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Effects of thermal pollution on benthic macroinvertebrate communities of a large Mediterranean River

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Abstract
The influence of a thermal discharge caused by the cooling system of a nuclear power station on benthic macroinvertebrate communities was assessed at the lower Ebro River (in Spain). Surveys collected from natural and artificial substrata and conducted at sites before and after the effluent were analyzed and in order to assess changes in community structure, Non-metrical Multidimensional Scaling (NMDS), Similarity Percentage Analysis (SIMPER) and 1-way Analysis of Similarities (ANOSIM) were performed. The relationship between macroinvertebrate assemblages and environmental variables was assessed with a multivariate distance-based linear regression model (DISTLM) and the model was visualized through a redundancy analysis (dbRDA). NMDS ordination was obtained with a stress of 0.12 and 0.11 for natural and artificial substrates, respectively. ANOSIM showed significant differences between Control and Impacted sites ($p<0.05$). Simper analysis showed that the mean dissimilarity between Control and Impacted sites was of 68.37 % for natural substrate and of 39.97 % for artificial substrate. DISTLM selected a set of explanatory variables (temperature difference, depth, total nitrogen and conductivity) with an 82.58 % of fitted variation for natural substrate, while for artificial substrate, the set of variables selected were temperature difference, total phosphorus and chlorophyll with an 83.13 % of fitted variation.

Macroinvertebrates assemblages showed sensitivity to thermal changes both in natural and artificial substrata, even though warming did not exceed 3 ºC. Factors that seemed to influence benthic macroinvertebrate assemblages the most were the thermal increase caused by the nuclear power station and seasonal variation in nutrients and conductivity.

Keywords: benthic macroinvertebrates, Ebro river, thermal pollution, nuclear

1. Introduction

Macroinvertebrates are commonly used in biomonitoring and are considered useful indicators of environmental alterations [1, 2, 3, 4]. Many groups are ubiquitous, which allows comparison between systems [5, 6] and although they are patchily distributed [7], the collection and identification is relatively easy. Many macroinvertebrates are rapid colonisers, which allow to identify environmental changes in short periods of time, while others have long life cycles (e.g, mussels), integrating environmental conditions over time.

Temperature has a significant role in most life traits and in several physiological functions of organisms; temperature also influences the morphology, physiology, behavior, growth, reproduction, and distribution of species [8, 9, 10]. Some potential effects of increasing temperature on species are higher rates of reproduction and growth, faster development, and shorter generation times [11, 12, 13]. Additionally, numerous changes in aquatic ecosystems have been recorded as consequence of increased temperature, which include: enhanced organic matter decomposition and nutrient cycling, increased primary production, longer growing seasons and reduced habitat for species of cool water [14]. Changes in community structure as response to thermal disturbances have been detected even with a temperature alteration of few degrees centigrade [15].

To generate thermal power, nuclear power stations use nuclear fission to heat water and drive steam turbines that then produce electricity; but this process requires large volumes of water for its cooling system in order to remove the waste heat produced. The increase in river water temperature caused by these thermal discharges has showed to alter biological and ecological components of aquatic ecosystems [16, 17, 18].

Nevertheless, the effects on biological communities can vary depending on the biological features of the environment and on the levels and quantity of heated discharge. Depending on the design and the operating units of the power plants, water temperature in effluent sites can increase by as much as 8 ºC [19]. However, in Europe, legislation requires that the temperature downstream of the effluent should not increase by more than 3ºC [20].

Many authors have studied the ecological effects of temperature in aquatic environments [17, 21, 22], and several have assessed the impact of thermal effluents on benthic communities [12, 23, 24, 25, 26]. Some studies have also analyzed the implications of climate change on macroinvertebrate assemblages in Mediterranean climate regions worldwide, and strong effects as a consequence of climatic variability are expected [27, 28, 29]. However, literature dealing with the effects of thermal pollution on benthic communities of large Mediterranean rivers is scarce, even though this type of alteration is frequent in the watersheds of the Mediterranean basin.

The aim of this study was to assess changes in the community structure of benthic macroinvertebrates inhabiting a river section influenced by the presence of a nuclear power station (Ascó NPS). This is one of the main anthropogenic factors exerting pressure on the lower Ebro River and has been subjecting the river to a sustained heating during the last 30 years, therefore providing an excellent opportunity for assessing the long-term effects of water warming on benthic communities.

2. Materials and Methods

2.1 Study Area

The Ebro is the Spanish River with the highest mean annual flow and one of the most important tributaries to the Mediterranean Sea. It is located in the NE of the Iberian Peninsula (Fig. 1), and its basin has a surface of 85 534 km^2 with a length of 928 km. The river flow is regulated by nearly 190 dams.

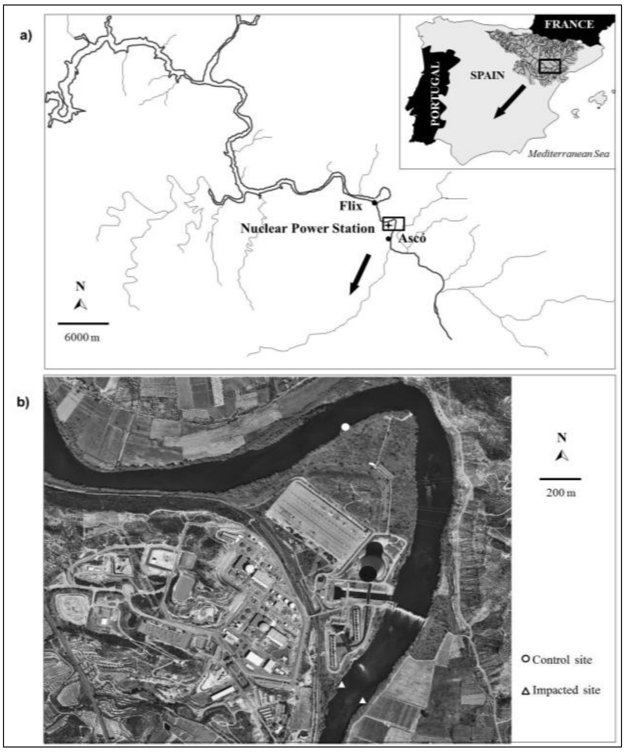


Fig 1: a) Map of the lower Ebro River showing the study area, b) location of sampling sites.

The lower part is regulated by two large reservoirs (Mequinensa with a capacity of 1534 hm^3 and Riba-roja with a capacity of 207 hm^3) built in 1964 and 1969 respectively for hydropower purposes. Downstream at Flix it is located a smaller reservoir with a capacity of 11.4 hm^3.

The Ascó nuclear power station is located on the right margin of the lower Ebro River, 10 km downstream the Flix dam, between Ascó and Flix towns, and at about 110 km from the river mouth (Fig. 1a). The power station was built in 1984 and has two reactors with a high electrical power output of about 2050 MWe and a thermal reactor power of about 5900 MWt. (data available at http://www.anav.es). The power station has a concession of 72.3 m^3/s of the Ebro's flow for its cooling system, and a weir was built to collect the river water to the condensers. After its use the water is returned to the river with an average thermal increase of 3 ºC [30].

The river at the study area has a total length of 2 km that comprise 1 km before and after the nuclear power station (41°12'0"N, 0°34'10"E), a mean width of 140 m and the substrate is dominated by gravel. At the lower Ebro River, studies about macroinvertebrate assemblages are scarce [31, 32].

2.2 Macroinvertebrate sampling and preparation

In order to compare benthic community features of a site non-impacted by the heated effluent with those under its influence, surveys at sites located before and after the effluent were conducted, and to minimize the potential influence of substrate heterogeneity, artificial substrates deployed over the same temperature gradient than natural surfaces were also analyzed.

Three sampling sites were selected: a control site (C), located upstream the nuclear power station, and two impacted sites (I1 and I2) covering the thermal plume, located downstream of the effluent outlet, on the right and left river margins respectively (Fig. 1b).

Substratum composition analysis of the sampling sites was based on Wentworth [33] scale according to the following fractions: fine gravel (0-8 mm), medium gravel (9-16 mm), coarse gravel (17-32 mm) and pebble (33-64 mm). Artificial substrates were built with a composition of 1 kg of fine gravel, 4 kg of medium gravel, 4 kg of coarse gravel and 1 kg of pebbles placed within a polypropylene bag with rectangular mesh (5 mm) with a bottom cover of 0.25 mm to avoid the loss of specimens.

Three sampling campaigns were conducted in August, October and December of 2013; and in every occasion, three replicates were collected at each site from natural and artificial substrata.

Surveys from the natural substrate were collected in the littoral zone using a Surber net (32x32 cm) with a mesh size of 500 μm; the riverbed was disturbed for 1 minute and the subsequent sample was deposited in a tray in order to pick up the attached fauna. Artificial substrates remained during a colonization period of 6 weeks and then were carefully extracted, washed and sieved in order to collect the specimens.

Invertebrate samples were preserved in 4% formaldehyde and taken to the laboratory to be sorted and identified under a stereomicroscope, according to Tachet *et al.* [34]. During the summer campaign the artificial substrates placed on site I1 were not recovered due to vandalism.

Physicochemical data were recorded for every sampling site and occasion. A YSI 556 multi-parameter probe was used to measure dissolved oxygen (mg/l), oxygen saturation (%), pH, salinity (ppt) and conductivity (mS/cm); current velocity at

60% of total water depth was recorded with a Braystoke BFM 001 current meter; total dissolved nitrogen (TDN), total nitrogen (TN), soluble reactive phosphorus (SRP), total phosphorus (TP) were measured according to Koroleff [35, 36]; and the total chlorophyll concentration was calculate using the colorimetric method [37]. At every sampling site, water temperature (°C) was monitored at intervals of 30 minutes during all the study period with a TCtemp1000 Madgetech data logger.

2.3 Data Analysis

Water temperature values recorded over the study period were analyzed to identify variations and trends, the difference of temperature between control and impacted sites was calculated (Diff_T) and the temperature variation at each site was represented by the standard deviation values (TempSD).

Differences in values of environmental variables between sites were tested by analysis of variance (ANOVA) with Tukey post hoc test performed using software SPSS 19 (SPSS Inc, Chicago, IL, USA).

Macroinvertebrate abundance is presented as relative percentages and it was square-root transformed in order to reduce the effect of highly variable population densities on ordination scores. All environmental variables that expressed concentration were logarithmically transformed before analysis to avoid skewed distributions.

Descriptive community parameters were calculated: Richness (S), Shannon-Wiener's diversity index (H', as $\log_e$) and Pielou's evenness index (J').

Sites were ordered in relation to their species composition using Non-metric Multidimensional Scaling (NMDS) and significant differences were identified using 1-way Analysis of Similarities test (ANOSIM), that hypothesizes for differences between groups of samples (defined a priori) through randomization methods on a resemblance matrix; ANOSIM provides an *R* statistic value that reflects the amount of dissimilarity associated with each group; *R* values close to one indicate very different composition, whereas values near to zero indicate little difference. Then, in order to identify resemblances between sample groups and to identify taxa that contributed to dissimilarity among sites, a Similarity Percentage Analysis (SIMPER) was performed.

Finally, the relationship between macroinvertebrate assemblages and environmental variables was assessed with a multivariate distanced-based linear regression model (DISTLM) [38] and a set of explanatory variables was identified. The model was visualized through a distance-based redundancy analysis (dbRDA) performed using PRIMER V6 software [39] with the add-on package PERMANOVA+ [40].

3. Results

3.1 Environmental characteristics

The average values for physicochemical parameters measured at each sampling site are shown in Table 1. Water temperature showed permanent higher values at impacted sites (Fig. 2) as consequence of the water heating produced by the cooling system of the nuclear power station, and showed significantly differences between control and impacted sites (ANOVA p=0.008) (C≠ I1, C≠I2, I1=I2). The mean values recorded over the study period were 20.54 °C (C), 23.04 °C (I1) and 22.98 °C (I2); while the mean difference of temperature recorded between C and I1 was 2.39 °C and 2.33 °C between C and I2. Water velocity showed mean values of 0.26 m/s at control site, and 0.13 m/s and 0.11 m/s at I1and I2 respectively; significant differences between control and impacted sites were found (ANOVA p=0.000) (C≠ I1,C≠I2, I1=I2). The other environmental variables measured (dissolved oxygen, pH, conductivity, soluble reactive phosphorus, total phosphorus, total dissolved nitrogen, total nitrogen and depth) showed no or only minor variation and did not present significant differences between sites.

Table 1: Values of physicochemical parameters measured at each sampling site. (T = temperature, Diff. T = temperature difference, TempSD = temperature variability, DO = dissolved oxygen, Cond = conductivity, SPR = soluble reactive phosphate, TP = total phosphorus, TDN = total dissolved nitrogen, TN = total nitrogen, Chl a = chlorophyll a).

	T (°C)	**Diff. T (°C)**	**TempSD (°C)**	**pH**	**DO (mg/l)**	**Cond (mS/cm)**	**SRP (µg/l)**	**TP (µg/l)**	**TDN (µg/l)**	**TN (µg/l)**	**Chl a (µg/l)**	**Depth (m)**	**Velocity (m/s)**	**Pebble (%)**	**Coarse gravel (%)**	**Medium gravel (%)**	**Fine gravel (%)**
Summer																	
C	22.30	0.0	0.41	8.1	8.46	0.84	46.6	381.0	1479.4	2457.6	2.95	0.83	0.18	12	38	34	16
I1	24.78	2.5	0.37	8.0	6.89	0.90	53.0	369.5	1400.8	2403.4	0.95	0.65	0.12	14	36	38	12
I2	24.55	2.3	0.36	8.0	6.71	0.89	36.7	598.7	1430.8	2111.7	1.29	0.78	0.07	13	37	36	14
Autumn																	
C	21.10	0.0	0.34	7.8	6.96	1.15	37.8	341.6	1337.4	2251.4	2.46	0.89	0.28	12	38	34	16
I1	23.57	2.5	0.41	7.9	6.73	1.15	32.9	195.8	1319.4	1999.9	1.95	0.66	0.12	14	36	38	12
I2	23.62	2.5	0.58	8.0	7.71	1.16	35.9	196.7	1376.4	2114.9	1.56	0.73	0.07	13	37	36	14
Winter																	
C	18.23	0.0	0.44	8.1	10.23	1.20	29.6	111.5	1587.7	3116.8	0.32	0.91	0.31	12	38	34	16
I1	20.76	2.2	0.51	8.0	9.30	1.21	34.6	196.1	1712.2	3120.4	0.83	0.90	0.16	14	36	38	12
I2	20.76	2.2	0.70	8.1	9.33	1.31	31.8	241.0	1522.5	3008.8	0.67	0.89	0.20	13	37	36	14

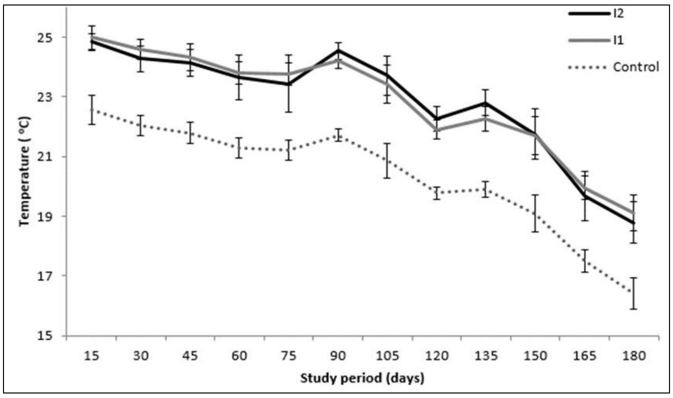


Fig 2: Water temperature recorded over the study period at control (C) and impacted (I1, I2) sites.

3.2. Macroinvertebrate assemblages

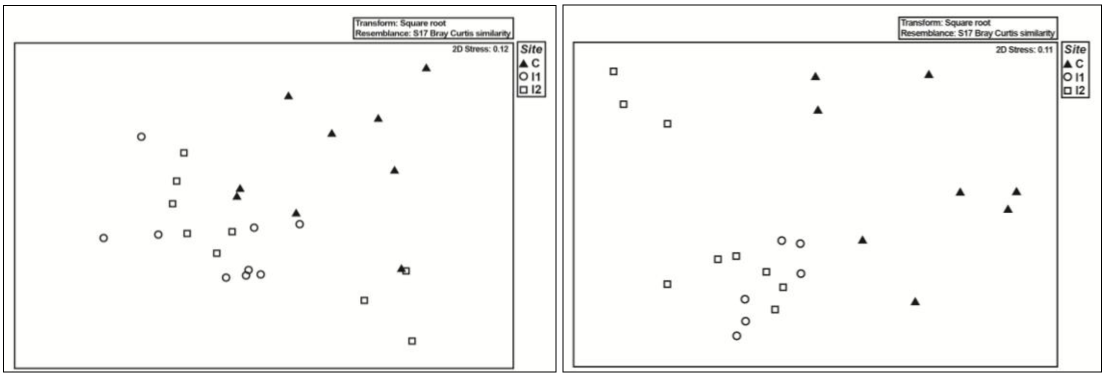
During the study period a total of 37 and 46 taxa were found in natural and artificial substrate, respectively (Appendix 1). Arthropoda was the dominant phylum and accounted for 41.73% of the total abundance. The most abundant taxa found in natural substrate were Nemertea (17.58%), *Corbicula* (14.41%) and Chironomidae (12.51%); while in artificial substrate were Dugesiidae (29.27%), Nemertea (14.09%) and Chironomidae (12.58%).

Seasonal changes were observed in the macroinvertebrate community along the study period. In natural substrate assemblages, Nemertea was the dominant taxa, sharing this dominance with Chironomidae and Ostracoda in summer; and with *Corbicula* in autumn and winter. While, artificial substrate assemblages were dominated by Dugesiidae, sharing the dominance with Chironomidae and Baetidae in summer, with Chironomidae and *Corbicula* in autumn, and with Nemertea and Hydropsychidae in winter.

Community descriptive parameters showed no significant differences (ANOVA $p>0.05$) between control and impacted sites.

The NMDS ordination (Fig. 3) displays the spatial distribution of the control (C) and impacted sites (I1, I2); the obtained stress value was 0.12 and 0.11 for natural and artificial substrata, respectively. For both types of substrate, the assemblage composition was analyzed with ANOSIM and showed significant differences between Control and Impacted sites (Table 2).

The Simper analysis (Appendix 2) for natural substrate assemblages showed that the mean dissimilarity between control and impacted sites was 68.37% and *Caenis*, Chironomidae and Nemertea were the taxa with highest percentage of contribution to dissimilarity between groups. While for artificial substrate, the mean dissimilarly was 39.97% and the taxa with the highest contribution were Chironomidae, *Corbicula*, Hydropsychidae, Nemertea, Dugesiidae, Gammaridae, Oligochaeta, and *Theodoxus fluviatilis*.



a) b)

Fig 3: Two dimensional NMDS plots based on Bray-Curtis similarities of square-root transformed macroinvertebrate abundance data. a) Natural substrate ordination. b) Artificial substrate ordination.

Table 2: Values of R statistic and significance level of differences between control (C) and impacted (I) groups, obtained by ANOSIM test for macroinvertebrate communities of natural and artificial substrata.

Groups	R statistic	Significance	
Natural Substrate			
Control, Impacted	0.304	0.003	**
Artificial Substrate			
Control, Impacted	0.484	0.001	***

Significance: * $p \leq 0{,}05$; ** $p \leq 0{,}01$; *** $p \leq 0.001$

The dbRDA analysis performed for natural substrate (Fig. 4), revealed that the set of variables selected by the DISTLM (Tº difference, depth, total nitrogen and conductivity) explained 82.58 % of fitted variation and 45.44 % of total variation in the two first axes; while the dbRDA performed on artificial substrate (Fig. 5), revealed that the set of variables selected by the DISTLM (Tº difference, total phosphorus and chlorophyll) explained 83.13 % of fitted variation and 37.13 % of total variation in the first two axes.

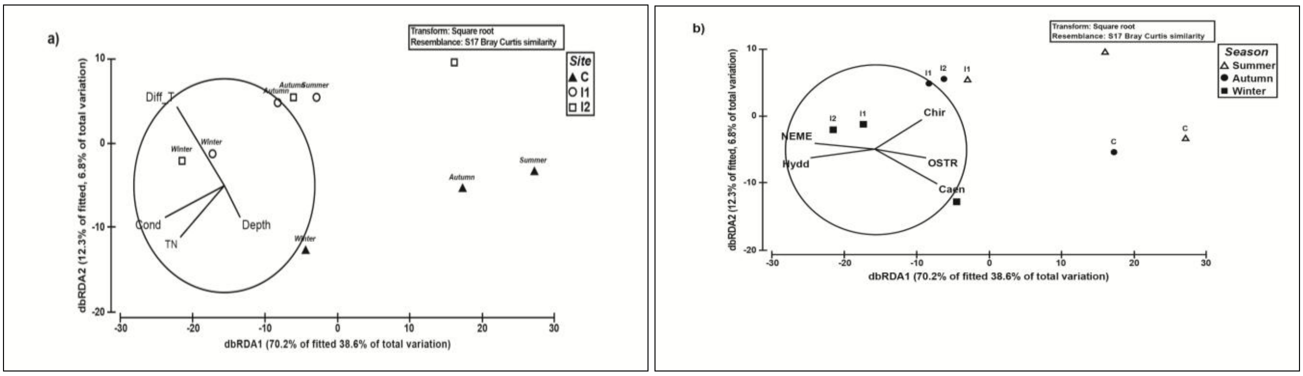


Fig 4: Distance-based Redundancy Analysis (dbRDA) ordination of natural substrate data: a) samples displayed by site and season and vectors showing correlation between explaining variables and dbRDA axes; b) Samples displayed by season and site and vectors showing correlation between the five taxa with highest contribution to the dissimilarity between control and impacted sites and dbRDA axes. (Chir = Chironomidae, OSTR = Ostracoda, Caen = Caenis, Hydd = Hydridae, NEME = Nemertea).

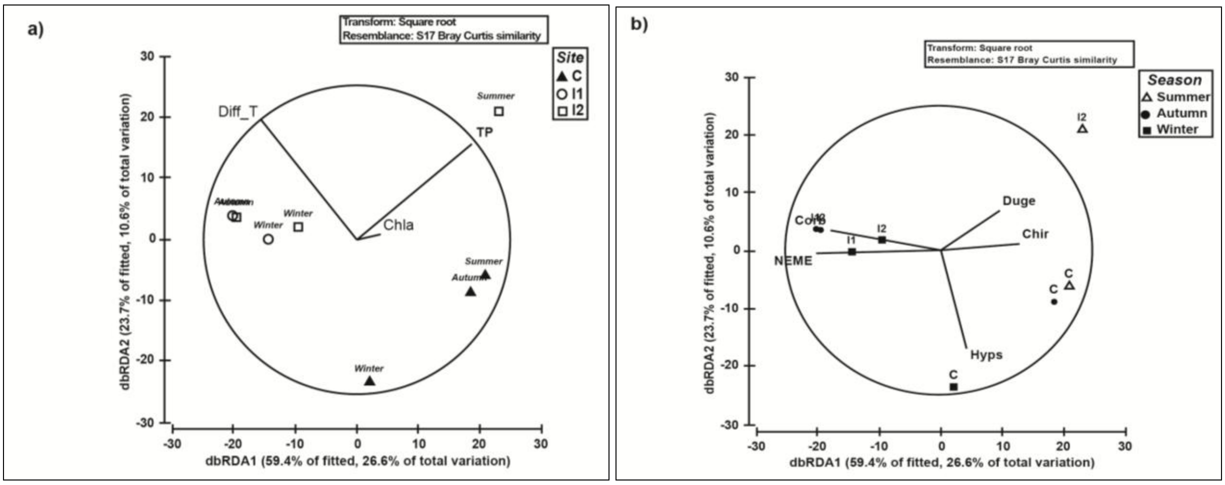


Fig 5: Distance-based Redundancy Analysis (dbRDA) ordination of artificial substrate data: a) samples displayed by site and season and vectors showing correlation between explaining variables and dbRDA axes; b) samples displayed by season and site and vectors showing correlation between the five taxa with highest contribution to the dissimilarity between control and impacted sites and dbRDA axes. (Duge = Dugesiidae, Chir = Chironomidae, Hyps = Hydropsychidae, NEME = Nemertea, Corb = Corbicula).

The first axis of the dbRDA plots of both natural (Fig. 4) and artificial (Fig. 5) substrata distinguished samples from control and impacted sites and in both cases the axis was strongly correlated with the difference in water temperature caused by Ascó nuclear power station. The second dbRDA axis, also of both natural (Fig. 4) and artificial (Fig. 5) substrata, was strongly correlated with a gradient of temperature and nutrient levels (total nitrogen for natural substrate and total phosphorus for artificial substrate) associated with the seasonal variation in the fluvial system. The five taxa with the highest contribution to the dissimilarity between control and impacted sites are represented in the dbRDA plots (Figs 4 and 5).

4. Discussion

The macroinvertebrate community of the lower Ebro River has been influenced by a sustained increase in water temperature over the last 30 years due to the presence of the Ascó nuclear power station. Results showed that the macroinvertebrate communities proved to be sensitive to water warming even though this alteration did not exceed 3 ºC. Most of the measured environmental variables did not differ between control and impacted sites; therefore, the differences detected in macroinvertebrate assemblages could be mostly attributable to the warming effect, either by its direct influence or by its interaction with other functional processes. These results are in agreement with other similar studies analyzing the consequences of thermal alteration on benthic assemblages [18, 26, 41, 42]. Although thermal pollution has often adverse effects on macroinvertebrates [23, 26, 42, 43], increases in abundances and higher values of richness have been also detected in other studies [44, 45, 46, 47]; however, it does not mean that these effects could be considered positive, since they evidence an alteration in the community structure, and mainly due to the lack of information about reference conditions in rivers.

Unlike other studies on the effect of thermal effluent plumes on macroinvertebrates in which the loss of stenothermic organisms reduces taxonomic diversity, while a few species become dominant [26, 41, 42], we found that diversity did not decrease due to thermal pollution. This can be explained by the structure of the macroinvertebrate community inhabiting the study area, which is characterized by tolerant taxa such as Oligochaeta, Chironomidae, Dugesiidae, Nemertea and *Corbicula*, reflecting the habitat degradation of the lower Ebro caused by decades of alteration in nutrient levels, the presence of large dams upstream and the existence of polluted sediments at the Flix reservoir [48, 49, 50]. Therefore, the community evidenced a previous level of affectation, which made difficult to detect significant changes in community diversity metrix.

Our results did not show a significant variation in the species pool and we only found slightly higher values in species richness and diversity indices at impacted sites where water temperature never exceeded 25 ºC. Higher water temperatures that do not reach macroinvertebrate lethal limits, approximately 32ºC, may enrich the community [51, 52].

Significant changes in community composition were detected between control and impact sites, and although temperature is not the only variable influencing the community, our results evidenced that warming is a determinant factor either through a direct effect or increasing other indirect effects on the structure of benthic communities.

Our results did not allow attributing the observed changes in community structure solely to the temperature alteration, but evidenced that warming is a determinant factor either through a direct effect or enhancing other indirect effects on the structure of benthic communities.

Higher water temperatures enhance the colonization of alien macroinvertebrate species [53], and experimental studies indicate a wider tolerance range and thus a higher competitive ability of non-indigenous species to water temperature in comparison with native species [54]. Thus, it is expected an increase of alien species in both richness and abundance in ecosystems where there is a water temperature increase. This phenomenon has been predicted to occur as a consequence of climate change in alpine streams [6, 55] and in marine intertidal systems [56], and could occur also in freshwater thermal plumes. In this study, nonnative taxa as *Corbicula* and *Physella* seems to thrive in the heated water, and abundances recorded were higher at impacted sites; these occurrences also have been previously documented in studies conducted in artificial thermal plumes [18, 57].

Natural and artificial substrata provided essentially the same picture of thermal influence, this agrees with several works where artificial substrata have proven their usefulness for the assessment of riverine ecosystems [58, 59].

We detected changes in macroinvertebrate community structure after a prolonged exposition to higher temperatures and our results agree with other studies carried out in rivers of Mediterranean climate regions [27, 28, 29], where significant responses in benthic communities related to long-term temperature increases have been identified. In one of them, Bonada *et al.* [27]**,** dealing with taxonomic and trait differences of macroinvertebrate assemblages, noted that climate change in Mediterranean climate regions may result in large changes in taxonomic composition; similarly, Daufresne *et al.* [28] observed gradual changes in macroinvertebrate community structure under climate change conditions, attributable to high temperatures associated with decreasing oxygen contents; and Lawrence *et al.* [29] also found significant differences in benthic communities and developed an indicator based on macroinvertebrate taxa to monitor the climate change effects.

Since Mediterranean region is going to be among the most impacted ones by climate change, and in particular by global warming [60], the challenge of understanding the consequences of warming on biodiversity remains as a main research subject in this region. A substantial warming (≈1.5ºC in winter and ≈2ºC in summer) might affect the Mediterranean region in the 2021-2050 period compared to the reference period (1961-1990), in an A1B emission scenario [60]. Consequently, the thermal gradient caused by the nuclear power station could provides an opportunity to contribute in the prediction of changes in benthic communities under global warming scenarios, minimizing in some way the difficulties that usually have field experimentation on warming effects.

However, it is difficult to predict the isolated effects of temperature on benthic communities because alterations in aquatic freshwater ecosystems are complex and may vary greatly as a function of climatic, hydrological and biological features of each study area. Furthermore, it is needed to note that invertebrate distributions are not only constrained by a maximum temperature, but rather by a long-term accumulated range of temperature [61, 62].

The information generated here could be useful to the better understanding of the warming effects on benthic communities of large Mediterranean rivers and hence could provide a baseline data for assessing the effects of warming under future projected scenarios.

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6. References

1. Chessman B, Williams S, Besley C. Bioassessment of streams with macroinvertebrates effect of sampled habitat and taxonomic resolution. Journal of the North American Benthological Society, 2007; 26(3):546-565.
2. Hellawell JM. Water and Man. In Biological indicators of freshwater pollution and environmental management. Springer Dordrecht, 1986, 456.
3. Hilsenhoff WL. An improved biotic index of organic stream pollution. Great Lakes Entomologist, 1987; 20:31-40.
4. Rosenberg DM, Resh VH. Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York, 1993, 488.
5. Allan JD, Castillo MM. Stream ecology. 2nd edition, Springer, New York, 2007, 430.
6. Hauer FR, Lamberti GA. Methods in stream ecology. Elsevier Inc, Oxford UK, 2011, 877.
7. Covich AP, Palmer MA, Crowl TA. The role of benthic invertebrate species in freshwater ecosystems: Zoobenthic species influence energy flows and nutrient cycling. Bioscience. 1999; 49(2,1):119-127.
8. Kishi D, Murakami M, Nakano S, Maekawa K. Water temperature determines strength of top-down control in a stream food web. Freshwater Biology. 2005; 50:1315-1322.
9. Stanford JA, Ward JV. Insect species diversity as a function of environmental variability and disturbance in stream systems. In: Barnes JR, Minshall GW. (Eds.), Stream Ecology Application and testing of general ecological theory. Springer, New York, 1983, 265-278.
10. Sweeney BW, Vannote RL. Influence of food quality and temperature on life history characteristics of the parthenogenetic mayfly, Cloeon triangulifer. Freshwater Biology, 1984; 14:621-630.
11. Arnell NW. Climate change and water resources in Britain. Climatic Change, 1998; 39:83-110.
12. Arthur JW, Zischke JA, Ericksen GL. Effect of elevated water temperature on macroinvertebrate communities in outdoor experimental channels. Water Research. 1982; 16(10):1465-1477.
13. Hughes L. Biological consequences of global warming is the signal already apparent?. Trends in Ecology and Evolution. 2000; 15(2):56-61.
14. Mulholland PJ, Best GR, Coutant CC, Hornberger GM, Meyer JL, Robinson PJ *et al*. Effects of climate change on freshwater ecosystems of the south- eastern United States and the Gulf Coast of Mexico. Hydrological Processes. 1997; 11(8):949-970.
15. Kaushal SS, Likens GE, Jaworski NA, Pace ML, Sides AM, Seekell D *et al*. Rising stream and river temperatures in the United States. Frontiers in Ecology

and the Environment. 2010; 8(9):461-466.
16. Caissie D. The thermal regime of rivers a review. Freshwater Biology, 2006; 51(8):1389-1406.
17. De Vries P, Tamis JE, Murk AJ, Smit GD. Development and application of a species sensitivity distribution for temperature-induced mortality in the aquatic environment. Environmental Toxicology and Chemistry. 2008; 27(12):2591-2598.
18. Langford TE. Ecological effects of thermal discharges. Elsevier, London, 1990, 426.
19. Laws EA. Aquatic pollution an introductory text. 2nd edn, John Wiley and Sons, New York, 1993, 740.
20. European Union. Directive 2006/44/EC of the European Parliament and of the Council of 6 September 2006, on the quality of fresh waters needing protection or improvement in order to support fish life. Official Journal of the European Communities. 2006; L264:20-31.
21. Hawkes HA. Ecological changes of applied significance induced by the discharge of heated waters. In Parker FL, Krenkel PA (Eds.), Engineering aspects of thermal pollution. Vanderbilt University Press, Nashville Tennessee, 1969, 15-57.
22. Verones F, Hanafiah MM, Pfister S, Huijbregts AJ, Pelletier GJ, Koehler A *et al* Characterization factors for thermal pollution in freshwater aquatic environments. Environmental Science & Technology, 2010; 44(24):9364-9369.
23. Durrett CW, Pearson WD. Drift of macroinvertebrates in a channel carrying heated water from a power plant. Hydrobiologia, 1975; 46(1):33-43.
24. Langford TE. Comparative assessment of thermal effects in some British and North American rivers. New York Academic Press, UK, 1972, 319-351.
25. Snoeijs JM, Prentice IC. Effects of cooling water discharge on the structure and dynamics of epilithic algal communities in the northern Baltic. Hydrobiologia, 1989; 184(1-2):99-123.
26. Wellborn GA, Robinson JV. Effects of a thermal effluent on macroinvertebrates in a central Texas reservoir. American Midland Naturalist. 1996; 136:110-120.
27. Bonada N, Doledec S, Statzner B. Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios. Global Change Biology, 2007; 13(8):1658-1671.
28. Daufresne M, Bady P, Fruget JF. Impacts of global changes and extreme hydroclimatic events on macroinvertebrate community structures in the French Rhône River. Oecologia. 2007; 151(3):544-559.
29. Lawrence JE, Lunde KB, Mazor RD, Beche LA, Mcelravy EP, Resh VH. Long-term macroinvertebrate responses to climate change: implications for biological assessment in mediterranean-climate streams. Journal of the North American Benthological Society. 2010; 29(4):1424-1440.
30. Prats J, Val R, Armengol J, Dolz J. Temporal variability in the thermal regime of the lower Ebro River (Spain) and alteration due to anthropogenic factors. Journal of Hydrology. 2010; 387(1):105–118.
31. Cid N, Ibáñez C, Prat N. Life history and production of the burrowing mayfly Ephoron virgo Olivier, 1791 Ephemeroptera Polymitarcyidae in the lower Ebro river a comparison after 18 years. Aquatic Insects. 2008; 30(3):163-178.
32. Muñoz I, Prat N. Macroinvertebrate community in the lower Ebro river (NE Spain). Hydrobiologia. 1994; 286(2):65-78.
33. Wentworth CK. A scale of grade and class terms for clastic sediments. The Journal of Geology. 1922; 30(5):377-392.
34. Tachet H, Richoux P, Bournaud M, Usseglio-Polatera P. Invertébrés d'eau douce: systématique, biologie, écologie. CNRS éditions, Paris, 2000, 588.
35. Koroleff F. Simultaneous persulfate oxidation of phosphorus and nitrogen compounds in water. In Grasshoff, K. (Ed.), Report of the Baltic Intercalibration Worshop. Annex Interim Commission for the Protection of the Environment of the Baltic Sea, 1977, 52-53.
36. Koroleff F. Determination of phosphorus. In: Grasshoff, K, Ehrhardt, M, Kremling, K. (Eds.), Methods of Seawater Analysis. Verlag Chemie, 1983, 125-132.
37. Jeffrey S, Humphrey G. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanzen. 1975; 167(2):191-194.
38. Mcardle BH, Anderson MJ. Fitting multivariate models to community data a comment on distance-based redundancy analysis. Ecology, 2001; 82(1):290-297.
39. Clarke KR, Gorley R. PRIMER v6 User manual/tutorial. Primer-E, Plymouth UK Chessman B, Williams S and Besley C. (2007), Bioassessment of streams with macroinvertebrates effect of sampled habitat and taxonomic resolution. Journal of the North American Benthological Society, 2006; 26:546-565.
40. Anderson MJ, Gorley RN, Clarke KR. PERMANOVA+ for PRIMER guide to software and statistical methods. PRIMER-E. Plymouth, UK, 2008.
41. Gibbons JW, Sharitz RR. Thermal ecology environmental teachings of a nuclear reactor site. Bioscience. 1981; 31(4):293-298.
42. Howell F, Gentry J. Effect of thermal effluents from nuclear reactors on species diversity of aquatic insects. In Gibbons JW, Sharits RR, (Eds.), Thermal Ecology. US Atomic Energy Commission. Springfield, Virginia, 1974.
43. Alston DE, Lawrence JM, Bayne DR, Campbell FF. Effects of thermal alteration on macroinvertebrate fauna in three artificial channels. In Thorp JH, Gibbon JW (Eds.), Energy and Environmental stress in aquatic systems. U.S. Department of Energy, Springfield, Virginia, 1978, 569-579.
44. Dahlberg MD, Conyers JC. Winter fauna in a thermal discharge with observations on a macrobenthos sampler. In: Gibbons, JW, Sharits, RR (Eds.), Thermal Ecology. US Atomic Energy Commission, Springfield, Virginia, 1974, 414-442.
45. Dusoge K, Wisniewski RJ. Effects of Heated Waters on Biocenosis of the Moderately Polluted Narew River macrobenthos. Polskie Archiwum Hydrobiologii, 1976; 23(4):539-554.
46. Gibbons JW. Thermal alteration and the enhancement of species populations. In Esch GW, McFarlane RW (Eds.), Thermal ecology II. 27-31, Energy Research and Develpment Administration. Springfield, Virginia, 1976, 27-31.
47. Poff NL, Matthews RA. Benthic macroinvertebrate community structural and functional group response to thermal enhancement in the Savannah River and a coastal plain tributary. Archiv für Hydrobiologie. 1986; 106(1):119-137.
48. Alcaraz C, Caiola N, Ibáñez C. Bioaccumulation of

pollutants in the zebra mussel from hazardous industrial waste and evaluation of spatial distribution using GAMs. Science of the Total Environment. 2011; 409(5):898-904.
49. Ibáñez C, Alcaraz C, Caiola N, Rovira A, Trobajo R, Alonso M, *et al*. Regime shift from phytoplankton to macrophyte dominance in a large river Top-down versus bottom-up effects. Science of the Total Environment. 2012; 416:314-322.
50. Suárez SA, Alcaraz C, Ibáñez C, Trobajo R, Barata C. Procambarus clarkii as a bioindicator of heavy metal pollution sources in the lower Ebro River and Delta. Ecotoxicology and Environmental Safety. 2010; 73(3):280-286.
51. Langford TE. Electricity generation and the ecology of natural waters. Liverpool University Press Liverpool. UK, 1983, 281-335.
52. Lessard JL, Hayes DB. Effects of elevated water temperature on fish and macroinvertebrate communities below small dams. River Research and Applications. 2003; 19(7):721-732.
53. Leuven RS, Slooter AH, Snijders J, Huijbregts AJ, Van Der Velde G. The influence of global warming and thermal pollution on the occurrence of native and exotic fish species in the river Rhine. In: Os AG (Ed.), NCR-days, sustainable river system. Centre for River Research. Netherlands, 2007, 62-63.
54. Wijnhoven S, Van Riel MC, Van Der Velde G. Exotic and indigenous freshwater gammarid species physiological tolerance to water temperature in relation to ionic content of the water. Aquatic Ecology. 2003; 37(2):151-158.
55. Durance I, Ormerod SJ. Evidence for the role of climate in the local extinction of a cool-water triclad. Journal of the North American Benthological Society. 2010; 29(4):1367-1378.
56. Sagarin, RD, Barry JP, Gilman SE, Baxter CH. Climate-related change in an intertidal community over short and long time scales. Ecological Monographs. 1999; 69(4):465-490.
57. Simard MA, Paquet A, Jutras C, Robitaille Y, Blier PU, Courtois R, *et al*. North American range extension of the invasive Asian clam in a St. Lawrence River power station thermal plume. Aquatic Invasions. 2012; 7(2):81-89.
58. Benzie JA. The colonisation mechanisms of stream benthos in a tropical river (Menik Ganga: Sri Lanka). Hydrobiologia. 1984; 111(3):171-179.
59. Khalaf G, Tachet H. Colonization of artificial substrata by macro-invertebrates in a stream and variations according to stone size. Freshwater Biology. 1980; 10(5):475-482.
60. IPCC. Climate Change The Physical Science Basis. Summary for Policymakers. Contribution of Working Group 1 to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. Cambridge, United Kingdom, 2013.
61. Hawkins CP, Hogue JN, Decker LM, Feminella JW. Channel morphology, water temperature, and assemblage structure of stream insects. Journal of the North American Benthological Society. 1997; 16(4):728-749.
62. Pritchard G, Harder LD, Mutch RA. Development of aquatic insect eggs in relation to temperature and strategies for dealing with different thermal environments. Biological Journal of the Linnean Society. 1996; 58(2):221-244.