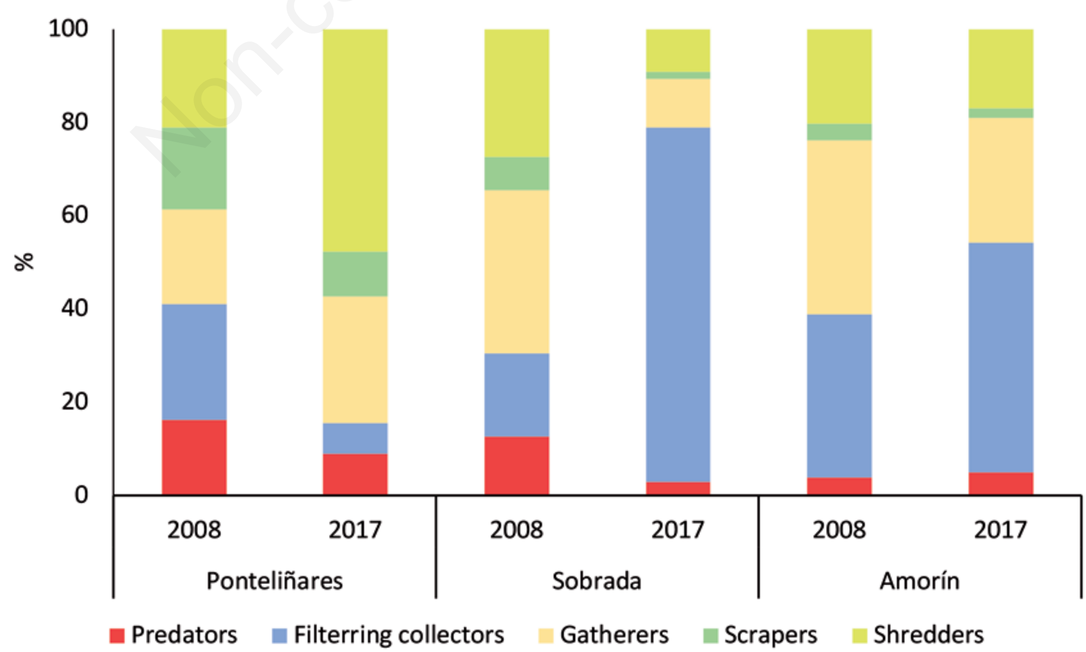

Fig. 3. Relative abundance of the different trophic groups in each sampling point in both years.

(greater than 2 in 66% of the samples). The lowest diversity was recorded in Sobrada in 2017 (1.24) and the highest in Sobrada as well in 2008 (2.90).

Regarding the ecological quality of the water, the IBMWP values shows that all sampling points have a good water quality independently of the year (Tab. 1), since all of them got the highest IBMWP class (Alba-Tercedor and Sánchez-Ortega, 1988). Although the ecological status of the river was still good in 2017, the value of the IBMWP suffered a sharp decrease since 2008 (especially in Sobrada, located downstream of the sewage treatment plant). The obtained results for the EQR index, compared with the reference values (Government of Spain, 2015), also show that most of the sampling points reached the highest ecological class; nevertheless, this index's score indicates a decrease of the water quality from 2008 to 2017.

Tab. 2 shows the value of the different environmental variables measured in the Furnia River for each sampling point (Ponteliñares, Sobrada, and Amorín) in 2008 and 2017. The most variable parameter between both sampling periods was the conductivity, which was higher in the samples of 2017. The Pearson correlation test was performed to assess the relation between the environmental variables and the diversity indexes. Although some of the environmental parameters were correlated with the diversity indexes ($r>0.5$), none of them was statistically significant ($p>0.05$). The result of the Principal Component Analysis (PCA) shows the different sampling points represented in an ordination space according to the physicochemical measurements and biological indexes (Fig. 4). According to this, Sobrada's sampling point (SB) was the most modified site through the years, while the other sampling points remained similar.

One-way PERMANOVA were tested to analyse whether there were differences between sites and between years (PL2008, SB2008, AR2008, PL2017, SB2017, AR2017) and also if there were a combined interaction effect. The matrix included all stations with all the data together in the study, the interaction among them, the effect of the years and the effect of the location. No significant differences among any of the groups were found ($p>0.05$) presented in the one-way PERMANOVA test (Tab. 3).

Tab. 1. Annual indexes values in each sampling site: abundance (N), richness (S), Shannon-Wiener (H´), EPT, Iberian Bio-monitoring Working Party (IBMWP), Iberian Average Score per Taxon (IASPT) and Equivalent Quality Ratio (EQR).

	Ponteliñares		Sobrada		Amorín	
	2008	2017	2008	2017	2008	2017
N	701	546	2811	4108	1717	976
S	37	27	53	26	40	25
H´	2.70	2.60	2.90	1.24	2.13	1.96
EPT	18	13	23	14	19	13
IBMWP	248	185	332	176	269	209
IASPT	6.7	6.85	6.26	7.04	6.72	6.74
EQR	1.10	0.82	1.48	0.78	1.20	0.93

Tab. 2. Environmental parameters measured in Furnia River.

	Ponteliñares		Sobrada		Amorín	
	2008	2017	2008	2017	2008	2017
Temperature (ºC)	13.2	13.7	13.1	14.3	13.1	14
pH	7.5	5.9	7.3	7.2	8.02	7.3
Conductivity (mS/cm)	35.7	51.2	42.2	64.9	47	58.3

Tab. 3. One-way PERMANOVA tests for total abundance of individuals found in all sites considering as factors: years (2008, 2017), locations (upstream, downstream of the sewage station and lowest course) and the interaction location per year (with location corresponding to upstream-downstream sites).

Source	SS	Within-group sum of squares:	F	p
Interaction (location x year)	7,09E+06	4,13E+06	1,073	0,471
Years	7,09E+06	5,73E+06	0,944	0,596
Locations	7,09E+06	4,13E+06	1,073	0,458

The similarity of the sampling points in both dates according to the community composition is represented in Fig. 5 using the nMDS. Thus, the stress obtained with the ordination was 0.02 showing a true presentation of the ordination. Fig. 6 shows the distribution of species according to river basin and location. There was not a clear separation into two groups. The most remarkable aspect is that the differences between the three sampling points were completely reduced through the years. This indicates that the river is getting more uniform over time, but more studies would be necessary to corroborate this fact and the reasons for it.

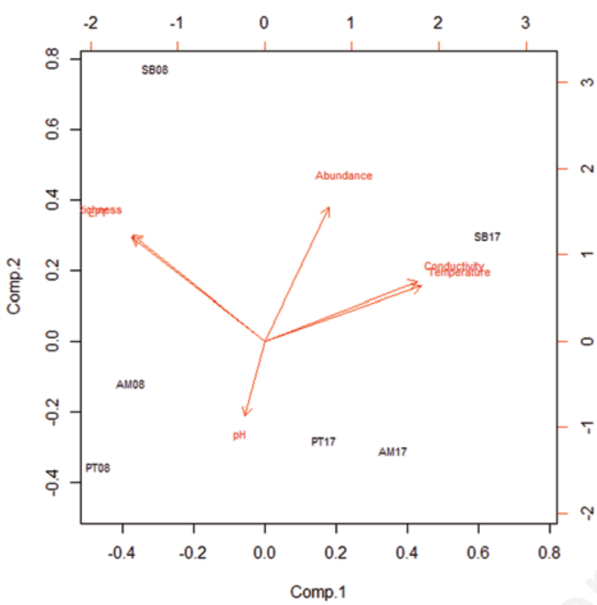

Fig. 4. Results of the Principal Component Analysis (PCA) considering the biological index and the physico-chemical measures obtained.

Although it would be expected that the macroinvertebrate community composition from each sampling point would be more similar among themselves, independently of the sampling dates, the obtained results show that the fauna composition was more similar between the different sampling points located along the river course, especially in 2017, when barely exist differences among sites.

DISCUSSION

Since macrobenthic fauna is considered a good biological indicator, it is remarkable that the fauna abundance was higher in 2008 than 2017 in both Ponteliñares and Amorín, but maximum was observed in 2017 in Sobrada (immediately downstream of the effluent). High abundance in Sobrada in 2017 is bound to the great number of black-fly larvae (Diptera: Simuliidae). Particularly, this family can develop in a wide range of streams, from pristine rivers with high concentration of oxygen to medium-high polluted rivers with low oxygen saturation (Docile *et al*., 2015). In Europe, some species of black-flies were found in rivers with just 10% oxygen saturation, so they can be useful as indicators of organic pollution (Carlsson,

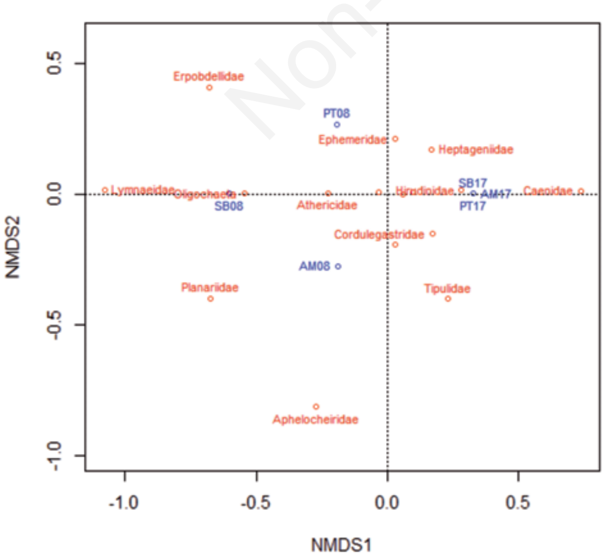

Fig. 5. Non-metric Multidimensional Scaling (NMDS) ordination plot, calculated by using the Jaccard distance and the Ward.D2 method (stress<0.02).

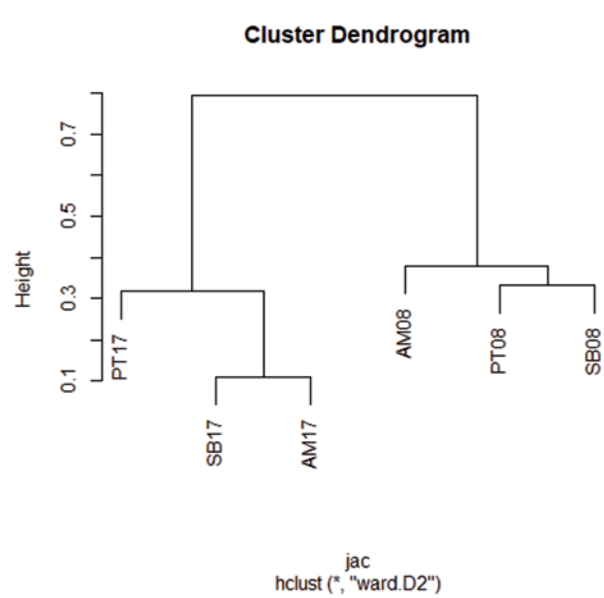

Fig. 6. Hierarchical cluster calculated by using the Jaccard similarity index and the Ward.D2 method.

1967; Bernotiene, 2015). Nevertheless, no significant statistical differences were found between the sampling points and between years ($p>0.05$). This could mean that the STP has a null or even a positive effect on the river, being able to minimize pollution from the discharge of wastewater.

Regarding the physical-chemical measures, an increase of the conductivity was found in all the 2017 sampling points, especially in Sobrada. This fact indicates that Furnia River is suffering an incipient degradation process of its water quality. This is also reflected by the disproportioned relative abundance of the different trophic groups found in Sobrada in 2017 (Fig. 3), since filtering organisms as Simuliidae are much more abundant than the remaining groups (Roy *et al*., 2003). Some authors showed that this kind of relative abundance is common in polluted streams because generalist organisms (filtering collectors) increase its abundance whereas specialist species as predators or shredders decrease themselves (Barbour *et al*., 1999; Ortiz *et al*., 2005; Pérez-Bilbao *et al*., 2013; Xu *et al*., 2014).

The negative effect that the start-up of the sewage treatment plant may be producing to the river is also supported by the diversity indexes calculated. In general, the biodiversity in the river has decreased in 2017, being one of the most common consequences of the different disturbances that affect streams (Strayer and Dudgeon, 2010). The richness in all the sampling points decreased as well, but specially in the points located downstream of the STP's effluent. The total number of taxa found in 2017 in Sobrada and Amorín decreased by 51% and 37.5% respectively compared to 2008, and the EPT index shows that wide range of sensitive species of Ephemeroptera, Plecoptera and Trichoptera has disappeared, being the most affected taxa as consequence of organic pollution (Xu *et al*., 2014). Pollutants from STP might have been affecting the biodiversity of the macroinvertebrates in the stream.

The values of the Shannon-Wienner index also support the suggested hypothesis since in 2008 all the sampling points reached a value greater than 2, whereas in 2017 the H´ only was higher than 2 in Ponteliñares, located upstream of the effluent. Freshwater ecosystems values lower than 2 for this index are associated with low diversity points (Magurran, 1989). The biodiversity loss through the years is represented by the general decrease of the diversity indexes and the water quality values for 2008 to 2017 in each sampling point of Furnia River. The IBMWP index in all the studied sites was higher in 2008; nevertheless, the water quality status stayed the same in 2017, corresponding to the highest category, which includes waterbodies with very clean waters (Alba-Tercedor and Sánchez-Ortega, 1988). The result is different considering the EQR index, because although all the sampling points experimented a decrease of the index value in 2017, just only in Sobrada implicated a worse status of its water quality category (RDL 817/2015,11 September). In the same line as several studies (Roy *et al*., 2003; Ortiz *et al*., 2005: Feeley *et al*., 2011), it was found that the most affected point by the STP inputs is the located immediately downstream of the effluent point, which in this case is Sobrada. However, the river's self-purification capacity allows to recover the water quality along the path. Likewise, Furnia River could be exposed as well to other perturbations that might affect the whole basin, since as the biodiversity loss also affected the sampling point located upstream of the effluent. Influence of meteorological factors in different years was not considered for the study; however it is a factor that might have affected heavily the river discharge and the macroinvertebrate response.

Furnia's river basin is occupied by a great extension of grape crops and other kind of agricultural production zones. There are many water abstractions, hydrological alterations and exotic species documented as possible source of pressure in the basin (MigraMinhoProject, http://migraminho.org/?lang=pt-pt). Miller *et al*. (2007) showed that benthic macroinvertebrate communities can be altered by the irrigation water withdrawal. In this way, increasing the taxa redundancy and resilience of the ecosystem it is necessary to guarantee a good conservation status of the invertebrate community what would make the stream more resistant to the different disturbances (Canobbio *et al*., 2009; Vidal *et al*., 2014). These results coincide with those obtained by other authors mentioned previously (Pérez-Bilbao *et al*., 2012).

CONCLUSIONS

The results show that Furnia River keeps a good quality water in all the sampling points considering the IBMWP index. The EQR index indicates that water quality in Sobrada in 2017 was worse than in 2008. Nevertheless, statistically significant differences were not found between the two sampling campaigns nor between the studied sites. The results obtained in this work prove that effluents from WWTP not always produce degradation process in the streams. The self-depuration capacity of the river allows a recovery of water quality and macroinvertebrate community downstream of the effluent. Nevertheless, the river's biodiversity decreased in 2017 in all the sampling points comparing to the previous results from 2008, what seems to indicate that other listed disturbances are probably affecting Furnia River. Thus, it would be necessary to carry out future studies in the river, including more physicochemical, hydro morphological and land use parameters to deeply analyse the possible causes that are threatening the good conservation of the Furnia River.

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